ورقة العمل المقدمة من مركز فقيه للأبحاث والتطوير الخاصة بالملتقى العلمي الثامن لأبحاث الحج

Implementation of new techniques for a specific and sensitive detection of pathogenic micro-organisms in drinking water

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ABSTRACT

Good quality drinking water is essential for well health status and critical for social and economical development. A Physio-Bio-Chemical investigation of one hundred drinking water sources (50 hotels reservoirs, 25 ground water wells and 25 neighborhood water treatment plants) was carried out in Makkah Al-Mukarramah. During this project, we implemented new techniques for a specific and sensitive detection of pathogenic micro-organisms.

Water contamination by fecal bacteria was explored simultaneously by filtration and most probable number (MPN) methods on selective media. Enterococci, *E. coli* and *Klebsiella* were detected in 100%, 96% and 88% of wells and in 52%, 18% and 4% of hotels reservoirs, respectively. Bacteria counts were significantly higher in ground water sources (P=0.00). Only two water treatment plants were supplying drinking water contaminated by fecal bacteria.

From each type of sources, highly contaminated ones were subjected to Parasitological analysis according to the U.S. EPA method 1623, UK DWI standard operating protocols and ISO/DIS 15553. An *in situ* filtration through Envirocheck capsules followed by immuno-magnetic separation and fluorescence detection method was implemented for the first time in KSA to detect *Giardia* and Cryptosporidium parasites. *Giardia* cysts and *Cryptosporidium* oocysts were detected in one and two sources out of ten, respectively.

Physiochemical parameters were measured *in situ*. Dissolved oxygen, salinity and conductivity were significantly low in water treatment stations samples with respective means of 0.09 mg/L, 0 ‰ and 114 µs/cm. The highest values of biochemical parameters, BOD and COD, were detected in ground water samples.

In summary, supplied water from all studied ground water sources is unsuitable for consumption. Hotels reservoirs become easily contaminated when wells water is used to overcome shortages in municipal desalinated water. Neighborhood water treatment stations remain the optimal choice as a source to obtain relatively safe drinking water in Makkah Al-Mukarramah.

INTRODUCTION

People can survive days, weeks or months without food, but only about four days without water. The body uses water for digestion, absorption, circulation, transporting nutrients, building tissues, carrying away waste and maintaining body temperature. The average adult consumes and excretes about 10 cups of water daily. Adults should drink six to eight cups of liquids per day (Kendall, 1992).

Arabian Gulf countries are located in an arid area with limited water resources. Hydrological investigations point to large resources of underground water, but they are saline and need to be desalted (Al-Mutaz and Al-Anezi, 2004). The best choice for providing fresh water in the Arabian Gulf countries is through sea water desalination with ground water as a back up (Al-Mutaz and Al-Anezi, 2004). Regarding the Kingdom of Saudi Arabia the major drinking water sources are ground water and desalinated sea water. In most occasions, ground water is mixed with desalinated water to increase its levels of total dissolved solids (TDS) before it is pumped into the water networks for consumption. However, in some areas where desalinated water is not available, ground water is still the major source of drinking water (Fayad and Tawabini, 1991). These regional averages mask the already severe water shortages in most of the cited countries. Per capita renewable freshwater supplies are as shown in table 1 (UN, 2003).

Countries	Total renewable freshwater (km^3/y)	Per capita renewable freshwater (m^3/year)		
		1970	2000	2030
Saudi Arabia	2.4	418	118	54
Iraq	96.4	10304	4201	2237
Jordan	0.9	555	183	96
Kuwait	0.02	27	10	6
Oman	1.0	1383	394	164
Bahrain	0.1	455	156	108
Qatar	0.1	901	177	129
Yemen	4.1	648	223	71

Table 1. Annual renewable freshwater available in Middle East countries for the years 1970 and 2000, and the estimated volumes for 2030.

