

Balneotherapy Features of Natural Thermal Source from Pertek (Turkey-Tunceli) Due to Its Some Chemical, Radioactivity and Biological Parameters

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Summary. It can also be defined as a discipline that reviews the factors of balneology or hydrotherapy, which are alternative treatment methods for public health, from chemical, biological and medical aspects. In this study, it was aimed to investigate whether the underground waters in the Pertek district of Tunceli province are suitable for balneology. Chemical, radioactivity analysis, microbiological quality, antimicrobial activities were determined in relation to natural groundwater and its ability to be a natural healing source was investigated. When the chemical content is examined, it was determined to be bromide amount is 0.24 mg / L, Iodine (I); 1.9 mg / L, sulfur (S); 0.02 mg / L, boron (B): 2676 µg / L, silicium (Si); 1.9 mg / L. With respect to the results, thermal water has the feature to be used in balneotherapy. According to the results of the radioactivity analysis, alpha (α) is 0.15 Bq / L; Beta (β) is <0.30 Bq / L and radon (Rn) is <13.4 Bq / L tritium (T) content is <10 Bq / L (<83.33 TB) and it is included in both mineral and young water group. This ground water and they comply with the standards in terms of microbiology (Total bacteria, coliform, *Escherichia coli*). Its antibacterial, antifungal and antidermatophyte properties prove to be a healing source. We confirmed the effectiveness of this healing water to both protect and promote human health as a drinking cure and reach the newest and most effective treatment modalities by using related water in Health and Thermal Tourism.

Key words: Balneotherapy; natural thermal water; chemical content; radioactivity value, microbiological quality; antimicrobial activity; Pertek

Introduction

Healing water is an important factor in both disease treatment and the protection of health (1). Besides diseases, thermal facilities are preferred for getting rid of stress, gaining vitality, youth, and beauty even. Increasing the elderly population especially in developed countries; adopting precautions that protect health and trying to lead a life for these principles make tourism establishments more interesting (2).

Turkey has rich thermal water and health resorts due to its remarkable position in terms of geothermal belt (3). Almost 529 thermo mineral sources are recorded as the name of thermal spring in literature; some of them have permission from the Ministry of Health. Some of the widely known thermal water

resources with minerals are in Ankara, Afyonkarahisar, Denizli, Konya, Yalova, Aydın, Muğla, İzmir, İstanbul, Tuzla, Manisa, Nevşehir, Tokat, Bingöl, anlıurfa, Amasya, Kahramanmaraş, Kırşehir, Rize. The temperature of waters in those hot springs differs in terms of physical, chemical and microbiological properties (4).

The use of water resources and mineral waters in human nutrition, especially in different stages of life, in physical activity and in the presence of some morbid conditions, is of great importance (5). There are many studies on both thermal resources waters that is the raw material of hot-springs and thermal tourism and the positive impacts of related waters on human health (6-14).

This paper has the first data in terms of determining some of the biological parameters of Pertek thermal water.

The tendency to alternative healing sources in the whole world; taking protective measures and trying to lead a life based on these principles have shown increase day by day. Healing waters that are mineral resource (15) by a great number of beneficial components and also valuable in terms of both thermal health and thermal tourism were evaluated within the scope of this study. Antimicrobial and anti-inflammatory properties of related waters emphasize the subject and purpose of this paper. In addition, with this study, to therapeutically effective foods for consumers who turn to natural products a quality alternative will be offered.

The necessity to research the new antimicrobial products is inevitable when the high resistance against antibiotics is considered. Bacterial, viral and fungal diseases seriously threaten the societies of developed countries especially. Natural products have potential in terms of having some therapeutic agents for several conditions including infectious diseases (16). It is an immemorial method to try to treat diseases by using underground sources (17). Balneology or hydrotherapy that is a dime a dozen treatment method is the alternative treatment for public health. Balneology can also be defined as a discipline that reviews factors of balneotherapy in terms of physical, chemical, biological, geological, hydrological, ecological and medical (18).

