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Microbiological Quality of Different Bottled Water Brands, Marketed in Trabzon, Turkey

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The microbiological quality of demijohn (19 L) and disposable bottled water products of six different brands were tested with membrane filter technique over a period of 6 months. Bacteriological quality of some of the demijohn bottled water samples was investigated after exposure to the sun light. Under this conditions, microbial contamination was mostly seen in the samples marked as C and E in terms of total coliforms and heterotrophic plate counts. Although their shelf-live was determined as one year, only the second sample (B) was evaluated as appropriate to standards determined by regulation on Intended Human Water Consumption and World Health Organization for 6 months. C and E were not consistent with standards beginning from 1st month. Eventually, in performed study, most of the samples tested were unsuitable for drinking purpose according to permitted levels, if they were consumed even a month after the date of manufacturing. Although, same investigations done for disposable bottled water disclosed that, such as packaged waters safer than the water in demijohn. Because, it was determined that 97 % of 36 tested water samples were found to appropriate to standards.

Key Words: Bacteriological quality, Demijohn, Disposable bottled water, Heterotrophic plate count.

INTRODUCTION

Water forms 50-60 % in weight of our body and has an active role in all the vital processes of our body for digestion, food elaboration and waste elimination. So, it is the most important resource for humans[1]. Water borne diseases originated from the consumption of unsafe water and unhygienic drinking water, continue to be one of the major health problems especially in developing nations[2]. The most dangerous form of water pollution occurs when faecal contaminants enter the water supply. Pathogenes such as *Salmonella* species, *Shigella species*, *Vibrio cholerae* and *Escherichia coli* being shed in human and animal faeces ultimately find their way into water supply through seepage of improperly treated sewage into ground water[3]. Other microbial indicators of possible faecal contamination are faecal *Enterococci* especially *Enterococcus faecalis* and *Clostridium perfringens* spores[4]. Microbial contamination by human or animal excreta is the most common reason for water to be considered unsafe for drinking because of the high probability of presence of pathogenic organisms. Coliform bacteria describe a group of enteric bacteria that includes *E. coli*, *Klebsiella* species and *Enterobacter* species[4]. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic bacteria, viruses and protozoans[5].

Bottled water can be defined as any potable water that is bottled and distributed or offered for sale and specifically intended for human consumption[6]. The format of the 19 L bottle (demijohn) is the most widespread[7]. Faulty design and construction of the water supply networks, insufficient water supply from city water distribution networks, problems in the taste, purity and odor of tap water and erratic power supply were caused to public distrust of tap water. In other words, growing population and shifting of the population from rural to urban areas have increased the sale and consumption of bottled water in most countries of the world[8,9]. So, during the past decade, there has been a considerable increase in the consumption of bottled water in Turkey as well as around the world and it is estimated that 70 % of the households in Turkey regularly utilize bottled water to meet their daily drinking water requirements. Turkish people consumed about 5.2×10^9 L of bottled water in 2002, which is *ca.* 78 L per capita[10]. The source of 89 % of bottled water sold in Turkey is from protected springs and the remaining is pumped from drilled wells tapping an aquifer. However, at present, only 20 % of natural spring water (still) resources and 1 % of natural mineral water (sparkling) resources are utilized by the Turkish bottled water industry. The industry's annual capacity usage averages around 35-55 % because of demand differences between winter/summer seasons and improving quality of tap water supplied by municipalities[10].

Turkish bottled waters are divided into several classes as natural spring water, drinking water, processed drinking water and natural mineral water[11]. The quantity of microbial flora of spring water is usually high. If these microorganisms are not adequately removed during processing and bottling, bacterial multiplication may occur for 1-3 weeks after bottling and the bacterial count can reach 10^3-10^4 bacteria per mL at 37 ºC[12]. In addition to natural contamination, the product can also deteriorate before it reaches the consumer. Consumers should be aware that bottled water is not necessarily safer than tap water because bottled and municipal water may contain the same microorganisms since both can originate from the same sources. Under improper and/or prolonged storage of bottled water, bacteria can grow to levels that may be harmful to human health[6]. Therefore, the bottled water industry has to exhibit strict quality standards in terms of microbial parameters, production processing, bottling, transportation and storage[13,14].