The holy city of Makkah is located at a latitude of 21_25.5′ North and a longitude of 39_48.5 East, approximately 75 Km to the east of Jeddah city. It lies among rocky mountainous foothills at an average elevation of approximately 300 m above sea level (Al-suba'I, 1984). According to its geographical nature, the temperature is high in most days of the year, it ranges between (27-47 °C), it has little rain, the average that can fall in a month or a year may drop in only one or two days, therefore this natural location has its significant effect on its little water reservation (The geographical encyclopedia of Islamic world , 1999).

Citizens and visitors of the holy city have access to several types of drinking water sources, bottled mineral and treated water, locally treated water from neighborhood water treatment plants, desalinated sea water and ground water like the famous ZamZam well.

The Zamzam well, which is located within the precinct of the Holy Mosque in Makkah is sacred to Muslims because of its miraculous origin. Water from Zamzam is used directly for drinking, occasionally by local population but also by millions of visitors. The peak seasons being the month of Ramadan and Hajj period, when Zamzam water is intensively used (Zamzam Studies and Research Centre, 2006). There are many other ground water sources in Makkah region which supplies water directly for consumption to the city population, to local water treatment plants and for its addition to desalinated sea water to increase its TDS. The main wells are located in Al_Rayan and Al_Kheif areas at the North, Al_Quobaiah and Al_Madheq at the North East, Al_Yamaneyah and Al_Zeemah at the East, and Wadi Nouaman at the South of the holy city.

Desalinated water is supplied to Makkah Al_Mokarramah tanks to giant pumping unites from Al_Shoaibah sea water desalination plant on the Red Sea (Saline Water Conversion Corporation) (SWCC). The Al_Shoaibah plant uses a Multi-stage Flash Distillation (MSF) technology to desalinate sea water to potable water by a process where sea water is heated in a heat exchanger and by condensing steam on a bank of tubes.

One of the main sources for drinking water used by Makkah's citizens are the small industrial neighborhood plants which treat raw ground water to a safe final unbottled drinking water sold locally. There are about 49 registered neighborhood plants in Makkah Al_Mukarramah controlled by municipal health services.

Waterborne illnesses, such as Hepatitis, Cholera, Salmonellosis, Cryptosporidiosis, Giardiasis etc. are generally caused by ingestion of water that was contaminated with pathogens derived from human or animal excreta (Ashbolt *et al.*, 2001). It is stated that all water contains impurities, such as protozoa oocyst, bacteria and viruses occurring naturally as result of human activity (Bustamante, 2005). However, significant risks to human health may also result from exposure to nonpathogenic, toxic and heavy metals contaminants that are often globally ubiquitous in waters (Ritter *et al.*, 2002). In summary, the diseases that may be acquired by ingestion of unsafe water are: i) intoxications caused by either chemical substances or toxins produced by microorganisms, ii) infections caused by microorganisms that produce enterotoxins during their growth in the intestinal tract and iii) infections caused by microorganisms that invade the intestinal tract and may travel to and affect other tissues (Roone *et al.*, 2004). New diseases caused by pathogens including Cryptosporidiosis, Giardiaois, pathogenic *E. coli* and a variety of enteric viruses emerged due to changing in nature of waterborne illnesses (Rochelle and Clancey, 2006).

Deaths from water-related diseases are inadequately monitored and reported. A wide range of estimates is available in the public literature, ranging from 2 million to 12 million deaths per year (Gleick, 2002). 1.4 billion annual episodes of diarrhea in children under five years of age kill about 2 million of these children (Medema *et al.* 2003) and as many as 4 billion cases of waterborne diseases occur globally and parasitic diseases accounted for approximately 16.4 million (Medema *et al.*, 2003, Hicks, 1998 and Neumann *et al.*, 2005). WHO verified 578 infectious disease outbreaks in 132 countries from 1998 until 2001 and cholera was the most frequent, with acute diarrhea as the fourth. Further behind were typhoid and paratyphoid fevers caused by *Salmonella typhi* and *S. paratyphi*, respectively (Ashbolt, 2004).

The objectives of this research was a Physio-Bio-Chemical investigation of one hundred drinking water sources (50 hotels reservoirs, 25 ground water wells and 25 neighborhood water treatment plants) in Makkah Al-Mukarramah. During this project, we implemented new techniques for a specific and sensitive detection of pathogenic micro-organisms according to the international standards.

