Evaluation of Microorganisms of Drinking Water of Rafha City, Northern Borders, Saudi Arabia

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The present studies included evaluation of pathogenic parasitic protozoans, bacteria, fungi and algae collected from drinking water of three sources; wells, tap and three re-purifying water-private companies; from Rafha City, Northern Borders, Saudi Arabia, to count and identify these contaminants. The results revealed three species of parasitic protozoans (*Giardia lamblia, Cryptosporidium parvum* and *Cyclospora cayetanensis*), three species of Coliform bacteria (*Proteus mirabilis*, *Escherichia coli*, *Enterobacter cloacae* and *E. aerogenes*) beside seven bacterial genera (*Bacillus, Brevibacillus, Staphylococcus, Streptococcus, Aeromonas, Acinetobacter and Pseudomonas*), five genera of fungi (*Aspergillus*, *Chrysoporium, Alternaria, Acremonium* and *Exophiala*) as well as three genera of algae in tap and the present three re-purifying water-private companies (*Anabaena*, *Microcystis* and *Oscillatoria*). The present results indicated that the three water sources of Rafha city are important contributor to transmission of contaminants to consumers. The present work recommends future field-application of selected specialized anti-parasites and anti-bacterial compounds to control, or at least reduce the present resulting contaminators to gain safe levels of drinking water, according to WHO-, or at least SASO- levels, to reduce the risk of propagation of the present microorganisms.

Key words: drinking water contaminants – protozoa – bacteria – fungi - algae.

Water is scarce and valuable resource in Saudi Arabia. Thereby, well or ground water is still and will continue to be one of the main sources of drinking water in Saudi Arabia, especially in rural and border areas.

The microbial contamination of drinking water constitutes a major issue worldwide, because it is still a major source of infection and mortality. Evaluation of the microbiological quality of drinking water aims to protect consumers from illness, that due to protozoon's parasites, bacteria, fungi, algae and viruses. Nowadays, prevention of contaminated drinking-water-related-illness is still an important challenge [1].

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Rafha-citizens basically obtained their drinking water from treated wells stored in public tanks and re-distributed through waterborne-pipes to houses. However, citizens are usually avoid using tap water as a source of drinking water, owing to their dis-satisfaction of taste, odour and colour of this water. Instead, they prefer drinking water-consumption of desalinated groundwater obtained from some desalinating-private companies others drink industrially bottled water.

The famous protozoan parasites contaminating drinking water are *Giardia*, *Cryptosporidium*, *Entamoeba histolytica* and *Cyclospora*, that cause severe gastrointestinal disorders; namely giardiasis, cryptosporidiosis, amoebiasis and cyclosporiasis respectively [2]. Indication of the presence of disease-causing bacteria in drinking water is the coliform bacteria, that are non-pathogenic but associated with many diseases; from which *Proteus*, *Escherichia coli* and *Enterobact*er; indicating faecal contamination of water. *Proteus mirabilis* is associated with urinary tract infections[3].

In this concept, the coliforms constitute common intestinal commensal bacteria. This group contains important pathogens, as *E. coli*, which is the most prominent and causes disease when the immune-system is suppressed[4]. *E. coli* considered as an indicator of fecal contamination when found in drinking water[5]. *Enterobacter* species act as opportunistic pathogens.

Many other bacteria are pathogenic, such as *Streptococcus* that colonizes the heart valves[6], *Aeromonas* that produces cytotoxic-enterotoxin causes tissue damage[7], *Acinetobacter* that causes urinary tract infections, pneumonia, endocarditis, wound infections, septicemia and meningitis[8] as well as *Pseudomonas* that causes bronchopneumonia, ecthyma gangrenosum, urinary tract catheterization, necrotising enterocolitis, hemorrhage, necrosis of the skin[9].

On the other hand, some fungi are pathogenic; such as *Aspergillus* that causes chronic pulmonary aspergillosis[10], *Chrysoporium* that cause hyalo-hyphomycosis[11], *Alternaria* that cause respiratory infections[12] and *Acremonium* that cause mycetoma, onychomycosis, and hyalohyphomycosis[13].

