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Bacterial quality control of domestic and imported brands of bottled water in Saudi Arabia

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Water is one of the most abundant and essential commodities of man occupying about 70% of the earth's surface and 60% of the human body therefore it should be continuously protected against microbial infections. Also, the mineral content in drinking water should be maintained within the acceptable range. Quality control of drinking water emerged with the invention of bottled drinking water. In this study, samples of bottled drinking water from Saudi markets were compared with tap water samples collected from different areas in Riyadh; both samples were tested for the presence of pathogenic bacteria. The bacterial isolates identified by the Biolog system (Hayward, CA, USA) include *Bacillus cereus*, *Staphylococcus* sp. and *Pseudomonas aeruginosa* in bottled drinking water, whereas tap water was mainly contaminated by *P. aeruginosa*. Bacterial contamination was highly observed in tap water samples and higher mineral content, determined by inductively coupled plasma-mass spectrometry (ICP-MS) was also observed in tap water. Bacterial cell count determined as CFU/ml was observed in bottled drinking water. Decreased water bacterial number was achieved with the solar disinfection system (SODIS) for one day with direct exposure to sunlight in polyethylene terephthalate (PET) plastic bottles. Thus water considered to be consumed by humans must maintain good microbial and mineral qualities within the acceptable ranges and must undergo effective treatment in order to reduce bacterial count and infection.

Key words: Solar disinfection system (SODIS), mineral content, *Bacillus cereus*, *Pseudomonas aeruginosa*.

INTRODUCTION

Water is one of the most abundant and essential commodities of man, occupying about 70% of the earth's surface, yet a greater percentage of the world's population, particularly developing countries live without access to safe water (Adriano and Joana, 2007). Water forms about 50 to 60% of our body weight and plays an active role in our entire vital body metabolism: It allows digestion, food elaboration and waste elimination. Although natural mineral waters have been consumed since Roman times, only the 20th century has seen the emergence of natural mineral water industry and the drinking of these products on a large scale as an alternative to tap water and non-alcoholic beverages (Stickler, 1989). In recent years, coping with the modern human lifestyle, there has been a tremendous increase in consumers demand for bottled mineral waters worldwide (Warburton, 1993; Venieri et al., 2006), including Saudi Arabia. Recently, more than 90 registered manufacturers providing and packaging drinking water were observed in Saudi Arabia.

Bottled water consumption in North America registered, in the last decade, an annual growth rate of 25% (Bharath et al., 2003). European Federation of Bottled Water (EFBW, 2006) estimated the consumption of

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bottled water during 2003 as 45,000 ml. Bottled water is often expected to be purer compared with tap water, although this is not necessarily the case. Public awareness about waterborne diseases and poor quality control of drinking water has been increased (Rosenberg, 2003). Both bottled and tap water can be oligotrophic environments with sufficient nutrients to maintain bacterial growth, releasing organic matter and providing additional substrates for the microbial growth. The presence of opportunistic pathogens such as *Pseudomonas aeruginosa* in mineral waters underscores the importance of caution regarding the safety of these products, especially for health compromised individuals.

The purpose of this study was to evaluate the quantitative and qualitative microbiological status of indigenous and imported bottled water sold in Saudi Arabia and to determine any changes occurring during storage at room temperature, with the aim of identifying optimal recovery conditions for the bacterial micro flora, with respect to media and incubation temperature and investigating the diversity of bacterial isolates using the Biolog system (Hayward, USA). Mineral contents for both bottled and tap water samples were determined by inductively coupled plasma-mass spectrometry (ICP-MS), where variable and higher concentrations of different anions and cations were observed in tap water rather than in bottled water samples. Water samples were further subjected to solar disinfection system (SODIS) resulting in a notable decrease in bacterial cell count present in water samples being tested.

