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AMELIORATION OF THE "TRUSKAVETSKA" BOTTLED WATER BY HYDROGEN-RICHING

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Annotation

Background and aim. The "Truskavetska" bottled water (TW) is officially classified as table water and is not considered medicinal. Recently, it rats experiment we found that enrichment of TW with Hydrogen generally has a favorable effect on its stress-limiting capacity, associated with antioxidant activity. We hypothesized that enrichment of TW with Hydrogen may increase its physiological/therapeutical activity, in particular in relation to patients with chronic pyelonephritis. Testing this hypothesis was the aim of this study.
Material and methods. The object of observation were 22 men with chronic pyelonephritis. We determined the leukocyturia and bacteriuria levels and recorded EEG, HRV, phagocytosis, lipids peroxidation as well as routine hematological and biochemical blood parameters. The survey was conducted twice: on admission and after week of drinking of Naftussya bioactive water (NW) or TW by 200 mL for 1 hour before meals three times a day, or TW by 100 mL, mixed before use with 100 mL of regular water, but enriched with Hydrogen.
Results. It was found that TW does not affect catalase activity and blood levels of α2-globulines and sialic acids, while NW significantly increases them; after enrichment of TW

with Hydrogen, it partially acquires the properties of NW, although it is still significantly inferior to it. On the other hand, Hydrogen gives TW the ability to reduce blood levels of MDA, bilirubin, prothrombin and eosinophils, which also brings it closer to NW. Instead, hydrogen-enriched TW even slightly outperforms NW in terms of enhancing the intensity of blood neutrophils phagocytosis, increasing serum lysozyme activity and urinary amylase excretion, on the one hand, and reducing leukocyturia and bacteriuria, blood levels of CIC and HDLP cholesterol, as well as vagal tone, on the other hand. In addition, enrichment of TW with Hydrogen eliminates caused by it the decrease in the blood content of erythrocytes and hemoglobin and even reverses the decrease in the entropy of EEG and HRV as well as PSD of theta-rhythm in occipital loci.

Conclusion. Enriching the low-activity "Truskavetska" bottled water with Hydrogen significantly increases its physiological activity to a level comparable to those of therapeutic Naftussya water, which is manifested in a favorable effect on leukocyturia and bacteriuria as well as phagocytosis, metabolism, and erythron in patients with chronic pyelonephritis.

Keywords: Hydrogen, "Truskavetska" bottled water, chronic pyelonephritis, EEG, HRV, phagocytosis, metabolism.

Introduction

The “Truskavetska” bottled water is officially classified as table water and is not considered medicinal. However, judging by the lack of publications, its physiological activity has not been studied. However, it is known that this water, like the famous Naftussya bioactive water [6,8,18,19,37,39,40,48,50], contains oil-like organic substances, but is devoid of microflora and the fatty acids produced by it. Recently, it was found that the preventive use of “Truskavetska” bottled water affects a number of post-stress parameters of the neuroendocrine-immune complex, metabolome, ECG and gastric mucosa of rats, similar to Naftussya water. At the same time, another constellation of parameters changes in the opposite way, on the basis of which the individual contributions of oil-like organic substances and the autochthonous bacteria/fatty acid complex to the stress-limiting effects of Naftusya water are estimated [36].

On the other hand, molecular hydrogen (H_2) is now recognized as a therapeutic gas for the treatment of many diseases [7,17]. Hydrogen rich water is used in clinical medicine to correct metabolic disorders and inflammation [3,9,20,27,28,32,45].

Recently, it rats experiment we found that the preventive use of “Truskavetska” bottled water enriched with Hydrogen minimizes the post-stressor increase in sympathetic tone and adrenal mass, and prevents the increase in catecholamines and corticosterone as well as plasma cells in the blood and rod-shaped neutrophils in the spleen. On the other hand, it prevents a post-stressor decrease in the intensity of macrophage phagocytosis and the bactericidal capacity of blood microphages, the content of lymphoblastes in the thymus, the activity of both antioxidant enzymes and vagal tone, and also minimizes the decrease in the content of eosinophils in the blood, non-alpha-lipoprotein cholesterol in the serum, and the mass of the spleen, in addition, the reduced content of plasma cells in the spleen reverses to an excess. Finally, the non-stress-responsive parameters of the control animals: the activity of AlT, CPhK, AsT and diene conjugates of the serum, the content of reticulocytes and Hassal’s bodies in the thymus - under the influence of these water increase to one degree or another. A strong canonical correlation was found between the activity of antioxidant enzymes, on the one hand, and metabolic-endocrine (R=0.959) and immune (R=0.959) sets, on the other hand. Thus, enrichment of "Truskavetska" bottled table water with Hydrogen generally has a favorable effect on its stress-limiting capacity, associated with antioxidant activity [51].