Some algal genera are pathogenic, as; *Anabaena*, *Microcystis* and *Oscillatoria*. These genera secrete toxins, as *Anabaena* and *Microcystis* that secrete microcystin-LR and cylindrospermopsin, leading to liver inflammation, pneumonia, dermatitis, kidney damage and tumor growth and anatoxin-a group[3]-toxin from *Anabaena* and *Oscillatoria* that causes nervous disorders[14].

SASO (Saudi Arabian Standards Organization) continuously evaluates drinking water standards for bottled, tap and well-waters to define a quality of water that re-inforce healthy population. These standards set limits for the permissible and maximum contaminants level of parasites and the indicator-microorganism that endanger the health of consumers [15]. A substantial number of these standards are originated from the accurate World Health Organization international-standards for drinking water [16].

The main objective of this study is to assess the parasitological and microbiological status of drinkable water of Rafha City in Saudi Arabia.

MATERIALS AND METHODS

Water sampling

The present studies were carried out from July to October, 2014, in Rafha City, Northern Borders, Saudi Arabia. The water-samples had been collected from three major water sources; ten wells, tap and three private desalinating companies; El-Shefaa, Bardy and El-Razaz. Aliquots of 100 ml from each water-sample were collected in sterilized conical flasks, provided with silica gel to keep dryness.

Enumeration of protozoans, bacteria, fungi and algae

Protozoan parasites

All samples preserved in 4% neutralized formalin solution, left to settle [17]. Then, supernatants were collected, filtered through 20 µm net mesh. Materials that retained by filter were then fixed with Lugol's solution. The protozoans were then counted by Sedwgwish Rafter counting method and identified (Cells/100ml), using Olympus binocular compound microscope.

To confirm identification of *Giardia lamblia* and *Cryptosporidium parvum*, aliquots of 100 ml of water samples were filtered through a 47 mm diameter, 0.450 ìm pore size membrane filter. Each material retained by the filter was fixed with Lugol iodine on a separate slide. Fresh preparations of 0.9% saline smear of samples were visually examined for parasitic cysts over approximately 100 fields and then subjected to cold acid-fast. Trichrome staining technique was then applied [18].

Bacteria-count

Coliform bacteria

Coliform bacteria were determined by incubation of samples into lactose broth as presumptive test. The test tubes are placed at 35ºC for 24 hours for gas production. To confirm the presence of coliform, gas produced in incubation into Brilliant Green Bile broth at 35ºC for 24 hours [19]. Water quality analysis was based on the most probable number of Cells/100 ml. The test had been repeated three times.

Aerobic and other facultative anaerobic bacteria

One ml of each water sample was inoculated and spread on blood agar and nutrient agar plates. Then, plates were incubated at 37°C for 18 to 24 h for determination of bacterial cell count, as cells/100 mL, and for isolation and differentiation of various bacterial strains depending on their morphological descriptions. Different isolated bacteria were further identified by Bilog system (Biolog, Hayward, CA, USA). Experiment had been repeated three times[20].

Fungi

Sabouraud Dextrose Agar (SDA) was used as a culture medium to reveal the presence of fungi and chloramphenicol is used to increase selectivity against commensal microorganisms. For isolation of fungi from water-contaminated specimens, SDA-medium should be inoculated and the plates incubated at 28°C in an inverted position with increased humidity. For isolation of fungi, two sets of media should be inoculated at 28°C and a duplicate set at 35°C. All cultures should be examined 7-10 days for fungal growth, and should be held for 5 weeks before being reported as negative[21]. The test had been repeated three times.

Algae

Aliquots of 100 ml from each water sample were allowed to settle overnight in sterilized conical flasks. Then after, solid materials from the bottom of the flasks were pipetted for examination. The experiment had been repeated thrice. Algal species had been identified using binocular microscope (Zeiss)[22].

