Middle-East Journal of Scientific Research 4 (4): 341-347, 2009
ISSN 1990-9233

Assessment of Bottled Water Quality Using Microbial Indicators

[1]G.A. Osman, [2]M.S. Ali, [1]M.M. Kamel and [3]A.Z. Al-Herrawy

[1]Bacteriology Labortary, Water Pollution Research Department,
National Research Center, Dokki, Giza, Egypt
[2]Agriculture Microbiology Department, National Research Center, Dokki, Giza, Egypt
[3]Parasitology Labortary, Water Pollution Research Department,
National Research Center, Dokki, Giza, Egypt

Abstract: The microbiological quality of bottled water from different manufacturing companies (A, B, C and D) was studied from May 2008 until April 2009. The bottled water were collected from the local markets in Greater Cairo, Egypt and stored under three different conditions for one month. The first condition was at room temperature, the second one was the exposure to direct sun light in boxes of glasses and the third condition was storing at 10°C. According to American Public Health Association, all water samples were examined microbiologically. The results showed that all the examined bottled water samples were free from total coliforms, faecal coliforms, faecal streptococci, sulphite reducing bacteria (clostridia), salmonellae group, total vibrios and free living amoebae. Total staphylococci, *Pseudomonas aeruginosa*, total yeasts and coliphage were recovered from some bottled water samples. The counts of *Pseudomonas aeruginosa*, yeasts, total staphylococci and total bacterial counts (at 22°C and 37°C) decreased in all the samples exposed to the direct sun light for one month. On the other hand, the counts of *Pseudomonas aeruginosa*, yeasts and total bacterial counts increased, while those of total staphylococci decreased in samples stored at room temperature. Generally, yeasts, total bacterial counts, total staphylococci and *Pseudomonas aeruginosa* were detected in bottled water samples stored at 10 °C for one month. In addition, coliphage were detected under the three different conditions only for one week of storage. The results of microbiological revealed that the bottled water is more safe when stored at refrigerated or cool places.

Key words: Bacterial indicators · Bottled water · *Staphylococci* · *Pseudomonas* · Yeasts · Coliphage, Free-living amoebae

INTRODUCTION

The microbiological quality of bottled water is of great interest as many consumers us it as an alternative to tap water and consider it to be better and safer. The quality of water is determined largely by bacteriological analysis. In bottled waters, the bottling process may be a source of additional contamination [1]. In addition, the common sources of contamination of bottled water are equipment, bottles and caps, exposure to air and contact with humans during the bottling process [1, 2]. Although the microbial quantity levels in processed water are often initially low, they can evolve rapidly to high levels during storage [3]. This rapid growth of bacteria after the water is bottled may be due to oxygenation of the water during processing, the increase in surface area provided by the bottle, the increase in temperature and the amount of nutrients arising from the bottle [4, 5]. Guerzoni *et al.* [6] reported that some bacteria can multiply on polyvinyl chloride of ultra marine blue dye in bottle plastic material. Another factor to be taken into account is whether the water is carbonated. The decrease in pH resulting from carbonation acts to prevent bacterial growth [7]. The multiplication of bacteria was observed 1 to 3 weeks after bottling bacterial counts ranged from 103 to 140 cfu /ml at 37°C in the absence of chlorine or ozone treatment [8]. The microbial contamination of bottled water occurred most likely due to improperly cleaned equipment and bottles, failure of ozonation or UV equipment or due to contamination of the water by workers [9].

Corresponding Author: G.A. Osman, Bacteriology Labortary, Water Pollution Research Department,
National Research Center, Dokki, Giza, Egypt.

The water sources from which the bottled water is produced must meet a number of microbiological and physicochemical standards. In addition, the water source should be free from parasites and pathogenic microorganism [2]. The approved sources for bottled water may be springs, wells, or other sources that have been analyzed and found to be safe with or without treatment [10]. The overall treatment of the water source depends on the initial quality of water [11]. Bottled water is generally not sterile and may contain bacteria from naturally occurring source as well as those introduced during manufacturing and consumer handling [12].

