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Microbiological Viewpoint to Pelotherapy from Turkey

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Introduction: In the therapeutic use of spas, it is important to ensure the effectiveness of removal of coliform pollution in peloids and water. It is aimed that a study on microbiological characterization in the thermal habitats located in Turkey.

Method: The study was conducted on water and mud samples taken from spas in different parts of our country. The Most Probable Number (MPN) values obtained as a result of analyses of 100 ml and 300 ml water and peloid samples taken from each station for determination of the types of iron and acidophilic bacteria. Furthermore, IC50 values were calculated from the graph drawn between the concentrations water and mud concentrations and % activity values. IC45, IC50 and IC90 values indicating toxicity for granulated and anaerobic mud cultures were identified.

Results: For human health, the infection dosage of the bacteria in an individual must be about 50 IC50%. In water and mud samples, mostly iron bacteria such as *Siderocapsa sp, Thiobaccillus ferrooxidans* and acidophilic varieties such as *Thiobaccillus acidophilus* and *Leptosprillum sp* were identified. According to the results of the study, except for the water samples taken from stations TW-5, 6 and 15, pH value was in the range of 6-7, which indicated that it was slightly acidic. Moreover, their chemical contents and physical properties and at the same time their hygienic and microbiological characteristics were tested repeatedly through analyses.

Conclusion: We intended to make suggestions about the usability of the mud by identifying the properties of the spa muds and waters through analyses and /or make the existing mud compounds more useful by making additions/improvements to it, and thus initiating preliminary studies to set a standard.

Keywords: Pollution, thermal sources, microbiologic analysis, spas, peloids

Introduction

Sanitas Per Aquas (SPAs) are used as supplementary methods in the treatment of different diseases in the world and in our country. SPA waters contain different inorganic and organic compounds and are in a "physi-cochemical dynamism" (1). Widespread use of an established SPA treatment in Anatolia due to the presence of many mineral water springs results from the fact that our country is located on Alpine-Himalayan belt, which is one of the world's largest geothermal belts (2, 3). Our country is quite rich in thermo-mineral water springs. Therefore, ensuring widespread use of hot and/or mineral waters both at home and abroad for medical purposes is of great significance in terms of public health and its contributions to health tourism in our

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country. To this end, physical, microbiological and hydrochemical analyses of the physical, chemical and even radioactive findings of thermal and/or mineral water springs in our country were made (4). Thus, all changes they have undergone in the historical process and their effects on human and environmental well-being were revealed. Moreover, micro-flora populations in peloids and waters were also determined.

Thermal zones are very exclusive habitats for microorganisms (extramophiles) that like living in abnormal conditions. Although activities of living organisms of this sort are the cause of many problems encountered in thermal systems, they bear the solutions to these problems within themselves. In this context, discovery of microorganism diversity in any thermal region was considered to be the primary condition for benefiting from these living bodies in thermal processes. Presence of some organic and inorganic compounds in water and mud usually arises from microbial flora. In addition to physical and chemical tests, unique microbiological tests must definitely be performed in order to define the existing flora in thermal springs.

In the study, total coliform diagnosis and count, total aerobic bacteria count, iron bacteria, sulfur oxidizer (*Thiobacillus* sp.) bacteria determination and count, sulfate reducing bacteria (*Desulfovibrio* sp.) count and diagnosis, acid forming (acidophilic) bacteria determination, 24 and 48 h aerobic IC_{50}, IC_{90} toxicity tests, anaerobic toxicity tests (ATA), determination of the specific methane generation speeds of anaerobic microorganisms, diagnostic analyses for Pseudomonas and Staphylococcus bacteria types were performed. In short, this study investigated the microbiological properties of the mud and water samples taken from SPAs where mud treatment is used heavily or which allow taking mud and water samples, and tried to establish whether the materials are suitable for therapeutic purposes or not.

