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Isolation and Identification of Microbial Communities from Different Habitats (Freshwater and Soil) in Aljouf Province, KSA

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The aim of this study was to undertake a preliminary assessment of the freshwater and soil samples of four different regions at Domate Elgandal Lake (Aljouf Governorate, Kingdom of Saudi Arabia) during summer and autumn seasons of one year (2014-2015). The Physico-chemical characteristics of the water and soil samples were evaluated. pH was fluctuated in normal ranges between 7.93-8.74 during the study, while sulfate, phosphorus, copper and manganese were recorded high values more than the acceptable ranges. Microbiological analyses indicated the total coliform was too numerous to count and, therefore, warrants more attention. Total coliforms, thermotolerant coliforms, *Escherichia coli*, *Enterococcus* spp., *Salmonella* sp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* were detected in 5% of the water samples. Cyanobacteria, Chlorophyceae and Bacillariophyceae were represented by 2, 2 and 7 genera, respectively, all over the water and soil samples. The Shannon-Weaver diversity index (*H'*) appears <1 in the soil sample during autumn season (0.64).

Key words: Physico-chemical, microbiological quality, microalgae, Domate Elgandal Lake, Saudi Arabia.

The scope of finding, developing and maintaining suitable water supplies has not been limited to modern times. It has had to be faced wherever large numbers of people have crowded together in small spaces; and therefore the popular indifference towards safe, clean water has prevailed (Sudeep and Hosmani, 2007).

Lakes are open water systems and preserve for production of drinking water, for fishing and other human impacts. The ecological nature of many lakes, however have desecrated, mainly as a consequence of eutrophication (Scheffer, 1998). The degree of variation of life form in an entire planet or within a given ecosystem and biome is defined as biodiversity (Mohammed et al., 2014). Groundwater represents an important source of drinking water and its quality is currently threatened by a combination of over-abstraction and microbiological and chemical contamination (Pedley and Howard, 1997; Reid et al., 2003). Depending on the source, raw water may contain a wide variety of harmless heterotrophic microorganisms such as *Flavobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* spp., *Chromobacterium*, *Achromobacter* spp. and *Alcaligenes* spp., as well as numerous unidentifiable bacteria. Traditionally, the microbiological quality of drinking water is assessed by monitoring non-pathogenic bacteria of faecal origin (faecal indicator bacteria). *E. coli* and *Enterococcus* spp. members are traditionally used as hygiene indicator bacteria and methods for their detection are essential elements of drinking water regulations all over the world (Reid et al., 2003).

However, bacterial pollution of water sources may occur and is mostly derived from

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watershed corrosion as well as drainage from sewage, swamps, or soil with high humus content. This type of hazard exists particularly in limestone areas where underground chambers or fissures may permit water to flow in the freely moving streams without substantial filtrations (Alotaibi, 2009). Soil algae help in aggregation of soil particles and sand grains to form microbial crusts (Hu et al., 2003) as a result of that, they stabilize the soil surface of bare eroded soils against further erosion and soil removal by wind, improve infiltration of water into the soil, reduce water loss, improve soil texture by aggregating soil particles, add organic matter to the soil, provide a favorable habitat for seed germination and have been suggested as biofertilizers as well.

The aim of this study was to determine the microbiological and physico-chemical condition of Domate Elgandal Lake which is one of several man made lakes in the arid region of the northern region of Saudi Arabia. An attempt was also made to identify the coliforms isolated from the examined water samples. The findings may be considered as a basis for water health policy decisions at different administrative levels in the study area.

MATERIALS AND METHODS

Study area

Domate Elgandal Lake (29° 23.465'N, 39° 40.397'E) is an artificial lake, which was formed in a low basin of water surrounded by high hills from all sides, over an area of 1.1 million square meters, shapes are not regular and depth varies from 4 to 17 meters (Al-Ruwaili, 2011) . At autumn and summer seasons, four locations along the lake were chosen, where, each location included water and dry soil.