Pseudomonas aeruginosa has been found in some mineral waters in various countries such as Brazil, Canada, France, Germany, Spain, United States and others [13]. Examination of drinking water for *Pseudomonas aeruginosa* is not recommended as a routine procedure, but it can be used as an indicator of good manufacturing processes and suitability for drinking water. Bacteria belonging to the genus *Pseudomonas* are widely spread in the environment and are often opportunistic bacteria, for many episodes of infections [13]. During the period of storage the growth of *Pseudomonas aeruginosa* may lead to a risk for consumers especially the immunologically weak persons, as well as very young or elderly ones [14]. In addition, Mardani *et al.* [15] reported that the initial microbial counts of the examined bottled water moderately increased during the increase of storage time at room temperature. Improvement of the quality of bottled water will be achieved only by improving the manufacturing processes and subsequent storage condition [16]. A survey of bottled water conducted in the United Arab Emirates, where about 90% of the population drink bottled mineral water, showed that out of 20-1.5 liters bottles, 40% were bacteriologically contaminated [17].

The prevalence of waterborne diseases in some cities has caused the business of bottled water to flourish, as many people believe that bottled water is completely safe [18]. It was reported that persistence of microbes in bottled water was greatly affected by several factors, of which temperature was the most important [19].

So, the objective of this study is to assess the effect of different storing temperatures on the viability and productivity of microbial contents of some bottled water of the Egyptian market.

MATERIALS AND METHODS

A total of 144 bottles (bottled water 1.5 liters volume each) were seasonally collected from (36 bottles each season) from Greater Cairo markets, in Egypt during one year period from May 2008 until April 2009. All examined bottled water pursued within were a month from a production date. Bottles were equally collected from produce of 4 different bottled water companies namely A, B, C and D. Each season bottles were equally divided into 3 groups (9 bottles from produce of each company under examination). Bottles of first group (12 bottles) were stored at room temperature for 30 days and acted as a control group. Bottles of second group (12 bottles) were exposed to direct sunlight for 30 days. Bottles of last group (12 bottles) were stored at 10°C for 30 days. Bottled water subjected to these different storage conditions were weekly tested fore the following biological parameters:

Enumeration of Classical Bacterial Indicators: Total bacterial count (at 22°C and 37°C), total coliform, feacal coliform and feacal streptococci were examined using pour plate and MPN methods according to APHA [20] for drinking water.

Enumeration of Coliphage: The host *E. coli* culture was prepared by inoculation of 10 ml trypticase soy broth containing 10% glycerin with a loop *E. coli* C (ATCC 137706), grown on triptcase soy agar [TSA] for 24 hours as a stock slant culture and incubated overnight at 35°C. About 1.0 ml of host *E. coli* C (ATCC 137706) culture was transferred to the tubes contain 5 ml concentrated bottled water (100ml) and 0.08 ml from Triphnyle tetrazollium chloride 1% (TTC). The content of this tube was added to another tube containing 10 ml TSA medium, then mixed and poured into petri dishes, after incubation at 35°C, the coliphage plaques were counted during 4 to 6 hrs of incubation [20].

Detection and Enumeration of *Pseudomonas aeruginosa*: Asparagine broth medium was used as a presumptive test. The positive tubes produced a greenish fluorescent color after exposure to long-wave ultraviolet light. These tubes were used to streak the surface of acetamide agar slants as a confirmatory test. Positive confirmed tubes gave the purple color indicating a high pH value after incubation at 37°C for 24 hours [20].

Detection of Total Yeasts, Salmonellae Group and Total Vibrios: This was carried out using membrane filter technique. A 100 ml of each type bottled water samples were separately filtrated through the membrane filter (0.45 ìm pore size and 47 mm diameter). The membrane was transferred onto selective media [20].

Detection and Enumeration of Sulphite Reducing Clostridia: Sulphite reducing bacteria were detected and enumerated in the tested bottled water samples according to Fewtrell *et al.* [21].