When the drinking water source distribution of houses in Turkey is investigated, 72.6 % of the water sources are mains water, 16.1 % of them are packed water (glass, plastic bottle water, demijohn water) and 4.9 % of them are quarter fountains[15]. Legal regulations are present about the water supply and control in Turkey. 17th of February 2005 dated and 25730 numbered one "Regulation about waters having the purpose of human consumption" aims at supply of water to the public which appropriate to technical and hygienic qualifications. Moreover, it's necessary for the water which is used as drinking water to be appropriate to TSE-266, the standarts of drinking water of Turkish Standarts Institution[16].

In this study, microbiological parameters of 54 water samples in refillable 19 L polycarbonate plastic bottles and 36 water samples in disposable pet bottle of 6 different brands selected randomly from various retail outlets in Trabzon were investigated and evaluated for permitted levels according to Regulation on Intended Human Water Consumption and World Health Organization (WHO).

EXPERIMENTAL

Six bottled drinking water brands all of different manufacturers were used for this investigation. Manufacture dates of bottled water samples were differ as October and November 2009. Except of the sample marked E, shelf-lives of others were determined as one year by manufacturer. The shelf-live of the E was six months. Eighteen of this 54 bottled water samples were directly exposed to sun light for three months between December 2009-February 2010. The others (36 bottled water samples) were stored at cool (*ca.* 20 ºC) for six months between December 2009-May 2010. Water samples in disposable bottles were tested in a period, the month of their manufacture date. All of the samples were tested in terms of total coliform bacteria (TC), fecal coliform (FC) bacteria, *Escherichia coli* (EC), spore-forming sulphite-reducing anaerobes (SR), *Pseudomonas aeruginosa* (PA), *Salmonella* (SL), *Staphylococcus aeurus* (SA) and total mesophilic aerobic bacteria (heterotrophic plate count, HPC), monthly storage.

Microbial water analysis was performed by using the most flexible method for qualitative and quantitative studies of bottled water because of it has an advantage that small numbers of organisms can be detected, because the amount of water passed through the membrane is restricted only by the amount of gross-suspended matter present in water; membrane filtration (MF) technique permits the analysis of water volumes, ranging from 1 mL to as much as 10 L[17]. Membrane filter counts may achieve the sensitivity of the multiple tube count, while retaining the accuracy of the colony count method[17,18].

To detect the presence of total coliforms, fecal coliforms, *E. coli*, *P. aeruginosa*, *Salmonella* spp. and *S. aureus*, a 250 mL of each bottled water sample was filtered through nitrocellulose membranes (0.45 µm pore size, 47 mm diameter) followed by plating on selective media. For TC, membranes were plated on Endo Broth followed by incubation at 35 ºC for 24 h. For EC and FC, membranes were transferred on MFC Endo Broth With Rosolic Acid and plates were incubated at 35 and 44 ºC for 24 h, respectively. To detect PA, SL and SA membranes were plated onto *Pseudomonas* agar base with cetrimide, chromogenic *Salmonella* agar and Chapman -14074 acetonitrile medium, respectively and all were incubated at 37 ºC for 24 h.

For the enumeration of spore-forming sulphite-reducing anaerobes (SR), 1 mL of water sample and mixing with melted sulphite polymyxin sulphadiazine agar were used. After 48 h incubation in anaerobic jar, developing colonies were counted. Furthermore, Plate Count Agar (PCA) was used for determination of total mesophilic aerobic bacteria (HPC) quantity. For this purpose two sets of plates were prepared for all samples. One set was incubated aerobically at 37 ºC for 48 h and the other set at 22 ºC for 72 h. The 37 ºC can provide an indication of fast growing bacteria, related to pathogenic types and 22 ºC an indication of characteristic bacteria that develop slowly[19]. All studies were performed three times at sterile conditions.