MATERIALS AND METHODS

Questionnaire design: A questionnaire was designed for collection of complete data concerning drinking water sources available in Makkah Al-Mukarramah and suburbs which supplies water for human consumption. These were separated in three categories, ground water wells, Hotel buildings reservoirs and local water treatment plants (WTP). Other data sheets were designed to record parameters measurements and experimental results of physio-chemical, biochemical, bacteriological and parasitological tests.

Samples collections: 100 sites were visited for water samples collection, 50 hotel buildings mainly from the central area of the holy city, 25 wells of ground water and 25 neighborhood water treatment plants. 2 carboys of 1L each were collected from each site for bacteriological and physio-biochemical laboratory analysis. pH, temperature, salinity and DO were measured in situ using a portative meter. For parasites exploration, a filtration built system was carried to the field for samples filtration. Samples and filters were transported to the laboratories in a cool box in presence of ice blocks to maintain low temperatures.

Microbiological analysis: Two parallel microbiological tests were performed for each sample. First two volumes of 100 ml each were filtered through sterile grid-marked 0.45 µm pore size. Following filtration, membranes containing trapped bacteria were placed, one on bile esculine agar and the other one on MacConkey agar media. The filters were then incubated for 24-48 h at 37±0.5°C. Following incubation, colonies were counted and further biochemical or subculture assays were conducted for species confirmation. Special interest was reserved to selected fecal contamination indicators. Results were interpreted, registered and statistically treated. In parallel, water samples were submitted to MPN cultures following the recognized standards, in total 10 tubes of 20 ml with two different volumes and a flask for 50 ml sample culture were prepared for each sample. After 48 h, cultures results were registered and negative ones discarded. Positive ones were submitted to further tests for bacteria identification following the standards.

Parasitological analysis: To investigate the presence of *Cryptosporidium sp* and *Giardia lamblia* oocysts and cysts, the most important waterborne parasites, a filtration system was home built for samples concentration in the field according to the parameters recommended by Pall Inc. company, manufacturers of Envirochek HV filters used in this case. According to the results obtained with bacteriological and biochemical analysis, 10 water sources were

selected for parasites investigation, because such parasitic exploration cannot be done routinely since it is laborious, time consuming and also they are very costly to perform as a routine tests.

The filtration system was carried to the water sources in different days. Depending on the tested water flow rate, 50 to 70L of samples was filtered *in situ*. Envirocheck capsules were then immediately preserved in a cool box in presence of ice blocks to maintain low temperature ambient and transported to the laboratory for their processing.

Once in the laboratory, protozoa oocysts and cysts, if present, were eluted from the filter capsules and centrifuged for their concentration. These were then separated from the rest of particles in the sediment by immuno-affinity using immunomagnetic beads carrying specific antibodies anti-*Cryptosporidium* and anti-*Giardia*. Following separation, the parasites were eluted from the beads and submitted to a direct immunofluorescent antibody test (IFAT) and UV fluorescence microscopy detection.

Physio-Chemical and Biochemical analysis: Dissolved oxygen (DO), conductivity, salinity, pH and temperature were measured *in situ* using glass electrodes combined instruments (WTW multi 340i). Biochemical parameters were determined once in the laboratory following universal standards: Biochemical oxygen demand (BOD) which measures the rate of oxygen uptake by micro organisms in a water sample at a fixed temperature (20°C) and over a given period of time (usually five days) in the dark with a very small amount of micro-organism seed is added to each sample being tested. BOD method was conducted using (WTW, BOD_5) in accordance to Standard Methods for the Examination of water and Wastewater (Method 507:1985 and Method 218B:1971) and the United States Environmental Protection Agency , 1979 (Method 405.1)