MATERIALS AND METHODS

Collection of drinking bottled and taps water samples

A total of 20 bottled mineral water samples stored at room temperature were bought from different grocery stores in Riyadh, kingdom of Saudi Arabia (KSA) and nine tap water samples were collected from different areas (west to center) in Riyadh city, KSA. Prior to analysis, 1 ml of each of the water samples, bottled and tap, was inoculated and spread on blood agar and nutrient agar plates (Oxoid, USA). Plates were then incubated at 37°C for 18 to 24 h for the determination of bacterial cell count as colony forming unit (CFU) and for the isolation and differentiation of various bacterial strains depending on their morphological description, respectively. Water samples were further analyzed for total fecal coli form contamination. Bacterial isolates showing different morphology were further identified by the Bilog system (Biolog, Hayward, CA, USA) (Kawther et al., 2007). All experiments were performed for a minimum of three times.

Inoculation of the Biolog plate

Pure bacterial strains exhibiting distinct morphology was selected for further analysis. A bacterial suspension of 0.5 Mac Far land from each of the bacterial isolates obtained was prepared. 150 µl of each suspension was inoculated into the 96 wells Biolog micro plate. The micro-plates were incubated at 37°C for 4 to 24 h according to the manufacturer's specification, prior to incubation the plates were read with the Biolog Microstation TM system and compared to database.

Solar water disinfection (SODIS)

Solar disinfection of drinking water (SODIS) is a simple and low cost technique used to disinfect contaminated drinking water. Transparent bottles [preferably polyethylene terephthalate (PET)] are filled with contaminated water and placed in direct sunlight for a minimum of 6 h (Boyle et al., 2008). Prior to incubation the bacterial count will decrease and the water is now considered safe to drink.

SODIS technique

In the present work, a 500 ml to 2 L bottle (usually made of polyethylene terephthalate or PET), is cleaned and filled with biologically contaminated water (World Health Organization, 2007). The bottle is then placed in the sun, on a SODIS table of corrugated metal sheet, for 1 day if it is sunny or two days if it is cloudy (The Water School, 2008). When the exposure period is completed, water can be safely consumed. If the source water has turbidity greater than 30 NTU pretreatment is recommended, since particulate matter in water can absorb ultra violet (UV) light and shade pathogens from being exposed to the sun's rays. One of the most important operational factors regarding SODIS is that, once treated, the water can be consumed directly without being transferred to another container, reducing as such, water recontamination.

RESULTS

Mineral content of bottled and tap water samples

Mineral content analysis by inductively coupled plasma (ICP) of all samples showed diverse results of mineral contents as well as the presence of some inorganic toxic substances such as phosphates, sulphates, nitrates, nitrites and aluminum (Table 1). Fifteen out of 20 (78%) bottled mineral water samples had neutral pH (pH 7.0 to 7.4) and four (22%) were slightly alkaline (pH > 7.4). All samples showed high sodium and calcium contents ranging within 13 to 20 ppm in 7 samples, which was above the recommended limit of 20 ppm. Eight samples had acceptable calcium level between 20 to 50 ppm, one sample had 76 ppm calcium content. Chloride concentration in 3 samples was above the standard limit > 30 ppm. Sulphate measured in two samples was between 33 and 50 ppm of sulphate, higher than the acceptable limit. Traces of aluminum contents were seen in all bottled samples (range: 0.004 to 0.019 ppm). Other anions and cations were within the acceptable ranges.

A comparison of the mean values of bottled drinking water and municipal tap water showed a highly significant difference, but both were below the optimum fluoride levels (0.75 to 1 ppm). 9 samples showed fluoride concentrations between 0.90, 0.92, and 0.94 mg/L, slightly below the optimal level of 1.00 mg/L (Table 1). As a result, tap water samples showed higher levels of mineral and toxic substances content especially sodium, potassium, calcium, magnesium, aluminum, chloride, bicarbonates and pH in comparison to bottled water samples being tested in this study. However; nitrate, fluoride and iron were higher in bottled mineral water compared to tap water.

