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Original Research

The Microbiological and Physico-Chemical Quality of Groundwater in West Thrace, Turkey

A. Aydin*

Istanbul University Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, 34320 Avcilar, Istanbul, Turkey

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Abstract

The aim of this study was to undertake a preliminary assessment of the groundwater quality of the West Thrace region. Forty groundwater samples collected from Edirne (Site 1) and Canakkale (Site 2) were assessed for their suitability for human consumption. Eight water samples (20%) had heterotropic plate counts exceeding the EU and Turkish Water Directive limit of 20 cfu/ml in drinking water and the maximum number of bacteria recorded as 44 cfu/ml. Total coliforms, thermotolerant coliforms, *E. coli*, *Enterococcus* spp., *Salmonella* sp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* were detected in 25%, 17.5%, 15%, 47.5%, 15%, 27.5% and 15% of the groundwater samples, respectively. Eleven (27.5%) samples exceeded the EU Water Directive value of 50 mg/l for nitrate, but 13 (32.5%) samples violated the Turkish standard of 45 mg/l nitrate in drinking water. pH values of all samples were between 5.5-8.5 limits. Conductivity of all samples were below Turkish and EU Water Directive levels. Five samples (12.5%) exceeded the Turkish Water Directive for total dissolved solids (TDS) in water.

Keywords: groundwater, microbiological quality, physico-chemical quality, public health, Thrace

Introduction

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 [1, 2].

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 unidentified or unidentifiable bacteria [3-5]. Traditionally, the microbiological quality of drinking water is assessed by monitoring non-pathogenic bacteria of faecal origin (faecal indicator bacteria) [3, 6, 7]. *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. In the recently adopted European Drinking Water Directive [8], only *E. coli* and *Enterococcus* spp. are defined as obligatory microbial parameters.

Nitrate is one of the most common groundwater contaminants in rural areas. It is regulated in drinking water primarily because high levels can cause methemoglobinemia or "blue baby" disease [9, 10].

Meteorological events and pollution are a few of the external factors which affect physico-chemical parameters such as pH, TDS and conductivity of the water. They have a major influence on biochemical reactions that occur within the water. Internal factors, on the other hand,

*e-mail: aliaydin@istanbul.edu.tr

include events which occur between and within bacterial and plankton populations in the water body [11].

Although the Thrace region occupies only 3% of Turkey, 15% of the population lives there. The amount of accessible water in the region is nearly 3.4 billion m^3, which consists of 2.8 billion m^3 of surface water (river, lake, etc.), 0.4 billion m^3 of groundwater and 0.2 billion m^3 other resources' water (12). The possibility of pollution in water supplies is increasing every day by the developing industry and enhancing population of the region, which shares a border with Europe. No studies have, however, been done on the water quality with regard to its microbial and chemical activity.

The aim of this study was to determine the microbiological and physico-chemical condition of groundwater sources from West Thrace in Edirne and Canakkale area and to compare with levels obtained with the EU Drinking Water Directive / Turkish Food Codex- Drinking Water Directive.

Materials and Methods

Sample Collections

Forty groundwater samples were obtained from West Thrace region Site 1 (Edirne province area) and Site 2 (Gelibolu area, Canakkale province) (Fig. 1) during spring 2003. The water samples were taken from wells, average depth 25-35 m. All water samples were collected in sterile glass Schott bottles (5 liter). All samples were stored and transported in a cool box kept below 4°C. Analyses were performed as soon as the samples were carried to the laboratory.

Microbiological Analysis

All samples were examined using the Membrane Filter (MF) Technique (Sartorius, 3 branch manifolds) for Heterotropic Plate Count (HPC), total coliforms (100 ml), thermotolerant coliforms (100 ml), *E. coli*, *Enterococcus*

