Microbial load as Pollution Indicator in Water of El-Khadra Lake at Wadi El-Natroun, Egypt

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Abstract: Occurrence and survival of some classical bacterial indicators, (salmonellae group, total staphylococci and *Pseudomonas spp.)* in water samples at surface and one meter depth of El-Khadra lake have been studied as well as, cyanobacteria and fish lagoons were included for comparison. The results showed that, fecal streptococci and *Pseudomonas spp.* are not present in surface and deep lake water samples respectively, while other bacteria tested are presented. Similarly, salmonellae group and fecal coliform were absent in all water samples from the fish lagoon and the deep lake samples. In addition, the high and low log average counts of total viable bacteria incubated at 37 ºC for 24 hours were 7.5 and 3.4 /100m in cyanobacteria lagoon and surface lake water samples respectively. On the other hand, the high log average counts of total viable bacterial incubated at 22 ºC for 48 hours was 7.3 /100m in cyanobacteria lagoon, while the low recorded 3.67 /100m in surface water samples. The statistical analysis (log average) showed that, some factors such as human activity, sun ray and sedimentation as well as biological activity play role on the bacterial distribution in all water samples tested.
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Key words: Lake water, Classical bacterial indicators, Salmonellae group, Total staphylococci and *Pseudomonas spp.*

1. Introduction:

Water must be safe and free of risk factors. Risk factors related to water pollution, can be divided into two basic categories: chemical and biological pollutants. Both categories derive from human activity which inevitably tends to modify water composition, with respect to its original state in nature (Grabow, 1996 and Payment *et al*, 1997). The lake water sources must be protected from contamination by human and animal wastes which contains a variety of bacterial, viral, protozoan pathogens and helminthes parasites.

The heterotrophic plate count (HPC), gives a valuable indication of general microbiological quality of water. The test is widely used to monitor the water pollution, as well as the deterioration of water quality during storage and distribution (Reasoner *et al*, 1989; Grabow, 1996 and WHO, 2001).

Generally, the European Union regulation require that the HPC be assessed at two recovery temperatures: 22 ºC for 72 hrs and 37 ºC for 24 hrs. The 37 ºC plate count was believed to give an indication of fast-growing bacteria more likely to be related to pathogenic types and 22 ºC. Plate count was used for enumeration of characteristic water bacteria that tend to develop slowly (Ramalho *et al,* 2001).

Coliform group that have a wide distribution in the environment and are not specific to faecal material. *Enterobacter* (*Aerobacter*) *aerogenes* and *Enterobacter cloacae* are frequently found on various types of vegetation in soil and in polluted water. All of these coliforms group, may be found in sewage and in polluted water environment (Edeberg *et al,* 1990). The value of selected indicators for assessment of faecal pollution as well as the distinction of faecal pollution of human and animal origin has been investigated (Jagals *et al,* 1995).

Several Studies for the survival of faecal coliforms are numerous (Dawe & Penrose, 1978 and LaBelle *et al*, 1980). Most investigations have involved either soil or marine environments and have concentrated only on reduction in bacterial numbers over time. Studies in recent years have frequently revealed much higher numbers of indicator and pathogenic bacteria in sediments than in overlying waters. Apparently, higher concentrations of indicator and pathogenic bacteria in the sediments are due to a combination of sedimentation, sorption (which provides protection from bacteriophage and microbial toxicants (Weiss, 1951 and Roper& Marshall, 1978), and the phenomenon of extended survival in sediments (Goyal and Adams, 1984). The water quality testing criteria in use at present do not take into account sediments as a potential reservoir of pathogens. The higher numbers of pathogens occurring in sediments, along with increasing usage of recreational waters, creates a potential health hazard from resuspension and subsequent ingestion (Matson *et al*, 1978 and Grimes, 1980). Thus, there is a need to obtain additional information on the survival of indicator and pathogenic bacteria in sediments and the factors which contribute to their survival.

Enterococci have also been related to human disease becoming firmly established as major nosocomial pathogens (McDonald *et al*, 1997). In addition, *Enterococcus* and *Streptococcus* have been proposed as indicators of faecal contamination in water because of their high abundance in feces and their long survival in the environment. Although the ratio of faecal coliforms to faecal streptococci have been ruled out as indicator (Pourcher *et al*, 1991 & Olajide, 2010). With respect to the relative proportions of faecal coliforms and faecal streptococci, faecal streptococci species profiles whether these characteristics can be used to distinguish between human and animal effluent (Sinton and Donnison, 1994).