Physico-chemical analyses

The physical and chemical variables selected for both soil extract (soil-water extract of 1:5 were prepared) and water samples were pH, anion concentrations, halogens and metal concentrations. The following chemical examinations were conducted by using Hach® spectrophotometer methods (model, DR 2800). Nitrate, nitrite, ammonia silica, sulfate and phosphorus as PO_4^{3-} concentrations were examined according to cadmium reduction (method 8039), diazotization (method 8507), salicylate (method 8155), silicomolybdate (method 8185) sulfaVer® 4 turbidimetric (method 8051) and ascorbic acid (method 8048), respectively. By using DPD method (diethyl-p-phenylene diamine) we determined bromine (method 8016), chlorine (method 8021) and iodine (method 8031). The metal concentrations as chromium (Cr^{6+}), copper, iron and manganese were measured by using 1,5-diphenylcarbohydrazide (method 8023), bicinchoninate (method 8506), 1,10-phenanthroline (method 8008) and periodate oxidation (method 8034) methods, respectively.

Microbiological analyses

Enumeration of different bacterial groups

All samples were examined using the Membrane Filter MF Technique (Sartorius, 3 branch manifolds) for Heterotropic Plate Count (HPC), total coliforms, fecal coliforms, fecal streptococci, Lactobacilli and Bifidobacteria, Fungi, Sulphite-reducing clostridia, *E. coli*, *Enterococcus* sp., *Salmonella* sp., *Pseudomonas aeruginosa*, and *Staphylococcus* sp. analyzed in 100 ml groundwater (Table 1).

Biochemical characterization and presumptive identification

After bacterial colonies had been counted, plates were selected for the identification of different bacterial isolates. Bacterial colonies differing in size, shape and color were randomly selected from the different plates and further isolated on nutrient agar (Scharlau, Spain) by the streak plate technique and incubated at 37°C for 24h. These were further purified by the same method at least three times before Gram staining was done. Oxidase tests were then done on those colonies which were Gram-negative. For those isolates that were found to be oxidase-positive, the 20NE API kit was used and the strips were incubated at 30°C for 24h. The 20E API kit was used for the oxidase-negative colonies and the strips were incubated at 37°C for 24h.

Species-specific PCR tests

Control DNAs from pure cultures of different strains were prepared by suspending a loop of overnight colonies in a tube that contained 500 µl sterile distilled water, boiling for 10 min and then centrifuging at 14 000 g for 5 min. An aliquot of the supernatant (5 µl) was used as the template in a final volume of 25 µl PCR mixture, which contained: 1X PCR buffer, 2 mM $MgCl_2$, 200 µM

each dNTP, 400 nM each primer and 0.25 U *Taq* DNA polymerase (Life Technologies). Specific PCR tests were carried out using different species-specific primers (Table 2) with DNA obtained from the bacterial pure cultures. Primers (MWG Biotech AG, Ebersberg, Germany) were prepared at a final concentration of 60 µM in deionized, autoclaved water. PCR was performed in a GenAmp system model 2400 (PerkinElmer, France), and all reactions were carried out following conditions previously provided by the authors (Table 2). Twenty-five microliters of the PCR product was electrophoresed at 100 V for 1 h on a 1.5% agarose gel (Roche Molecular Biochemicals) in 0.5x Tris-acetate-EDTA buffer stained with 0.1 µg of ethidium bromide (Sigma Chemical Co., St. Louis, Mo.)/ml. The smaller amount of ethidium bromide used ensured a high contrast between faint bands and the background. A 1-kb DNA ladder (Invitrogen, Merelbeke, Belgium) was used as a molecular size marker.

Sampling, isolation and culturing of algae

The water samples were collected from the lake by using water sampler to collect surface water (at 0-20 cm depth) in sterile glass Schott bottles (5 liter) from the different location. On return to the laboratory, portion of the water samples from each stand were preserved by using Lugol's iodine solution. These samples were examined microscopically for the presence of at least on algal species. The soil collection was carried out according to John (1942), the surface soil layers, normally to a depth of 2 cm were removed with a knife and were freed from gravels and debris and collected in sterile plastic bags. Each sample (water or soil) represented a mixture of four random samples from each stand and then mixed carefully to give one homogenous sample. The collected samples were kept in an ice box and transported to the laboratory for the subsequent studies as soon as the samples were carried to the laboratory. The moist plate technique recommended by Jurgensen and Davey (1968) was applied on air dried soil samples for cultivation of algae that might be persisting in the form of spores, hormogonia, akinetes or any other stages. Different growth media as Z-medium (Staub, 1961), soil extract medium (Starr and Zeikus, 1993) and Allen's medium (Allen, 1968) were used for isolation and cultivation of the different soil algal genera. The inoculated media were incubated in constant light intensity (4000 Lux) at 32°C for preferential isolation of the blue-green algae and at 25°C for the eukaryotic algae until good growth had been obtained (3-6 weeks). Both water and soil algal species were identified according to the systems proposed by Desikachary (1959); Chapman (1962); Baker and Bold (1970) and Prescott (1962 & 1978). Diatoms were cleared and identified according to Cox (1996).