What made the study unique was that we intended to make suggestions about the usability of mud by identifying the properties of the SPA muds and waters through analyses and /or make the existing mud compounds more useful by making additions/improvements to it, and thus initiating preliminary studies to set a standard.

Study Design

The study was conducted on water and mud samples taken from SPAs in different parts of our country (Table 1). The sample volume was about 100 ml and 300 ml for all tests in sterile conditions and adequate quantities. Since it was necessary to begin microbiological analyses without delay to prevent unforeseen changes in micro-bacterial population after the samples were collected, and to obtain more reliable and accurate results, the samples were kept in cooling systems during their transportation to laboratories so that they could be analyzed within an hour.

In cases where colony formation was limited, the count was made using liquid medium. The method most frequently used to this end is MPN and counting of many microorganisms in the food industry is performed using this method. The MPN values obtained as a result of analyses of 100 ml and 300 ml water samples taken from each station (hot spring) were shown in the table; at the same time the types of iron and acidophilic bacteria were also identified. For microbiological analyses, the tests in question were conducted by

expert researchers at experienced laboratories (9 September University in Turkey) accredited by TÜRKAK and Ministry of Environment, partly at Faculty of Engineering Department of Environmental Engineering Environmental Microbiology Laboratory (toxicity analysis) and at another accredited laboratory, namely Refik Saydam Public Health Institute Environmental Microbiology Laboratory.

The results were interpreted using a multi-disciplinary method. In peloid therapy, the whole body or a local part of the person receiving the SPA treatment to make use especially of the thermal properties of peloid is covered with mud. Therefore, parameters that impair conditions of peloids fit for health are mainly coliform and fecal coliforms and other bacteria that might lead to contagion through contact. On top of them are patho-gene bacteria and fungi such as *Pseudomonas aeroginosa, Staphylococcus aureus* and *Candida albicans*. During the bacteriological analysis of the muds, using samples of 10 gram dry mud diluted at various proportions, numbers of types of bacteria were calculated depending on the rate of dilution.

Microbiological Analyses

Presence/transformation of organic and inorganic compounds present in water and mud is usually affected by microbial flora. For example, in defining some elements such as the amount of sulfur, iron etc. in water and mud, microorganisms that are present or may be present in the environment constitute an indicator prototype for diagnosis in the long run. Total coliform diagnosis (total and fecal coliform), total aerobic bacteria count, 24 and 48 hours aerobic IC_{50}, IC_{90} toxicity tests etc. in water and mud samples are extremely important for the determination of microorganisms needed for microbiological diagnosis to investigate the properties of thermal muds and waters and their therapeutic use. Moreover, the study also identified the sulfur oxidizing (*Thiobacillus* sp.) bacteria determination and count, iron bacteria, and acid forming (acidophilic) bacteria determination and count.

Anaerobic Toxicity Tests (ATT)

Glucose was put in serum bottles with Vanderbilt mineral medium containing no acclimated anaerobic and granulated mud and water and mud samples in increasing concentrations were added. IC_{50} (water and mud concentration inhibiting 50 % of activity) values were calculated from the graph drawn between the concentrations water and mud concentrations and % activity values. IC_{45}, IC_{50} and IC_{90} values indicating toxicity for granulated and anaerobic mud cultures were identified.

Results

The MPN values obtained as a result of analyses of 100 ml and 300 ml water samples taken from each station (hot spring) indicate the range shown in the table; at the same time the types of iron and acidophilic bacteria were also identified (Tables 1, 3). According to this, a microflora composed of *Thiobaccillus acidophilus, T.thiooxidans, T. ferrooxidans and Siderocapsa sp*. is predominant in thermal waters. Moreover, since bacteria of *Thiobacillus* sp. type are sulfur oxidizing bacteria, this situation can also be regarded as an indicator providing information about the sulfur content of the water because presence of some organic and inorganic compounds in water and mud usually arises from microbial flora. Because, the limit of the waters containing low sulfur content was determined according to

what extent they cause erythema on the skin. Waters with medium-level sulfur are the most frequently used ones for treatment. Waters of high sulfur are used in certain inflammatory and dystrophic diseases.