Thus, our study will contribute to development of alternative treatment approach as therapeutic food in seeking health in natural thermal resources in spite of developing technology. It is beneficial to conduct a study in this issue to show the importance of hot springs in medical tourism; provide alternative treatment modalities and sustainability of tourism in general.

This study determined microbiological quality and sulfur, iodine, silicon, bromine, boron and radioactivity values of the water of Pertek thermal facility that have positive and treater impacts on human health.

Materials and Methods

Preparing water samples for analysis

It was taken from the Singeç site of Pertek district of Tunceli province without overflowing into a sterile bottle by pouring pressurized water for at least 30

seconds. Samples were carried to the laboratory within 6-8 hours by keeping in refrigerator or isotherm containers with cool- pack at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. The duration between sampling and analysis was not over 12 hours. Samples were carried to the laboratory under cold chain conditions.

Determination of some chemical contents

The sulfur, silicium, bromide, boron contents of thermal water were made by the following methods. Sulfur content of thermal water (mg / L) by SM4500 S-2-D sulfide methylene blue method (19), iodine content (mg / L) by (mg / L) by artek engineering environmental laboratory, Silicium content (mg / L) by SM3111D standart methods- methals by flame atomic absorption spectrometry (20), Bromide content (mg / L) by SM4110B Standart method determination of anion by ion chromatography with chemical suppression of eluent conductivity (21), Boron content ($\mu\text{g} / \text{L}$) by EPA 200.7 Determination of metal and trace elements water and wastes by inducively coupled plasma atomic emission spectrometry (22).

Radioactivity quality

We got service from the Artek engineering environmental laboratory for determining radioactivity parameter and microbiological quality (total coliforms, total aerobic and mesophilic, *Escherichia coli*) of thermal water samples.

Microbiological analysis

In this study, membrane filtration method was used for the detection and enumeration of TSEN ISO 9308-1, *E. coli* and coliform bacteria. Membrane filtration system was sterilized before working. The species-specific Tergitol medium was wetted with 3.5 mL sterile distilled water according to the instructions given by the manufacturer. A sterile membrane filter with a pore diameter of $0.45\ \mu\text{m}$ was placed on the porous plate of the membrane filtration system using sterile forceps. The funnel system was carefully placed on the table and locked. 250 mL of the sample to be studied was filtered through a sterilized membrane

filtration system over a membrane filter that holds bacteria. The sample was vacuum filtered. The funnel was unlocked and placed on Tergitol medium immediately to prevent air contamination with membrane filter sterile forceps, without leaving any air between the membrane filter and the medium and left for incubation at $(36 \pm 2)^\circ\text{C}$ de $(21 \pm 3)^\circ\text{C}$ for hours. Yellow orange colored colonies on the membrane filter and lactose positive bacteria forming yellow spots under the membrane filter were counted. All colonies with an oxidase negative reaction were counted as coliform bacteria, and all colonies with an oxidase negative and indole positive reaction were counted as *E. coli*. After considering the characteristic colonies counted on the membrane filter and the results of the verification tests, the numbers of coliform bacteria and *E. coli* present in 250 mL of the sample were calculated in accordance with TS EN ISO 9308-1 (23).

Total bacteria count (aerobic and mesophilic) was performed with SM9215B standard methods heterotrophic plate count (24).

Antimicrobial effects

Water samples. In January and September, the antimicrobial activities of water samples were investigated by transmitting water samples to the laboratory under sterile conditions as soon as possible, protected from light in a refrigerator at $5^\circ\text{C} \pm 3^\circ\text{C}$ and in ice-battery isothermal containers.

Microbial strain. The bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* clinical isolate), yeast (*Candida albicans* RSKK 02007, *Candida glabrata* RSKK 014019, *Candida tropicalis* RSKK 02011, dermatophyte fungi (*Epidermophyton floccosum* RSKK 14024, *Trichophyton rubrum* RSKK 03022) were the species tested in the current study. The bacteria strains tested were obtained from Infectious Diseases Clinical Microbiology Laboratory, Göztepe Hospital, Istanbul-Turkey Medical Park; the yeast and dermatophyte fungi were obtained from the Refik Saydam National Drug Culture Collection.