The presence of *Pseudomonas aeruginosa* in water indicates that the source has become polluted either by organic material or contamination. De Victorica and Galvan (2001) reported that *Pseudomonas aeruginosa* is used as indicator of health risk association with drinking water.

Pseudomonas aeruginosa is considered as opportunistic bacteria expresses virulence factors which are related to serious infections in human especially in immuno-compromised individuals and special precautions may be required to limit the exposure of these susceptible populations (Warburton 2000). It is also, widespread in natural and industrial environments and is able to grow in water (Leclerc and Da Costa, 1998).

Staphylococci have been recently investigated as possible indicator for pollution of swimming pool waters and other aquatic environment (De Araujo *et al*, 1990 and WHO, 2003). Because the staphylococci had a higher resistance to the chlorine level in pool waters than coliforms and streptococci, they were isolated when coliforms and streptococci were absent (Favero *et al*, 1964 and Antai, 1987).

The enumeration of either total staphylococci or specific *Staphylococcus aureus* appears to provide a useful index for the water quality level of the aquatic sources. Staphylococci are salt tolerant which survive in the marine environment (Gabutti *et al*, 2000 and Kamel, 2005).

Salmonellae grow at temperatures ranging from 7 to 48°C, at pH 4–8, and at water activities above 0.93 (Baird-Parker 1990). Salmonellae are capable of prolonged survival in faecal materials, in slurry, or on pasture (Wray & Sotka, 1977). The fact that salmonellae are able to survive and readily multiply in the environment is an important factor in the transmission and spread of salmonellosis. Examples quoted by Williams (1984) illustrate this: salmonellae will live for 28 months in naturally infected avian faeces; *S. heidelberg* was recovered from contaminated poultry litter, grit, feed, and dust held for extended periods at room temperature (the poultry litter was positive at 7 months); *S. thompson* survived 4–5 weeks in old poultry litter and 8–20 weeks in new litter; and *S. typhimurium* survived in urban garden soil in England for at least 280 days (WHO, 2003).

The specific objectives of this study are to describe the relative association of classical bacterial indicators as well as some pathogenic bacteria in Lake water samples during winter 2010 at Wadi El-Natron, Egypt and in lagoons for growing cyanobacteria and fish.

2. Materials and methods

Sample collection. Lake water samples were collected monthly during winter season 2010, from El-Khadra lake at Wadi El-Natron, Egypt. Water samples were collected in 1 liter sterile glass bottles and then transferred from the sites to the lab in ice box. Water sampling was taken at 0, 30 and 100 cm deep from El-Khadra lake. Water samples were taken also from cyanobacteria lagoon and fish lagoon to assess the pollution load.

Microbial load of water samples

Collected water samples were analyzed for total viable microbial count and total count of different bacterial indicators using the poured plates and the most probable number (MPN) technique, respectively.

Poured plates technique (APHA, 2005).

Method for decimal dilution of water samples was used for determination of total bacterial load, on nutrient agar (APHA, 2005). The plates were incubated for 1-2 days for fast growing bacteria at 37°C and 2-3 days at 22°C for characteristic water bacteria (APHA, 2005).

The most probable number technique [MPN] (APHA, 2005).

The most probable number technique was carried out for estimation of some microbial indicators in the tested water samples using special presumptive and confirmed tests for each indicator. During presumptive test, 5ml of each appropriate three decimal dilutions of raw water samples were used to inoculate five tubes (20 x 1.5cm^2) each containing 5ml of proper medium (single strength), and the tubes were incubated at 37°C for 48 hours. The positive presumptive tubes were used to inoculate the confirmed test which detected the bacterial indicators as following:-

- Total coliform; Lauryl tryptose broth medium was used for presumptive test. The positive tubes which showed gas and acid were used to inoculate brilliant

green lactose bile broth medium (BGB), as a confirmed test. The production of gas and acid was recorded as positive confirmed test for total coliforms (APHA, 2005).