Statistical Methods

Analysis of Variance "ANOVA"

The mean data of analysis of variance "ANOVA" between four categories; protozoans, bacteria, fungi and algae, had been achieved to obtain significant differences, using the statistical SPSS-program

RESULTS

Protozoan parasites

The present result showed four protozoan-species, as cysts, *Giardia lamblia* (Diplomonadida, Hexamitidae), *Cryptosporidium parvum* (Eucoccidiorida, Cryptosporidiidae), *Cyclospora cayatenensis* (Eucoccidiorida, Eimeriidae) and *Entamoeba coli* (Archamoebae).

Table (1) display average counts the highest values in water of the different wells (5.8, 21, 0.7& 15.1 cells/100 ml for *G. lamblia*, *C. parvum*, *C. ayetanensis* and *E. coli* respectively) whereas *C. cayetanensis* revealed the highest value in tap water, whereas all species were completely absent in water of the three private companies. On the other hand, frequency % revealed the highest values; of *G. lamblia* and *E. coli* in all sources of water (28.57, 27.27 and 33.33% in well-, tap and companies-water respectively), highest values of *C. cayetanensis* in tap and the three companies-water (27.27 and 33.33% respectively) and the highest value of *C. cayetanensis* in well-water (28.57%) (table 2). The statistical analysis of variance "ANOVA" revealed highly significant differences in the two species *G. lamblia* and *C. parvum* (Table 7(.

Bacteria

Coliform Bacteria

The present work reported four gram-negative coliform bacteria-species; facultative anaerobic *Proteus mirabilis*, *Escherichia coli*, *Enterobacter cloacae* and *E. aerogenes*.

Table (1) displays mean counted numbers, as CFU/100 ml, where the highest values of *P. mirabilis*, *E. cloacae* and *E. aerogenes* had been found in well-water (1318.7, 34.2 & 92.5 respectively), beside the highest value of *E. coli* in El-Razaz Company-water (700).

On the other hand, frequency% revealed the highest values; *P. mirabilis* in well-water (22.73%), *P. mirabilis* and *E. aerogenes* in tap water (11.11%), *P. mirabilis* in El-Shefaa company (42.86%), *E. cloacae* in Bardy company (33.33%) and *E. coli* in El-Razaz company (42.86%) (table 3).

Aerobic Bacteria

Gram negative bacteria

Beside the previous coliform bacteria; aggregated gram negative *Acinetobacter haemolyticus* and coccobacillus gram-negative *Pseudomonas aeruginosa* resulted.

The mean counts, as CFU/100 ml, displays the highest values of *A. haemolyticus* and *P. aeruginosa* in well-water (16.7 & 100000 respectively) (Table 1).

The frequency% revealed the highest values; *P. aeruginosa* in wells (11.36%) and *A. haemolyticus* in Bardy company (11.11%) (table 4).

Table 1. Average counted-number of protozoan parasites, bacteria, fungi and algae (mean count/100 ml) which obtained from water-resources of Rafha city, Saudi Arabia