The number of bacteria colonies were determined and reported as colony-forming units per millilitre (cfu mL^{-1}).

RESULTS AND DISCUSSION

The microbial quality of bottled water is of great interest as many consumers use it as an alternative to municipal water and consider it to be better and safer. Bottled water is generally of good quality for drinking, but if not properly protected during bottling, transit and storage, could be a subject of contamination[20]. Therefore, in this study the microbiological quality of 54 refillable and 36 disposable bottled water samples comprising 6 different brands were investigated. For investigation of sunlight during storage before using, 18 of demijohn bottled water samples were exposured to sun light for 3 months and analyzed monthly. The others were monthly tested over a period of 6 months. The water samples in disposable bottle were analysed in month in which they had been manufactured and bought.

When the samples exposured to sun light investigated, it was easily seen that SL and SR bacteria were not detected in all studied brands for three months. EC was detected in only the samples marked as C and E after three months. Microbial contamination was mostly seen in these (C and E) in terms of TC, particularly (Table-1).

TABLE-1
MICROBIOLOGICAL ANALYSIS RESULTS OF BOTTLED WATER SAMPLES EXPOSURED TO SUN LIGHT FOR THREE MONTHS

Brand code	Time (month)	HPC		TC[1]	FC[1]	EC[1]	SL[1]	PA[1]	SA[1]	SR[1]
		37 ºC 48 h	22 ºC 72 h							
A	1	43	76	0	0	0	0	0	0	0
	2	51	82	0	0	0	0	0	0	0
	3	168	>500	50	1	0	0	14	0	0
B	1	0	3	0	0	0	0	0	0	0
	2	0	1	0	0	0	0	0	0	0
	3	3	4	0	0	0	0	0	0	0
C	1	59	107	12	0	0	0	2	0	0
	2	8	115	64	0	0	0	0	2	0
	3	23	151	37	24	1	0	5	4	0
D	1	0	58	0	0	0	0	0	0	0
	2	78	126	1	0	0	0	0	0	0
	3	0	132	4	0	0	0	0	0	0
E	1	5	127	24	0	0	0	7	0	0
	2	34	148	61	0	0	0	4	0	0
	3	19	196	112	8	3	0	17	9	0
F	1	0	0	0	0	0	0	0	0	0
	2	0	72	0	0	0	0	0	0	0
	3	2	136	1	0	0	0	0	0	0

[1]TC: Total coliforms; FC: fecal coliforms; EC: *Escherichia coli*; SR: spore-forming sulphite-reducing anaerobes; PA: *Pseudomonas aeruginosa*; SL: *Salmonella*; SA: *Staphylococcus aeurus*; HPC: total mesophilic aerobic bacteria.

The microbial contaminations of bottled water could be influenced by factors such as their raw water source, treatment process employed and hygienic practices observed in production[21]. Ineffectiveness or malfunctioning of the treatment process employed could also result in the presence of coliform bacteria in the samples of water. According to Edberg[22] no treatment process or method used in mass production of drinking water yields a sterile product, it only produces a safe product devoid of pathogenic organisms. Appropriate treatment processes should therefore be utilised for production of quality and safe packaged drinking waters. In samples marked as B and F, TC, FC, EC, SL, PA, SA and SR bacteria species were not detected all over the test period. It was seen that from the Table-2, the count of bacteria had been increasing after 4 and 5 months for these samples, respectively. For 6 months, only the second sample (B) was evaluated as appropriate to standards determined by Regulation on Intended Human Water Consumption and WHO. C and E were not consistent with standards beginning from first month. In a similar study for bottled waters consumed in Trinidad, of the 344 samples tested 18 (5.2 %), 5 (1.5 %) and 26 (7.6 %) were positive for total coliforms, *E. coli* and *Pseudomonas* spp., respectively[23].