- Faecal coliform estimation was carried out by inoculation in the EC broth tubes from positive BGB broth medium tubes, then incubated at 44.5°C for 24 hours (APHA, 2005). The positive tubes containing gas production were used to detect the count per 100 ml sample (MPN index / 100ml) and streak the eosin methylen blue agar medium (EMB) plates, then incubated at 37°C for 24 hours. Metallic sheen colonies considered as a positive confirmed results for *E. coli* presence (APHA 2005).
- Faecal streptococci; Azide dextrose broth was used as presumptive test without fermentation tubes. The positive tubes were turbid, (APHA, 2005), then used to inoculate ethyl violt azide broth medium, (EVA) as a confirmed test. The positive results were turbid after incubation at 37°C for 48 hours (Gerhardt, *et al*, 1981). The positive tubes were used for the confirmed test which detected by streaking on m-Enterococci medium (APHA, 2005).

Salmonellae groups was counted from inoculated buffer peptone water (British Standard Institute, 2002) tubes (incubated at 37°C for 24 hours) which used as MPN technique (Morinigo *et al*, 1992). One loop from these tubes were streaked on the plates of bismuth sulphate agar as a confirmed test. After incubation at 37°C for 48 hours, typical black colonies with or without metallic sheen and blackening extended beyond the colonies considered as confirmed positive results for the presence of salmonellae.

Staphylococcus sp was determined by streaking one loop from buffer peptone water tubes on the surface of mannitol salt agar plates, then incubated at 37°C for 24 hours. The growing colonies had yellow zones, flat and 1.2 mm diameters (APHA, 2005).

Pseudomonas sp. was detected in the lake water samples using asparagine broth medium as a presumptive test. The positive tubes produced a greenish fluorescent color after exposing to long-wave ultraviolet light were used to streak the surface of acetamide agar slants as confirmed test. a positive confirmed tubes with the purple color indicated high pH value after incubation at 37°C for 24 hours (APHA,2005).

3. Results and Discussion

The bacteriological quality of the collected water samples was evaluated by monitoring of the total bacterial counts, fecal bacterial indicators and some pathogenic bacteria in five different water body sites at Wadi El-Natron, Egypt.

The results in table (1) show that; generally the high log average counts of total viable bacterial counts were increased at 37°C and 22°C in water samples taken at 100 cm depth of the lake than that at 0 and 30 cm depth. Whereas, the high log average counts recorded 6.47 cfu at 22°C the low log average was 2.9 cfu at 37°C / 100ml in the water samples taken at 100 cm. These results agree with Mansour and Sidky, (2003) where they found total viable bacterial counts in Qarun lake water samples and Rayan lake water $5.8x10^6$ and $8.1x10^5$ respectively. Also Ali, *et. al.* (2008) counted average of the total bacteria at 37°C and they found that the counts reached 2.1 x 10^6/ 100ml in lake water sample. This may be due to the effect the sunlight (UV ray), organic substances, biological activity and sedimentation (Olayemi, 1993). Phillip *et al*, (1988) suggest that the decline in lake water of bacteria that are resistant to starvation may be a result of protozoan grazing and that the extent of growth of introduced species may be limited by the supply of available carbon and sometimes of nitrogen and phosphorus, and by predation by indigenous protozoa. In addition, Olayemi (1993) found that sun light was shown to cause sublethel injury to some bacteria from10^5 to 10 for *St. bovis* and 10^6 to10^5 for *E.coli* 1n 14 days. On the other hand, he noticed more decrease in the counts of these bacteria with exposure to UV irradiation from 10^5 to 10^2 and from 10^4 to not detected for *St. bovis* and *E.coli* respectively.

In 100 cm deep water samples of this lake the faecal streptococci were recorded log average 1.43 cfu / 100ml, while it was absent in the surface (0 cm) and sub-surface (30 cm) water samples. On the other hand, total coliforms were detected, with the high and low log average count 1.57 and 1.3 cfu / 100ml respectively. Moreover, faecal coliforms was present in surface and sub-surface water samples while they were absent in the bottom water samples. The average log numbers of fecal coliforms was 0.43 cfu / 100ml, these results conflicted with U.S. Environmental Protection Agency (2003). Ola et al, (2006) confirmed our investigation where they found that *E. coli* levels in three sites out of studied 11 sites of Michigan lake shore was less than the recommended U.S. Environmental Protection Agency (2003) limits, 235 cfu/100 ml while the others ranging from 0 to 6,900 cfu/100 ml. in addition, the authors suggested that *E. coli* and enterococci survival and growth in the direct sun light. Moreover, the ability of *E. coli* to survive for several days in aquatic sediment in situ suggests that faecal coliforms in water may not always indicate recent faecal contamination of that water but rather reuses pension

of viable sediment-bound bacteria Lalibertet and Grimes, (1982).