Detection of Staphylococci by Using Membrane Filter Technique: After filtration of the bottled water samples, the membrane was transferred onto the surface of manitol salt agar plates and then incubated at 37°C for 24 hours. The growing colonies were flat with yellow zones, measuring 1.2 mm in diameter [20].

Detection of Free Living Amoebae: Free-living amoebae were detected in bottled water according to the method of Ali and Al-Herrawy [22]. Briefly, water samples (one liter volume each) were reparatory filtrated through nitroacetate membrane filters (1.2 im pore size and 0.47 mm diameter). After filtration, the membrane was inverted face to face on the surface of non-nutrient (NN) agar plates previously seeded with 0.1 ml living *E. coli*. The inoculated plates were incubated at 22°C for 7 days with daily examination for the presence of any amoebic growth [23].

RESULTS AND DISCUSSION

Bottled water quality is often related to the degree of bacterial and chemical contamination. The count of bacteria in bottled water is generally dependent on the disinfection process of natural spring from which bottled water is produced [17]. According to the increase of demand and consumption of bottled water in Egypt, samples of bottled water were obtained from four different companies in Greater Cairo during May 2009 until April 2010 and examined for total viable count of bacteria (at 22°C and 37°C), total coliform, feacal streptococci, staphylococci, *Pseudomonas aeruginosa*, sulphite reducing bacteria, salmonellae vibrios groups, yeast and coliphage as well as free-living amoebae.

Data presented in Table 1 showed that the average of initial total bacterial counts increased at 22°C and 37°C with the increase of storage time at room temperature. The average counts of total bacteria were at 22°C was higher than those at 37°C. The average total bacterial counts at 22°C increased from 216 and 153 to 325 and 299 cfu/100 ml produce C and D bottled water companies, respectively. Also, the average total bacterial counts of bottled water at 37°C increased from 205 and 207 cfu /100 ml for produce B and C companies, respectively. These results are in agreement with those obtained by Emmanuel *et al.* [24] who found that the mean log of initial total bacterial counts were 0.10 and 0.30, but after storing bottled water for a month at room temperature, the count reached 0.39 and 0.45 cfu/100 ml at 22°C and 37°C, respectively. In addition, Leclerc H. and Moreau [25] reported that bottled water generally have high bacterial counts as a result of natural biological process resulting mainly from multiplication of these bacteria that were present in low numbers in the water source. Moreover the heterotrophic bacteria are capable of multiplying in low-nutrient water especially at room temperature which is considered as the optimum temperature for multiplication [26,27].

The obtained data in the present work showed that, although the classical bacterial indicators, sulphite reducing bacteria, salmonellae and vibrios groups as well as free-living amoebae were not detected in all sample, staphylococci, *Pseudomonas aeruginosa*, total yeasts and coliphage were present. These results are in agreement with those obtained by Abou-Ali [28] who reported that faecal indicator microorganisms were not observed in tested bottled water, while the most prevalent bacteria were *Bacillus sp.* and *Pseudomonas aeruginosa*.

Regarding to the additional microbial indicators used for testing bottled water, in the present study data given in Table 2 revealed that all kinds of bottled water contained staphylococci, *Pseudomonas aeruginosa*, yeast and coliphage. The presence of these microorganisms indicated that the pollution could be originated from organic materials or domestic wastewater contamination during the bottling process [29,30]. The highest average values of *Staphylococcus spp.* were noticed in type D followed by type B at room temperature being 45 and 41 cfu / 100 ml, respectively. Other works reported that staphylococci can be considered as a principal bacterial indicator showing a significant relation with physico-chemical character and phytoplankton biomass [31]. In addition, the detection of *Candida albicans* and *Staphylococcus spp.* was suggested as complementary tests for the evaluation of water pollution [32, 33].