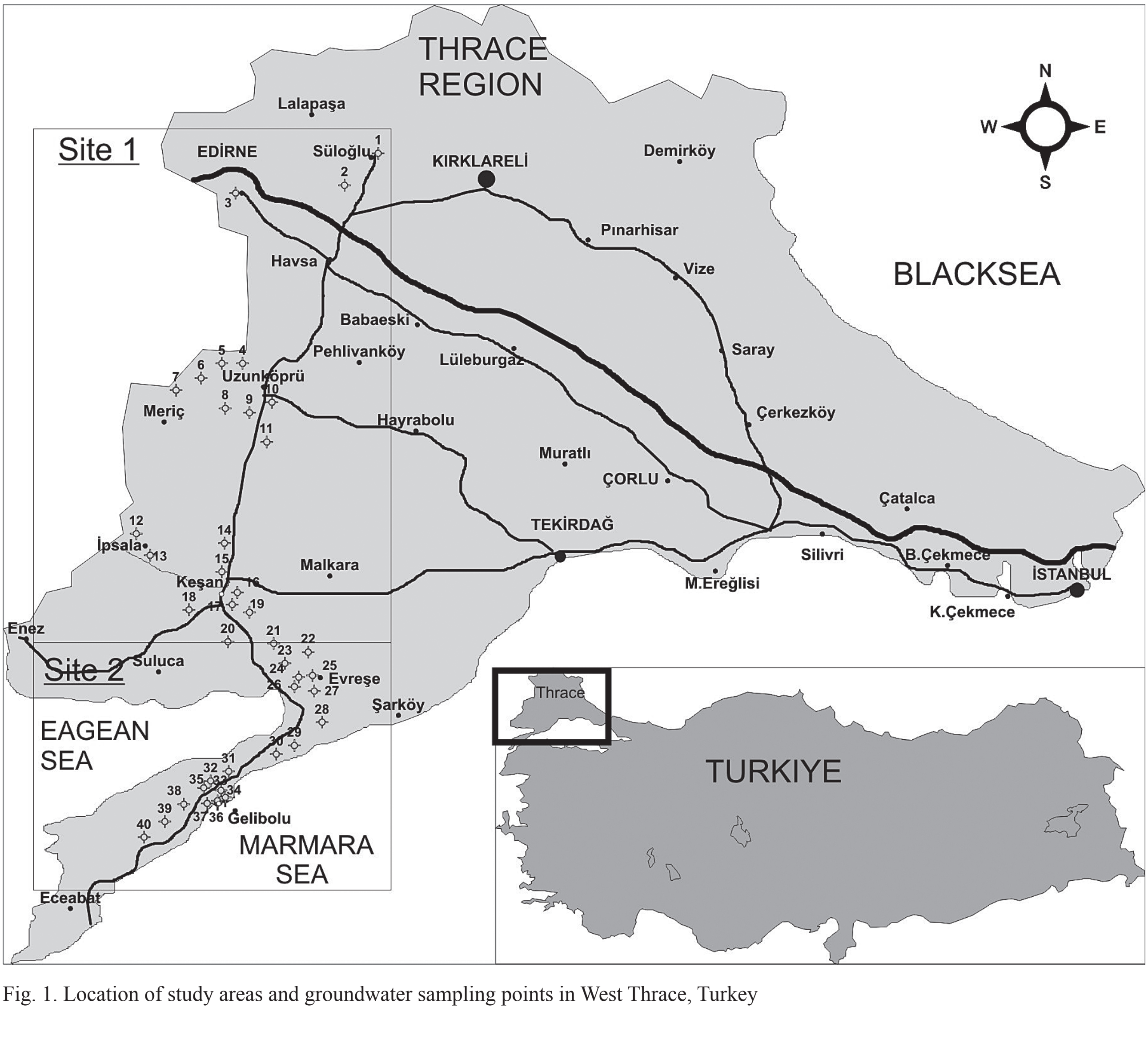

Fig. 1. Location of study areas and groundwater sampling points in West Thrace, Turkey

sp., *Salmonella* sp., *Pseudomonas aeruginosa* and *Staphylococcus* sp. analyzed in 250 ml groundwater according to APHA [13], Slanetz and Bartley [14] and United States Pharmacopeia [15] (Table 1).

Physico-Chemical Analysis

Groundwaters were analyzed for pH, conductivity, TDS and nitrate ion. The pH, conductivity and TDS were measured with a pH, conductivity, and ion-selective meter (Sartorius PP 50, Germany). Nitrate concentration was measured using test kits (Merck, Spectroquant 1.14773) relating to the nitrospectal method. In concentrated sulfuric acid, nitrate ions react with a benzoic acid derivate to form a red nitro compound, the concentration of which is determined photometrically at 517 nm (Thermospectronic Aquamate 2000E UV visible spectrophotometer, USA).