Based on the above, we hypothesized that enrichment of "Truskavetska" bottled water with Hydrogen may increase its physiological/therapeutical activity, in particular in relation to patients with chronic pyelonephritis. Testing this hypothesis was the goal of this study.

The study was conducted within the framework of a triune neuro-endocrine-immune complex [15,25,29,33,36,39,42] and functional-metabolic continuum [14] adopted at the Truskavetsian Scientific School of Balneology.

Material and methods

Participants. The object of observation were 22 men (age 26÷61 years), residents of the city of Truskavets', with chronic pyelonephritis.

The survey was conducted twice: on admission and after week of drinking of Naftussya bioactive water or "Truskavetska" bottled water by 200 mL for 1 hour before meals three times a day, or "Truskavetska" bottled water by 100 mL, mixed before use with 100 mL of regular water, but enriched with Hydrogen.

Rich by Hydrogen water produced by chemist Viktor S. Sorokendya (LLC BE FRESH ORGANIC, Dnipro, Ukraine). Hydrogen concentration: 420 ÷ 460 μg/dm^3 (0.42 ÷ 0.46 ppm), redox potential: -350 ÷ -375 mV. The measurement of hydrogen concentration was carried out using the device "Dissolved hydrogen analyzer MARK-501", manufactured by LLC "VZOR", Nizhny Novgorod, RF. Factory No. 266. The redox potential was measured with a portable ORP-meter HM ORP-200.

Procedure / Test protocol / Skill test trial / Measure / Instruments.

The day before, samples of morning urine was collected, in which was determined the leukocyturia and bacteriuria levels and as well as amylase and medium-mass molecules. Unified methods are applied [4].

Systolic (Ps) and diastolic (Pd) blood pressure was measured by tonometer "Omron M4-I" (Netherlands) in a sitting position.

To assess the parameters of heart rate variability (HRV) we recorded during 7 min electrocardiogram in II lead (software-hardware complex "CardioLab+HRV", KhAI-MEDICA, Kharkiv). For further analyses the following parameters HRV were selected [6,9,24,49]. Temporal parameters (Time Domain Methods): the standard deviation of all NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater than 50 msec (pNN_{50}). Spectral parameters (Frequency Domain Methods): absolute (msec^2) and relative (% of total) power spectrum density (PSD) bands of HRV: high-frequency (HF, range 0,4÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,04÷0,015 Hz) and ultralow-frequency (ULF, range 0,015÷0,003 Hz) [2,5,16,43].

Simultaneosly EEG recorded a hardware-software complex "NeuroCom Standard" (KhAI MEDICA, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on tassels the ears. Two minutes after the eyes had been closed, 25 sec of artifact free EEG data were collected by computer. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz) as well as absolute (μV^2/Hz) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device.

In addition, we calculated for HRV and each locus of EEG the Shannon's Entropy (h) of normalized PSD using Popovych's IL [15,34] equations:

$hEEG = -[PSD\alpha \bullet \log_2 PSD\alpha + PSD\beta \bullet \log_2 PSD\beta + PSD\theta \bullet \log_2 PSD\theta + PSD\delta \bullet \log_2 PSD\delta]/\log_2 4;$

$hHRV = -[PSHF \bullet \log_2 PSHF + PSLF \bullet \log_2 PSLF + PSVLF \bullet \log_2 PSVLF + PSULF \bullet \log_2 PSULF]/\log_2 4.$

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by the phagocytosis index (PhI), the microbial count (MC) and the killing index (KI) for Staphylococcus aureus (ATCC N25423 F49). In addition, the serum level of Lysozime (by the test of bacteriolysis of Micrococcus lysodeikticus) and Complement (by 50% hemolysis in the complement fixation reaction) was determined [26,35,38].

