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Microbiological Quality and Physciochemical Parameters of Alexandria Drinking Water and Zamzam Water

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Water is the most precious natural resource on our planet. Drinking water should be pure and free of contaminants to ensure proper health and wellness. The purpose of the present study was to analyze the microbial and chemical composition of potable tap water and compare it with that of Zamzam water. Water samples were examined using standard methods of analyses following the World Health Organization (WHO) and United States Environmental Protection Agency (EPA) for drinking water, with respect to total dissolved solids (TDS), electrical conductivity (EC), pH, concentrations of calcium, magnesium, potassium, sodium, chloride, fluoride, sulphate, and nitrate. The two sources of water were examined for the presence of potential pathogens, which include viable counts for total viable bacterial count (TVB) and presence of coliform in water. The isolated strain of drinking water approved to be of family Enterobacteriaceae by the most probable number (MPN) technique. Suspected colonies were confirmed and identified as *E. coli* by cultural, morphological, staining characteristics and biochemical identifications. *E. coli* isolate was tested for its resistance to ten different antibiotics by the disc diffusion method. Also, the occurrence of filamentous fungi together with bacteriological parameters was assessed in this study, *Aspergillus* spp. was the most frequently isolated fungal species. The data suggested that the drinking water quality deterioration in Alexandria was unpalatable due to poor sanitation and unawareness about personal hygienic practices. On other hand there wasn't any sign of microbiological growth for Zamzam water samples and free from any contaminant.

Keywords: Potable tap water, Zamzam water, Microbial water quality, *E. coli,* physicochemical analysis.

Water is essential to sustain life, and a satisfactory supply must be made available to consumers[1]. Its quality plays an important role for the safety of food. Efficient surveillance and check strategies are important for executing a high quality management of this resource[2,3]. The Enterobacteriaceae is a large family of Gram-negative bacteria that includes many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. The Enterobacteriaceae, which are common and widespread in the environment, include the coliform bacteria, which are used as indicator organisms in evaluating faecal pollution in various water bodies[4]. *E. coli* is considered a more specific indicator of faecal contamination than faecal coliforms since the more general test for faecal coliforms also detects thermotolerant non-faecal coliform bacteria[5]. *E. coli* may be washed into creeks, rivers, streams, lakes, or groundwater and can be easily enter surface water in fecal material where it persists for weeks. Therefore, *E. coli* serves as an indicator of faecal contamination of water and its presence in water resources refers to possible occurrence of bacterial enteric pathogens[6]. Standard techniques used for detection of *E. coli* from water samples are based

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on cultivation on selective growth media in combination with conventional biochemical tests[7-9].

Fungi are ubiquitous organisms that are widely distributed in nature. It has also demonstrated conclusively that filamentous micro fungi grow and sporulate on the inner surfaces of water pipe and in soft sediments within the water distribution system[10]. Several fungal genera have been shown to be allergenic, such as *Aspergillus*, *Alternaria* and *Cladosporium*[11-14]. Several studies have suggested an important role for waterborne fungi to endanger human health[15-17]. There are several species of *Aspergillus* which cause infection to the human especially *Aspergillus fumigatus*. Aspergillosis is opportunistic respiratory infection which causes about 40% of fatal nosocomial infections. *Aspergillus* spp. infections are transmitted by water[18].

According to Arab historians, the Zamzam well has been in use for around 4000 years. In 1971, the Ministry of Agriculture and Water Resources sent samples of Zamzam for investigations to the European laboratories to test the potability of the water. The results showed that Zamzam water has a special physique that makes it advantageous water[19], also conducted a special research and examined the extent of purity of Zamzam water and found that it has a wonderful physique that makes it different from other drinkable liquids because it is naturally pure and sterile that has no germs in it[20]. Zamzam water has always maintained the same composition and taste ever since it came into existence. Water tastes different at different places, biological growth and vegetation usually takes place in most wells. This makes the water unpalatable owing to the growth of algae causing taste and odor problems. But in the case of the Zamzam water well, there wasn't any sign of biological growth[21]. The main source of Zamzam water is pure by it self but the leakage of underground water and external usage of people cause its pollution. For this purpose ultraviolet rays is being used as a safe mean for sterilization.

The main difference between Zamzam water and other water (city water) was in the quantity of calcium, sodium and magnesium salts, the content of these was slightly higher in Zamzam water[22]. Fluoride as an antimicrobial work is based on the ability of these compounds in inhibiting enolase, phosphopyruvatehydratase, is a glycolytic enzyme responsible for the catalysis of the conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP). This enzyme is an enzyme that plays a role in the metabolism of microbial growth in general[23]. Moreover, the remarks of the European laboratories showed that the Zamzam water was fit for drinking[24]. Drinking Zamzam water enhanced antioxidant power and reduced HbA1c significantly in type 2 diabetic patients. Further research is needed in this area to confirm the results and explore the mechanism behind HbA1c lowering effect produced by Zamzam water[25]. Zamzam water is considered as the richest of all waters in the world in calcium[26]. Zamzam water has never been chemically treated or chlorinated as in the case with water pumped into the cities[27]. Its hydrogen component is 7.75, indicating that it is alkaline to some extent[28]. According to[29] and after matching up the chemical analysis to the specifications of World Health Organization[30], results proved the beneficial effect of Zamzam water on the body. Total dissolved solids (TDS) affect taste of drinking water. Water with content above 1500 mg/liter can taste poor. Generally, TDS levels of less than 500 mg/L are acceptable to households[31].The aim of this study was to analyze the drinking tap water quality in respect to microbial contamination and physicochemical composition comparing it with that of Zamzam water and discover any microbial pathogens in water as a source of biological environmental health hazard.