Table (2) shows the counts of all types of bacteria tested and it shows that the bacteria were excesses in water samples which collected from cyanobacteria and fish lagoons than lake in water samples. The bacterial count log average in water samples from cyanobacteria lagoon at 22°C, 37°C showed that total coliforms, fecal coliforms and fecal streptococci were 7.37, 7.5, 3.3, 2.13 and 2.13 / 100ml respectively. With regarded to the water samples collected from fish lagoon recorded 4.47, 4.3, 1.97, 1.13 and 1.43 cfu / 100ml for total bacterial counts at 22°C, 37°C, total coliforms, fecal coliforms and fecal streptococci respectively.

Table (1): The average log of the viable count of the total bacterial load as well as classical bacterial indicators in the tested water samples during winter season of 2010 at El-Khadra Lake, Wadi El-Natron, Egypt.

**Site of water samples	Date	Samples number	Log number of colony forming unit (cfu) / 100 ml				
			Total bacterial count at:-		Bacterial indicators *(MPN-index)		
			22 °C	37 °C	TC	FC	FS
Surface lake (0 cm)	12/1/2010	1	3.5	2.9	1.3	0	0
	9/2/2010	2	3.7	3.6	1.7	1.3	0
	9/3/2010	3	3.8	3.7	1.7	0	0
		Average	3.67	3.4	1.57	0.43	0
Sub-surface lake (30 cm)	12/1/2010	1	3.6	3.8	1.8	0	0
	9/2/2010	2	3.9	5.2	3.5	1.3	0
	9/3/2010	3	3.8	5.3	1.3	0	0
		Average	3.77	4.67	2.2	0.43	0
One meter deep of lake (100 cm)	12/1/2010	1	6.9	4.8	1.3	0	1.3
	9/2/2010	2	6.1	5.1	1.3	0	1.3
	9/3/2010	3	6.4	5.3	1.3	0	1.7
		Average	6.47	5.07	1.3	0	1.43

Note:
* TC: Total coliforms FC: Faecal coliforms FS: Faecal streptococci

Table (2): The average log off the viable count of the total bacterial load in water of El-Khadra Lake at Wadi El-Natroun, Egypt used in growing cyanobacteria and fish outside the water body of the lagoon.

Site of water samples	Date	Samples number	Log number of colony forming unit (cfu) / 100 ml				
			Total bacterial count at:-		Bacterial indicators (MPN-index)		
			22 °C	37 °C	TC	FC	FS
Cyanobacteria lagoon	12/1/2010	1	7.1	7.5	3.5	2.2	2.1
	9/2/2010	2	7.4	7.3	3.2	2.1	2.2
	9/3/2010	3	7.6	7.7	3.2	2.1	2.1
		Average	7.37	7.5	3.3	2.13	2.13
Fish lagoon	12/1/2010	1	4.3	4.7	1.7	0	1.3
	9/2/2010	2	4.5	4.8	2.1	1.7	1.3
	9/3/2010	3	4.6	4.5	2.1	1.7	1.7
		Average	4.47	4.3	1.97	1.13	1.43

This phenomenon may be due to the nutrients availability for growth of the bacteria. Niemi and Niemi (1991) and Putheti & Leburu (2009) reported that domestic and industrial wastewater, agriculture waste environment are sources of faecal bacterial to rivers. In these lagoons the water was found to be contaminated with pathogenic microorganisms, some of which originate

from animal wastes (Ho & Tam, 1998). Contamination of water sources by wastewater can pose a health risk due to the presence of pathogenic microorganism agents in water used for recreation, drinking and fishing Olajide, (2010). In Uruguay (Laguna de Rocha) piccini (2006) found that bacterial counts (4.3 x 10^5cfu / ml) were higher average three times in the brackish southern part of the lagoon than the freshwater, in addition they suggested that this may be a consequence of better growth conditions. Moreover, they reported that the lagoon receives domestic waste from a small town through its main tributary

Table (3) demonstrates the occurrence of some pathogenic bacteria (Salmonellae groups, *Staphylococcus sp.* and *Pseudomonas sp.*) in lake water samples [except at 100 cm depth of the lake for *Pseudomonas sp* and Salmonellae groups in fish lagoon water samples (table 4)]. The water samples from the lake water showed that Salmonellae groups were decreased and the log average count recorded 2.37, 2.17 and 1.57 cfu / 100ml for the surface, the surface, sub-surface and the bottom respectively.