State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract [12]) and malondyaldehide (test with thiobarbituric acid [1]), as well as the activity of antioxidant enzymes: catalase of serum (by the speed of decomposition hydrogen peroxide [23]) and superoxide dismutase of erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH [10,30]).

Finally, according to the protocol, routine hematological (hemoglobin, erythrocytes, reticulocytes, hematocrit, erythrocyte sedimentation rate, thrombocytes, prothrombin) and biochemical blood parameters: albumins, alpha-1, alpha-2, beta- and gamma-globulins, urea, uric acid, creatinine, glucose, sialic acids, alkaline phosphatase, amylase, alanine and aspartic transaminases, medium-mass molecules, high-, low-, and very-low-density lipoproteins cholesterol were determined.

The analyzes were carried out according to the instructions described in the manuals [4,13]. The analyzers “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets.

Data collection and analysis / Statistical analysis.

Statistical processing performed using a software package “Microsoft Excell” and “Statistica 64 StatSoft Inc”.

Results and discussion

Adhering to the Truskavetsian Scientific School's analytical algorithm [25,34,39], the actual/raw parameters were normalized by recalculation by the equations:

$Z = (V – N)/SD = (V/N – 1)/Cv$, where

V is the actual value; N is the normal (reference) value; SD and Cv are the standard deviation and coefficient of variation respectively.

Reference values are taken from the database of the Truskavetsian Scientific School of Balneology (EEG, immunity) or instructions (HRV, metabolism).

In the first stage of the analysis, through screening, variables were identified whose average levels after consuming at least one of the waters were significantly different from the basal ones. The selected 26 variables were combined into 7 patterns (Table 1).

Table 1. The patterns of effects of the "Truskavetska" Water **(**TW**)**, the "Truskavetska" Water plus Hydrogen (TWH_2) and Naftussya Water (NW). See also Table 3

Variables	**Effect of TW (11)**	**Effect of TW H_2 (4)**	**Effect of NW (3)**
MC of Monocytes	0,86	0,96	1,09
MMM Excretion	0,37	1,13	1,01
PhI of Neutrophils	0,83	0,35	0,45
Catalase	0,02	1,67	2,99
A_2-globulines	0,33	1,37	2,37
Sialic acids	-0,23	0,47	2,05
Malondyaldehide	0,14	-0,36	-0,78
Bilirubin	-0,01	-0,47	-0,64

Prothrombin Ind	0,07	-0,4	-1,09
Eosinophils	1,27	-1,3	-1,78
MC of Neutrophil	0,69	0,95	0,69
Lysozyme	0,71	1,23	0,59
Amylaseuria	1,62	1,93	1,18
HDLP Cholesterol	-0,55	-1,00	-0,72
SDNN	-0,45	-0,67	-0,62
Leukocyturia	-0,93	-1,31	-0,47
Bacteriuria	-1,18	-1,82	-0,90
CIC	-0,94	-1,75	-1,14
Erythrocytes	-0,42	0,12	0,31
Hemoglobin	-0,25	0,12	0,60
P3 PSD Entropy	-0,45	0,33	0,25
P4 PSD Entropy	-0,3	0,52	0,18
O2-θ PSD	-0,27	0,64	0,00
O2 PSD Entropy	-0,35	0,65	0,37
HRV Entropy	-1,28	0,00	-0,19
O1-θ PSD	-0,19	1,45	0,02

Further, profiles of patterns were created (Fig. 1).

It was found that all three variants of drinking balneotherapy enhance phagocytosis and increase urinary excretion of medium-mass molecules to approximately the same extent. "Truskavetska" Water (TW) does not affect catalase activity and blood levels of α2-globulines and sialic acids, while Naftussya Water (NW) significantly increases them; after enrichment of TW with hydrogen, it partially acquires the properties of NW, although it is still significantly inferior to it. On the other hand, hydrogen gives TW the ability to reduce blood levels of MDA, bilirubin, prothrombin and eosinophils, which also brings it closer to NW.