MATERIALS AND METHODS

Sample Collection and Examination

Potable tap water samples were collected randomly from two different localities within Alexandria in the period between January and May 2014 and two samples of Zamzam water were collected from the Al Haram pipes. Prior to sampling, water was allowed to run for several minutes to avoid external contamination. Collected water samples about 200 ml were immediately transported in clean sterile glass bottles to the laboratory of Microbiology, Faculty of Science, Alexandria University, Egypt. For the Zamzam water, two samples were collected from the Al Haram pipes. Samples were examined individually,

subjected to Microbiological study and Physicochemical analysis.

Microbiological Study

Bacteriological examination

The two water sources were tested for the presence and enumeration of coliform bacteria and total viable bacterial count (TVB).

Enumeration of total viable bacteria

For the enumeration of TVB pour plate count method was chosen, using 1 ml of water sample and mixing with melted water plate count nutrient agar tempered at 37°C. Sets of plates were prepared for all samples and incubated aerobically at 37°C for 48 hrs, experiments were conducted in duplicate. All colonies were counted as colony forming units (CFU) per milliliter of the water sample.

Detection and enumeration of *coliforms*

For detection and enumeration of Enterobacteriaceae *spp. (coliform)*, the most probable number (MPN) technique was carried out[32]. One milliliter from the sample was used to inoculate two test tubes containing Macconkey broth with Durham's tube[33]. The tubes were incubated at 44°C for 24–48 hrs. The production of acid yellow color and gas (appear in durham tube) from lactose indicate the presence of lactose fermenters. A loop full of the positive tubes was cultured on eosin methylene blue (EMB) reference agar and incubated at 37°C for 24 hrs based on the occurrence of a green metallic sheen that appears on the surface of the bacterial colonies[34], which indicated the presence of faecal coliforms. Suspected colonies were confirmed as *E. coli* using standard biochemical test methods.

Identification of lactose fermenter

Cultural, morphological and staining characteristics

The primary identification of the isolate was carried out on the basis of cultural characteristics, cellular characteristics, and microscopic observations. The detection of which aid in the identification and classification of bacteria those were found morphological identical[35]. Cultural characteristics or colonial morphology of bacteria grown on the nutrient, blood and MacConkey agar media were recorded. Gram's staining method was performed to study the cellular morphology. The first step in screening the lactose fermenting Enterobacteriaceae isolates for potentially pathogenic features consisted of testing their ability to grow on human blood agar media. Pure 24 hrs bacterial cultures were streaked aseptically on blood agar plates and incubated at 37°C for 24 hrs. The observation of green zones around the colonies suggested α- haemolysis. Clear zones around the bacterial colonies indicated β- haemolysis; and no reference was made to as γ-haemolysis[36]. MacConkey agar is culture medium designed to grow Gram-negative bacteria and stain them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose).

Biochemical identification

Several biochemical tests were performed and done to detect *E. coli*[37], at Genetic Engineering and Biotechnology Research Institute, Scientific Research and Technology Applications City, Egypt. The biochemical tests performed were sugar fermentation tests, catalase tests, indole test, methyl red (MR) test, Vogue's Proskauer(VP) test, lactose fermentation, urease production and citrate utilization[38].

Determination of susceptibility to antimicrobials

The investigation of antimicrobial susceptibility of the bacterial isolate was carried out by the Kirby-Bauer quality-controlled disk diffusion method[36]. Antibiotic disks were placed on the inoculated plates with sterile forceps on Muller-Hinton agar (Hi-Media, India)[39]. The plates were incubated at 37ºC for 24 hrs. The following antibiotics were tested (Dose strength): penicillin (P; 10µg), ampicillin (AMP; 10µg), amikacin (AK; 25µg), meropenem (MEM; 10µg), ceftriaxone (CRO; 10µg), ciprofloxacin (CIP; 10µg), erythromycin (E; 15µg), streptomycin (STR; 10µg), gentamicin (GEM; 10µg), and tetracycline (TET; 25µg). These antimicrobial agents were chosen on the basis of their importance in treating human or animal *coliform* infections and their use as feed additives to promote growth in animals and on the basis of their ability to provide diversity for representation of different antimicrobial agent classes[40]. The mean zones of inhibition were finally measured, including the diameter of the disk using a ruler to the nearest millimeter and recorded.

Fungal Examination

On Sabouraud's dextrose agar (SDA)

media, the two water sources were plated and incubated at 28ºC for 72 hrs. Fungal colonies that developed were subcultured onto fresh SDA for pure, single colony isolation and identification. The identification of filamentous fungi was based primarily on the macroscopic and microscopic morphology.