Table (4) shows the occurrence of the same pathogenic group in the lake water transported to special tanks to grow cyanobacteria and fish. Nearly the log counts of Salmonellae groups were stable in all water samples collected from cyanobacteria lagoon while absent in fish lagoon. The log average counts of *Staphylococcus sp* were 1.7 / 100ml in cyanobacteria lagoon. Pe´rez *et al,* (2004) reported that protozoa can be grazing bacteria where they found to reduce the survival of another pathogenic bacterium, *Vibrio cholerae*, in brackish waters (Christoffersen, 2004).

Table (3): The log average viable count of some pathogenic bacteria in lake water samples during winter 2010 at Wadi El-Natron, Egypt.

* Site of water samples	Samples number	Date	Number of cell forming unit (cfu) / 100 ml		
			Salmonellae group	Total staphylococci	*Psudomonas spp.*
Surface lake	1	12/1/2010	2.3	4.4	2.4
	2	9/2/2010	2.6	4.1	1.9
	3	9/3/2010	2.2	4.6	2.6
	Average		2.37	4.37	2.3
Sub-surface lake (30 cm)	1	12/1/2010	2.1	4.6	1.3
	2	9/2/2010	2.2	4.5	1.7
	3	9/3/2010	2.2	4.4	1.3
	Average		2.17	4.5	1.43
One meter depth	1	12/1/2010	1.3	4.5	0
	2	9/2/2010	1.7	4.5	0
	3	9/3/2010	1.7	5.1	0
	Average		1.57	4.7	0

The results show that *Pseudomonas sp.* were absent in one meter deep of lake water samples while detected in others. This may be due to the competition between the microbes in aquatic environmente. Schallenberg *et al,* (2005) demonstrated that *Daphnia carinata* (40 cell /liter) were grazing type bacteria (*Campylobacter jejuni*) where it declined 2 orders (from 10^7 to 10^5/ ml) of magnitude in day.

The log counts of Salmonellae groups were stable in all water samples collected from cyanobacteria lagoon while decline in fish lagoon. These results conflict those of Khatun1, et al 2007, who found the Salmonellae groups (in the average of 3.3 x 10^6 cfu / 100ml.) in swamp water sample used in growing the fish during November 2001 to October 2002. On the other hand, these results agree with those of Sangu, et al (1985) who reported that the total population of bacterial species specially *Escherichia coli, Aerobacter aerogenes, Staphylococcus aureus*, *Pseudomonas sp.* and *Micrococcus sp*. were considerably high in Keetham Lake (India) water sample.

In this investigation, the count log average of *Pseudomonas* sp. Recorded high values being 3.6/100ml in water samples collected from fish lagoon (table 4). On the other hand, the results show the log counts of *Staphylococcus* sp. were from 1.7 to 4.7/100ml which detected in water samples from cyanobacteria lagoon and one meter deep of the lake (table 3&4).

The *Pseudomonas sp.* Were absent in one meter deep of lake water samples while detected in others (table 3). These results agree with Ahmed and Naim, (2006) where they found that *Flavobacterium* sp., *Micrococcus* sp., *Streptococcus* sp., *Burkholderia*

glumae and *Pasteurella* sp. were present in some seasons of the year as well as *Pseudomonas fluorescens* and *Salmonella* sp. were present only in winter, whereas *Pasteurella pneumotropica* was found only in summer. In addition, results agree with Naim and Ahmed, (2004) where they found that. *Flavobacterium* sp. and *Pseudomonas* spp. were dominant only in the winter. This may be due to ambient seasonal temperature variation could account for some of the bacterial population variation.

The counts and the type of bacteria in the fish lagoon indicated special variation which might be due to the intestinal flora of fish is characterized by both the day-to-day and individual-to-individual variations (Sugita, 1990).