The absence of faecal indicator bacteria in most brands of selected bottled water samples could be attributed to better hygienic practices. These include use of protective sealed caps on bottles, improved and hygienic filling system and use of non-returnable plastic containers. At the end of the 6 month period, all brands except for B failed to meet the WHO drinking water standard of zero coliform per 100 mL water making them unsuitable for human consumption. Furthermore, in the case of these samples the monthly storage seemed to affect the microbial safety, since HPC levels increased. But, it should also be taken into account that the samples of the study were kept monthly after bottling, conditions that allow proliferation of their aututrophic microbial flora[24-26]. Increasing levels of HPC is consistent with literature. It is stated in literature that, bottled (non-carbonated) water generally have high HPCs, since the elimination of micro-organisms by disinfection or sterilisation is not permitted[27,28]. The presence of high numbers of heterotrophic bacteria in bottled water may be due to the natural microbial flora of the source water. These bacteria can multiply after bottling, resulting in high numbers of HPCs[29]. While bottled water may initially meet bacteria standards, the lack of a residual disinfectant (chlorine) and prolonged periods of storage at room temperature or higher may result in elevated HPC bacteria counts by the time the water is consumed[29]. Microbial numbers reach a peak after a week of storage and remain fairly constant thereafter[29]. Nevertheless, HPC were kept in low numbers and although there has been considerable discussion as to the health importance of these organisms their measurements are always recommended[30].

The number of bacteria recovered at the source is generally very low, around 10 CFU mL^{-1}, but there are many reports that viable counts increase, notably in uncarbonated water, to 10^4-10^5 CFU mL^{-1} after 1-2 weeks of storage[12,20,31,32]. According to results of a study, 54.4 % of the examined bottled mineral water samples had failed to comply with the standard set by the Taiwan legislation on bottle mineral water (HPC 200 CFU mL^{-1})[32]. According to WHO, 2002 report, a high HPC concentration does not itself present a risk to human health. Nevertheless HPCs are used as good indicators of the overall quality of production[33,34]. When compared with the results of demijohn bottled water samples, it was determined that the disposable bottled water samples belonging to selected brands had not contained TC, FC, EC, SL, PA, SA and SR bacteria species. Only, total mesophilic aerobic bacteria growing was observed in some of the investigated samples at 37 ºC for 48 h and at 22 ºC for 72 h. In Table-3, HPC concentrations of disposable water samples were summarized. While the bacterial growing was only observed in the samples marked as A, C and E, which had been bought in specified date,

TABLE-2
MICROBIOLOGICAL ANALYSIS RESULTS OF BOTTLED WATER SAMPLES FOR SIX MONTHS

Brand code	Time (month)	HPC		TC[1]	FC[1]	EC[1]	SL[1]	PA[1]	SA[1]	SR[1]
		37 ºC 48 h	22 ºC 72 h							
A	1	19	54	0	0	0	0	0	0	0
	2	139	>200	48	0	0	0	1	0	0
	3	>250	>250	0	0	0	0	17	0	0
	4	>500	>500	55	0	4	0	12	0	0
	5	>500	>500	114	0	0	0	27	41	0
	6	>500	>500	>500	0	1	0	24	30	0
B	1	0	2	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	1	0	0	0	0	0	0	0	0
	5	4	24	0	0	0	0	0	0	0
	6	4	31	0	0	0	0	0	0	0
C	1	38	89	7	0	0	0	0	1	0
	2	54	62	27	0	0	0	0	0	0
	3	91	97	65	0	0	0	0	9	0
	4	115	172	146	0	0	0	0	21	0
	5	124	>500	168	0	0	0	15	38	0
	6	146	>500	174	14	3	0	19	24	0
D	1	4	14	0	0	0	0	0	0	0
	2	5	47	5	0	0	0	0	0	0
	3	54	71	3	0	0	0	0	0	0
	4	21	84	0	0	0	0	0	0	0
	5	87	>200	0	0	0	0	2	4	0
	6	125	>200	3	0	0	0	6	5	0
E	1	2	67	32	1	0	0	0	3	0
	2	43	150	39	0	0	0	0	0	0
	3	>200	120	44	0	0	0	7	5	0
	4	7	>500	>200	0	0	0	12	8	0
	5	>500	>1000	>500	0	0	0	14	0	0
	6	>500	>1000	>500	13	1	0	8	31	0
F	1	0	0	0	0	0	0	0	0	0
	2	1	4	0	0	0	0	0	0	0
	3	3	8	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	5	23	32	0	0	0	0	0	0	0
	6	14	>200	2	0	0	0	0	0	0