Waters with very powerful and ultra powerful sulfur, on the other hand, are not used in the treatment of humans. Acidophilic bacteria like *Thiobacillus acidophilus* play a role on the acidity of the mud. Thermal muds of acidic characteristic are an undesirable situation, so determination of the presence/number of this bacteria is important. According to the results of the analysis, acidophilic bacteria were identified profusely in thermal water and mud samples. Since waters whose pH values vary between 0 and 7 are acidic, the acidic medium will also influence micro-flora population in the medium. According to the research results, except for the water samples taken from the stations TW-5, 6 and 15, pH values varied between 6 and 7, which indicates that it is slightly acidic. Thus, the number and types of acidophilic bacteria increased. The number of total germs at 37 ^{o}C 24 hours should be 20 according to the formal norms. However, it was determined to be 170 at TW-14 and 100 at TW-15. The number of total germs at 36 ^{o}C 24 hours should be 5 according to the formal norm. However, it was found to be 0 at TW-16. IC_{50} and IC_{90} were sample dilution rate that inhibits 50 % and 90 % of the total bacteria and coliforms at 95 % reliability range.

Bathroom and swimming pool waters must not have any coliforms at 100 ml. Especially TW-8 coliform bacteria content was found to be high in the samples examined, but others were within standard limits. Bacteriological analyses must be conducted in bathroom and pool waters. Bathroom and swimming pool waters must be disinfected regularly with chlorine and the amount of free chlorine remains in such waters must not drop below 0.4 ppm/lt. Water disinfection is necessary to eliminate pathogenic microorganisms that may be found in such waters and can cause diseases.

As for the types of bacteria found in thermal muds, in addition to bacteria that need to be found in 100 gr according to aforementioned parameters, iron bacteria such as *T.ferrooxidans, Leptothrix sp., Siderocapsa sp.,* and acidophilic bacteria types such as *T. acidophilus, T. thiooxidans,* and *Leptosprillum sp.* were observed in the mud samples taken within the scope of the project. According to the results obtained from peloid and water samples that were taken from different regions where thermal water springs existed and that were subjected to analysis regularly, iron bacteria such as *Siderocapsa sp, Thiobaccillus ferrooxidans* and acidophilic bacteria such as *Thiobaccillus acidophilus* and *Leptosprillum sp* were predominantly identified.

The total number of germs at 37 ^{o}C 24 hours should be 20 according to formal norms. However, it was found to be more than 300 at TW-19 and 20. According to the results of the analysis given in Tables 4 and 5, the water sample numbered TW-19 is not compatible with the relevant regulations in terms of the total germ count (24 hours) parameter at 37 ^{o}C. (The Hot Spring Regulation published in the Official Gazette dated 24.07.2001 and no. 24472 amendment published in the Official Gazette 28.02.2006/26094). The water sample TW-15/1 at 36 ^{o}C and 37 ^{o}C does not conform to relevant regulation in terms of the parameters total germ count (24 hours), total coliform bacteria and fecal coliform bacteria.

Table 1. Results of microbiological analysis in thermal waters.