Macroscopic examination

On SDA media the texture, surface of the colonies, color and pigment at reverse (underside) appeared in positive fungal growth were detected.

Microscopic examination

A small portion of the fungal growth was mixed with drops of Lactophenol Cotton Blue (LPCB) on slide. The slide tested by low and high power for presence of macroconidia, microcondia, spores and hyphae[41]. The phenotypic identification of fungi according to Microscopic characters is based on morphological characteristics such as septation of hypha, and the formation, morphology, patterns and branching frequency of conidiophores[42].

Physicochemical Analysis

The analysis was carried out within 48 hrs. The analysis was for sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), chloride (Cl), fluoride (F), nitrate (NO3), and sulphate (SO4). The pH, the total dissolved solids (TDS), and electrical conductivity (EC) were also measured. The analyses were carried out at Central Laboratory Unit, Faculty of Science, Moharram Bek, Alexandria University, Egypt.

Apparatus

The hydrogen ions concentration (pH) of water samples has been measured by using pH electrode, turbidity by optical absorption. The total dissolved salts and electrical conductivity for water samples have been determined at the temperature of laboratory by Conductivity TDS. OC. meter with TDS in mg/l and EC in µS/cm units. The calcium and magnesium concentrations have been calculated from titration method by the calibration with standard solution (EDTA-Na) in mg/l[43]. The sodium and potassium concentrations have been measured by Flame Photometer, the readings were recorded and calibration curve was prepared[44]. The water samples were run and the concentration was calculated in mg/l. The analysis of chloride (Cl), sulphate (SO4), nitrate (NO3), and fluoride (F) have been registered by means of Ion Chromatography (IC) the concentration of the samples were recorded as mg/l[44]. The average calculations were estimated for each source.

RESULTS

Microbiological Parameters

Bacteriological examination

The water samples of the two sources were tested for the presence and enumeration of total viable bacteria count (TVB) and coliforms. The average count of drinking water samples varied between 2.5–5.0 x 10^2 CFU/ml Table (1). The diagnostic colonies with the same morphological appearance. The most probable number (MPN) technique was used to detect *E. coli* as an Enterobacteriaceae member. The production of (acid yellow color) and gas (appear in durham tube) from lactose indicate *E. coli* positive. Tap drinking water samples were contaminated with *E. coli* which identified by using cultural and biochemical techniques. According to the pollution status as per the described limit of World Health Organization[30], Table (2) the drinking water samples

Table 1. Total viable bacterial count (CFU ml^{-1}) of the samples tested at 37°C, and incubated for 48hrs

Sample type	Total viable bacterial count (CFU ml^{-1})
Tap water	
Sample (1)	100 < TVB < 1000
Sample (2)	TVB ≥100
Zamzam water	
Sample (1)	-ve
Sample (2)	-ve

Table 2. The pollution status of drinking water on the basis of *E. coli* contents (WHO 1984)

E. coli (CFU/ml)	Water pollution status
10,000	Heavily polluted
1000	Polluted
100	Slightly polluted
10	Satisfactory
3 or less	Potable
TVB	Tap water samples
2.5–5.0 x 10^2	Slightly polluted - Polluted

were under the category between slightly polluted and polluted exceeded the acceptable recommendation level. But in the case of the Zamzam water samples, there wasn't any sign of biological growth and free from any contaminant, indicated that samples were negative with no single colony of any type.

Identification of bacterial isolate

Cultural, morphological and staining characteristics

The summary of cultural, morphological and staining characteristics of bacterium recovered from drinking tap water is given in (Table 3). The isolated strain was grown in the nutrient agar, eoisn-methylene blue (EMB) agar, blood agar and MacConkey agar medium. The cultural and staining characteristics of the bacterium isolate indicating that the potentially pathogenic isolate was *Escherichia coli*. *E. coli* was gram-negative and grew well on commonly used media as eoisn-methylene blue agar Fig. (1a) green metallic sheen colonies were detected, on blood agar medium Fig. (1b) the observation of clear zones around the colonies suggested β-haemolysis of *Escherichia coli*, and on MacConkey agar medium Fig. (1c) the pink colony pigment is due to lactose fermentation.

Biochemical identification

A series of selective biochemical tests for *E. coli* identification were performed (Table 4). The isolated strain fermented five sugars like sucrose, fructose, glucose, maltose and lactose produced acid with gas. Acid production was indicated by the color change from reddish to orange yellow color and the gas production was manifested by the appearance of gas bubbles in the inverted Durham's tubes. The isolate was found positive in catalase, methyl-red test, indole test, and negative for VP test, urease test, citrate utilization.

Determination of antimicrobial susceptibility

The antibiotic – resistance profiles of representative *E. coli* isolate was tested regarding its resistance to 10 different antibiotics: penicillin (P; 10µg), ampicillin (AMP; 10µg), amikacin (AK; 25µg), meropenem (MEM; 10µg), ceftriaxone

(a) **(b)** **(c)**

Fig. 1. Representation of plates showing:
a-Green metallic sheen colonies of *Escherichia coli* on EMB agar.
b- β-haemolysis of *Escherichia coli* on blood agar.
c- *Escherichia coli* on MacConkey agar, pink colony pigment is due to lactose fermentation.