Table 1. Average composition of anions and cations from 18 commercially available bottled mineral water in Riyadh, Saudi Arabia.

Samples	Production country	Production date	Expired date	Positive cations (ppm)					Negative anions					pH
				Ca	Mg	K	Na	Fe	Bicarbonate	Chloride	Nitrate	Fluoride	Sulphate	
1A	Saudi Arabia	23-9-2010	22-9-2011	21.2	4.5	1.2	20	0.01	37.5	20	7	0.8	32	7.7
2B	Saudi Arabia	27-2-2011	26-2-2012	14.4	3	1.5	12.3	0	24	17.5	2	0.9	28	7.2
3C	Saudi Arabia	20-2-2011	19-2-2012	23	13	1.25	19	0.01	28	20	4.5	0.8	31	7.2
4D	Saudi Arabia	18-2-2011	17-2-2012	20	13	1.25	18.5	0.01	27	22	4	0.8	30	7.2
5F	Saudi Arabia	15-3-2011	14-3-2012	17	7	2.5	20	0	80	27	2	1	12	7.5
6G	Saudi Arabia	05-1-2011	1-4-2012	15	10	0.1	10	0	26	45	0.1	0	0	7.2
7H	Saudi Arabia	01-7-2010	30-7-2011	2.4	0.5	0.8	24.6	0.02	40	30	5	0.75	12	7.5
8K	Saudi Arabia	31-1-2011	30-1-2012	8.8	2.4	1.5	21	0.01	30	24	1.4	-	23	7.2
9P	Saudi Arabia	25-2-2011	24-2-2012	36	4.2	0.2	18	0.02	42	66	0.1	0.9	22	7.1
10Q	Saudi Arabia	04-9-2010	9-3-2011	20	14	1.5	18	0.01	27	20	4	0.9	27	7.2
11S	Saudi Arabia	23-1-2011	22-1-2012	8.4	1	1.4	29	0	7	47	7	0.9	16	7.4
12U	Saudi Arabia	01-3-2011	30-3-2012	10	4.4	1	16.7	0	20	17	3	0.8	35	7
13V	Saudi Arabia	19-12-2010	18-12-2011	15	5	0.2	19	0.02	50	15	0.1	0.8	50	7
14W	Saudi Arabia	02-3-2011	3-1-2012	9.5	3.5	1.7	19	0	24	26	1.2	1	22	7.5
15X	Saudi Arabia	04-1-2011	3-1-2012	5	13	1	16	0.01	1.3	27.5	0.1	1	51	7
16Y	Saudi Arabia	23-1-2011	22-1-2012	8	3	2	18	0	28	32	2.5	0.8	36	7.2
17Z	Saudi Arabia	13-2-2011	2-12-2012	19	3	1.8	19	0	39	33	2.8	1	27	7.5
181	Saudi Arabia	25-3-2011	24-3-2012	13	4	0.8	20	0	30	30	5	0.8	20	7.1

Microbial quantification

All brand bottled water samples showed negative fecal coli form test. Bacterial analysis using Biolog system (Hayward, USA) revealed that two out of the twenty commercially tested bottled mineral water in Riyadh, Saudi Arabia were mostly contaminated by *Bacillus cereus, Pseudomonas* spp. and *Staphylococcus* spp. Tap water obtained from different areas in Riyadh showed contamination with *Pseudomonas aeruginosa, Staphylococcus* spp. *and Corynebacterium* spp.

Twelve bacterial isolates had been identified by Biolog from both water samples and were tabulated (Table 2).

SODIS treatment

Our work showed one day SODIS treatment with a direct exposure to sunlight in PET plastic bottles to be 92% effective; however, two days SODIS treatment was 100% effective. This effect was revealed by a drastic reduction in the bacterial cell count as indicated by Table 3 and shown in Figures 1 and 2, giving promising cheapest technique for the treatment of drinking water and tap water as well, consequently reducing the occurrence of waterborne pathogenic infection and the presence of coliforms; making water safer to use and drink.