Microdilution method (MIC). McFarland 0.5 turbidity suspension will be prepared from microorganisms growing in liquid media. Microdilution

broth method will be used in this study to determine the minimum inhibition concentration (MIC) of water samples against microorganisms. Mueller Hinton Broth (Accumix® AM1072) will be used for bacteria; Saboraud Dextrose Broth (Himedia ME033) media will be used for yeast and dermatophyte fungi. 90 µL of medium will be added to the wells in the first row of microtiter plates and 50 µL medium to each of the other wells. 11th-row wells will be utilized as control and 100 µL of medium will be added (25). 8th-row wells will be used as reproduction control. 10 µL of extract will be added to the wells in the first row and serial dilution will be performed 6 times. 50 µL microorganism suspension will be added to each well so as to be 5×10^5 CFU / mL for bacteria and $0.5\text{-}2.5 \times 10^3$ CFU / mL for yeast and dermatophyte fungi. Plates with bacteria will be incubated at $37 \pm 1^\circ\text{C}$ for 24 hours, plates with yeast added at $25 \pm 1^\circ\text{C}$ for 48 hours. 50 µL of 2 mg / mL 2, 3, 5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) will be added to each well and incubated at 37°C for 2 hours to make growth visible at the end of the incubation period. The first wells with no color change will be accepted as MIC values. The test will be repeated three times.

Results

Chemical content

According to physicochemical analysis results of samples of Pertek thermal main well in Pertek district of Tunceli province whose medical and balneological evaluation was performed in terms of both drinking water and hydrotherapy. Related water is in the group of "Thermo mineral waters with calcium and magnesium bicarbonate". Related water exceeds a threshold value that is accepted as valid for water drinking cure with mineral in terms of carbon dioxide, bicarbonate and fluoride values. It was also found that related water samples are perfect from the point of agricultural or organic pollution. However, there are no sulfur, iodine, silicon, bromine, boron, and radioactivity values. This is because we did not conduct a balneological evaluation regarding these factors. Moreover, a study regarding microbiological quality was not performed as well.

It was mentioned to people from surrounding provinces about the advantages of related water in bathing and drinking cure for degenerative joint disorders, inflammatory rheumatic diseases, chronic back pain, rehabilitation after surgery, psoriasis, eczema, acne, diabetes and skin lesions due to immobility. It was also mentioned to people from surrounding provinces about the advantages of related water in drinking cure for kidney and urinary tract stones, chronic and recurrent ulcers, functional disorders of the stomach and small intestine, urinary tract infections, asthma, bronchial disorders. There is necessity for further scientific studies to confirm the benefits of such usages (15).

However, it is mentioned in research that Pertek district, too, is in health tourism in Turkey (26). A related study is important in terms of researching sulfur, iodine, silicon, bromine, boron and radioactivity values and microbiological quality of this healing source. We did not regard a repetition analysis because the confirmed high values with calcium, magnesium, bicarbonate and carbon dioxide positive have impacts on the human health.

Table 1 shows the chemical content of Pertek thermal water: Bromide (Br) amount is 0.24 mg / L; Iodide (I) is 1.9 mg / L; Sulfur (S) is 0.02 mg / L; Boron (B) is 2676 µg / L; Silicium (Si) is 1.9 mg / L.

Radioactivity content

Humans and the environment are two inseparable concepts. The element that people benefit at maximum is the water in nature. One of the crucial properties that control the usage of water is radioactivity. The World Health Organization (WHO) and American Environmental Protection Agency (EPA) accept radioactivity limits as 0.5Bq / L for total alpha;

Table 1. Important chemicals in Pertek natural thermal water (Turkey-Tunceli)

Pertek natural thermal water chemical content	
Bromur (Br)	0.24 mg/L
Iodure (I)	1.9 mg/L
Sulfur (S)	0.02 mg/L
Boron (B)	2676 µg/L
Silicium (Si)	28.9 mg/L