Statistical Analysis

To compare the nitrate value of sites 1 and 2 groundwaters, independent sample t-tests were performed using SPSS 8.0 program package [16].

Results

Table 2 compares EU Drinking Water Directive (EU DWD) [8] and Turkish Food Codex-Drinking Water Directive (TFC-DWD) [17] for the results of the microbiological analysis of groundwater.

A total of 40 samples had pH levels below the TFC-DWD. The highest pH level was 8.4 and the lowest was 6.5 in Site1. The conductivity of all samples was below action levels according to EU DWD (2500 µS/cm) and TFC-DWD value of 2000 µS/cm for drinking water. The highest and lowest conductivities were 1460 µS/cm and 463 µS/cm, respectively, in Site 1. Five (12.5%) groundwater samples exceeded the TFC-DWD [13] for TDS in drinking water (Table 3). The highest TDS level was 949 mg/l and the lowest was 300.9 mg/l in Site 1.

Discussion

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).

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

Analysis	Referans Method	Culture Media	Incubation Conditions (Temperature, Hours)	Interpretation
Heterotropic Plate Count	Standard Methods for The Examination of Water and Waste Water (1998)	TTC Agar (Meat Extract-Peptone Medium, Sarto No: 14055)	30°C; 48 h	Their colonies are stained red by TTC reduction.
Total Coliforms	Standard Methods for The Examination of Water and Waste Water (1998)	ENDO Agar (Sarto No:14053)	35-37°C; 24 h	Bacteria develop sharply contoured, dark red, mucoid or nucleated colonies.
Thermotolerant Coliforms		ENDO Agar (Sarto No:14053)	44.5°C; 24 h	Bacteria develop sharply contoured, dark red colonies.
E. coli	Standard Methods for The Examination of Water and Waste Water (1998)	ENDO Agar (Sarto No:14053)	37°C; 24 h	Bacteria have a greenish metallic sheen, finally IMViC test.
Enterococcus spp.	Slanetz and Bartley (1957)	Azide Agar (Sarto No: 14051) BHI (Brain Heart Infusion) Broth	37°C; 24-48 h	Bacteria form small reddish brown colonies (approx. 1mm ∅) with smooth peripheries, BHI with ose, finally catalase, gram strain.
Salmonella sp.	United States Pharmacopeia (1995)	Bismut Sulfit Agar (According to Wilson and Blair) (Sarto No: 14057) Selenite Cystine Broth	37°C; 18-48 h	Selenite cystine broth (enrichment) to plate (streak with an inoculating loop) the sample on MF, light-colored colonies with brown to black centers.
Staphylococcus spp.	United States Pharmacopeia (1995)	Chapman Agar (Mannitol-sodium chloride-phenol red medium, (Sarto No: 14047)	37°C; 48 h	*S. aureus*; golden yellow to orange-colored colonies with a yellow zone; *S. epidermis* whitish colonies
P. aeruginosa	United States Pharmacopeia (1995)	Cetrimide Agar (Sarto No: 14075)	37°C; 48 h	Blue colonies with 1-2 mm diameter and blue zones, oxidase test, gram strain

Table 2. Compared EU Drinking Water Directive and Turkish Food Codex-Drinking Water Directive on the results of the microbiological analysis of groundwater (n: 40).

Parameter	EU DWD*	TFC-DWD**	Positive Samples		
			(Total) (%)	Site 1 (%)	Site 2 (%)
Heterotropic Plate Count (37°C)	20 /ml	20 /ml	8*** (21-44/ml) (20)	5 (12.5)	3 (7.5)
Total Coliforms	0 in 100 ml	0 in 100 ml	10 (25)	6 (15)	4 (10)
Thermotolerant Coliforms	Not mentioned	0 in 100 ml	7 (17.5)	3 (7.5)	4 (10)
E. coli	0 in 250 ml	0 in 100 ml	6 (15)	3 (7.5)	3 (7.5)
Enterococcus spp.	0 in 250 ml	0 in 100 ml	19 (47.5)	13 (32.5)	6 (15)
Salmonella sp.	Not mentioned	0 in 100 ml	6 (15)	4 (10.0)	2 (5)
Staphylococcus spp.	Not mentioned	0 in 100 ml	11 (27.5)	7 (17.5)	4 (10)
P. aeruginosa	0 in 250 ml	0 in 100 ml	6 (15)	4 (10)	2 (5.0)

*EU Drinking Water Directive 1998; ** Turkish Food Codex-Drinking Water Directive, 2003; *** Samples higher than permitted level (cfu/ml);

Table 3. The results of physico-chemical analysis of groundwater supplied from two sites and comparison with EU Drinking Water Directive and Turkish Food Codex-Drinking Water Directive (n: 40).