Chemical oxygen demand (COD) tests commonly used to indirectly measure the amount of organic compounds in environmental chemistry. COD tests were conducted in the laboratory to determine the amount of organic pollutants found in water and in consequent to determine the quality of water in each source. The values are expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. COD method was conducted using Reactor Digestion Method: Method 8000- EPA approved for water, wastewater and seawater. (Hach spectrophotometer) in accordance to the standards Methods for Examination of Water and Wastewater, 20th Editiom, 2003

RESULTS AND DISCUSSION

Bacteriological screening of water sources:

Although, public health surveillance programs are relatively insensitive for detecting waterborne pathogens, diseases originated by drinking unsafe water have been estimated to cause more than two million deaths and four billion annual cases of diarrhea (Steinberg *et al.*, 2004; Neumann *et al,* 2005). Thus, nowadays in many countries, drinking water is ranked as food, and high standards are being set for its quality and safety. The accurate and strict requirements such as bacterial contents should be nil or very low for environmental ones and no pathogenic or even non-pathogenic other microorganisms should be detectable (Szewzyk *et al*, 2000).

In this project, we carried out an integrated biological and physiochemical investigation the main drinking water sources used by the population and visitors of Makkah Al-Mukarramah, buildings reservoirs which receives both municipal and ground waters, waters sold by neighborhood water treatment plants (WTP) and ground water wells. 100 samples were examined for the presence of bacteria, in particular fecal contamination indicators. Water sources and reservoirs found to be contaminated with *E. coli*, *Enterococcus faecalis*, *Klebsiella* and other non-identified bacteria species are summarized in table 2.

		Tested samples	*E. coli*	Enterococci	*Klebsiella*	Non-coliforms
Wells	Wells (A)	20	19/20 (95%)	20/20 (100%)	17/20 (85%)	20/20 (100%)
	Wells (B)	5	5/5 (100%)	5/5 (100)	4/5 (80%)	5/5 (100%)
Hotels		50	9/50 (18%)	26/50 (52%)	2/50 (4%)	20/40 (40%)
Water Stations		25	0/25 (0%)	2/25 (8%)	0/25 (0%)	3/25 (12%)
Total		100	33/100 (33%)	53/100 (53%)	23/100 (23%)	48/100 (48%)

Table 2: Incidence of total bacteria and bacteria species contamination among investigated water samples from the three different drinking water sources.

* Wells (A): Al-Rayan, Al-khaeef, Zaemah and Yamaneeh; Wells (B): Wadi Nooman regions.

E. coli, Enterococci, *Klebsiella*, *Pseudomonas aeruginosa*, *proteus* and other non-identified bacteria species were identified in analyzed drinking water samples. The presence of total coliforms (including *E. coli* and *Klebsiella*) and Enterococci was a strong indication of fecal contamination in many sources, especially ground water ones. Enterococci were the most frequent bacterial indicator, found in 53% of samples distributed among water samples origins in this order: 25/25 (100%) of wells, 26/50 (52%) of hotels reservoirs and 2/25 (8%) of WTP, but in visibly different counts which means were 13988, 557 and 1.5 /100 ml, respectively. Followed by non-coliforms bacteria which were detected in 48% of sources, *E. coli* and total coliforms bacteria found in 33% and *Klebsiella sp.* in 23% of water sources.

When relative bacteria counts were compared between the three types of sources, these were found to be significantly ($P = 0.00$) high in wells where 23 samples were contaminated by more than 180 *E. coli*/100 ml and the average counts of coliforms and Enterococci bacteria among the 25 tested wells were 1312 and 13988 organisms/100ml, respectively. In the literature it is reported that ground water may show high levels of bacteria contamination due to septic systems, aquifers contamination, wells degradation, inadequate wells construction allowing easy infiltration of contaminated materials around the wells (Bourne, 2001) (figure 1).

28 out of 50 (56%) investigated hotel samples were found to be contaminated by one or more bacteria species. High differences in bacteria counts and types were observed between investigated reservoirs because of the origin of water and the presence or absence of internal filtration units in each hotel. But we have to note that only 11 hotels using filtration systems maintained the systems on a regular basis and 7 hotels either did never or at irregular basis. Furthermore, water reservoirs cleanness maintenance is important for sanitation. In our survey, we found that 33 (66%) hotels treated their water reservoirs every year and remaining hotels either after many years or never did.