1 Bq / L for the total beta. These limit values were determined with reference to the idea of radiation dose that a human takes for the whole life does not cause a loss in health. WHO's recommended values have directly reflected Turkish Drinking Water Standards (TSE 266). Balneologically use of related water besides drinking is common despite its negative impacts of radioactivity such as lung and other respiratory diseases and skin and digestive system cancers. Even if the mineralization of radioactive waters is low, they are healer at the same time. It needs to determine radioactive elements and radioactivities if related waters should be known before use. Waters with elements like Radon, Uranium, Thorium are radioactive waters. Such waters have been utilized in rheumatic, metabolic, endocrine, neurological, gynecological, gastrointestinal, and circulatory system diseases. It is observed in studies for patients with ankylosing, spondylitis, degenerative neck pain that therapies like radon baths and inhalation cause positive impacts such as a reduction in pain and a significant increase in movement. In other words, natural resources that are low dose ionized radioactivity source have positive impacts in several system diseases (27). According to the study results, alpha is 0.15 Bq / L; beta is <0.30 Bq / L, and radon is <13.4 Bq / L. This is proof of the issue that related waters can also be used as balneological besides drinking (Table 2).

Microbiological quality

Coliforms were enumerated in EMS method in laboratory as microbiological analysis; results were evaluated based on TS266. It was found at the end of the enumeration that this underground water has no coliform (Table 3).

Table 2. Radioactivity content of Pertek natural thermal water (Turkey-Tunceli)

Radioactivity content	Natural thermal water (Bq/L)
Alpha (α)	0.15
Beta (β)	<0.30
Tritium (T)	<10
Radon (Rn)	<13.4

Table 3. Microbiological quality of Pertek natural thermal water (Turkey-Tunceli)

Microbial communities (log CFU/g)	January and September
<i>Escherichia coli</i> cfu/250 mL	0
Total Bacteria cfu/ mL	13000
Total Coliform cfu/250 mL	0

It is emphasized that waters should be in accord with limit values of TSE (Turkish Standards Institute), WHO (World Health Organization) and EPA (American Environmental Protection Agency) to be used as drinking water (10). Values at the end of the analyses were compared with the values of WHO, EPA, TS266. It is concluded based on the data that related thermal water does not pose danger for public health.

Since coliforms are inherently in the intestine system of humans and homothermic animals, they were evaluated as the best indicators of fecal contamination in the beginning.

The number of coliform bacteria in 100 mL of microbiologically thermal water of high quality should be less than 2.2. It also is expected that related bacterias should be more than 100 per mL when they are incubated in an agar plate for 24 hours (12). It can be said with reference to the data above, there is not microbiological contamination to this resource in terms of coliform.

A study that used Health Directorate Public Health Laboratory results shows us related thermal water is not microbiologically proper even if it is thermal and in a thermal region. Since the smell of the water has the property of thermal water in terms of physical characteristics, the study was continued and regionally more important data were reached (4).

The thermal water resource that we used in this study is mostly preferred water for drinking and several bath cures of the people in Singeç location of Pertek district of Tunceli Province. Our microbiological test results confirm that this water has the proper content.

A vast number of hot springs in our country have been utilized by the public for both traditional applications and medical expectations. Many different medical applications like microdermabrasion, botulinum toxin and cosmetology procedures, photorejuvenation

in thermal facilities today. Especially, thermal water treatments can be recommended for dermatological diseases with the medical treatment psoriasis, atopic dermatitis, pruritus, lichen ruber planus, rosacea, acne, seborrheic dermatitis and ichthyoses can be shown as the examples for related diseases.

So and so, besides being low-cost and natural also independent of toxic effects; determining the positive impacts of this water on human health makes this paper is a preliminary survey for the next studies by preferring it instead of high dosing strategies of drugs. We also think that Pertek thermal water that is researched as in-vitro should be researched for in vivo animal models as well.