Parameter	EU* Drinking Water Directive (MAC**)	Turkish*** Food Codex Drinking Water Directive (MAC)	Site 1 (Edirne) The mean concentration (± SD)**** mg/l (n: 20)	Site 2 (Canakkale) The mean concentration (± SD) mg/l (n: 20)	Samples higher than permitted level (Total)		Samples higher than permitted level Area			
							Site 1		Site 2	
					EU DWD (%)	TFC DWD (%)	EU DWD (%)	TFC DWD (%)	EU DWD (%)	TFC DWD (%)
pH	Not mentioned	5.5-8.5	7.44 ± 0.12	7.17 ± 0.13	0	0	0	0	0	0
Conductivity	2500 µS/cm	2000 µS/cm	901.40 ±50.26	884.45 ± 61.28	39 (97.5)	0	20 (50)	0	19 (47.5)	0
TDS	Not mentioned	600 mg/ l	456.60 ±24.31	450.65 ± 30.80		5 (12.5)		2 (5.0)		3 (7.5)
Nitrate (NO_3^-)	50 mg/ l	45 mg/ l	52.25 ± 10.85^a	27.45 ± 5.19^b	11 (27.5)	13 (32.5)	7 (17.5)	9 (22.5)	4 (10.0)	4 (10.0)

*EU Water Directive 1998 ** MAC: Maximum Acceptable Concentration; *** Turkish Food Codex-Drinking Water directive, 2003; **** Means with different letters in a same line are significantly different from one another (P<0.05).

In rural areas, drinking water generally supplied groundwater through individual or community wells [18].

The HPC is used to estimate the total amount of bacteria in water and indicates the overall microbial status of the water [3, 10]. Eight (20%) groundwater samples, 5 (12.5%) samples on Site 1; 3 (7.5%) samples on Site 2, exceeded the EU DWD and TFC-DWD value in drinking water for HPC (Fig. 2a). Possible sources of this contamination may be intensive population and the Meric River.

Total coliforms, thermotolerant coliforms and *E. coli* were detected at rate of 25% (10 samples), 17.5% (7 samples), and 15% (6 samples) of the groundwater samples at the same region wells, respectively (Fig. 2b). Total coliforms, thermotolerant coliforms, *E. coli* and *Enterococcus* spp. are bacteria whose presence indicates that the water may be contaminated by human or animal wastes [4]. Fresh human and animal faeces contain between 10^2 and 10^4 fold more thermotolerant coliforms per gram than *Enterococcus* spp. [19]. Disease-causing microbes