Sample Number	Sample amount (ml)	ATA analysis[1] (inhibition %)		Total bacteria[2] IC_{50} and IC_{90} (inhibition %)		Total coliform[3] IC_{50} and IC_{90} (inhibition %)	
		24 hour	48 hour	24 hour	48 hour	24 hour	48 hour
2011 YEAR							
TW9	300	IC_{50}=%28.5	IC_{50}=%57	IC_{60}=%44.3 IC_{90}=%13.8	IC_{60}=%88.5 IC_{90}=%27.5	IC_{50}=%26.5 IC_{90}=%7	IC_{50}=%53 IC_{90}=%14
TW10	300	IC_{50}=%29.5	IC_{50}=%59	IC_{60}=%29 IC_{90}=%37.8	IC_{60}=%55.5 IC_{90}=%20.5	IC_{65}=%42.2 IC_{90}=%14	IC_{90}=%28 IC_{65}=%84.4
TW11	300	IC_{50}=%20.5	IC_{50}=%41	IC_{60}=%48 IC_{90}=%24.8	IC_{60}=%71 IC_{90}=%34.5	IC_{50}=%41.2 IC_{90}=%8.7	IC_{90}=%17.5 IC_{50}=%82.3
TW12	300	IC_{50}=%29.1	IC_{50}=%58.2	IC_{50}=%22 IC_{90}=%12.01	IC_{50}=%80.5 IC_{90}=%40	IC_{60}=%40 IC_{90}=%11.1	IC_{90}=%22.2 IC_{60}=%80
TW13	300	IC_{50}=%24.5	IC_{50}=%48.96	IC_{50}=%34.2 IC_{90}=%4.01	IC_{50}=%68.5 IC_{90}=%8.15	IC_{50}=%30.5 IC_{90}=%7	IC_{90}=%14 IC_{50}=%61
TW14	300	IC_{75}=%33.1	IC_{75}=%66.2	IC_{50}=%44.6 IC_{90}=%8.7	IC_{50}=%89.2 IC_{90}=%17.4	IC_{50}=%40.6 IC_{90}=%9.3	IC_{90}=%18.5 IC_{50}=%81.2
TW15	100	-	-	-	-	-	-
2012 YEAR							
TW1	500	IC_{45}=%14,3	IC_{45}=%24,6	IC_{90}=%41	IC_{90}=%80	IC_{30}=%21	IC_{30}=%49
TW1/1	500	IC_{45}=%80	IC_{45}=%90	IC_{99}=%25	IC_{99}=%50	IC_{90}=%33	IC_{90}=%60
TW2	500	IC_{45}=%38	IC_{45}=%60	IC_{50}=%53	IC_{50}=%83	IC_{50}=%6,55-	IC_{50}=%13
TW3	500	IC_{45}=%16,7	IC_{45}=%32	none	None inhibition	IC_{58}=%35	IC_{58}=%70-
TW4/1	500	IC_{45}=%49	IC_{45}=%80	None inhibition	None inhibition	IC_{50}=%70	IC_{58}=%99
TW5/1	500	IC_{45}=%15	IC_{45}=%28	IC_{50}=%99.65	IC_{50}=%99.99	IC_{50}=%65-	IC_{50}=%99.99
TW7	500	None inhibition	None inhibition	IC_{50}=%79	IC_{50}=%96	IC_{50}=%15	IC_{50}=%30
TW8/1	500	IC_{45}=%24	IC_{45}=%48	IC_{50}=%84	IC_{50}=%99.99	IC_{50}=%94	IC_{50}=%100
TW9/1	500	IC_{45}=%32	IC_{45}=%65	IC_{50}=%89	IC_{50}=%99.99	IC_{50}=%45	IC_{50}=%90
TW15	500	IC_{45}=%24	IC_{45}=%48	None inhibition	None inhibition	IC_{50}=%1	IC_{50}=%37
TW15/1	500	IC_{45}=%34	IC_{45}=%64	IC_{45}=%78	IC_{45}=%99.99	IC_{50}=%7	IC_{50}=%16
TW16	500	IC_{42}=%35	IC_{42}=%70	IC_{50}=%36	IC_{50}=%72	IC_{50}=%20	IC_{50}=%40
TW16/1	500	IC_{45}=%27	IC_{45}=%54	IC_{40}=%24	IC_{40}=%48	None inhibition	None inhibition
TW17	500	IC_{45}=%40	IC_{45}=%80	IC_{50}=%84	IC_{50}=%99.99	IC_{50}=%12	IC_{50}=%24
TW18	500	IC_{45}=%23.9	IC_{45}=%56	IC_{50}=%39	IC_{50}=%72	None inhibition	None inhibition
TW18/1	500	IC_{45}=%33.7	IC_{45}=%67	IC_{50}=%100	IC_{50}=%100	IC_{50}=%3	IC_{50}=%78
TW18/2	500	IC_{45}=%42	IC_{45}=%80	IC_{50}=%98.1	IC_{50}=%100	IC_{50}=%4	IC_{50}=%98
TW19	500	IC_{45}=%26.5	None inhibition	IC_{50}=%71	IC_{50}=%99.99	None inhibition	None inhibition
TW20	500	IC_{40}=%43.4	IC_{40}=%86	None inhibition	None inhibition	IC_{50}=%38	IC_{50}=%76
TW20/1	500	IC_{40}=%21.7	IC_{40}=%43	IC_{50}=%47	IC_{50}=%96	IC_{50}=%33	IC_{50}=%66