Antimicrobial effect

Many health resorts have been used for traditional applications and medical expectations in Turkey. Today, many different medical applications such as microdermabrasion, botulinum toxin and cosmetology procedures, photorejuvenation are applied in thermal facilities. Thermal water treatments can be recommended along with medical treatment for diseases especially in the field of dermatology. These include psoriasis, atopic dermatitis, pruritus, lichen ruber planus, rosacea, acne, seborrheic dermatitis and ichthyoses.

Besides, being low-cost and natural and also independent of toxic effects; determining the positive impacts of this water on human health makes this paper is a preliminary survey for the next studies by preferring it instead of high dosing strategies of drugs.

In this study, serial dilution was made starting from 100 μ L to 1.875 μ L for the Minimal inhibition concentration value of Pertek thermal water. This situation reveals that this healing water can be used by public in terms of meeting medical expectations

and its antimicrobial effect on bacteria, yeast and dermatophytes that cause significant health problems. There was no effect on *K. pneumoniae* only in the study (Table 4, Figure1).

Pertek thermal water is effective at different ratios on *E. coli* (MIC value; 12.5 μ L) causing urinary tract infections; *S. aureus* (MIC value; 100 μ L) causing diseases with different clinical stages; *E. faecalis* which causes hospital infections and gains increasing

resistance in the first well (MIC value; 100 μ L); *Candida* species causing fungal infections (MIC value; 50 μ L) and the most common dermatophytes causing dermatophytosis (MIC values; 50, 12.5 μ L). Our findings are the first on this issue and can be utilized as a source for next studies.

The effectiveness of this natural resource will be verified in order to protect and improve human health and also reach the latest and most effective treatment