No coliforms were detected in samples collected from neighborhood WTP. Never the less, two samples were found to be contaminated with enterococci bacteria but with only 30 enterococci organisms/100ml in one sample and 5 organisms/100ml in the other one, this last sample was also found to be contaminated with 1140 non-coliforms bacteria/100ml. Other two samples were contaminated with non-coliforms bacteria but also with very low counts, 3 and 1 organisms/100ml in each (figure 1). The presence of fecal bacteria, even in very few

samples from WTP, have to be considered as an important issue since water from such treatment plants is announced as totally clean and safe, and because it is intensively used for human consumption.

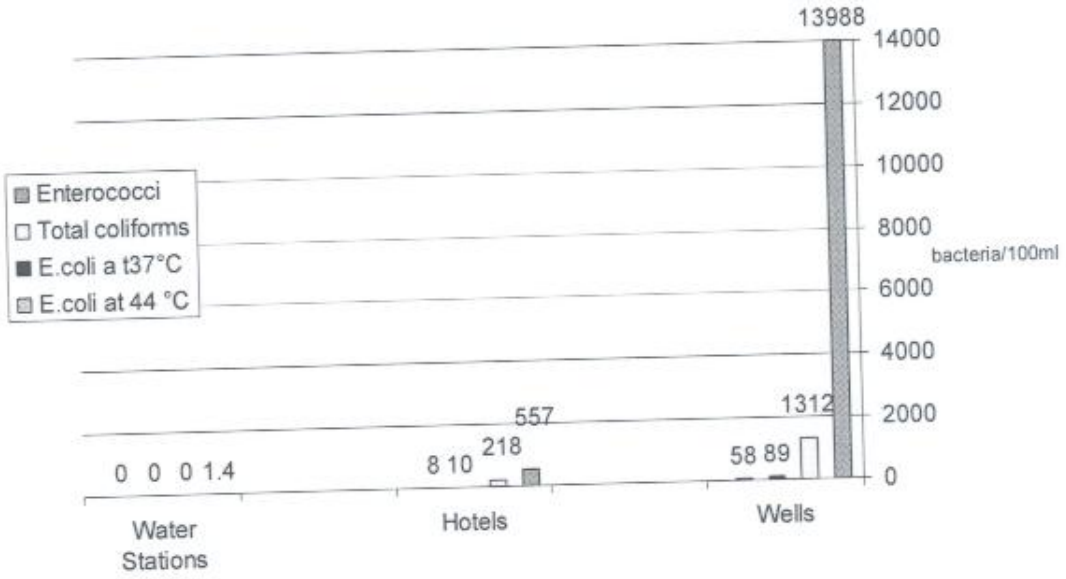

Figure 1: Mean counts of coliforms bacteria, enterococci and *E. coli* (incubated at 37°C and 44°C) /100ml of investigated drinking water samples.

The presence of enterococci in 53/100 (53%) of samples, identified as the most frequent bacteria in investigated Makkah Al-Mukarramah water sources, is in accordance with their high resistance to environmental changes. Baker and Herson (1999) reported that *Enterococcus fecalis* was able to survive for prolonged time under conditions of complete starvation and high temperature in relation to other indicators. Also, Easton *et al.* (2005) observed, when studying the persistence of bacterial indicators to environmental conditions changes, that *E. coli* die-off rates were more significant than die-off rates of enterococci bacteria when temperatures increased from a basic measurement by about 13°C.

Parasitological analysis of water sources:

Parasitological analysis was carried out for the detection of *Cryptosporidium sp.* oocysts and *Giardia sp.* cysts in 10 water sources, 2 hotel reservoirs, 2 WTP and 6 ground water wells (1 from Al-Kheef, 2 from Al-Riyan, 1 from Al- Kobaeeh and 2 from Wadi-Noaman regions) which were found to be highly contaminated by enteric pathogens. 50-70L of water, were filtered in the field through 1µm pore size capsule filters. Elution, immuno-separation and IFAT were run in the laboratory. Results of parasitological assays are summarized in table 3.