Species No.	Total isolated-species	Average count / 100 ml				
		Wells	Tap water	Private companies		
				El-Shefaa	Bardy	El-Razaz
	I. Protozoan parasites:					
1	*Giardia lamblia*	5.8	3	1.3	2	2.3
2	*Cryptosporidium parvum*	21	17	4.3	7.7	7.3
3	*Cyclospora cayetanensis*	0.7	0.7	0	0	0
4	*Entameba coli*	15.1	7.3	6	12	10.7
	II. Coliform bacteria-group "Facultative Anaerobes":					
	Proteus mirabilis					
1	*Escherichia coli*	1318.7	33.3	35	0	0
2	*Enterobacter cloacae*	192.5	370	33.3	66.7	700
3	*E. aerogenes*	34.2	0	0	6.7	0
4	III. Total bacteria:	92.5	3.3	0	36.7	0
	a) Aerobic bacteria:					
	Bacillus subtilis					
1	*Brevibacillus brevis*	8.3	0	0	0	0
2	*Staphylococcus hominis*	92.5	0	0	0	0
3	*Acinetobacter haemolyticus*	0	333.3	0	0	0
4	b) Other facultative anaerobic Bacteria:	**16.7**	**3.3**	**3.3**	**0**	**0**
	Bacillus cereus					
	Bacillus anthracis					
5	*Staphylococcus aureus*	11.7	0	3.3	3.3	3.3
6	*Streptococcus sanguis*	0.8	33.3	0	0	66.7
7	*Aeromonas hydrophila*	166.7	333.3	0	0	0
8	*Pseudomonas aeruginosa*	166.7	0	33.3	0	0
9	IV. Fungi:	17.5	0	0	0	0
10	*Aspergillus fumigatus*	100000	0.33333	0.33333	0	0
	Aspergillus flavus					
1	*Chrysoporium tropicum*	1.3	0	0	0.7	0.3
2	*Alternaria alternate*	3.3	3	4	2.3	3.3
3	*Acremonium* sp.	1.1	0	0	0	0
4	*Penicillium* sp.	0.9	20.3	1.3	1.7	0
5	*Cladosporium cladosporioides*	1	1.3	0	0	0
6	*Rhodotorula mucilaginosa*	0	0	0	0	4
7	*Exophiala jeanselmei*	0.1	0	0	0	0
8	V. Algae:	0.3	0	0	0	0
9	Diatoms	0.7	0.3	0	0	0
	Cyclotella sp.					
1	*Synedra* sp.					
1	*Melosira* sp.	0	3	0	0	0
2	*Cymbella* sp.	0	5	0	0	0
3	*Fragilaria* sp.	0	5.3	0	0.3	0
4	*Nitzschia* sp.	0	4.3	0	0	0.7
5	*Merismopedia tenuissima*	0	6	1.3	0.3	0
6	*Anabaena sphaerica*	0	0.3	0.3	0	0
2	*Microcystis aeruginosa*	0	10	10.7	0	0.7
3	*Oscillatoria limnetica*	0	0.3	0	0	0
4		0	0.7	0	0	0
5		0	0	0	0.7	0.3

Gram positive bacteria

Obligate aerobe *Bacillus subtilis*, rod shaped *Brevibacillus brevis* and clusters of spherical *Staphylococcus hominis* resulted.

The highest values of mean counts of *B. subtilis* and *B. brevis*, as CFU/100 ml, detected in wells (8.3&92.5 respectively) *S. hominis* in tap water (333.3) (Table 1).

Table 2. Frequency% of protozoan parasites in different water sources, Rafha city, Northern Borders, Saudi Arabia

Types of water	*Giardia lamblia*	*Cryptosporidium parvum*	*Cyclospora cayatenensis*	*Entamoeba coli*
Well water	28.57	14.29	28.57	28.57
Tap water	27.27	27.27	18.18	27.27
El-Shefaa Co.	33.33	33.33	00.00	33.33
Bardy Co.	33.33	33.33	00.00	33.33
El-Razaz Co.	33.33	33.33	00.00	33.33

Table 3. Frequency% of coliform-group bacteria in different water sources, Rafha city, Northern Borders, Saudi Arabia

Types of water	Isolated coliform groups "negative gram"			
	Proteus mirabilis	*Escherichia coli*	*Enterobacter cloacae*	*Enterobacter aerogenes*
Well water	22.73	13.64	09.09	06.82
Tap water	11.11	33.33	00.00	11.11
El-Shefaa Co.	42.86	14.29	00.00	00.00
Bardy Co.	00.00	22.22	33.33	22.22
El-Razaz Co.	00.00	42.86	00.00	00.00

Table 4. Frequency % of aerobic and anaerobic bacteria in different water sources, Rafha city, Northern Borders, Saudi Arabia