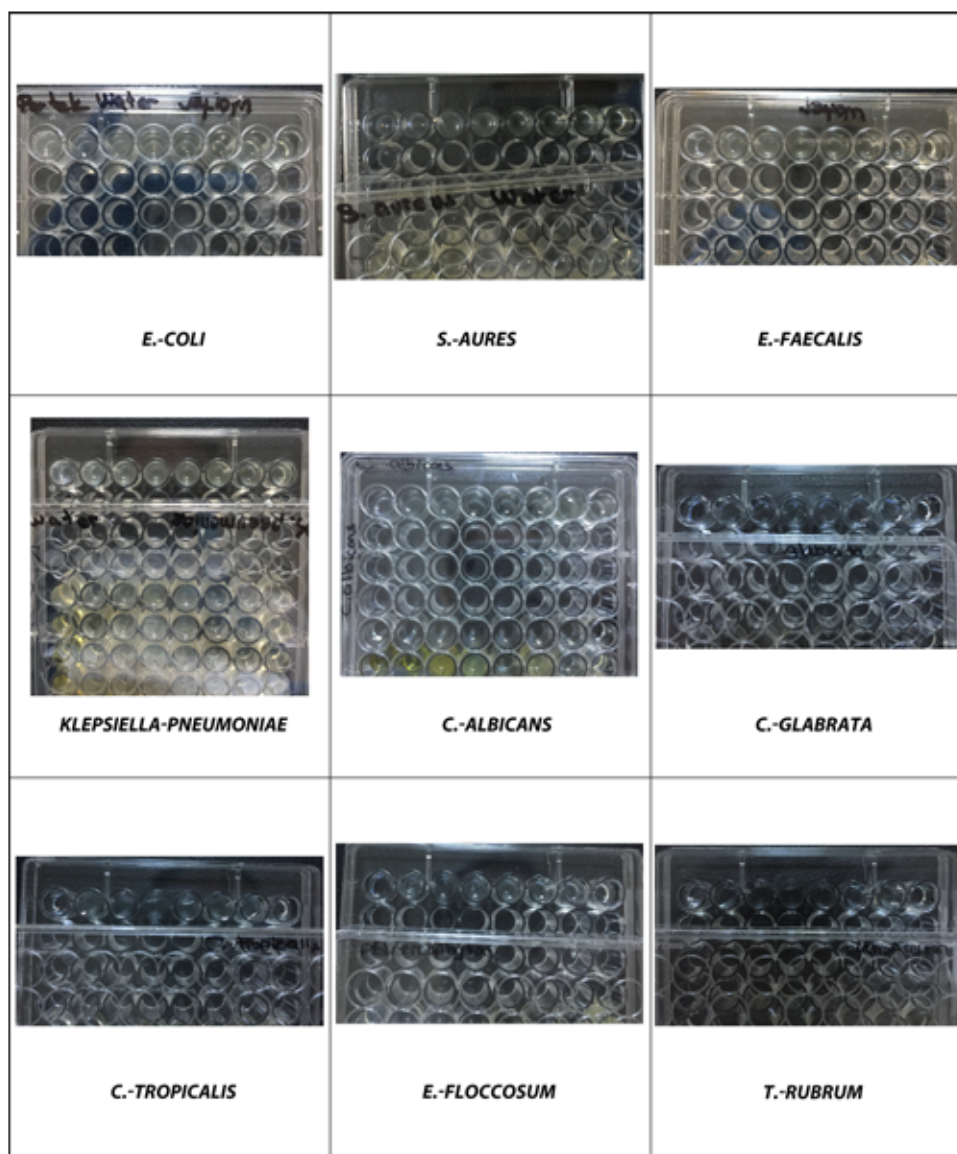


Figure 1. There are eight wells in microplatte. First well, thermal water and microorganism cultures. The wells in microplatte were diluted from 2nd to 8th wells. Liquid medium from 2nd to 8th wells + (100, 50, 25, 12.5, 6.25, 3.75, 1.875 microliter pertek thermal water + microorganism)

Table 4. The inhibitory effect of Pertek thermal water by the minimum inhibition concentration (MIC in 100 μ L)

Microorganisms	Inhibition area (μ L)
	MIC values
<i>E. coli</i>	12.5
<i>S. aureus</i>	100
<i>E. faecalis</i>	100
<i>K. pneumoniae</i>	-
<i>C. albicans</i>	25
<i>C. glabrata</i>	50
<i>C. tropicalis</i>	50
<i>E. floccosum</i>	50
<i>T. rubrum</i>	12.5

methods. This natural source also contains some therapeutic natural compounds such as antimicrobial, anti-inflammatory and wound healing, including infectious diseases. The importance of the study will be determined in terms of not causing residue problems of this natural resource and its reliability in terms of public health, especially as it is an important geothermal value.

Discussion

Natural waters dissolve the elements and substances in the soil during their underground cycles. Among these, calcium, magnesium and chlorine are among the elements that play a role in human nutritional physiology and that must be taken in a certain amount daily for a healthy life. In addition, these waters can contain trace elements such as fluoride, iron, iodine, selenium, copper, zinc, which have important roles in some basic vital events in humans such as respiration, excretion and digestion, substance synthesis and energy production, together nutrition. Previously, these waters were used only where they came out, but later, developments in science and technology made it possible to bottle them.

The tradition of using bottled natural mineral waters to meet physiological water needs is still common in many developed and developing countries. In our country, natural mineral waters are bottled under the names of mineral water, mineral water soda and soda.

However, bottled mineral water consumption is below the European level (28).

For expressions, the bromide amount rarely exceeds 1 mg/Lt in drinking waters (29). According to this expression, Pertek thermal water is also in the iodized water group in terms of its chemical properties.

Iodine is a strong antibacterial, antiparasitic, antiviral and anti-cancer agent (30). Özbek et al. (3) highlighted in their study that the German spa association accepts healing waters whose natural temperature is over 20 °C as 'Thermal springs'; they also term waters with some special minerals that are over the least values as 'Special Balneological Waters'.

Waters containing iodine above 1mg / L are called iodic waters. Since the amount of iodine of Pertek thermal water is 1.9 mg / L, it can be used in balneotherapy.

Regarding therapeutic properties of Taraklı thermal water and iodized waters on respiratory, heart, blood circulation and eye disorders, the amount of silicon (silicon) is 35.8 Mg/ L; the amount of boron is 2.3 Mg / L (31).