[1]TC: Total coliforms; FC: fecal coliforms; EC: *Escherichia coli*; SR: spore-forming sulphite-reducing anaerobes; PA: *Pseudomonas aeruginosa*; SL: *Salmonella*; SA: *Staphylococcus aeurus*; HPC: total mesophilic aerobic bacteria.

bacterial growing was not observed in the samples marked as B, D and F.

The presence of total coliforms and *E. coli* indicates incidence of contamination and potential presence of pathogenic enteric microorganisms[23]. Presence of *P. aeruginosa* was detected after two months, in sample A. Unlike this in B and F, fertility of *P. aeruginosa* was not detected during the study period. From the literature it is known that, among the species of *Pseudomonas* isolated, the most important was *P. aeruginosa*, considering that to date in Greece it was the only one unacceptable criterion in bottled water and was used as a process management indicator in the production. Its presence means contamination during the bottling process or that the source had become polluted by organic material[26,30,35]. *P. aeruginosa* is an opportunistic pathogen that is known to cause urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections, particularly in patients who are severely immunocompromised, those with catheters, open wounds or cystic fibrosis. A particular feature of *P. aeruginosa* is its ability to grow in low-nutrient water. Besides being a primary cause of disease, *P. aeruginosa* is often monitored as an indicator of other bacterial contamination of fecal origin[36].

Increasing of microbial contamination in the samples exposuring to sunlight was more faster than samples stored at cool. Because, the 16 % of samples stored at cool was appropriate to standards for six months. However, the 16 % of samples exposured to sunlight was appropriate to standards for only three months. Microbiological quality of bottled and tap water have been widely investigated by the researchers all over the world. However, in the literature the number of studies on effect of sun light to the water during storage in terms of contribution of microbiological contamination are scarcely any. In that respect, this study has been evaluated so important. Furthermore, this study displayed that the using of bottled water in disposable containers are safer than bottled water in refillable containers. Because, the 97 % of water samples in disposable containers were considered to appropriate as a result of microbiological analysis.

Worldwide, sales of bottled water increase every year[37] because of the general belief that it is safe and free of all impurities[23]. Nevertheless, several studies have shown that bottled

TABLE-3
MICROBIOLOGICAL ANALYSIS RESULTS OF DISPOSABLE BOTTLED WATER SAMPLES

Brand code	Time (month)	HPC	
		37 ºC 48 h	22 ºC 72 h
A	December	–*	–
	January	–	–
	February	–	2
	March	–	–
	April	–	–
	May	–	–
C	December	2	8
	January	–	–
	February	17	109
	March	–	–
	April	–	–
	May	3	19
E	December	–	–
	January	–	–
	February	–	–
	March	5	44
	April	–	–
	May	24	23

[a]Any bacterial growing was observed. HPC, total mesophilic aerobic bacteria.

water is not sterile as it may contain various pathogens like coliforms, *Pseudomonas* spp., *E. coli*, Campylobacter or even mycobacteria[23,32,34,38-43].

Conclusion

Briefly, most of the samples tested in this study were unsuitable for drinking according to permitted levels, if they were consumed even a month after date of manufacture. Therefore, at least, we should prefer the bottled water in disposable containers, because of it is spended in a short time. The community must be made conscious of buying and using of bottled water, surely. In the light of this study, another similar study on these selected bottled water brands or different brands should be performed by changing storage conditions such as temperature, moisture and light. Furthermore, investigation of chemical quality of these water samples will be useful for consumers.

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