Types of water	Anaerobic bacteria					
	Bacillus cereus	*Bacillus anthracis*	*Staphylococcus aureus*	*Streptococcus sanguis*	*Aeromonas hydrophila*	*Pseudomonas aeruginosa*
Well water	13.64	2.27	00.00	00.00	6.82	11.36
Tap water	00.00	00.00	33.33	00.00	00.00	00.00
El-Shefaa Co.	00.00	00.00	00.00	42.86	00.00	00.00
Bardy Co.	11.11	00.00	00.00	00.00	00.00	00.00
El-Razaz Co.	14.29	42.86	00.00	00.00	00.00	00.00

Types of water	Aerobic bacteria			
	Bacillus subtilis	*Brevibacillus brevis*	*Staphylococcus hominis*	*Acinetobacter haemolyticus*
Well water	2.27	6.82	00.00	4.55
Tap water	00.00	00.00	11.11	00.00
El-Shefaa Co.	00.00	00.00	00.00	00.00
Bardy Co.	00.00	00.00	00.00	11.11
El-Razaz Co.	00.00	00.00	00.00	00.00

Table 6. Frequency % of algae in different water sources, Rafha city, Northern Borders, Saudi Arabia

Types of water	*Merismopedia tenuissima*	*Anabaena sphaerica*	*Microcystis aeruginosa*	*Oscillatoria limnetica*	Diatoms *Cyclotella*	*Synedra*	*Melosira*	*Cymbella*	*Fragilaria*	*Nitzschia*
Well water	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Tap water	10.0	00.3	00.7	00.0	03.0	05.0	05.0	04.0	06.0	00.3
El-Shefaa Co.	10.7	00.0	00.0	00.0	00.0	00.0	00.0	00.0	01.3	00.3
Bardy Co.	10.7	00.0	00.0	00.0	00.0	00.0	00.0	00.0	01.3	00.3
El-Razaz Co.	00.0	00.0	00.0	00.7	00.0	00.0	00.3	00.0	00.3	00.0

Table 5. Frequency % of fungi in different water sources, Rafha city, Northern Borders, Saudi Arabia.

Types of water	*Aspergillus fumigatus*	*Aspergillus flavus*	*Chrysoporium tropicum*	*Alternaria alternate*	*Acremonium*	*Penicillium*	*Cladosporium cladosporioides*	*Rhodotorula mucilaginosa*	*Phialophora*
Well water	38.89	22.22	16.67	05.56	05.56	00.00	05.56	03.00	00.00
Tap water	00.00	12.50	00.00	37.50	25.00	00.00	00.00	00.00	12.50
El-Shefaa Co.	00.00	50.00	00.00	25.00	00.00	00.00	00.00	00.00	00.00
Bardy Co.	25.00	50.00	00.00	25.00	00.00	00.00	00.00	00.00	00.00
El-Razaz Co.	20.00	40.00	00.00	00.00	00.00	40.00	00.00	00.00	00.00

The frequency % scored only *B. subtilis* and *B. brevis* in well-water (2.27 & 6.82 respectively) while *S. hominis* isolated from tap water (11.11) (Table 4).

Anaerobic bacteria

The present results revealed gram positive-facultative species included *Bacillus cereus*, *B. anthracis* and *Staphylococcus aureus* and gram negative-facultative *Aeromonas hydrophila* and gram-positive-facultative aerobic *Streptococcus sanguis*.

The highest values of mean counts, as CFU/100 ml, detected *B. cereus*, *S. sanguis* and *A. hydrophila* in wells (11.7, 166.7 & 17.5

Table 7. Statistical analysis of variance (ANOVA) of counts of protozoan parasites, bacteria, fungi and algae-species (CFU or cells/100 ml water), from different water sources of Rafha City, Saudi Arabia (N.S = non-significant - P>0.001 = highly significant)