Bottled water can be an oligotrophic environment with sufficient nutrients to maintain autochthonous bacterial growth [34] and growth of bacteria in stored bottles has been reported as a result of specific bottling-materials [35] that can release organic matter and provide additional substrates for the microbial growth during storage period [36]. Data in Table 2 showed that the

Table 1: Average numbers of total viable bacterial counts (cfu/100ml) in four brands (A. B. C. and D) of bottled water samples exposed to direct sun light, at room temperature and/or stored at 10°C for a month during May 2008 until April 2009

		Total viable bacterial counts /100ml at:					
		22 °C			37 °C		
Sample	Time	The sun	The room	at 10 °C	The sun	The room	at 10 °C
A	0	72	105	88	112	118	135
	1w	68	144	95	88	113	109
	2w	46	149	103	69	125	107
	3w	33	168	92	35	133	99
	4w	18	177	98	31	145	103
B	0	177	189	158	188	205	184
	1w	124	211	124	166	217	111
	2w	103	224	133	126	223	122
	3w	66	218	128	103	234	117
	4w	49	236	116	98	265	109
C	0	204	216	199	181	207	188
	1w	188	281	162	177	216	121
	2w	152	293	125	151	233	135
	3w	103	312	136	118	246	127
	4w	88	325	129	104	258	118
D	0	166	153	173	153	144	135
	1w	133	185	112	143	168	105
	2w	117	198	118	124	177	98
	3w	102	263	107	118	195	113
	4w	91	299	114	109	221	115

W = week

Table 2: Average counts of additional microbial indicators in four brands (A. B. C. and D) of bottled water samples exposed to direct sun light, at room temperature and/or stored at 10°C for a month during May 2008 until April 2009

		Additional microbial indicators / 100 ml											
		Total staphylococci			*Pseud. aerginosa* MPN-index			Yeast CFU			Coliphage PFU		
Sample	Time	The sun	The room	at 10°C	The sun	The room	at 10°C	The sun	The room	at 10°C	The sun	The room	at 10°C
A	0	10	9	9	7	12	15	24	28	32	4	6	4
	1w	4	7	7	4	23	18	15	41	22	0	0	0
	2w	1	5	11	2	33	16	9	47	18	0	0	0
	3w	0	1	12	0	73	21	7	52	25	0	0	0
	4w	0	0	8	0	70	19	0	58	19	0	0	0
B	0	36	41	33	23	33	29	32	35	42	6	8	9
	1w	28	39	35	16	46	24	19	45	33	0	0	0
	2w	16	24	29	9	70	32	11	51	27	0	0	0
	3w	6	19	28	7	79	33	8	63	35	0	0	0
	4w	0	13	31	0	94	38	0	72	36	0	0	0
C	0	26	31	28	36	49	47	29	36	42	9	9	7
	1w	18	19	29	49	70	44	24	42	29	0	0	0
	2w	6	11	24	33	94	41	16	55	25	0	0	0
	3w	1	7	35	27	110	49	12	71	31	0	0	0
	4w	0	2	23	0	130	39	0	77	38	0	0	0
D	0	49	45	37	70	79	57	36	41	48	10	11	8
	1w	31	32	33	49	110	49	29	49	31	0	0	0
	2w	19	23	39	34	130	52	7	61	37	0	0	0
	3w	9	12	35	26	130	55	5	69	34	0	0	0
	4w	0	4	32	0	135	46	0	79	38	0	0	0

W = week

Total coliform, feacal streptococci, salmonellae group, total vibrios and sulphite reducing clostridia as will as free living amoebae were not detected at zero time and after week storage.

average counts of *pseudomonas aeroginosa* increased from 79 and 49 MPN-index/100 ml to be 135 and 130 MPN-index/100 ml for products of the companies D and C, respectively during storing for a month room temperature. These results are confirmed with those obtained by Tamagnini and Gonzalez [37] who stated that *Pseudomonas aerugionsa* can multiply and reach to very high numbers after water bottling. Also, during the period of storage the growth of *Pseudomonas aeruginosa* may lead to a risk for consumers especially the immunologically weak persons and very young or elderly ones [14].