Statistical Analyses

TWINSPAN, Two Way Indicator Species Analysis (Hill, 1979), was applied for the classification of stands and species into groups based on the relative abundance of the species (reported as a percentage of the total count). The physicochemical analyses data were compared using one-way ANOVA (IBM SPSS Statistics 20). Shannon- Wearver Diversity Index (*H'*) was calculated to qualify the water quality of the lake. $(H') = -"\{n_i/N\} \ln\{n_i/N\}$, where: n_i is the relative abundance of species (i) in proportion format, ln is the natural logarithm (Shannon and Wearver, 1963).

RESULTS AND DISCUSSION

Physiochemical analyses

Table 3 showed a summary of compliance rates of tested water and soil samples at autumn and summer seasons. The pH was within the recommended limit for no risk (pH between 6 and 9). Sulfate (SO^{4-}) was recorded about 4.5 folds as the normal recommended range in the soil sample during summer season, also water samples had high values of sulfate during summer and autumn (98.5 and 106.5 mg/L, respectively). Another major aspect of non-compliance was observed in phosphorus (PO_4^{3-}), copper and manganese content when examined in soil samples during autumn season.

Microbiological analyses

Out of our tested water samples, total coliform (TC) and fecal coliform (FC), 6% and 9% of the samples exceeded the WHO limits for TC and FC, respectively, as shown in table 4. These percentages were up to 15.5% and 7.1%, respectively. Enteric bacteria isolated in total were *Escherichia coli* (42%), *Enterococcus faecalis* (34%), *Klebsiella* (12%), *Citrobacter* (3%) and *Enterobacter* (1%). In all water samples, the most frequently isolated bacteria from different sources were *E. coli* (8%) and *E. faecalis* (5%) with the

highest contamination. This was followed by *Klebsiella* with the highest contamination (3%). Not frequently isolated bacteria were *Citrobacter* and *Enterobacter* that showed steady frequencies of (1%) (Table 4).

The geographical areas were thought to be different in terms of risks for waterborne transmission of zoonotic enteric diseases because of the origin of the water that supplies their population (i.e. surface and groundwater). In rural areas, drinking water generally supplied groundwater through individual or community wells (Bigras-Poulin et al., 2004). Safe water is essential for life and health. Thus, in emergency situation the safety of the water must be assured right through for consumption at home, since interruption in the supply may be disastrous. For these reasons,

Total coliform was detected with considerable variation in different water samples. High prevalence of total coliform in well was observed, this may be due to the act of wind blowing which carries impurities and pathogens to uncovered storage containers, storing of water in dirty environment, poor personal hygiene and unsanitary practices such as leaving containers open on ground exposed to children, insects and animals. Several studies highlighted the

Table 1. The incubation conditions and microbiological media that are used in microbiological analysis.

Indicator	Culture Media	Incubation condition	
		Hours	Temperature (°C)
Heterotrophic Plate Count	Plate count agar	48	30
Total Coliforms	MacConkey agar	24	37
Fecal coliforms	EMB agar	24	37
Faecal streptococci	mEnterococcus agar	24	37
Sulphite-reducing clostridia	Sulphite-cycloserine-azide Agar	24	37
Lactobacilli and Bifidobacteria	Lactobacilli MRS Agar	48-72	30 (Anaerobe)
E. coli	ENDO Agar	24-48	44.5
Enterococcus spp.	Bile esculin agar	24	37(Anaerobe)
Salmonella spp.	Bismut Sulfit Agar	18-48	37
Staphylococcus spp.	Mannitol salt agar base	48	37
P. aeruginosa	Cetrimide Agar	48	37
Fungi	Malt Extract Agar	72	30