Table 3. Summary of cultural and staining characteristics of bacterium isolated from tap water samples

Cultural characteristics				Staining characteristics			
Nutrient agar	Eoisn-methylene blue agar	Blood agar	MacConkey agar	Shape	Arrangement	Gram's staining reaction	Identified orgnism
Creamy, circular, entire, raised, smooth, shiny and non pigmented colonies	Green metallic sheen colonies	Large, red, round, colonies with Beta-haemolysis	Colonies pink to red with bile salt precipitate surrounding the colonies	Short rod shaped	Clumps and Singles	Pink short rod, gram negative bacilli	*Escherichia coli*

(CRO; 10µg), ciprofloxacin (CIP; 10µg), erythromycin (E; 15µg), streptomycin (STR; 10µg), gentamicin (GEM; 10µg), and tetracycline (TET; 25µg). The mean diameter of inhibition zones were measured in mm (millimeters) and the results were recorded in Fig. (2). Overall, the largest diffusion zones (indicating greater susceptibility). *E. coli* susceptibility was in the order: gentamicin > streptomycin > meropenem> ceftriaxone > amikim >ciprofloxacin > tetracycline > erythromycin > ampicillin > penicillin. Inhibition zones with diameter less than 10 mm were considered as having no antibacterial activity. Diameters between 10 and 14 mm were considered moderately active, and those with >14 mm were considered highly active. Accordingly inhibition zones growth of penicillin, ampicillin, erythromycin were considered as highly resistant zones, and zones of gentamicin, streptomycin, meropenem, ceftriaxone, were considered as highly susceptible.

Fungal examination

Only one fungal isolate was detected in drinking tap water samples. Generally phenotypic identification of fungi is based on the morphological features of the colony and microscopic examinations. Macroscopic characteristics such as colony form, structure, size and color are also important Fig. (3a). The colony color appears white at the beginning and turns to yellow. Microscopic characteristics is based on morphological features Fig. (3b). From the macroscopic and microscopic examinations, we could conclude that the fungal isolate that revealed from drinking tap water samples was *Aspergillus* spp. *Aspergillus* colonies have different colors which can be used in their identification[45]. Some species of specific colors are given in (Table 5), indicating that the fungal isolate may be *A.*

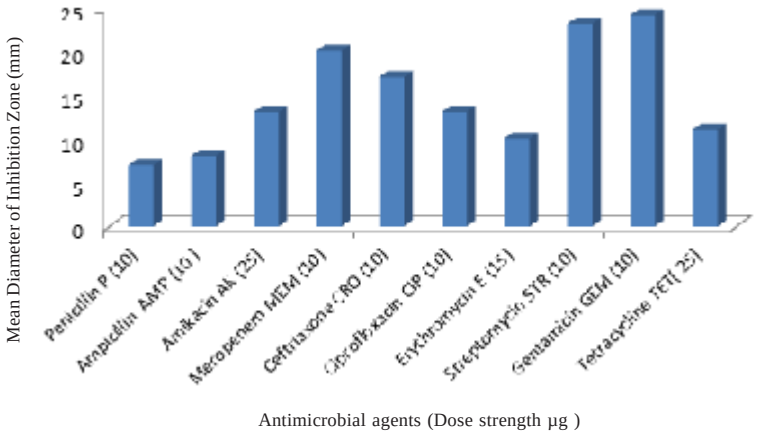

Fig. 2. Antimicrobial susceptibility patterns of *E. coli* by agar disc diffusion method

Table 4. Biochemical characterization of *E. coli*

Sugar fermentation	Result	Biochemical reaction	Result
Sucrose	+	Catalase test	+
Fructose	+	Indol test	+
Glucose	+	Methyl red test	+
Maltose	+	Voge's Proskauer test	-
Lactose	+	Urease test	-
		Citrate utilization	-

N.B: + = Positive, - = Negative

versicolor. In case of Zamzam water samples, there wasn't any sign of biological growth and free from any contaminant, indicated that samples were negative with no single colony of any type.

Physical and Chemical Analysis

The average laboratory investigations for the physicochemical analysis of Zamzam water compared to drinking water were shown in (Table 6). Zamzam water showed highly significant readings in all inorganic elements: Na, Ca, Mg, K, Cl, F, NO_3, TDS and hence higher electrical conductivity than drinking water, except for pH which was almost similar in tap water (7.40) to that of Zamzam water (7.75).

DISCUSSION

The obtained results revealed that *E. coli* was found in drinking tap water samples indicates recent sewage or animal waste contamination[46,47]. The same results have been reported by[48], who isolated *E. coli* from 14% of water samples obtained from 50 mosques in Tripoli, Libya. *E. coli* contamination in the present study was found to be higher than that recorded by[49] which represent 10.7%. The Ministry of Health's Annual Report on Drinking-Water in New Zealand showed unacceptable levels of *E. coli* were found in the water of 72,000 or 2 percent of people accessing a registered water supply[50]. So, it was concluded from the present study, that samples taken from tap water exceeded the maximum bacteriological limits according to WHO standards brands. This may originate from the water source, sanitation conditions of process, unhygienic[51]. On the other hand in Zamzam water well, there isn't any sign of biological growth[52,53]. The main source of Zamzam water is pure by itself but the leakage of underground water and external usage of people cause its pollution. For this purpose, before distribution of Zamzam to consumers at the King Abdul Aziz station filling point is treated by a series of sand filters, micro filters and ultraviolet disinfection; therefore the source of Zamzam water is generally of good quality for drinking. But if water is not properly protected during filling up, it could be subjected to contamination[54].