No	Source of variation	Species	df	SS	MS	F	P-value
I-	Protozoan parasites:						
1	Between 5 sources	*Giardia lamblia*	4	35.19	17.6	19.49	0.001> P
2	"Wells,	*Cryptosporidium parvum*	4	611.88	305.94	19.92	0.001> P
3	Tap,	*Cyclospora cayetanensis*	4	1.62	0.81	8.02	0.01> P
4	El-Shefaa,	*Entamoeba coli*	4	159.45	79.73	6.96	0.01> P
	Bardy & El-Razaz	II- Bacteria:					
	Private Companies"						
a)	Coliform bacteria "facultative anaerobes":						
1		*Proteus mirabilis*	4	4773169	2386585	13.87	0.001> P
2		*Escherichia coli*	4	232239	116120	2.18	N. S
3		*Enterobacter cloacae*	4	2411.34	1205.67	48.34	0.001> P
4		*Enterobacter aerogenes*	4	18464.6	9232.32	5.62	0.05> P
b)	Aerobic bacteria:						
5		*Bacillus subtilis*	4	166.53	83.27	2.4	N. S
6		*Brevibacillus brevis*	4	20535	10267.5	2.47	N. S
7		*Staphylococcus hominis*	4	266661	133331	2.4	N. S
8		*Acinetobacter haemolyticus*	4	573.63	286.81	6.26	0.01> P
c)	Other facultative anaerobic bacteria:						
1		*Bacillus cereus*	4	2026.69	1013.35	1.68	N. S
2		*Bacillus anthracis*	4	10569.4	5284.69	4.75	0.05> P
3		*Staphylococcus aureus*	4	266663	133332	2.26	N. S
4		*Streptococcus sanguis*	4	62669.3	31334.7	7.78	0.01> P
5		*Aeromonas hydrophila*	4	735	367.5	9.54	0.01> P
6		*Pseudomonas aeruginosa*	4	2.36×10^{11}	1.18×10^{11}	1.97	N. S
		III- Fungi:					
1		*Aspergillus fumigatus*	4	3.32	1.66	4.74	0.05> P
2		*Aspergillus flavus*	4	4.46	2.23	0.18	N. S
3		*Chrysoporium tropicum*	4	2.8	1.4	2.39	N. S
4		*Alternaria alternate*	4	903.3	451.65	2.27	N. S
5		*Acremonium*	4	5.05	2.53	2.63	0.10> P
6		*Penicillium*	4	38.4	19.2	5.49	0.05> P
7		*Cladosporium cladosporioides*	4	0.26	0.13	2.35	N. S
8		Total yeasts	4	8062.66	4031.33	1.07	N. S
9		*Exophiala jeanselmei*	4	1.07	0.54	1.93	N. S
							IV- Algae:
1		Total Diatoms	4	287.17	143.58	0.35	N. S
2		*Merismopedia tenuissima*	4	333.59	166.79	11.77	0.001> P
3		*Anabaena sphaerica*	4	0.25	0.12	2.08	N. S
4		*Microcystis aeruginosa*	4	0.94	0.47	8.48	0.01> P
5		*Oscillatoria limnetica*	4	0.95	0.48	4.29	0.05> P

respectively), *B. anthracis* in El-Razaz company-water (66.7) and *S. aureus* in tap water (333.3) (Table 1).

The detected highest values of frequency %; *B. cereus* and *B. anthracis* in El-Razaz company-water (14.29 & 42.86 respectively) whereas *S. aureus*, *S. sanguis* and *A. hydrophila* isolated from tap water (33.33), El-Shefaa company-water (42.86) and well-water (6.82) (Table 4).

Regarding bacterial counts, as cells/100 ml, the statistical analysis of variance "ANOVA" revealed highly significant differences in the coliform bacteria *Proteus mirabilis* and *Enterobacter cloacae* (Table 7).

Fungi

The present mycoflora that isolated from drinking water of the target three sources are nine genera; *Aspergillus fumigatus*, *Aspergillus flavus*, *Chrysoporium tropicum*, *Alternaria alternate*, *Acremonium* sp., *Penicillium* sp., *Cladosporium cladosporioides*, *Rhodotorula mucilaginosa* and *Exophiala jeanselmei*-yeast.