Table 2. Primers used in this study for the species-specific PCR assays

Target	Primers	Sequence (5'-3')
Escherichia coli	Eco 223Eco 455	ATCAACCGAGATTCCCCCAGTTCACTATCG GTCAGTCAGGAG
Enterococcus faecalis	Efs 03Efs 04	CTGTTGTTAGAGAAGAACAAGGACGTGGA CAACGCTTGCCACCTA
Enterococcus faecium	Efm 07Efm 08	AAGTCGAACGCTTCTTTTTCCACCAAGTG TTATCCCCTTCTGATG
Pseudomonas aeruginosa	Paer16SHPaer16SIR	AGGGCAGTAAGTTAATACCTTGCTGCCACC TCTACCGTACTCTAGCTCAG
Serratia marcescens	Smar16SVSmar16SWR	GGGAGCTTGCTCACTGGGTGGCGAGTAAC GTCAGTTGATGAGCGTATTA
Staphylococcus aureus	STAA-AuISTAA-AuII	TCTTCAGAAGATGCGGAATATAAGTCAAAC GTTAACATACG
Staphylococcus epidermidis	STAE-EpISTAE-EpII	TCTACGAAGATGAGGGATATTTCCACCAT ATTTTGAATTGT
Staphylococcus saprophyticus	fStSaprStSap	TCAAAAAGTTTTCTAAAAAATTTACACGG GCGTCCACAAAATCAATAGGA

contamination of water storage vessels within the household which reflect the quality of water consumed. Contamination of water sources is due to human activities, and poor sanitation and also may be due to leaching of faecal matter from pit latrines used all over the wells (Jain, et al., 2009).

Faecal coliform were observed exceeding the standard zero limits in water in all seasons and different sources throughout this study. According to the WHO/ SASO guidelines, faecal coliform should not be present in 100 ml water sample. Faecal coliform are found abundantly in the intestine of human and warm-blooded animals and their presence in different source is of no doubt reflect faecal contamination since they represent a more specific indicator of faecal contamination. Faecal coliform exceeding the limits indicates recent contamination of the water sources with faecal

Table 3. Physico-chemical characteristics of the study areas that representing the water and soil habitat.

Parameter		Normal range mg/L Domate Elgandal Lake samples			
		Water		Soil	
		Summer	Autumn	Summer	Autumn
pH		7.93±0.39	8.36±0.11	8.1±0.53	8.74±0.38
Nitrate (NO^{3-})	(0.3 - 30.0)	0.29±0.021	0.25±0.07	16.45±3.82	2.03±0.04
Nitrite (NO^{2-})	(0.002 - 0.300)	0.00	0.00	0.10±0.02	0.12±0.05
Ammonia (NH_3)	(0.01 - 0.50)	0.05±0.014	0.08±0.02	0.12±0.04	0.02±0.004
Silica (SiO_2)	(1 - 100)	11.87±2.171	11.88±0.78	11.85±2.76	1.6±0.28
Sulfate (SO^{4-})	(2 - 70)	98.5±14.8	106.5±9.2	321±8.5	66±1.4
Phosphorus (PO_4^{3-})	(0.02 to 2.50)	1.5±0.32	0.12±0.03	3.95±0.23	0.06±0.02
Bromine (Br_2)	(0.05 - 4.50)	0.03±0.004	0.03±0.007	0.07±0.007	0.07±0.01
Chlorine (Cl_2)	(0.02 - 2.00)	0.04±0.007	0.02±0.004	0.04±0.014	0.02±0.001
Iodine (I_2)	(0.07 - 7.00)	0.03±0.007	0.03±0.01	0.05±0.02	0.08±0.007
Chromium (Cr^{6+})	(0.01 - 0.70)	0.01±0.004	0.02±0.007	0.01±0.002	0.04±0.01
Copper (Cu)	(0.04 - 5.00)	2.31±0.184	2.5±0.55	10.8±0.89	3.64±0.75
Iron (Fe)	(0.02 - 3.00)	0.01±0.004	0.03±0.007	0.00	0.02±0.014
Manganese (Mn)	(0.1 - 20.0)	4.08±1.167	12.85±2.3	41.9±3.54	11.83±1.7

Table 4. Microbiological counts of water samples in Domate Elgandal Lake, Aljouf province, Saudi Arabia.