DISCUSSION

One of the greatest concerns for water consumers with respect to the quality of drinking water is contamination with pathogenic microorganisms. Certain microorganisms, including various bacteria, viruses and parasites, are well-known water contaminants, of which several may lead to waterborne disease and epidemics. Bacteria are probably the most frequently studied group of microorganisms with respect to drinking water quality; *P. aeruginosa* particularly is usually considered an indicator of contamination during the bottling process (Rosenberg, 2003).

Fast and accurate monitoring of chemical and

Table 2. Enumeration with the percentage of occurence of 12 bacterial species isolated from bottled and taps water samples using the Biolog system.

Isolates code	Bacterial species	% of occurrence
J	*Acinetobacter iwoffi*	25
R	*Bacillus cereus*	50
N	*Bacillus subtilis*	20
W	*Bacillus spp.*	1
O	*Corynebacterium*	20
Z	*Flavobacterium*	5
B,S	*Pseudomonas spp.*	15
W	*Staphylococcus*	10
P	*Nocardia barsiliensis*	3
J	*Pseudomouna aeruginosa*	60
A	*Rathayibacter tritici*	2

Table 3. Bacterial mean colony count obtained from three repeated experiments of bottled water, storage water and one day SODIS water treatment.

	Bottled water	Storage water	SODIS water
Sample	Bacterial count		
A	13	30	
B	26	60	8
C	25	50	1
D	3	-	-
E	15	25	-
F	1	-	-
G	1	1	-
H	1	20	-
I	11	21	-
J	45	90	-
K	1	1	-
L	18	30	-
M	44	55	-
N	2	7	-
O	10	19	-
P	31	3	-
Q	1	5	-
R	13	23	-
S	12	80	-
T	3	8	-
U	1	4	-
V	50	76	9
W	1	5	2
X	-	-	-
Y	30	-	-
Z	1	4	-
1	-	-	-
% Microbes	19	80	1
Wavelength UV	-	-	600-620 nm

Samples A to T: bottled drinking water samples. Samples U to 1: tap water samples collected from different areas in Riyadh.

Table 4. Bacterial count obtained from tap, bottled and SODIS treated water samples.

Treatments	Microbial number	
	Bacterial mean	**Std. error**
Isolation from tap water	13.3951[b]	1.73363
Isolation from bottled water	22.9012[a]	3.02651
Isolation from SODIS water	0.7407[c]	0.25017
F-value	30.32**	
P-value	0.000	