Table (1) revealed that 8, 4, 2, 3 and 3 colonies of fungal genera were isolated from the present three water-sources; wells, tap, El-Shefaa, Bardy & El-Razaz company respectively. The highest values of mean counts of fungal genera, as CFU/100 ml, were for *Aspergillus flavus* (3.3, 4 & 2.3 for wells, El-Shefaa and Bardy companies respectively), *Alternaria alternate* (20.3 for tap water) and *Penicillium* (4 for El-Razaz).

It is worthy of mentioning that the fungus *A. flavus* appeared in all five water-sources followed by *A. alternate* in four sources.

Concerning counts of the fungi, as CFU/100ml, the statistical analysis of variance "ANOVA" revealed low significant differences between the four sources; wells, tap and the three companies concerning *Aspergillus fumigatus* and *Penicillium* whereas rest of fungi show non-significant differences (Table 7).

Algae

Six algal genera had been isolated from four different water-sources except wells; *Merismopedia tenuissima*, *Anabaena sphaerica*, *Microcystis aeruginosa*, *Oscillatoria limnetica* and diatoms, including; *Cyclotella* sp., *Synedra* sp., *Melosira* sp., *Fragilaria* sp., *Cymbella* sp. and *Nitzschia* sp.

The highest values of the resulted mean counts of algae, as cells/100 ml, included; *M. tenuissima* from El-Shefaa and Bardy companies-water (10.7 for both), *A. sphaerica*, and two diatoms *Melosira* and *Fragilaria* from tap water (0.3, 5 & 6 respectively). The collected species from El-Razaz company-water included; *O. limnetica* (0.7), the diatoms *Cymbella* sp. and *Fragilaria* sp. from tap water (4.3 & 6 respectively). *M. aeruginosa* and the diatoms *Cyclotella* sp., *Synedra* sp., *Melosira* sp. and *Nitzschia* sp. from tap water (7, 3, 5, 5.3 & 0.3 respectively) (Table 1).

The highest values of frequency % of algae-samples are; in tap and El-Shefaa and Bardy companies-water for *M. tenuissima* (1.7, 10, 10.7 and 10.7) and in El-Razaz company-water for *Oscillatoria limnetica* (0.7) (Table 6).

Respecting algal counts "cells/100 ml", the statistical analysis of variance revealed highly significant differences in the alga *M. tenuissima* between the present four sources (Table 7).

DISCUSSION

The present work revealed several pathogenic microorganisms in three sources of water; 10 wells, tap and three private desalinating water-companies; including three parasitic protozoan-cysts, three genera of *Coliform* bacteria-group and some aerobic and anaerobic bacteria, five genera of fungi as well as three genera of algae.

In this respect, results of Al-Turki[23] reinforced the present results where he revealed that microbiological water quality results showed that 20% of the samples examined are contaminated with coliform bacteria (*Escherichia coli*, and *Enterobacter aerogenes*), indicating the necessity of water-sanitation of Hael's water prior to use.

WHO estimated that 1.8 million people die each year as a result of severe diarrhea caused by drinking contaminated water, where developing countries have the prevalence of giardiasis in patients with diarrhea is about 20% [24].

Concerning parasitic cysts of *Giardia lamblia*, *Cryptosporidium parvum* and *Cyclospora cayatenensis*, they contaminate water-sources causing severe gastrointestinal disorders. The maximum score of the present parasitic protozoans is for *C. parvum* and minimum count for *C. cayetanensis*.

Giardia-cysts resist chlorine leads to giardiasis, its symptoms are flu and severe gastrointestinal disorders, detected in 81% of raw water samples and 17% of filtered water samples in the United States[25]. In this respect, WHO declared that chlorine disinfection of drinking-water has limitations against the protozoan pathogens, in particular *Cryptosporidium*[26]. *C. parvum* is protozoan parasite causes Cryptosporidiosis in humans. Oocysts of *C. parvum* in 87% of raw water samples and 27% of drinking water samples in 15 Canadian regions[25]. Le Chevallier *et al.* reinforced the present counts of *C. parvum*-cysts which is higher than those of *G. lamblia* in all water-sources[25].