[1] The sample dilution rate inhibiting 50 % the methane gas created by methane bacteria (%). [2] The sample dilution rate inhibiting 50 % and 90 % of the total bacteria (%). [3] The sample dilution rate inhibiting 50 % and 90 % of the total bacteria (%) statistical analysis is in the 95 % reliability range. **TW:** Thermal Water Sample, **TW1:** first analysis result of sample, **TW1/1;** sample taken from the same station the second time, repeatedly; **TW18/2**: second evaluation.

Table 2. Results of microbiological analysis in thermal waters.

Microbiologic Parameters	TW 1	TW 2	TW 3	TW 4	TW 5	TW 6	TW 7	TW 8	TW 9	TW 10	TW 11	TW 12	TW 13	TW 14	TW 15	TW 16	TW 17
Iron bacteria count and species (EMS/ml sample)	0.23	1.5	0.75	0	0.036	0.072	0.092	0.074	0.11	2.1	>11	1.2	0.14	0.43	0.43		
Acidophilic bacteria count and species (EMS/ml sample)	0.75	2.1	2.9	0	2.1	0.35	0.036	0.23	0.036	0.23	11	0.14	0.23	1.5	1.2		
36 and 37 °C total jerm count - 24 hour	0	0	0	>300	0	0	0	0	13	5	29	>300	>300	170	100	0	0
Total coliform bacteria	0	0	0	0	0	1	0	81	0	0	0	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Fecal coliform bacteria	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	25	0	0	3	0	0	8	0	0	49	0	0	0	0	0
Staphylacoccus aureus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Sulphite reduction anaerobic bacteria	0	0	0	0	0	1	12	0	17	0	0	41	5	0	0	0	0

Note: EMS is the most probable count in material and is in the 95 % reliability range (a unit that form colonies).

Table 3. Results of microbiological analysis in thermal waters.

Microbiologic parameters	TW 1	TW 1/1	TW 2	TW 3	TW 4/1	TW 5/1	TW 7	TW 8/1	TW 9/1	TW 15	TW 15/1	TW 16	TW 16/1	TW 17	TW 18	TW 18/1	TW 18/2	TW 19	TW 20	TW 20/1	TSI 36 ±1°C
Iron bacteria count & species (EMS/ml sample)	0.53	>24	>24	>24	>24	>24	>24	>24	>24	0	0	0.11	0	0.03	0	0	0	0	0.29	0.16	0
Acidophilic bacteria count & species (EMS/ml sample)	0.03	0	0	0	0	0	0	0	0	0	0	0.036	0.23	0	0	0	0	0	0.15	2.1	0
36 and 37 °C total jerm count - 24 hour	0	0	0	>300	0	0	0	0	0	0	>300	>300	>300	170	>300	>300	>200	>300	>300	>300	0
Total coliform bacteria											310	0	0	0	0	0	0	0	0	0	0
Escherichia coli											250	0	0	0	0	0	0	0	0	0	0
Fecal coliform bacteria count											250	0	0	0	22	0	0	0	0	0	0
Pseudomonas aeruginosa											0	0	0	0	0	0	0	0	0	0	0
Staphylacoccus aureus											0	0	0	0	0	0	0	0	0	0	0
Sulphite reduction anaerobic bacteria											15	0	5	0	17	36	24	0	0	0	0

Note: EMS is the most probable count in material and is in the 95 % reliability range (a unit that form colonies)
0: There was no growth.