It is necessary to assess the microbial pollution load which is likely related to the chemical composition of the water in lake throughout the seasonal variations to which the lake water is exposed. This particularly important when the lake water is used for production of special biomaterials for potential commercial uses.

Table (4): The log average viable count of some pathogenic bacteria in water of El-Khadra Lake at Wadi El-Natron, Egypt used in growing cyanobacteria and fish outside the water body of the lagoon.

Site of water samples	Samples number	Date	Number of cell forming unit (cfu) / 100 ml		
			Salmonellae group	Salmonellae group	Salmonellae group
Cyanobacteria lagoon	1	12/1/2010	1.3	1.7	3.1
	2	9/2/2010	1.3	1.3	1.9
	3	9/3/2010	1.3	2.1	2.2
	Average		1.3	1.7	2.4
Fish lagoon	1	12/1/2010	0	4.1	3.2
	2	9/2/2010	0	4.2	3.5
	3	9/3/2010	0	4.4	4.1
	Average		0	4.23	3.6

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4. References:

1. Ahmed, H.and Naim, M. (2006). Seasonal changes in bacterial flora of fish pond sediments in Saudi Arabia. J. Appl. Aquacult. 18: pp. 35 – 45.
2. Ali, F. K.; El-Shafai, S. A.; Samhan, F. A. and Khalil, W. K. (2008). Effect of water pollution on expression of immune response genes of *Solea aegyptiaca* in Lake Qarun. Afri. J. Biotechno. 7: pp. 1418- 1425.
3. Antai, S. P. (1987). Incidence of *Staphylococcus aureus*, coliforms and antibiotic-resistant strains of *Escherichia coli* in rural water supplies in Port Harcourt. J. Appl. Bacteriol., 62: 371 – 375.
4. APHA [American Public Health Association], (2005). Standard Methods for the Examination of Water and Wastewater 21th ed. APHA, Inc. Washington, D C.
5. Baird-Parker, A. C. (1990). Food borne salmonellosis. The Lancet, 339: pp. 1231 – 1235.
6. British standard Institute (BSI), (2002). BSEN ISO 6579 incorporating corrigendum. No. 1. Microbiology of food and Animal Feeding Stuffs-horizontal method for the detection of salmonella spp. London: BSI
7. Christoffersen, K.; Ahl, T. and Nybroe, O. (2004). Grazing of nonindigenous bacteria by nano-sized protozoa in a natural coastal system. J. Microbial Ecol. 30: pp. 67 - 78.
8. Dawe, L. L., and Penrose, W. R. (1978). Bactericidal" property of seawater: death or