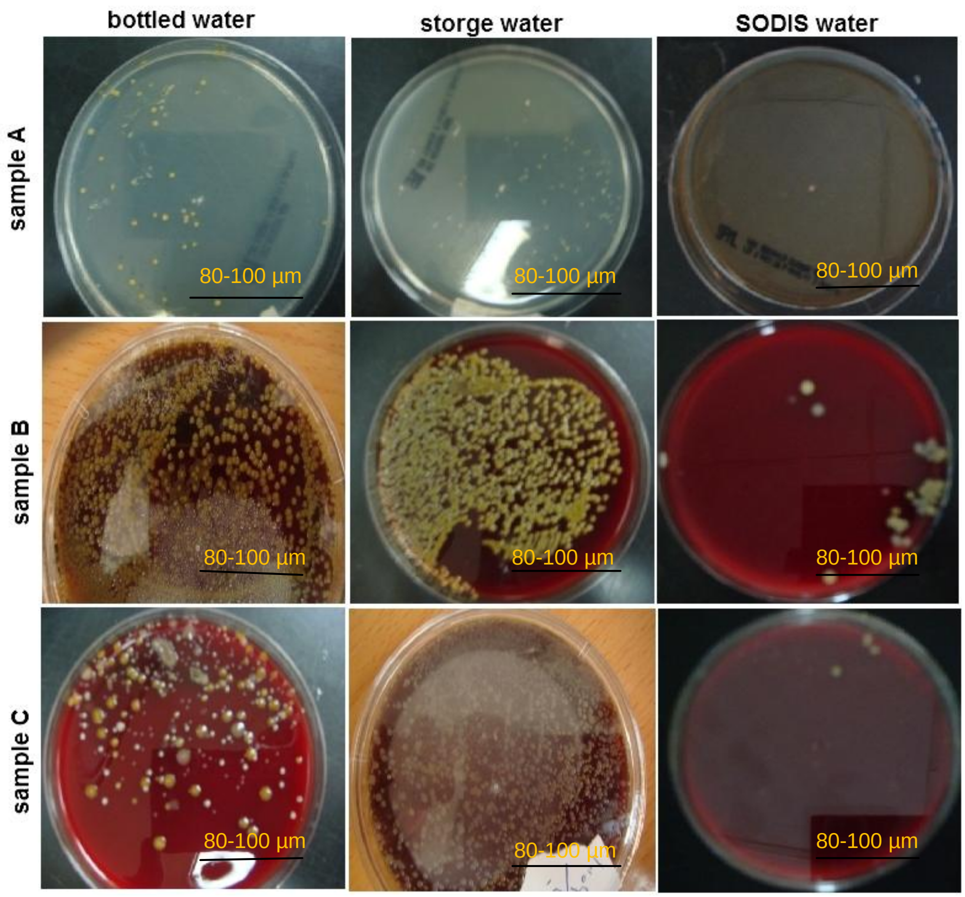

Figure 1. Microbial count of tap or storage water, bottled and SODIS treated water on different selective culture media chocolate, nutrient and blood agar (sample A, sample B, sample C). Scale 1:80 to 1:100.

microbiological parameters in drinking water is essential to safeguard the consumer and to improve the understanding of treatment and distribution systems (Berney et al., 2008). With the significant increase in bottled mineral water consumption over the last decade, there has been a growing concern about the micro-biological quality of such products. Polluted water is an important vehicle for the spread of diseases. Rare published information up to our knowledge is available on microbiological characteristics of drinking water in Saudi Arabia. El Safey (2005) indicated that numerous micro-organisms including bacteria, fungi and algae are present in water, forming a complex ecosystem. Among these organisms, bacteria are responsible for most drinking water contamination and diseases that are intrinsic to the public health.

Some parameters such as water pH, depth, oxygen and some heavy metals etc. determined in our study affect concentrations of water fungi and bacteria. These compounds vary in type and concentration, depending on