Parameter	World Health Organization (WHO)[b]		Saudi Arabian	Percentage of
	Normally found in ground water	Health based guideline by the WHO	Standards Organization (SASO) standards[c]	non-compliant samples (%)
Heterotrophic Plate Count 37°C		20	No guideline	10
Total Coliforms	-	0	0	9
Fecal coliforms	-	0	0	6
Faecal streptococci	-	0	0	0
Sulphite-reducing clostridia	-	0	0	0
Lactobacilli and Bifidobacteria	-	0	0	0
E. coli	-	0	0	0
Enterococcus spp.	-	0	0	0
Salmonella spp.	-	0	0	0
Staphylococcus spp.	-	0	0	0
Pseudomonas aeruginosa	-	0	0	0
Fungi	-	0	0	0

[a] All values are expressed in mg L^{-1} except specified otherwise
[b] World Health Organization (WHO)
[c] Saudi Arabian Standards Organization

matter and hence the possible presence of intestinal pathogens. According to the WHO guidelines, *E. coli* or faecal coliform bacteria should not be detectable in any water intended for drinking. Laboratory analysis of water samples in this study showed that faecal matter heavily contaminated water sources. Poor sanitary practices are also one of the causes of water sources contamination; there are many animals, especially Camels, left free, hence contaminating water with their faecal matters when washed away with water run-off from the land. It is well known that where basic sanitation is lacking, there is more likelihood of indicator bacteria from faeces being introduced into stored water. In terms of public health significance, *E. coli* has frequently been reported to be the causative agent of traveler's diarrhoea, urinary tract infection, haemorrhagic colitis and haemolytic uraemia syndrome. Moreover, *Klebsiella* is associated with pneumonia and upper respiratory tract infection. However, *Enterobacter* and *Citrobacter* species have also been previously reported as causes of cystitis, enteritis, pneumonia, diarrhoea and food poisoning. These results indicate a contamination of the domestic water supply in the water network by wastewater. This could be due to leakage from the wastewater sewage system, openly flowing sewage, and seepage pits into the water pipelines, as some of the pipes in the water networks are old and cracked. This would particularly happen during the cease periods of water pumping in these pipes, as negative pressure develops inside them. It could also be due to breakage in the water distribution system, thus promoting bacterial biofilm growth. Biofilms were reported to develop in water distribution systems.

Algae Analysis

The algal species of the water and soil samples that identified at summer and autumn seasons from Domate Elgandal Lake were plotted in table 5. Three major algal groups were present and classified as Cyanophyceae, Chlorophyceae and Bacillariophyceae. Eleven algal genera

Table 5. Relative abundance (%) and Shannon-Weaver index (H') of the studied area which represent the soil and water habitats

Algal species	water				soil			
	Summer		Autumn		Summer		Autumn	
	%	*H'*	%	*H'*	%	*H'*	%	*H'*
Cyanophyceae								
Oscillatoria formosa Bory de Saint-Vincent							33.33	0.37
Oscillatoria tenuis C.Agardh					100	2.92	66.67	0.27
Phormidium sp.	5.41	0.158						
Chlorophyceae								
Chlamydomonas cienkowskii L.S.Cienkowski			55.91	0.325				
Chlamydomonas polypyrenoideum G.W.Prescott			23.66	0.341				
Chlorella vulgaris Beijerinck	16.22	0.295						
Bacillariophyceae								
Cavinula jaernfeltii Hustedt	21.62	0.331						
Cyclotella meneghiniana Kützing			3.23	0.111				
Encyonema gracile Rabenhorst			2.15	0.083				
Frustulia rhonboides Ehrenberg			4.30	0.135				
Navicula accomoda Hustedt			1.08	0.049				
Navicula gregaria Donkin	5.41	0.158						
Navicula radiosa Kützing	13.51	0.270	5.38	0.157				
Nitzschia amphibia Grunow	8.11	0.204	2.15	0.083				
Nitzschia closterium Ehrenberg	8.11	0.204	2.15	0.083				
Nitzschia communis Rabenhorst	13.51	0.270						
Synedra ulna Ehrenberg	8.11	0.204						
Total number of species	9		9		1		2	
Total	100	2.094	100	1.366	100	2.92	100	0.64

including seventeen species of algal species were isolated and represented as follows, two genera including three species of Cyanophyceae, two genera including three species of Chlorophyceae, and seven genera including eleven species of Bacillariophyceae. *Chlamydomonas cienkowskii* was the most dominance species (55.9%), which recorded in water sample during autumn season and followed by *Cavinula jaernfeltii* (21.6%) but represented at summer season. Meanwhile, soil samples were represented by only one species (*Oscillatoria tenuis*) during summer season, whereas at autumn season it represented by two species (*O. formosa* and *O. tenuis*)