Table 4. Results of microbiological analysis in peloid samples.

Peloid sample number	Sample amount (ml)	ATA analysis [1] (inhibition%)		Total bacteria [2] IC_{50} and IC_{90} (inhibition%)		Total coliform [3] IC_{50} and IC_{90} (inhibition%)	
		24 hour	48 hour	24 hour	48 hour	24 hour	48 hour
TP1	100	IC_{70}=%35.5	IC_{70}=%71	IC_{50}=%30.6 IC_{90}=%7.1	IC_{50}=%61.2 IC_{90}=%14.2	IC_{50}=%34.5 IC_{90}=%9.5	IC_{90}=%19 IC_{50}=%69
TP2	100	IC_{50}=%41.8	IC_{50}=%83.5	IC_{50}=%49 IC_{90}=%20.1	IC_{50}=%88 IC_{90}=%24	IC_{50}=%37.6 IC_{90}=%16	IC_{90}=%19 IC_{50}=%75.2
TP3	100	IC_{50}=%25.8	IC_{50}=%51.5	IC_{50}=%43 IC_{90}=%10	IC_{50}=%86 IC_{90}=%20	IC_{70}=%43.1 IC_{90}=%16.2	IC_{90}=%32.4 IC_{70}=%86.2
TP5	100	IC_{50}=%30.6	IC_{50}=%61.2	IC_{50}=%44.2 IC_{90}=%10.5	IC_{50}=%88.4 IC_{90}=%21	IC_{80}=%41 IC_{90}=%22.2	IC_{90}=%44.5 IC_{80}=%82
TP6	100	IC_{50}=%21.7	IC_{50}=%43.3	IC_{50}=%15.1 IC_{80}=%4.1	IC_{50}=%30.3 IC_{80}=%8.2	IC_{80}=%39 IC_{90}=%21.6	IC_{90}=%43.3 IC_{80}=%78
TP7	100	IC_{70}=%32	IC_{50}=%43.3	IC_{50}=%43.5 IC_{90}=%10	IC_{50}=%87 IC_{90}=%20	IC_{80}=%38 IC_{90}=%20.6	IC_{90}=%41.2 IC_{80}=%76.1
TP8	100	IC_{50}=%37	IC_{50}=%74	IC_{50}=%27.2 IC_{90}=%6.2	IC_{50}=%54.4 IC_{90}=%12.3	IC_{60}=%77 IC_{90}=%26	IC_{90}=%26 IC_{60}=%77
TP9	300	IC50=%27.8	IC50=%55.6	IC65=%44.1 IC90=%13.5	IC65=%88.3 IC90=%27	IC70=%35.5 IC90=%14.6	IC90=%29.1 IC70=%71
TP10	300	IC50=%28.7	IC50=%57.3	IC50=%32.5 IC90=%14	IC50=%85 IC90=%30	IC50=%43.5 IC90=%11.6	IC90=%23.2 IC50=%87
TP11	300	IC50=%32.6	IC50=%65.2	IC50=%28.5 IC90=%7.5	IC50=%57.1 IC90=%15	IC50=%42.5 IC90=%12	IC90=%24 IC50=%85
TP12	300	IC50=%31.2	IC50=%62.3	IC50=%42.5 IC90=%9	IC50=%85 IC90=%18	IC50=%35.5 IC90=%7.5	IC90=%15 IC50=%71
TP13	300	IC50=%23.1	IC50=%46.2	IC50=%38.7 IC90=%8.7	IC50=%77.4 IC90=%17.5	IC50=%45 IC90=%9	IC90=%18 IC50=%90
TP14	300	IC50=%18.8	IC50=%37.5	IC50=%26 IC90=%7.3	IC50=%52 IC90=%14.5	IC60=%38.7 IC90=%16.5	IC60=%77.5 IC90=%17
TP15	300	IC50=%18.8	IC50=%37.5	IC50=%26 IC90=%7.3	IC50=%52 IC90=%14.5	IC50=%33 IC90=%6	IC90=%12 IC50=%66
TP16	300	IC45=%20	IC45=%40	None inhibition	None inhibition	None inhibition	None inhibition
TP17	300	IC45=%55	IC45=%100	IC50=%39	IC50=%76	IC50=%27	IC50=%54
TP18	300	IC45=%28	IC45=%50	IC50=%39	IC50=%79	None inhibition	None inhibition
TP19	300	IC45=%13.2	IC45=%26	IC50=%30	IC50=%60	None inhibition	None inhibition
TP20	300	IC40=%25	IC40=%50	IC50=%42	IC50=%84	IC50=%22	IC50=%44