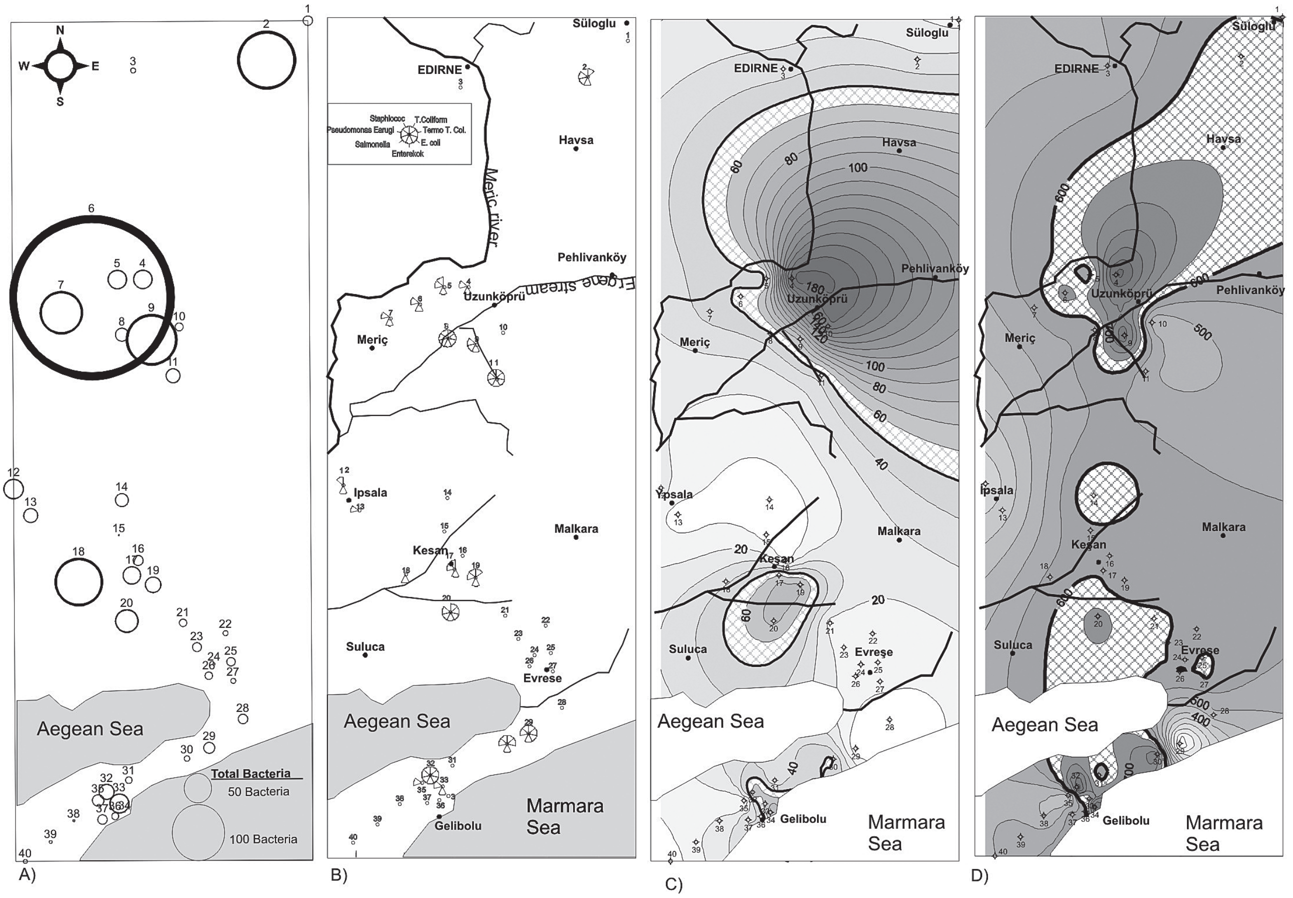

Fig. 2. Spatial variation of a) HPC, b) Other Bacteria, c) Nitrate (mg/l) and d) TDS (mg/l) in West Thrace.

(pathogens) in these wastes can cause diarrhea, cramps, nausea, headaches, or other symptoms. These pathogens may pose a special health risk for infants, young children, and people with severely compromised immune systems [10].

Enterococcus spp. was detected more often than either thermotolerant coliforms or *E. coli* [20]. Geldreich [21] suggested that *Enterococcus* bacteria are more numerous in faecal material than the other bacteria and more resilient in non-enteric environments, which may have accounted for these bacteria being more often detected and at a larger concentration in groundwater samples than thermotolerant coliforms. *Enterococcus* spp. was detected in approximately 47.5% and *E. coli* was detected in 15% of the groundwater samples. In a previous study, Demir et al. [22] found that 36.2% of samples contained *E. coli* and 42.5% of them contained *Enterococcus* spp. after investigating 22 groundwater, 64 spring water, 2 pond and 6 network water samples in the Kesan region (inside Site 1). When all the data were taken into account, it was observed that indicator bacteria were more prevalent in agricultural and urban areas, causing faecal contamination. This finding was similar to reports published by other researches [20, 23] (Fig. 2b).

According to TFC-DWD there should not be any pathogenic *Staphylococcus* sp. in 100 ml. of drinking water. In this study, *Staphylococcus* sp. was observed in 11 (27.5%) groundwater samples, while Demir et al. [22] cited that they found *Staphylococcus* sp. in 34% of their samples. Krapac et al. [20] identified more than 17 bacteria genera or species in groundwater samples collected near swine lagoons. They detected the same bacteria which were determined near the pits but also identified additional *Staphylococcus* sp. and *Enterococcus* spp. in their groundwater samples.