The Shannon and Weaver diversity index is an important aspect indicating the distribution of phytoplankton and their relation to pollution (Sudeep and Hosmani, 2007). Three water quality classes are defined for Shannon-Weaver diversity index by Wilhm (1975) as shown in table 6, which implies that the high *H'* value suggests the more healthy ecosystem (less pollution) while the low *H'* value suggests poor diversity in a community and a less healthy ecosystem (more pollution). Also, Magurran (2004) concluded that, Shannon-Weaver index was applied to compare diversity of the algal species, where typical values are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4. The present study revealed that, the maximum *H'* value was 2.92 (moderately polluted) in the soil sample during summer season. Meanwhile, during autumn the soil sample recorded the smallest *H'* value (0.637) among all studied samples. It means that, this sample is heavily polluted and has poor diversity in a community and a less healthy ecosystem all over the examined samples. It can be positively correlated with the vigorous increasing in sulfates, phosphorus, copper and manganese contents in the soil samples. Whereas in case of water samples the *H'* values were 2.094 and 1.366 during summer and autumn seasons, respectively.

Table 6. Water quality classes for Shannon-Weaver index (Wilhm, 1975)

Shannon-Weaver index (*H'*)	Class	Condition
> 3	I	clean
1 – 3	II	moderately polluted
<1	III	heavily polluted

The TWINSPAN classification analysis was done based on the relative abundance of the recorded identified species in the selected 4 stands. It was clear that the Dendrogram cluster analysis (Fig. 1) classified the investigated habitats into two major groups with different species composition at level number 2. The first one was with the indicator species *O. tenuis*. The second group comprised the remaining stands which represented with *Phormidium* sp. as indicator species.

CONCLUSION

Domate Elgandal Lake is one of the most popular areas in Aljouf Governorate. Human impacts were the main threat to this area and the efficient control over these activities is one of the main topics for our research. This takes place through some strategies and approaches to protect and expand the available natural resources base; aiming to increase productivity, reduce migration and improve the living condition in this area. In general, the results revealed a variation in physicochemical characteristics of the investigated area which could be attributed to the discharges

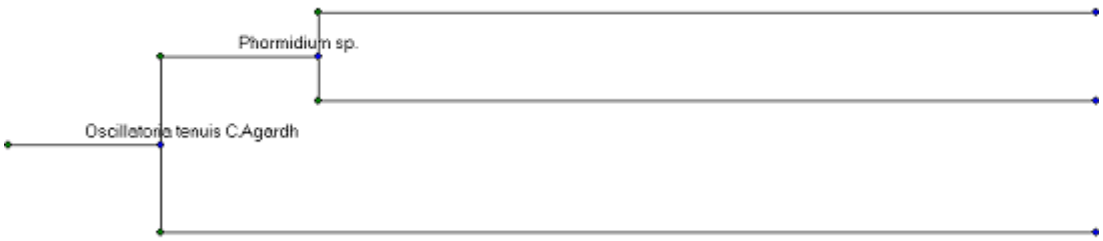

Fig. 1. The Dendrogram resulting from the cluster analysis with 4 algal vegetation groups as derived from automatic classification.

of different drains into the lake, and this conclusion is in agreement with Mansour and Sidky (2003).

In this study two environmental samples collected from Domate Elgandal Lake of Aljouf province, Saudi Arabia was analyzed. It was found that some microbiological and some chemical values determined from waters were above the limits set by WHO and SASO. The non-compliance rate is much higher for water pumped through the network and for water from rain-fed cisterns. The existence of indicator bacteria in high amounts demonstrates that there may be pathogenic bacteria such as important pathogens like *E. coli*. The comparison of different well groups spatially showed an increasing pattern of bromine, cupper, and sulfate concentration and microbial concentrations at the wells around Aljouf region. The people in these rural communities are therefore at higher potential risk of contracting water-borne and/or sanitation- related diseases. In conclusion, it is necessary to apply strong preventions immediately to save water supplies in a region which is rapidly developing and constitutes a location for increasing migration.

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