The fermentation reaction of *E. coli* isolate in sucrose, fructose, glucose, maltose and lactose sugars was positive. The other biochemical

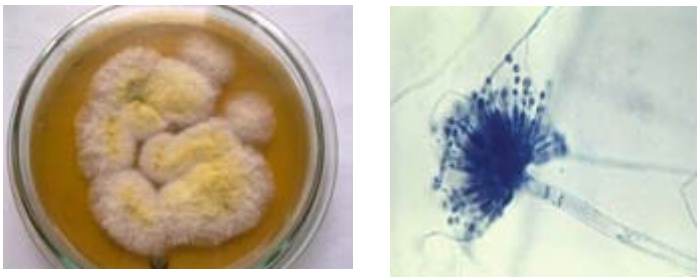

Fig. 3.

a- Macroscopic colony features **b-** Microscopic appearance

Table 5. The color of the colony in various *Aspergillus* species

Species	Surface Color
A. clavatus	Blue-green
A. flavus	Blue-green to gray
A.fumigates	Yellow-green
A.glaucus group	Green with yellow areas
A.nidulans	Green, buff to yellow
A.niger	Black
A.terreus	Cinnamon to brown
A.versicolor	White at the beginning turns to yellow, tan, pale green or pink

reactions Catalase, Methyl-red, Indole tests were also positive. The result of sugar fermentation agreed with the findings of [55,56]. These respective authors reported that although *E. coli* ferments sugars but it partially fermented sucrose and maltose. Variation of the results might be due to genetic factors and nature of inhabitant of the organisms.

The emergence of antimicrobial resistance in bacteria has been problem throughout the world[57]. The resistance of *E. coli* isolates to oflaxacin followed by cefdixin and ciprofloxacin, the gentamicin, chloramphenicol, streptomycin were the most effective[58]. It has been reported that beta-lactamase producing *E. coli* which had become resistant to ceftriaxone can become sensitive to the same antibiotic when the inhibitor sulbactam is added[59]. The excellent susceptibility to streptomycin, meropenem and variable susceptibility to aminoglycoside[60].

Waterborne fungi have been suspected as a source for allergic reaction in sensitive individuals and they may contribute to produce mycotoxins in water[61]. The recorded results here showed that *Aspergillus* was the most frequently isolated fungal species in drinking tap water. This finding is consistent with the works conducted by[62-64]. Twenty one percent of drinking water samples have been contaminated with *Aspergillus* spp[17]. *Aspergillus* spp. were recorded by[65] to be more as 70% of all drinking water samples examined. *Aspergillus* spp. are opportunistic and can cause health problems in immune compromised patients[18].

Table 6. Summary of the results obtained in this study for physical and chemical parameter

Parameter	Zamzam water	Tap water
Appearance	Clear	Clear
Odor	Acceptable	Acceptable
Turbidity	Nil	Nil
pH	7.75	7.40
Total Dissolved Solids(TDS)	329.0	207.0
Conductivity (µS/cm)	156.7	95.6
Chloride (Cl)	21.3	17.2
Fluoride (F)	0.64	0.43
Sulfate (SO_4)	1.21	1.57
Nitrate (NO_3)	1.60	1.40
Sodium (Na)	12.6	8.0
Potassium (K)	40.0	7.0
Calcium (Ca)	96.0	75.2
Magnesium (Mg)	31.7	17.1

Chemical results are in parts per million (ppm) or milligrams per liter (mg/l).

Comparison of the concentration of chemical constituents of drinking tap water in Alexandria and Zamzam water with respect to the drinking water standards[66], and to EPA showed that Zamzam water demonstrated highly significant readings in all inorganic elements including higher levels of fluoride when compared to tap water. Exposure to fluoride in drinking water has been shown to be beneficial for oral and general health, especially in relation to dental caries and osteoporosis[67-70]. The water contains fluorides that have an effective germicidal action[19,67]. The World Health Organization[30], regards 1.5 ppm as the upper limit of fluoride exposure that is appropriate. The pHs of all water samples showing slightly alkaline behavior. However, It was stated that alkaline drinking water plays an important role in ridding the body of mercury and other toxins[71]. Consequently, alkaline water has been used for improving bone density and healing[72]. It was indicated that Zamzam water is alkaline natural water which makes it potentially capable of enhancing antioxidant power[73].

Calcium is the most abundant and the most important mineral in the body. If the body does not have about two parts magnesium for every one part calcium, the calcium becomes pollution for the body while magnesium helps keep bone from becoming brittle[74]. Ionic calcium in water is the best form to use, being the only physiologically active form of this element to insure its proper absorption by the bones and teeth. The quantity of calcium and magnesium salts were slightly higher in Zamzam water than in drinking water[75].