In a study, it was stated that iodized waters in the form of bath applications normalize high blood pressure in arteriosclerosis and are used in some rheumatic diseases, bone diseases, gynecological diseases and chronic upper diseases (32).

Zengin and Eker (29) conducted a study and gave the Kuzuluk thermal water analysis report. According to related report, amount of iodure is <1 Mg/Lt; sulfur is <0.05 Mg / L; Bromur is <0.2. Due to these properties, Kuzuluk thermal water is used to treat dermatologic, digestive, and nervous system disorders. According to Kıranşan et al. (33) iodure is 0.46 in Bingöl Kös health resorts.

Boron content limit is accepted as 3 mg / L for irrigation water; 2 mg / L for drinking water. So high values for drinking waters cause stomach and intestinal ailments. Pertek thermal water is fit for the maximum acceptable limits of drinking water standards (34).

It was also demonstrated that radon balneotherapy has biological effects such as modulation in the anti-inflammatory, antioxidant peripheral immune system (35).

CO₂, radon (Rn), and sulfur (H₂S) are the most known elements that are absorbed by the skin. Radon has an analgesic effect and affinity for nerve tissue (36).

Radon concentration in Karakoç station thermal waters is 0.29-10.26 Bq / L; the same concentration in Cumalı station thermal waters is 01-16.67 B / L (37).

Tritium densities in mineral waters vary between 1 TB and 25 TB. This related density varies between 1 and 2 TB in 'age-old' waters. 'Young' waters have higher values. Radioactivity of 0.12 Bq / L (0.0186 Mev of ray) corresponds to the waters with 1 TB (38).

With reference to the data above, since tritium content is <10 Bq / L (<83.33 TB) in radioactivity analysis for Pertek thermal waters, it is called the young water.

Regarding the drinking and city waters standards in terms of microbiological, chemical and physical, Boron 1 mg/ L total coliform:0, *E. coli*:0 should be for the primary standards; alpha emitters should be (Bq / L) 0.1; beta emitters should be (Bq / L) 1 for the secondary standards (Bq / L) (4).

It can be said with reference to the results (alpha 0.15; beta <0.30) we obtained that alpha, beta radioactivity, radon, tritium level of Pertek thermal waters are proper for both drinking and using in balneotherapy. Values at the end of the analyses were compared with the values of SKKY, WHO, EPA, TS266. For the results, Pertek thermal waters have positive impacts on drinking and balneotherapy.

Enterobacter aerogenes, *Klebsiella pneumoniae* and *Citrobacter freundii* are in both nature and plants; however, they cannot be used as the indicator of fecal pollution because of reproducing in soil and mixing in the waters. These bacteria, too, are called total coliform. *E. coli* from fetal contamination is the most important indicator bacteria that show the bacterial hygiene of drinking water (39).

Thermal waters of Pertek thermal facility are commonly used by the people of Karahayit region for both thermal treatment and tourism. It is important to research the contributions of this environment to health (40).

This is the first study that scrutinized the antibacterial, antifungal, antidermatophyte properties of these healing sources. Subject and scope of this study is these healing waters that are valuable in terms of both thermal health and thermal tourism. Related waters also have many antimicrobial, anti-inflammatory properties due to minerals (15).

For this reason, we think that this mineral water will be beneficial to the society in the future by bottling this mineral water as therapeutic food and marketing it abroad to benefit everyone due to its superior qualities. Because we believe that meeting the daily needs of certain minerals, supporting metabolic processes and affecting the functions of some systems in terms of physiological and healing will be realized thanks to this mineral water.

Conclusion

It is thought with reference to the analyses about reviewing biological activities and microbiological quality of natural healing thermal waters that this study can light the way for next *in vivo* studies in terms of being a healing source for many diseases.

This water sample whose balneological and biological properties were determined by us has positive and therapeutic effects on human health via both drinking and external ways. In this sense, due to the proper level of sulfur, iodine, silicon, bromine, boron compounds of this water, this paper will be an inspiration to future studies.

It will be beneficial to make research in terms of showing the importance of hot springs in terms of medical tourism and also the sustainability of tourism in general.

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