With regarding to the additional microbial indicators, data given in Table 2 showed that the average counts of yeasts which increased in all samples to be 58, 72, 77 and 79 cfu/100 ml for companies A, B, C and D, respectively during one month storage at room temperature. These results are in agreement with Yamaguchi *et al.* [38] who found that 22 bottles (36.6 %) from 60 bottles contained yeasts and it could be transferred in the distribution system in Brazil. In addition, determination of *Candida albicans* and *Staphylococcus spp.* were suggested to be considered as complementary tests for the evaluation of water pollution [32, 33]. The presence of yeasts in different types of tested bottled water is considered new indicators for pathogenic microorganisms. Although the bacterial indicators and some pathogenic bacteria were absent, yeasts were detected in Egyptian drinking water by Samhan [39] in log number of count ml ranged from 0.15 to 0.78 cfu / 100 and also by Shaban and El-Taweel [40] with a range from 3 to 8 cfu / 100 ml.

Data presented in Table 2 showed that the average counts of coliphage completed disappeared after storing at room temperature and sunlight in the four products (A, B, C and D) of bottled water samples after one month storing period. These results are in agreement with those obtained by Gassilloud *et al.* [41] where they found that the enteric viruses were reduced 4 logs from 5 logs after only 19 days at 10, 20 and 35°C of mineral ground water contamination. Also, Ehlers *et al.* [42] reported detectable coliphages in two different bottled water products out of 10 undetectable coliphages in 10 different bottled water products after 3 months storing period. The presence of phage, which is typically associated with human and animal excreta, indicates the potential presence of enteric viruses [43]. Moreover, coliphage was used by El-Abagy *et al.* [29] as a more specific index of faecal pollution. On other hand, generally, the average counts of detectable the microbes of all bottled water samples in the present work decreased in samples exposed to the sunlight for a month. Also, *Pseudomonas aeruginosa* survived more than other tested microorganisms. These results are in harmony with Obiri-Danso *et al* [44] who used sunlight to sterilize contaminated drinking water in Kumasi, Ghana.

Generally, the counts of all tested microorganisms were stable except the phages during the storage period in cooling conditions. These data comply with APHA [20]. On other hand, the counts of all tested microorganisms in water samples which exposed to sunlight were decreased during the storage period and completely disappeared at the end of storage period. Although the sunlight causes elimination of microbes from bottled water during exposure of bottled water to direct sunlight inhibits or even kills the microbes after storage period, hazardous and undesirable risks could happen due to prolonged photo-degradation and leaching of some carcinogenic chemical compounds from the plastic materials of the bottles [45]. Therefore, it was recommended that bottled water should be stored in a cool, dry places in the absence of sunlight to avoid health hazards from microbial and/or chemical contamination [46].

REFERENCES

1. Davies-Colley, R., R. Bell and A. Donnison, 1994. Sunlight inactivation of faecal coliforms and faecal streptococci in sewage effluent attributed to sea water. Appl. Environ. Microbiol., 60: 2049-2058.
2. Warburton, D.W., B. Harrison, C. Crawford, R. Foster, C. Fox, L. Gour and P. Krol, 1998. A further review of the microbiological quality of bottled water sold in Canada: 1992-1997 survey results. Intl. J. Food Microbiol., 39: 221-226.
3. Stickler, D.J., 1992. The microbiology of natural water. J. Roy. Soc. Health, 4: 118-124.
4. Warburton, D.W., B. Browen and A. Konkle, 1992. The survival and recovery of *Pseudomonas aeruginosa* and its effect upon Salmonellae in water: Methodology to test bottled water in Canada. Can. J. Microbiol., 40(12): 987-992.
5. Warburton, D.W., 2000. Microbiology for screening bottled water for the presence of indicator and pathogenic bacteria. J. Food Microbiol., 17: 3-12.
6. Guerzoni, M.E., R. Lanciotti, M. Sinigaglia and F. Garidini, 1994. Analysis of the interaction between autochthonous bacteria and packaging material in PCA-bottled mineral water. Microbiol. Res., 149(2): 115-122.