Note: EMS is the most probable count in material and is in the 95 % reliability range (a unit that form colonies)
TP: Thermal Peloid Sample.

Table 5. Results of microbiological analysis in peloid samples.

Microbiologic parameters	TP 1	TP 2	TP 3	TP 5	TP 6	TP 7	TP 8	TP 9	TP 10	TP 11	TP 12	TP 13	TP 14	TP 15	TP 16	TP 17	TP 18	TP 19	TP 20	TSI 36 ±1°C
Iron bacteria count and species (EMS/ml sample)	0.23	0.085	0.93	0.085	0.24	0.092	1.2	0.030	0.062	0.092	0.062	1.2	0.11	0.155	0.2	2.9	1.5	0.062	0	0
Acidophilic bacteria count and species (EMS/ml sample	>11	0.24	0.065	0.036	0.28	0.126	2.9	0.21	0.030	0.38	0.14	0.11	0.062	0.27	0.75	0.43	2.14	0.43	0.20	0
Total colonies bacteria count (kob/g-ml)	>3.3x 10^4	>3.3x 10^4	500	>3.3x 10^4	<10	2x10^4	1.1x 10^5	>3.3x 10^5	>3.3x 10^5	>3.3x 10^5	>3.3x 10^5	8.9x10^4	1.5x10^5	5.1x10^4	>3.3x 10^5	>3.3x 10^5	3.7x10^3	1.9x10^6	2.5x10^6	0
Total coliform bacteria (EMS/g-ml)	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	1.1 x10^5	23	23	<3	>1.1 x10^3	>1.1 x10^3	43	<3	>1100	0
Escherichia coli	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	43	<3	<3	<3	>1.1 x10^3	>1.1 x10^3	<3	<3	>1100	0
Pseudomonas aeruginosa	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	0
Staphylococcus aureus	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<100	0	<100	0
Candida albicans	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	0	6x10^2	0

On the other hand, samples TW-18/1 and TS18/2 do not conform to the relevant regulation in terms of the parameter total germ count at 37 °C (24 h). According to the formal norms, the number of total germs at 36 °C (24 hours) should be 5 (Table 5). However, it was found to be more than 300 at samples TW-15/1,19,18,18/1, and 20, whereas it was 200 at TW-18/2. However, the water sample TW-18 does not conform to formal norm in terms of parameters of total germ count at 37°C (24 h), and total coliform bacteria. IC_{50} and IC_{90} are sample dilution rates that inhibit 50% and 90% of total bacteria and coliforms at 95% reliability range.

In the ATA test, the inhibition percentages based on sample dilutions inhibiting 50% of the gas created by methane bacteria at the end of 48 hours are higher in comparison to those at 24 h. Moreover, higher inhibition was observed at the end of 48 hours (IC_{50}=27.8% at the end of 24 hours; whereas it was IC_{50}=55.6% at the end of 48 hours). Likewise, inhibition percentages based on sample dilutions inhibiting 50% of microorganisms at the end of 48 hours were higher in total bacteria and total coliform bacteria. The rectilinear line we drew for 2 microorganisms based on sample dilutions regarding the inhibition percentages did not intersect some of 50% values, IC_{60} and IC_{90} values were given.