Conductivity is a proxy indicator of total dissolved solids, and therefore an indicator of the taste or salinity of the water. There is little direct health risk associated with this parameter, but high values are associated with poor taste, customer dissatisfaction and complaints[76,77]. High conductivity water, for example, can cause excessive scaling in water pipes, heaters, boilers and household appliances. The conductivity of water varies considerably by geological region,

owing to differences in the mineral and chemical properties of the water body. The value of electrical conductivity (EC) is almost half the value of TDS as proved in many studies[78], which is acceptable for drinking water.

CONCLUSION

Quantitative results revealed the presence of pathogenic organisms and water quality risk factors due to the damaged water and environmental sanitation infrastructure. Continued water quality monitoring, the application of household based disinfectants, and healthy domestic hygiene practices are highly recommended in similar circumstances. Also, residents of the area must endeavor to cultivate better sanitation habits and ensure that their surroundings and water sources are not indiscriminately polluted. The data clearly suggests that people of this region are under severe threat of water–related diseases and health risks. Zamzam water is 100 percent natural. There wasn't any sign of biological growth and free from any contaminant, and dose not chemically treated in any way. This study led to the conclusion that Zamzam water has a rich essential mineral profile. Zamzam water may provide safer alternative nutritional strategy to solids in labor. This approach is used successfully for pilgrims on Hajj and Umrah.

REFERENCES

1. Alhassan, H., Kwakwa, P.A. When water is scarce: the perception of water quality and effects on the vulnerable. *Journal of water, Sanitation and Hygiene for Development*., 2014; **4**: 43-50.
2. Alzahrani, A.M., Gherbawy, Y.A. Antibiotic resistance in *Escherichia coli* strains isolated from water springs in Al-Ahsa Region. *African J Microbiol Res*., 2011; **5**(2): 123-130.
3. Farkas, A., Drãgan-Bularda, M., Ciatarâº, D., et al. Opportunistic pathogens and fecal indicators in drinking water associated biofilms in Cluj, Romania. *J Water Health.* , 2012; **10**: 471-483.
4. Figueras, M.J., Borrego, J.J. New perspectives in monitoring drinking water microbial quality. *Int J Environ Res.* Public Health., 2010; **7**: 4179-4202.
5. Francy, D.S., Donna, N., Myers, T., et al. *Escherichia coli* and faecal coliform bacteria as indicators of recreational water quality. *Water Res*. Investigations Report., 2013; **93**: 4083.
6. **Katouli, M. Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. *Iran J Microbiol*., 2010;** 2**(2): 59–72.**
7. Augoustinos, M.T., Grabow, N.A., et al. An improved membrane filtration method for enumerating faecal coliforms and *E. coli* by a fluorogenic â-glucuronidase assay. *Water Sci Technol*., 1993; **27***:*267–270.
8. Eckner, K.F. Comparison of membrane filtration and multiple-tube fermentation by Colilert and Enterolert methods for detection of waterborne coliform bacteria, *Escherichia coli*, and Enterococci used in drinking and bathing water quality monitoring in Southern Sweden. *Appl Environ Microbiol*., 1998; **64***:* 3079–3083.
9. Rompré, A., Servais, P., Baudart, J., et al. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *J Microbiol Methods*., 2002; **49***:* 31–54.
10. Sammon, N.B., Harrower, K.M., Fabbro, L.D., et al. Three potential sources of microfungi in a treated municipal water supply system in sub-tropical Australia. *Int J Environ Res*., Public Health., 2011; **8(3)**: 713-732.
11. Black, P.N., Udy, A.A., Brodie, S.M. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy*., 2000; **55**: 501-504.
12. Bowyer, P., Fraczek, M., Denning, D.W. Comparative genomics of fungal allergens and epitopes shows widespread distribution of closely related allergen and epitope orthologues. *BMC Genomic*., 2006; **7**: 251.
13. Hedayati, M., Pasquallotto, A.C., Warn, P. A., et al. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology*., 2007; **153**: 1677.
14. Simon-Nobbe, B., Denk, U., Schneider, P.B., et al. NADP-dependent mannitol dehydrogenase, a major allergen of *Cladosporium herbarum*. *J Biol Chem*., 2006; **281**: 16354-16360.
15. Anaissie, E.J., Kuchar, R.T., Rex, J.H., et al. Fusariosis associated with pathogenic *Fusarium* species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. *Clin Infect Dis*., 2001; **33**: 1871.
16. Anaissie, E.J., Stratton, S.L., Dignani, M.C., et al. Pathogenic molds (including *Aspergillus* species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. *Blood.*, 2003; **101**: 2542-2546.