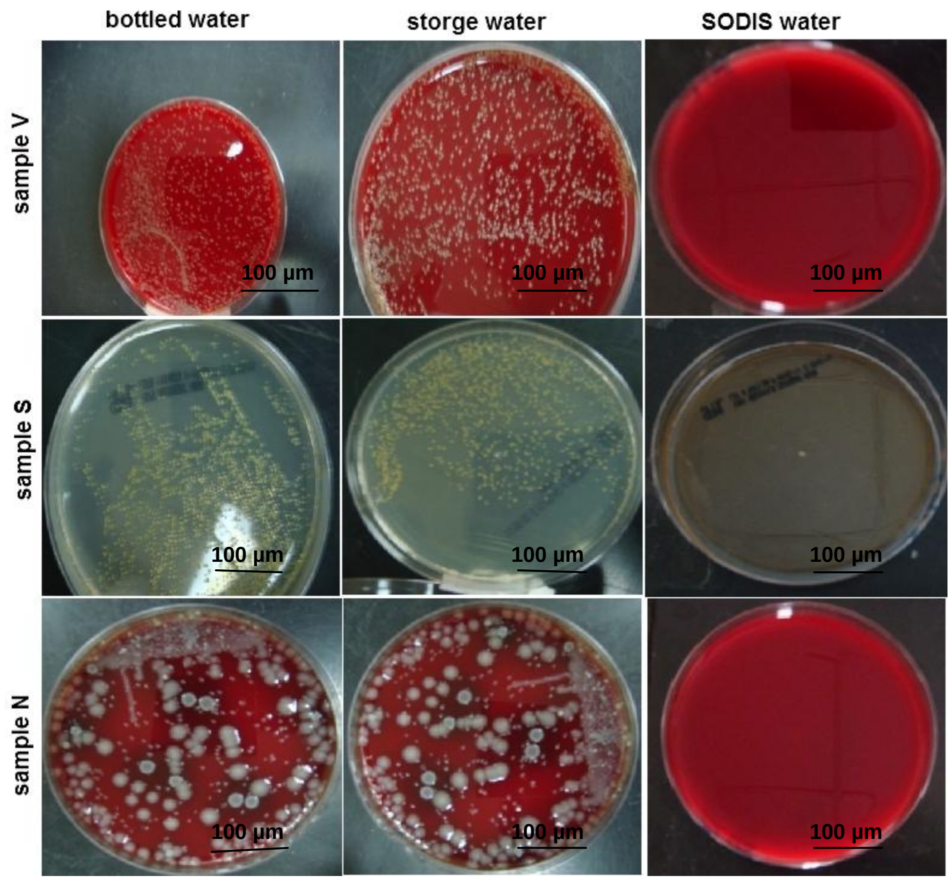

Figure2. Microbial count of tap, bottled and SODIS treated water samples after treatments on different selective culture media, chocolate, nutrient and blood agar (sample V, sample S, sample N). Scale 1 to 100.

the water source. Moreover, if the samples are concentrated to detect the microorganisms present at low densities, then minerals, organic components and biomass will also be concentrated, thus altering samples composition; making them more complex, this goes in agreement with our findings where higher concentrations of cations and anions would increase the pH levels and hence increase the bacterial count.

The presence of opportunistic pathogens such as *P. aeruginosa* in mineral waters underscores the importance of caution regarding the safety of these products, especially for health compromised individuals; however, the risk should by no means be exaggerated. Bottled and tap water can be an environment with sufficient nutrients to maintain bacterial growth. This latter, in stored bottles, has been reported to be affected by specific bottling-materials which during storage can release organic matter and provide additional substrates for the microbial growth (Rosenberg, 2003). Fluctuations in the bacterial count and species were mainly due to the variety of bottle types being used. However, in the attempt of decreasing the bacterial count, SODIS was selected for the treatment of tap and bottled drinking water samples. Following one day exposure of water samples in PET bottles to direct sunlight, the water now is safe to drink as the viable pathogen load can be significantly decreased. SODIS harnesses light and thermal energy to inactivate pathogens via a synergistic mechanism (McGuigan et al., 1998).

SODIS mineral water samples tested for one day with direct exposure to sunlight were found in this study to have low bacterial count due to the carbonation widely used in decreasing the water pH giving as such an antibacterial effect (Venieri et al., 2006). Ultraviolet light (300 to 400 nm; UVA and UVB) inactivates a large part of germs. However, the mechanism is not yet understood. SODIS reduces fecal contamination levels from 1 million bacteria per ml to zero in < 1.5 h, with consistency of the samples being studied; SODIS is completely effective against pathogens causing cholera, dysentery, typhoid, salmonellosis, gastroenteritis, cryptosporidi oasis, giardiasis and polio. This finding somewhat contradicts the recommendations provided by the Swiss Federal Institute for Environment and Technology (EAWAG, 1997) where Wegelin et al (2001) recommended five hours of exposure under bright sun or up to 50% cloudy sky, or two consecutive days fewer than 100% cloudy sky to be effective for disinfecting water.

The aim of this study was to determine the quality of bottled water commercially available in Saudi markets in comparison with tap water samples collected from different areas over Riyadh, Kingdom of Saudi Arabia. For each sample, microbial parameters and chemical indicators of contamination were evaluated. It was found that tap water contains higher mineral concentrations than does bottled water samples.