When we take into account the IC_{50} and IC_{90} values at the end of 24 and 48 hours, IC_{50} value obtained for total coliform at the end of 24 hours was 42.5 % whereas IC_{90} was 12 %; in contrast, when the sample dilution rate was 12 %, IC_{90} was high, but the dilution rate was 42.5 %, inhibiton was lower (IC50).

Maximum number of colonies in the sample taken from the SPA

Number of colonies (in the agar-agar or agar-gelatine mixture at 20-22 ^{o}C in 72 hours) is 20/ml. Number of colonies (in the agar-agar mixture at 37^{o}C in 24 hours) is 5/ml. At 37^{o}C, in 250 ml sample, there must not be anaerobes in coliform bacteria, fecal coliforms, *Pseudomonas aeruginosa*, and in 50 ml sample, again there must not be sulphate reductase sporophyte anaerobes. Far higher quantities of bacteria *E.coli* were found in samples P-12, 16, 17 and 20 in terms of coliform bacteria. E. *coli,* one of the most frequently encountered bacteria, is an intestine-based microorganism and generally indicates that the product, environment or equipment where microorganism exists has been contaminated with human excrement. Peloids of these SPAs bear the risk of contamination and necessary disinfection should be performed. On the other hand, detailed controls should be conducted in other SPAs as <3 value was obtained there. *C. albicans* was seen only in P-20 while *S. aureus* was seen only in P-18 and 20.

Discussion

SPAs are used as supplementary methods in the treatment of different diseases in the world and in our country. Microbiological analyses should be performed at the SPAs in question in terms of relevant bacterial/fungal contents and problems in this regard should be eliminated. The immune systems of individuals who have applied for SPA treatment are more vulnerable to infections than healthy people. Moreover, in addition to the presence of patients who come with many different health problems, hot and humid environments may cause or increase formation of pathogens such as bacteria, viruses, fungi etc. Therefore, all kinds of environments in SPAs should definitely be inspected to ensure that the air breathed, places where baths are taken (pool/bath-tub), the water drunk, and the mud used for treatment conform to the specified standards.

Muds in peloidotherapy applications are used for their thermal properties and people who receive mud treatment are covered with mud wholly or locally, or enter pools containing mud and apply the mud on their bodies. Therefore, it has been pointed out that parameters that might impair the hygienic conditions of peloids and thermal waters are mainly coliforms and fecal coliforms, as well as bacteria of the groups *Pseudomonas* and *Staphylococcus* (5, 6). *P. aeruginosa* is a Gram-negative, aerobic and ubiquitous environmental bacterium, present in moist areas and surface waters which are possible cause of exposure to the bacteria (7, 8).

Potential microbial risks at SPAs may or may not be of fecal origin. Even in places where there is no fecal contamination, *E.coli* bacteria may exist. *Escherichia coli* is an important indicator species among bacteria populations of fecal origin, and its content must be lower than 20/g (9). The bacteria are complied with the limit in most of peloids, except TP-12, 16, 17, and 20.

SPAs are health institutions where cures including use of mineralized thermal waters and mud baths, drinking and inhalations, as well as a combination of climatic cure, physical treatment, rehabilitation, mechanotherapy, physical education, massage, psychotherapy, diet and supplementary treatments are performed. This pilot study has tried to emphasize that therapeutic procedures

serving these purposes should be considered more particularly for the regions used in the study, and that these SPAs should be routinely inspected in terms of their compliance with regulations and equipment. In terms of pathogenic microorganisms, nearly all of the samples accordance with the microbiological limits proposed by Turkish legislation. Few some SPA water and peloids are need to be eliminated from the pathogenic bacteria.

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