17. Warris, A., Gaustad, P., Meis, J.F., et al. Recovery of filamentous fungi from water in a paediatric bone marrow transplantation unit. *J Hosp Infect*., 2001; **47**: 143-148.
18. Graybill, J.R. The echinocandins, first novel class of antifungal in two decades: Will they live up to their promise? *International J. of Clinical Practice*., 2001; **55(9)**:633-638.
19. The institute of the Custodian of the Two Holy Mosques. Analytical report of Zamzam water during the Ramadan and Hajj Seasons 1425H. *Al Hajj research centre*., 2005; Om Al Qura university.
20. Analytical report of Zamzam water during the Ramadan and Hajj Seasons. Cited from the institute of the Custodian of the Two Holy Mosques. *Al Hajj research centre.*, 2005; Om Al Qura university.
21. Zamzam Studies and Research Centre. Cited from The Saudi Geological Survey. 5th June; 2005.
22. Koshak, Y.H. Zamzam, First edition, *Dar Alelm for Publications.*, Jeddah.1983; **19**:126.
23. Haili, Ma., Xiaohu, Wu., Meng Yang, et al. Effects of fluoride on bacterial growth and its gene/protein expression. 2014; (100): 190 – 193.
24. Analytical report of Zamzam water. Cited from the annual report of the ministry of agriculture and water resources; 1971.
25. Bamosa, A., Elnour, A., Kaatabi, H., et al. Zamzam Water Ameliorates Oxidative Stress and Reduces Hemoglobin A1c in Type 2 Diabetic Patients. *J. Diabetes Metab*., 2013; **4**:3.
26. Hussain, T., Ahmed, M. Zamzam water: Scientific research findings. *Al-Islaah publications*; 2010. Imam Muslim on authority of AbiTharr. (2473).
27. Nabulsi, B. Zamzam Water. *Food and Cure*; 2007.
28. Saudi Geological Society & Central-Mosque. Zamzam well, its water& its history; 2006.
29. Yaqub, G., Hamid, A. Comparison of water quality and bottled. *Journal of Applied Sciences in Environmental Sanitation.*, 2014; **9**: 57-65.
30. World Health Organization. Guidelines for drinking water quality & recommendations., 1984. Geneva WHO.Vol. 1.
31. US Environmental Protection Agency (EPA). Code of Federal Regulations 40 Part 141. National Primary Drinking Water Regulation, 1986. U.S.A.
32. Christopher, R., Sechul, Z., Daniel, A., et al. Surface Water Treatment for Communities in Developing Countries; 1981.
33. APHA. Standard methods for the examination of water and waste water, 20th edition. *American Public Health Association*, Washington, D.C.,1981.
34. Hossain, Z., Sultana, P., Deb, S., et al. Multidrug resistance in large-plasmid-associated presumptive enterohaemorrhagic *Escherichia coli* isolated from contaminated lake water. *Bangladesh J Microbiol.* ,2012; **28**: 33-40.
35. Greenberg, A.E., Trussell, R.R., Clescri, L.S. Standard Methods for the Examination of Water and Waste water; 1985.16thedn. APHA, AWWA, WPCF, New York, USA.
36. Atlas, R.M. Principles of Microbiology; 1997. 2nd ed., W.C. Brown Publishers.
37. Holt, J.G., Krieg, N.R., Sneath, P.H., et al. Bergey's Manual of Determinative Bacteriology. In: Williams K, Wilkins O,(Eds.), *Baltimore*; 1993. USA, 9p.
38. Cheesbrough, M. District laboratory practice in tropical countries (part 2). Cambridge University Press, UK; 2000. p. 62-70.
39. Bauer, A.W., Kirby, W.M., Sherris, J.C., et al. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*., 1966; **45**: 493-496.
40. Krumperman, P. H. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of foods. *Appl Environ Microbiol*., 1983; **46**: 165-170.
41. Chei, J.O., Kalhatkar, A. Medical Laboratory Science. Theory and Practice. McGraw Hill, New Delhi; 2000.p. 1067 – 1069, 1072 – 1075.
42. McClenny, N. Laboratory detection and identification of *Aspergillus* species by microscopic observation and culture. *J Md Mycol*., 2005; **1**:S125-S128.
43. Smith, J.W. National Drinking Water Mission, Department of Rural Development, Government of India.1988; 53-59.
44. APHA. Standard methods for the examination of water and wastewater, 14th edition. American public Health Association, Washington, D.C., 1976.
45. Deryck, D.P. *Aspergillus*. Health Implication & recommendations for Public Health Food Safety. *Internet Journal of Food Safety.*, 2006; (8):19-23.
46. US Environmental Protection Agency (EPA). Preparing your drinking water consumer confidence report: Revised guidance for water suppliers, 2001. Retrieved November 26, 2008.
47. Bharath, J., Mosodeen, M., Motilal, S., et al. Microbial quality of domestic and imported brands of bottled water in Trinidad. *Int J Food Microbiol.*, 2003; **81**: 53-62.
48. Ghenghesh, K.B., Belhaj, K., Algaui, A., et al.

Bacteriological quality of drinking water obtained from mosques in Tripoli, Libya, *The Libyan Journal of Infectious Diseases*., 2007; **1**: 49-53.