7. Moreira, L., P. Agostinho, P.V. Morais and M.S. daCosta, 1994. Survival of allochthonous bacteria in still mineral water bottled polyvinyl chloride (PVC) and glass. J. Appl. Bacteriol., 77: 334-339.
8. Gonzalez, C., C. Gutierrez and T. Grande, 1987. Bacterial flora in bottled uncarbonated mineral drinking water. Can. J. Microbiol., 33: 1120-1125.
9. Venieri, D., A. Vantarakis, G. Kominou and M. Papapetropoulou, 2006. Microbiological evaluation of bottled non-carbonted (still) water from domestic brands in Greece. Int. J. Food Microbiol., 107: 68-72.
10. Ramalho, R., J. Cunha, P. Teixeira and P.A. Gibbs, 2001. Improved methods for enumeration of heterotrophic bacteria in bottled mineral waters. J. Methods, 44: 97-103.
11. Warburton, D.W. and J.W. Austin, 1997. Bottled Water. Chapter, 34 in: Microbiology of Food Chapman and Hall, London.
12. Cabral, D. and V.E. Fernendez-Pinto, 2002. Fungal sportage of bottled mineral water. Intl. J. Food Microbiol., 72: 73-76.
13. Schindler, P.R., H. Vogel and W. Back, 1995. Recommendation for changing microbiological examination parameter in filling bottled water to comply with the mineral and drinking water regulation. Gesundhert Swesen, 32: 391-393.
14. Legnani, P., E. Leoni, S. Rapuano, D. Turin and C. Valenti, 1999. Survival and growth of *Pseudomonas aeruginosa*, in natural mineral water: 5-Years Study. Intl. J. Food Microbiol., 53: 153-158.
15. Mardani, M.L., S.N. GachKar, A. Peerayeh, B. Asgari, H. Hajikhani and R.A. Amiri, 2007. Surveying common bacterial contamination in mineral bottled water in Iran. Iranian J. Clin. Infections Diseases, 2: 13-15.
16. Warburton, D.W., P.L. Peterkin, K. Weiss and M. Johnston, 1986. Microbiological quality of bottled water sold in Canada. Can. J. Microbiol., 32: 391-393.
17. Nsanze, H., Z. Babarinde and H. Al Kohaly, 1999. Microbiological quality of bottled water in the UAE and the effect of storage at different temperature. Environ. Int., 25(1): 53-57.
18. Chaggu, E.J., 2004. Sustainable Environmental Protection Using Modified Pit-Latrines. Ph.D. Thesis. Wageningen University, Netherlands.
19. World Health Organization, 2003. Guidelines for Drinking-Water Quality-Chapter, pp: 7-17.
20. APHA [American Public Health Association], 2005. Standard Methods for the Examination of Water and Wastewater 21th Ed. APHA, Inc. Washington, DC.
21. Fewtrell, L., D. Kay, M. Wyer, A. Godfree and G. ONeill, 1997. Microbiological quality of bottled water. Water Sci. Technol., 35(11-12): 47-53.
22. Ali, M.A. and A.Z. AL-Herrawy, 2001. Effect of retention time on removal of virus and amoeba during different water treatment technologies. Egypt. J. Appl. Sci., 16: 1-16.
23. Al-Herrawy, A.Z., 1992. *In vitro* cultivation of agents of amoebic meningoencephlititis isolated from water and sewage. Ph. D. Thesis, Fac. Vet. Med., Alexandria Univ.
24. Emmanuel, N.K., N.K. Aikaterini and A.F. Georgios, 2008. Monitoring microbiological quality of bottled water as suggested by HACCP methodology. J. Food Control, 19(10): 957-961.
25. Leclerc, H. and A. Moreau, 2002. Microbiological safety of natural mineral water. FEMS Microbiol. Rev., 26: 207-222.
26. Warburton, D.W., 1993. A review of the microbiological quality of bottled water sold in Canada. Part 2. The need for more stringent standards and regulations. Can. J. Microbiol., 39: 158-168.
27. Morais, P.V., C. Mequita, J. Andrade and M. Da-Costa, 1997. Investigation of persistent colonization by *Pseudomonas aeruginosa*,-like strains in a spring water bottling plant. J. Appl. Environ. Microbiol., 63: 851-856.
28. Abou-Ali, K.E., 1997. Studies on microbiological quality of some bottled and tap water samples in Egypt. J. Biotechnol., 1: 27-35.
29. El-Abagy, M.M., B.J. Dutka and M.M. Kamel, 1988. Incidence of coliphage in potable water supplies. Appl. Environ. Microbiol., 54(6): 1632-1633.
30. Hunter, P.R., 1993. The microbiology of bottled natural mineral water. J. Appl. Bacteriol., 74: 345-352.
31. Ali, G.H., G.E. El-Taweel, M.M. Ghazy and M.A. Ali, 1999. Microbiological and physcio-chemical evaluation of Nile river water quality. Egypt. J. Appl. Sci., 14: 12-38.
32. Evans, J.B., 1977. Coagulase-positive staphylococci as indicators of potential health hazards from water. In Bacterial Indicators / Health Hazards Associated with Water (Hoadly and Dutka, eds) pp: 126-130, Philadephia, PA. ASTMSTP 635.