Cyclospora cayetanensis is cyst-forming coccidian protozoan that causes a self-limiting diarrhea named cyclosporiasis, with symptoms range from watery, loose stool, weight loss, cramping, fatigue, vomiting, fever and nausea [2].

The maximum score of coliform bacteria are for *Proteus* and the minimum for *Enterobacter*. *Proteus* species causes wound infections, septicemia and pneumonia whereas *E. coli* produce potentially lethal toxins and causes food poisoning. Moreover, Uro-pathogenic *E. coli* is responsible for 90% of urinary tract infections [27]. *Enterobacter* causes opportunistic infections, where the urinary and respiratory tracts are the sites of infection[28].

Concerning other gram positive bacteria, *Acinetobacter haemolyticus* causes pneumonia, bacteremia and meningitis[29], *Pseudomonas aeruginosa* infects urinary tract, burns, wounds and causes blood infections[9].

Gram positive bacteria, included *Bacillus subtilis* and *Staphylococcus hominis* cause diseases in severely immune-compromised patients, *Bacillus cereus* causes severe nausea, vomiting, and diarrhea, *Bacillus anthracis* leads to anthrax disease[30], *Staphylococcus aureus* causes scalded-skin syndrome[31], *Streptococcus sanguis* causes sub-acute bacterial endocarditis[32] and *Aeromonas hydrophila* produces aerolysin cytotoxic enterotoxin leads to tissue damage[9].

The studies revealed 5 pathogenic fungal species isolated. The maximum count of fungal-species are for *Aspergillus flavus* and minimum for *Cladosporium cladosporioides*.

The most common pathogenic species are *Aspergillus fumigatus* and *A. flavus*. The latter species produces aflatoxin-toxin that contaminating foods and considers carcinogen leads to allergic disease while *A. fumigatus* causes allergic disease leads to chronic pulmonary infections[33]. *Alternaria alternate* causes respiratory infections and asthma in humans with compromised immunity[34].

Hyalo-hyphomycosis that caused by *Acremonium* includes arthritis, osteomyelitis, peritonitis, endocarditis, pneumonia, cerebritis, and subcutaneous infection[35].

Exophiala jeanselmei causes mycetoma, localized cutaneous infections, subcutaneous cysts, endocarditis, cerebral and disseminated infections, beside phaeohyphomycosis[36].

The highest value of the algae are for *Merismopedia tenuissima* and minimum for *Anabaena sphaerica*. *Anabaena sphaerica* and *Microcystis aeruginosa* secrete both microcystin-LR and cylindrospermopsin toxins that leading to liver inflammation, pneumonia, dermatitis, kidney damage and tumor growth. Anatoxin-a group[3]-toxin is also secreted by *Anabaena* and *Oscillatoria limnetica*, that causes nervous disorders[14].

Concerning standard levels of microorganisms in drinking water, EPA's MCLG declared that detection of protozoans, especially *Cryptosporidium*, is difficult and not technically feasible for routine analysis of human drinking water. The recommendation considered the proper way to control pathogenic protozoans is using an effective water treatment technique, such as reverse osmosis or ozonation.

According to EPA MCL, coliform must be less than one/100 mL. In this concern, the microbiological guidelines and standards for drinking water for *E. coli* are zero CPU count/100 ml[37]. In this respect, average count/100 mL of all water sources of the present work revealed considerable higher levels than those of EPA MCL and an apparent risk on Rafha's citizens-health.

The present investigation indicated that water sources of Rafha city are important contributor to transmission of contaminants to consumers.

The present work recommends a future-field-application of selected specialized anti-parasites and anti-microbial to control the present resulting contaminators to gain considerable safe levels of drinking water.

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