49. Golas, I., Filipkowska, Z., Lewandowska, D., et al. Potentially pathogenic bacteria from the family Enterobacteriaceae, *Pseudomonas* spp. and *Aeromonas* spp. In water designated for drinking and household purposes. *Polish J of Environmental Studies.*, 2002 ;**11(4)**: 325-330.
50. Danya, L. Unacceptable levels of *E. coli* in drinking water. *Fairfax New Zealand Limited Privacy*; 2011.
51. Tamagnini, L.M., Gonzalez, R.D. Bacteriological stability and growth kinetics of *Pseudomonas aeruginosa* in bottled water. , 2006; 83: 91-94.
52. The annual report of the ministry of agriculture and water resources, 1971. Analytical report of Zamzam water.
53. Mashat, B.H. The microbiological quality of sabil free drinking water in Makkah Al-Mukarramah. JKAU: *Met Env& Arid Land Agric Sci*., 2010; **21**: 87-100.
54. Yahya, H.K. Zamzam, First edition, *Dar Alelm for Publications*., Jeddah.1983; **3** (19) 126.
55. Beutin, L., Geier, D., Zimmeronann, S., et al. Epidemiological relatedness and clonal types of natural populations of *E. coli* strains producing shiga toxins in separate population of cattle of sheep. *Appl Environ Microbiol*., 1997; **63**: 2175-2180.
56. Sandhu, K.S., Clarke, R.C., McFadden, K., et al. Prevalence of the *eaa*Agene in verotoxigenic *E. coli* strain in dairy cattle in South-West Ontario. *Epidemio and Infect*., 1996; **116**: 1-7.
57. Cohen, M.L. Changing patterns of infectious disease. *Nature*., 2000; **406**: 762-767.
58. Tambekar D.H., Hirulkar, N.B.,, KaliKa M.V., et al. Prevalance of thermotolerant *E. coli* in drinking water and its multidrug-resistance. *Res J Microbial*., 2006; **1**: 458-462
59. Abdul, J., Abdul, W., Naseem, R., et al. Comparative susceptibilities of *E. coli* to ceftriaxone alone and in combination with sulbactam. *Pak J Med Res*., 2005; **44**:12-14.
60. Alhussain, J., Zahrani, A., Naeem, A. Susceptibility patterns of extended spectrum - Lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* isolated in teaching hospital. *Park J Med Res*., 2005; **44**: 64-67.
61. Hageskal, G., Lima, N., Skaar, I. The study of fungi in drinking water. *Mycol Res*., 2009; **113**: 165-172.
62. Arvanitidou, M., Kanellou, K., Constantinides, T.C., et al. The Occurrence of Fungi in Hospital and Community Portable Waters. *Letters in Applied Microbiology*.,1999; **29**, 81 – 84.
63. Arvanitidou, M., Spaia, S., Velegrak, A. High Level of Recovery of Fungi from Water and Dialysate in haemodialysis units. , 2000; **45** 225 -230.
64. Gunhild, H., Ann, K., Peter, K. Diversity and Significance of Mold species in Norwegian Drinking water. *Appl Environ Microbiol.*, 2006; **72**(12): 7586-7593.
65. Anaissie, E.J., Stratton, S.L., Dignani, M.C., et al. Pathogenic *Aspergillus* species recovered from a hospital water system: a 3-year prospective study. *Clin Infect Dis*., 2002; **34**:780-789
66. World Health Organization. Guidelines for Drinking Water Quality – I.Recommendations.1993; 2nd edn. World Health Organization, Geneva.
67. Brambilla, E. Fluoride. Is it capable of fighting old and new dental disease? An overview of existing fluoride compounds and their clinical applications. *Carie Res.*, 2001; 35 suppl **1**:6-9.
68. Kaminsky, L.S., Mahoney, M.C., Leach, J., et al. Fluoride benefits and risks of exposure. *Crit Rev Oral Biol Med*., 1990; **1**(4):261 – 81.
69. Ripa, W.L. A half century of community water fluoridation in the US. Review and commentary. *J Public Health Dent*., 1993; **53**:17-44.
70. Pervianen, K., Nordlling, H., Ainamo, J. Occurrence of dental caries and gingivitis in low, medium and high fluoride areas in Finland .*Community Dent Oral Epidemiol.*, 1977; **5**: 287-291.
71. Kellas, W., Dworkin, A., Kellas, W. Surviving the Toxic Crisis. *Comprehensive Health*, first ed. 1996; 523.
72. Wynn, B., Krieg, M. A., Aeschlimann, J.M. Alkaline mineral water lowers bone desorption even in Ca sufficiency: alkaline mineral water and bone metabolism. *Bone*., 2009; **44**: 120–124.
73. Shomar, B. Zamzam water: concentration of trace elements and other characteristics. *Chemosphere*., 2012; 86: 600-605.
74. Remer, T. Animal proteins cause calcium leached from the bones. *Am J Clin Nut*., 1994; **59:** 1356 –61.
75. Al-Weheb, A.M., Fahad, A. H. Effect of Zamzam water on the microhardness of initial caries like lesion of permanent teeth, compared to casein phosphopeptide-amorphous calcium phosphate agents. *Journal of Baghdad college of dentistry.*, 2012; **24**, 128-132.
76. Howard, G., Pedley, Barrett, M., et al. Risk factors contributing to microbiological contamination of shallow groundwater in

Kampala. Uganda, *Water Research*., 2003; **37**, 3421-3429.

77. World Health Organization. Guidelines for Drinking-water Quality. Volume 1.Recommendations.2004; (Third ed.). World Health Organization, Geneva, Switzerland.

78. Argo, B. Understanding pH management and plant nutrition. J. International phalaenapsis Alliance. 2003; **13**: 1.