Salmonella sp. was found in 15% of the analyzed groundwater samples. One possible explanation would be that water was contaminated from different resources (septic tanks, waste water and livestock) and if they are used, they may cause various illnesses amongst both humans and animals [3]. In a similar way, Demir et al. [22] found *Salmonella* sp. in 11.7% of the water supplies they observed, and they stated that water in those supplies ought not to be used.

The predominant bacteria in the field blanks were *Pseudomonas* sp. and *Bacillus* sp. [20]. *P. aeruginosa* was detected in 15% (6 samples) of the groundwater samples. The presence of opportunistic *Pseudomonads* in the water carries the potential for problems in an immunocompromised population. Shallow groundwater samples commonly contained *Pseudomonas* sp., *Bacillus* sp., which occur in both soil and fecal material, and may not be indicative of livestock manure. There had been no relationship between the occurrences of the relatively large concentrations of *Enterococcus* spp. that the samples were collected. The source of the enteric bacteria is currently unknown, but could have resulted from non-livestock animals, pit leakage, or migration of bacteria from manure application to crop fields.

The comparison of different well groups spatially showed an increasing pattern of TDS and Nitrat concentration at the wells around Edirne (Uzunköprü, Kesan) and the Canakkale (Gelibolu) region (Fig. 2 c-d).

Excessive levels of nitrate in drinking water may cause serious illness and sometimes death. Nitrates have the potential to cause shortness of breath, "blue babies" syndrome in infant diuresis, an increase in starchy deposits and hemorrhaging at the spleen [10]. Eleven (27.5%) samples exceeded the EU guideline value of 50 mg/l for nitrate, but 13 (32.5%) samples violated the Turkish standard of 45 mg/l nitrate in drinking water. The differences of nitrate levels between groundwater of Site 1 and 2 were significant ($p<0.05$). Site 1 (Edirne area) has intensive agriculture areas. It was reported that groundwater was contaminated from nitrate fertilizers and manures used in agriculture [9, 24, 25]. Furthermore, nitrate is used by microorganisms as food resources. In addition, high nitrate levels are often accompanied by bacterial and pesticide contamination [26] (Fig. 2c).

Physical parameters such as pH, conductivity and TDS have a major influence on bacterial population growth. pH values ranging from 3 to 10.5 could favor both indicator and pathogenic microorganism growth [11]. Previously indicated pH levels seem to support bacterial growth. Conductivity measures the ability of water to conduct in electrical current, and is directly related to the TDS [27]. TDS represents the amount of inorganic substances (salts and minerals). High TDS is commonly objectional or offensive to taste. A higher concentration of TDS usually serves as no health threat to humans until the values exceed 10,000 mg/l [24]. Individual wells and localized aquifer zones yielding lower TDS and conductivity values as well as elevated turbidity, correlated with wells and aquifer zones with elevated bacterial contamination [28] (Fig 2d).

Conclusions

In this study 40 groundwater samples collected from West Thrace were analyzed. It was found that all microbiological and some chemical values determined from groundwaters were above the limits set by EU DWD and TFC-DWD. The existence of indicator bacteria in high amounts demonstrates that there may be pathogenic bacteria such as important pathogens like *E. coli*, *Salmonella* sp. which were present so that it is necessary to disinfect the groundwater before human use. The high number of indicator microorganism counts observed reflected the poor quality of water being used by these communities served by groundwater. The comparison of different well groups spatially showed an increasing pattern of TDS, nitrate concentration and microbial concentrations at the wells around Uzunköprü, Kesan and Gelibolu region. The people in these rural communities are therefore at higher potential risk of contracting water-borne and/or sanitation-related diseases.

The microbiological and physico-chemical quality that adversely affected the quality of groundwater is likely to arise from a variety of sources, including land application of agricultural chemicals and organic wastes, infiltration of irrigation water, septic tanks, and infiltration of effluent from sewage treatment plants, pits, lagoons and ponds used for storage.

In conclusion, it is necessary to apply strong preventions immediately to save groundwater supplies in a region which is rapidly developing and constitutes a location for increasing migration.

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