Water is referred to as "hard," when it contains more minerals than ordinary water, particularly calcium (Ca) and magnesium (Mg). Hardness is expressed in terms of calcium carbonate ($CaCO_3$). Water from 0 to < 60 mg/L hardness is considered soft, 60 to < 120 mg/L as medium hard, 120 to < 180 mg/L as hard, and above 180 mg/L very hard (Environment Canada, 1997). Comparing bottled mineral water and tap water contents analyzed in this study, tap water showed higher levels of mineral and toxic substances content, particularly sodium (13 to 22.5 ppm), potassium, calcium (20 to 50 ppm), magnesium, aluminum, chloride (> 30 ppm), bicarbonates and pH values between 7.5 to 7.7; nitrate, fluoride and iron levels were higher in bottled mineral water compared to tap water.

In addition, tap water was found to have higher bacterial count when compared to bottled water. This bacterial count becomes significantly reduced with the use of SODIS water treatment 1 to 2 days under direct sunlight exposure; giving new cheapest and promising protocol for cleaning water to become safer to use and drink.

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REFERENCES

Adriano AB, Joana SB (2007). The quest for safe drinking water: An example from Guinea-Bissau (West Africa). Water Res. 41:2978-2986.
Berney M, Vital M, Hülshoff I, Weilenmann HU, Egli T, Hammes F (2008). Rapid cultivation-independent assessment of microbial viability in drinking water. Water Res. 42:4010-4018.
Bharath J, Mosodeen M, Motilal S, Sandy S, Sharma S, Tessaro T, Thomas K, Umamaheswaran M, Simeon D, Adesiyun AA (2003). Microbial quality of domestic and imported brands of bottled water in Trinidad. Int. J. Food Microbiol. 81:53-62.
Boyle M, Sichel C, Fernandez-Ibanez P, Arias-Quiroz GB, Iriarte-Puna M, Mercado A (2008). Bactericidal effect of solar water disinfection under real sunlight conditions. Appl. Environ. Microbiol. 74:2997-3001.
El-Safey EM (2005). Microbiological quality of drinking water cooling system in some trains solid in Egypt. EJB, 20:193-218.
EAWAG Sandec (1997). SODIS News no. 1. Internal Report.
Environment Canada. (1997). Surface water quality in Canada—an overview. Water Quality Branch, Inland Waters Directorate.
European Federation of Bottled Water (EFBW) (2006). The industry in figures http://www.efbw.org/about/industry.
Abed KF, Alwakeel SS (2007). Mineral and microbial contents of bottled and tap water in Riyadh, Saudi Arabia. Middle-East J. Sci. Res. 2(3-4):151-156.
McGuigan KG, Joyce TM, Conroy RM, Gillespie JB, Elmore Meegan M (1998). Solar disinfection of drinking water contained in transparent plastic bottles: characterizing the bacterial inactivation process. J. Appl. Microbiol. 84(6):1138-1148.
Rosenberg FA (2003). The microbiology of bottled water. Clin. Microbiol. Newslett. 25(6):41-44.
Stickler DJ (1989). The microbiology of bottled natural mineral waters. J. R. Soc. Health 109(118e):124.
Venieri D, Vantarakis A, Komninou G, Papapetropoulou M (2006). Microbiological evaluation of bottled non-carbonated (still") water from domestic brands in Greece. Int. J. Food Microbiol. 107:68-72.
Warburton DW (1993). A review of the microbiological quality of bottled water sold in Canada, Part 2. The need for more stringent regulations. Can. J. Microbiol. 39:158-168.
Wegelin M, Canonica S, Alder AC, Marazuela D, Suter MJF, Bucheli TD (2001). Does sunlight change the material and content of polyethylene terephthalate (PET) bottles? Aqua 50(3):125-133.
World Health Organization (2007). Managing Water in the Home: Accelerated Health Gains from Improved Water Supply. Accessed from the World Wide Web on November 4, 2008n.