33. World Health Organization, 2004. Guidelines for Drinking Water Quality, 3rd Edition, vol. 1, Recommendation.
34. Jeena, M.I., P. Deepa, K.M. Mujeeb Rahiman, R.T. Shanthi and A.A.M. Hatha, 2006. Risk assessment of heterotrophic bacteria from bottled drinking water sold in Indian markets. Intl. J. Hygiene and Environ. Health, 209: 191-196.
35. Criado, M.V., V.E. Fernandez-Pinto, A. Badessari and D. Cabral, 2005. Conditions that regulate the growth of moulds inoculated into bottled mineral water. Intl. J. Food Microbiol., 99: 343-349.
36. Evandri, M.G., P. Tucci and P. Bolle, 2000. Toxicological evaluation of commercial mineral water bottled in polyethylene terephthalate: A cytogenetic approach with *Allium cepa*. Food Additives and Contaminats, 17: 1037-1045.
37. Tamagnini, L.M. and R.D. Gonzalez, 1997. Bacteriological stability and growth kinetics of *Pseudomonas aeruginosa* in bottled water. J. Appl. Microbiol., 83: 91-94.
38. Yamaguchi, M.U., R.C. Rampazzo, S.F. Yamada-Ogatta, C.V. Nakamura, T. Ueda-Nakamura and B.P. Filho, 2007. Yeasts and Filamentous Fungi in Bottled Mineral Water and Tap Water from Municipal Supplies. J. Brazilian Archives Biolo. Tech., 150(1): 1-9.
39. Samhan, F.A., 1998. Microbial content and some factors affecting survival of bacteria in drinking water at Greater Cairo. M. Sc., Thesis. Faculty of Science, Cairo University, Egypt.
40. Shaban, A.M. and G.E. El-Taweel, 2002. Fate of new indicators of pollution and pathogenic bacteria during water treatment systems. Egypt. J. Microbiol., 37(1): 57-69.
41. Gassilloud, B., L. Schwartzbrod and C. Gantzer, 2003. Presence of viral genomes in mineral water: a sufficient condition to assume infectious risk. J. Appl. Microbiol., 69(7): 3965-3969.
42. Ehlers, M.M., W.B. van Zyl, D.N. Pavlov and E.E. Müller, 2004. Random survey of the microbial quality of bottled water in South Africa. Water SA, 30(2): 203-210.
43. Grabow, W.O.K., 2001. Bacteriophage: Update on application as models for viruses in water. Water SA., 27: 251-268.
44. Obiri-Danso, K., E. Emevor, L.A. Adnoh and K. Jones, 2005. Effect of sunlight, transport and storage vessels on drinking water quality in rural Ghana. J. Sci. Technol., 24: 32-44.
45. McGuigan, K.G., T.M. Joyce and R.M. Conroy, 1999. Solar disinfection: use of sunlight to decontaminate drinking water in developing countries. J. Med. Microbiol., 48: 785-787.
46. Food Marketing Institute, 1999. The food keeper. Food Marketing Institute, Washington, D.C.