Sample	Volume filtrated (L)	No. of *Cryptosporidium* oocysts	No. of *Giardia* cysts
1 (H 5)	70	0	0
2 (H 50)	70	0	0
3 (W 51)	50	2	6
4 (W 53)	50	0	0
5 (W 65)	50	5	0
6 (W 70)	50	0	0
7 (W 71)	50	0	0
8 (W 75)	50	0	0
9 (WTP 89)	70	0	0
10 (WTP 99)	70	0	0

Table 3: *Cryptosporidium* oocysts and *Giardia* cysts detected in investigated water sources.

Samples taken from hotels and WTP were found to be negative for both parasites, *Giardia* and *Cryptosporidium* (Table 3). It is reported in the literature that such water reservoirs may contain parasitic organisms even if in few numbers. Casually in the 2 samples analyzed in our study there was no parasites but all samples should be tested. Abo-Shehada *et al.* (2004), in a similar survey found by parasitological analysis of 255 water samples taken from cisterns in Bani-Kenanah, Jordan a very low incidence (2%) of *Cryptosporidium* in such type of water reservoirs, although fecal bacteria contaminations incidence was about 50%.

Parasitological investigation of ground water sources, revealed a contamination by both parasites, *Cryptosporidium* and *Giardia* in only one well. Another well was found to be contaminated by *Cryptosporidium* oocysts only (Table 3). A study carried out by Karanis *et al.*, (2006) on several drinking water sources including ground water wells, revealed contaminations by *Cryptosporidium* oocysts in 16% and *Giardia* in 11% of studied wells. In contrast an investigation carried out in Ohio, on 16 water samples taken from wells (Fong *et al.*, 2007) showed no contamination by *Cryptosporidium parvum* and *Giardia lamblia*, fact which was explained by the absence of any possible filtration of surface water to these concrete wells.

Physio-chemical and Biochemical screening of water sources:

Biochemical oxygen demand (BOD) which measures the rate of oxygen uptake by micro-organisms in water was determined. Mean values of BOD obtained for hotel reservoirs samples, ground water wells and WTP samples were 2.8mg/l, 6.5 mg/l and 3.7 mg/l, respectively (Figure 2). Slightly higher values of BOD were obtained in waters from WTP compared with hotels samples, probably due to the utilization of row water from contaminated wells. We also observed that wells water samples BOD values varied from a region to another, in Arrayan, Al-Kaeef, Zaemah and Yamaneeh a mean of 7.4 mg/l was obtained and only a mean of 3.9 mg/l was obtained with Wadei Nooman samples.

Chemical oxygen demand (COD) parameter was determined in the laboratory to identify the amount of organic pollutants found in water and consequently to determine the quality of water in each source. Mean values of COD determined for hotel reservoirs, ground water sources and WTP samples were 4.5 mg/l, 10.5 mg/l and 6 mg/l, respectively (Figure 3). In comparison with other studies carried out on river waters, where COD values oscillated from 32.2 mg/l to 42.3 mg/l (Salomoni *et al.*, 2006), we can clearly observe that COD values in ground and desalinated waters are much lower when compared with surface waters which are normally in poor sanitation conditions and exposed to agriculture activities and livestock contaminants.

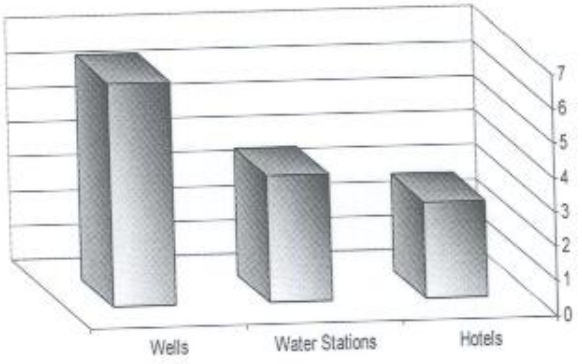

Figure 2: Biochemical Oxygen Demand (BOD) measurements means of water samples from the different types of drinking water sources.