debilitation?. J. Appl. Environ. Microbiol., 35: pp. 829-833.
9. De Araujo, M. A.; Guimaraes, V. F.; Mendonca-Hagler, L. C. and Hagler, A. N. (1990). *Staphylococcus aureus* and faecal streptococci in fresh and marine waters of Rio De Janeiro, Brazil. Rev. Microbiol., 21(2): 141–147.
10. De Victoria, J. and Galvan, M. (2001). *Pseudomonas aeruginosa* as an indicator of health risk in water for human consumption. Wat. Sci. & Tech., 43(12): 48– 52.
11. Edberg, S. C.; Allen, M. J.; Smith, D B. and Kriz , N. J. (1990). Enumeration of total coliforms and *Escherichia coli* from Source Water by the Defined Substrate Technology. Appl. Environ. Microbiol., 56(2): 366 – 369.
12. Favero, M. S.; Drake, C. H. and Randall, G. B. (1964). Use of staphylococci as indicators of swimming pool pollution. Pub. Hlth. Rep., 79: 61 – 70.
13. Gabutti, G.; De Donno, A.; Bagordo, F. and Montagna, T. (2000). Comparative survival of faecal and human contaminants and use of *Staphylococcus aureus* as an effective indicator of human pollution. Mar. Poll. Bulletin, 40(8): 697-700
14. Gerhardt, P.; Murray, R. G.; Costilow, N. R.; Nester, W. E.; Wood, A. W.; Krig, R. N. and Phillips, B. G. (1981). American Society for Microbiology Manual of Methods for General Bacteriology, Washington, D.C.
15. Goyal, S. M., and Adams, W. N. (1984). Drug-resistant bacteria in continental shelf sediments. J. Appl. Environ. Microbiol., 48: 861- 862.
16. Grabow, W. O. K. (1996). Waterborne diseases: Update on water quality assessment and control water SA., 22: 193 – 202.
17. Grimes, D. J. (1980). Bacteriological water quality effects of hydraulically dredging contaminated upper Mississippi River bottom sediment. J. Appl. Environ. Microbiol., 39: 782 - 789.
18. Ho, B. S. W. and Tam, T. Y. (1998). Giardia and Cryptosporidium in sewage contaminated river waters. Wat. Rec., 32: 2860 – 2864.
19. Jagals, P.; Grabow, W. O. K. and De-Villiers J. C. (1995). Evaluation of indicators for assessment of human and animal faecal pollution of surface runoff. Wat. Sci. Techol. 31 (5-6): 235 – 241.
20. Kamel, M. M. (2005). Evaluation of various selective and modified media for recovery of *Staphylococcus aureus* from aquatic environments. Egypt. J. Appl. Sci., 20(8A): 11 – 20.
21. Khatun1, H.; Afza1, R.; Hossain1, M.; Hussain, M.; Khan1, A.; Rahman1, M. and Ashrafi N.(2007). Load of *Aeromonas salmonicida* in swamp water and it's effect on tilapia (*Oreochromis mossambicus*). Bio-Sci. 15: 165 - 168.
22. LaBelle, R. L.; Gerba, C. P.; Goyal, S. M. J.; Melnick, L.; Cech, I. and Bogdan, G. F. (1980). Relationships between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. J. Appl. Environ. Microbiol., 39: pp. 588 - 596.
23. Leclerc, H. and Da Costa, M.S. (1998). The microbiology of natural mineral waters. In Technology of Bottled Water (ed. D.A.G. Senior and P. Ashurst), pp. 223–274, Academic Press, Sheffield.
24. Lalibertet, P. and Grimes, D. (1982). Survival of *Escherichia coli* in Lake Bottom sediment. J. Appl. Environ. Microbiol., 43: pp. 623 - 628.
25. Mansour, S. A. and Sidky, M. M. (2003). Ecotoxicological Studies. 6. The first comparative study between Lake Qarun and Wadi El-Rayan wetland (Egypt), with respect to contamination of their major components. Food Chem. 82: pp. 181 – 189.
26. Matson, E. A., Hornor, S. G. and Buck, J. D. (1978). Pollution indicators and other microorganisms in river sediment. J. Wat. Pollut. Cont. Fed., 50: 13 - 19.
27. McDonald, C. L.; Kuehnert, M. J.; Tenover, F. C. and Jarvis, W. R. (1997). Vancomycin-resistant enterococci outside the health-care setting: prevalence, sources, and public health implications. J. Emerg. Infect. Dis., 3: 311–317.
28. Morinigo, M. A.; Muňoz, M. A.; Cornax, R. Martinez-Manzanares, E. and Borrego, J. J. (1992). Presence of indicators and Salmonella in natural waters affected by outfall wastewater discharges. Wat. Sci. Technol., 25: 1 – 8
29. Naim ,M. and Ahmed, H. (2004). Seasonal variation of bacterial flora in ponds in Saudi Arabia used for tilapia aquaculture. J. Appl. Aquacult. 16: pp. 53 – 61.
30. Niemi, R.M. and Niemi, J. S. (1991). Bacterial pollution of waters in pristine and agricultural lands. J. Environ. Qual. 20: 620 – 627.
31. Ola A. Olapade, Morgan M. Depas, Erika T. Jensen, and Sandra L. McLellan (2006). Microbial communities and fecal indicator bacteria associated with *Cladophora* mats on beach sites along Lake Michigan shores. J. Appl. Environ. Microbiol., 72: pp. 1932–1938.
32. Olajide A. A. (2010). Characterization of surface and ground water with reference to microbiological study in Akungba-Akoko, Ondo