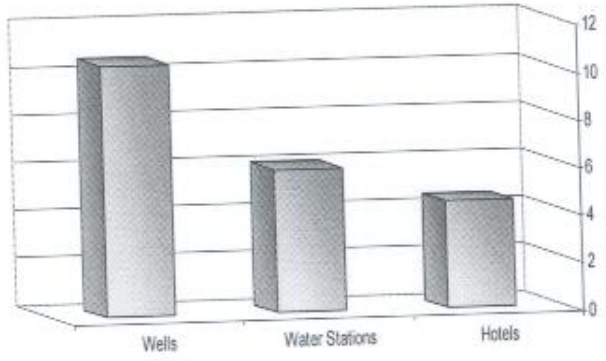

Figure 3: Chemical Oxygen Demand (COD) means obtained for water samples from wells (10.5 mg/l), hotels (6.49 mg/l) and neighborhood water treatment stations (3.6 mg/l).

Temperature is often cited as the most important environmental factor influencing survival of enteric bacteria, protozoa and viruses. Except for samples collected from WTP, which registered temperature values from 17°C to a maximum of 22°C, other visited water sources were at ambient temperatures at the moment of samples collection, ranging from 35 to 43°C in hot days. Fluctuations depended also on the time of visits. Temperatures must increase to higher values in summer time. We can suppose that the effect of higher temperatures in some samples may influence the persistence of sensitive bacteria (Easton *et al.*, 2005).

Also dissolved oxygen is considered one of the most important parameters in an aquatic environment. Low levels of DO in water are signs of possible pollution since low concentrations allow growth of reducing-bacteria causing taste and odor problems. But in ground water, low levels of DO are normal. Furthermore, warm water is much less capable of holding oxygen gas in solution than cool water. All registered DO values are lower than normal values expected in surface water and even in open wells, where other investigators reported higher values (i.e: 3.42 mg/l) (Efe *et al.*, 2005) Low DO obtained values (Table 4) are due to the fact that all waters in Makkah are originated either from desalination or ground water aquifers without a significant ability of aeration and also due to the high temperatures which decrease the ability of oxygen inclusion.

No dissolved salts were detected in water samples collected from WTP. Salinity means in hotels and ground water samples were approximately in the same range with 0.78‰ and 0.61‰ values, respectively. This small difference was due to the fact that, exceptionally very high values were registered in some hotel institutions, reaching 5.9 ‰ in one sample and other 7 samples registered salinity values over 1.5 ‰. And in ground water samples, a maximum value of 1.3‰ was registered and only in two wells out of 25.

Conductivity measurement means were 932.64 µs/cm in ground water sources, 567.26 µs/cm in hotels reservoirs and 217 µs/cm in WTP samples. Almost similar values of salinity and conductivity were reported by other researchers after investigation of 53 groundwater sources in Morocco (Mourabit *et al.*, 2002) where salinity measurements fluctuated between minimum and maximum values of 0.1‰ and 1.0‰ and conductivity measurements from 136 µS/cm to 1880 µS/cm.

pH values ranged from 6.5 to 8.2 with no remarkable differences between the three types of water sources. Means of Values for the other physio-chemical parameters, temperature *in situ*, conductivity, dissolved oxygen (DO) and salinity are shown in table 4.

	Hotels		Wells		WTP	
	Mean	SD	Mean	SD	Mean	SD
Temperature(°C)	32.2	1.67	35.5	2.55	18.51	0.71
Conductivity (us/cm)	567.26	511.90	932.64	564.76	217	85.55
pH	6.7	0.53	6.9	0.31	6.9.	0.24
DO (mg/L)	0.167	0.05	0.178	0.075	0.09	0.01
Salinity (‰)	0.78	0.181	0.612	0.39	0.00	0.00

Table 4: Means of physiochemical parameters values obtained during drinking water sources survey in Makkah Al-Mukaramah. SD: Standard Deviation; DO: Dissolved Oxygen.

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