State. J. Microbiology and Antimicrobials Vol. 2(1). pp. 11- 127.
33. Olayemi, A. B. (1993). Survival of *Escherichia coli* and some pathogenic bacteria in pond water exposed to solar and UV-irradiations. Biosci. Res. Commun., 5: pp. 11 – 127.
34. Pe´rez, M., Macek, M. and Galva´n, M. (2004). Do protozoa control the elimination of *Vibrio cholerae* in brackish water?. Int. Rev. Hydrobiol., 89: 215 – 227
35. Payment P.; Siemiatycki J.; Richardson L.; Renaud G. and Prevost M. (1997). A prospective epidermislogical study of gastrointestinal health effects due to the consumption of drinking water Int J. Environ. Hlth. Res., 7: 5 – 31.
36. Phillip, R.; John, P. and Martin, A. (1988). Factors affecting the survival and growth of bacteria introduced into lake water. Arch. of Microbiol., 150: 320 - 325.
37. Piccini, C.; Conde, D.; Alonso, C.; Sommaruga, R. and Pernthaler, J. (2006). Blooms of single bacterial species in a coastal lagoon of the southwestern Atlantic Ocean. J. Appl. Environ. Microbiol., 72: pp. 623-628.
38. Pourcher A. M.; Devriese, L. A.; Hernaders, J. F. and Delattre, J. M. (1991). Enumeration by a miniaturized method of *Escherichia coli, Streptococcus bovies* and Enterococcus indicates of origin of faecal pollution of waters. J. Appl. Bacteriol., 70: 525 – 530.
39. Putheti R. and Leburu R. (2009). Role of probiotics and their influence in different physico-chemical and microbiological studies of water – a case study. Intern. J. Fisher Aquacult. 1(1): 1- 4
40. Ramalho, R.; Cunha, J.; Teixeira, P. and Gibbs, P. A. (2001). Improved methods for enumeration of heterotrophic bacteria in bottled mineral waters. J. of Methods 44: 97 - 103.
41. Reasoner, D. G.; Blannon, J. C.; Geldreich, E. E. and Barnick, J. (1989). Nonphotosynthetic pigmented bacteria in a potable water treatment and distribution system. Appl. Environ. Microbiol., 55(4): 912-921.
42. Roper, M. M., and Marshall, K. C. (1978). Effects of salinity on sedimentation and of particulates on survival on bacteria in estuarine habitats. J. Geomicrobiol., 1: 103 - 116.
43. Sangu, R.; Sharma, K; Pathak, P and Sengar, S. (1985). Characteristics of water of Keetham Lake. J. Environ. and Ecol. 3: pp. 386 - 389.
44. Schallenberg, M.; Bremer, P.; Henkel, S.; Launhardt,A. and Burns, C. (2005). Survival of *Campylobacter jejuni* in water: effect of grazing by the freshwater crustacean *Daphnia carinata* (Cladocera) J. Appl. Environ. Microbiol., 71: pp. 5085 - 5088.
45. Sinton L.W. and Donnison A. M. (1994). Characterization of faecal streptococci from some New Zealand effluents and receiving waters. New Zealand J. Mar. & Freshwater Res., 28: 145–158.
46. Sugita, H; Miyajima, C ; Kobiki, Y and Deguchi, Y.(1990). The daily fluctuation and inter-individual variation of the faecal flora of carp, Cyprinus carpio L. J. Fish Biol. 36: pp. 103 - 105.
47. U.S. Environmental Protection Agency (2003). Bacterial water quality standards for recreational waters. EPA-823-R-03-008. U.S. Environmental Protection Agency Office of Water, Washington, D.C.
48. Warburton, D. W. (2000). Microbiology for screening bottled water for the presence of indicator and pathogenic bacteria. Intern. J. Food Microbiol., 17: 3 – 12.
49. Weiss, C. M. (1951). Adsorption of *Escherichia coli* on river and estuarine silts. Sew. Ind. Wast. 23: 227 - 237.
50. WHO [World Health Organization] (2001). Water Quality: Guidelines, Standards and Health. Edited by Lorna Fewtrell and Jamie Bartram. Published by IWA Publishing, London, UK. ISBN-900222-280 World Health Organization.
51. WHO [World Health Organization] (2003). Heterotrophic Plate Counts and Drinking-water Safety. Edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker, A. Glasmacher. Published by IWA Publishing, London, UK. ISBN: 1 84339 025 6.
52. Williams, J. E. (1984). Paratyphoid infections. In Diseases of Poultry, 8th edn (ed. M.S. Hofstad), chapter 3, Iowa State Univ. Press, Ames, IA.
53. Wray, C. and Sojka, W. J. (1977). Reviews of the progress of dairy science: bovine salmonellosis. J. Dairy Res.

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