Instead, hydrogen-enriched TW even slightly outperforms NW in terms of enhancing the intensity of blood neutrophils phagocytosis, increasing serum lysozyme activity and urinary amylase excretion, on the one hand, and reducing leukocyturia and bacteriuria, blood levels of CIC and HDLP cholesterol, as well as vagal tone, on the other hand. In addition, enrichment of TW with hydrogen eliminates caused by it the decrease in the blood content of erythrocytes and hemoglobin and even reverses the decrease in the entropy of EEG and HRV as well as PSD of theta-rhythm in occipital loci.

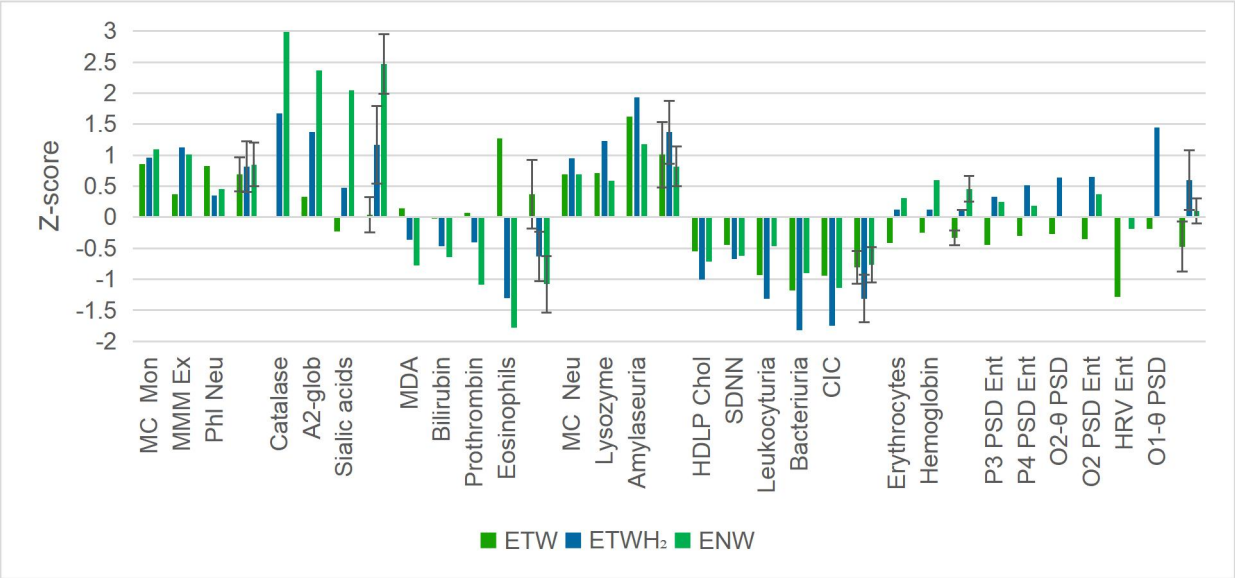


Fig. 1. The patterns of effects of the "Truskavetska" Water **(**ETW**)**, the "Truskavetska" Water plus Hydrogen ($ETWH_2$) and the Naftussya Water (ENW)

Since the content of electrolytes and trace elements in TW, as potential physiologically active factors, is almost the same as that in ordinary drinking water, the effects found in this study can be attributed to its organic substances, in particular agonists of aryl hydrocarbon receptors [8,36,42], which are expressed by neurons, endocrinocytes, immunocytes, and other cells of the body [11,31,41]. The algebraic differences between the effects of NW and TW reflect the essential effects of fatty acids and the autochthonous hydrocarbon-oxidizing bacteria producing these substances, which are absent in the composition of TW. Instead, the essential effects of hydrogen are simulated by calculating the algebraic differences between TW enriched with hydrogen and without it (Table 2 and Fig. 2).

Table 2. The patterns of essential effects of the Aryl hydrocarbons (Ah), the Hydrogen (H_2) and Bacteria&Fatty acids (Bac+FA) complex. See also Table 3

Variables	**Effect of Ah (11)**	**Effect of H_2 (4)**	**Effect of Bac+ FA (3)**
Catalase	0,02	1,65	2,97
Sialic acids	-0,23	0,70	2,28
A_2-globulines	0,33	1,04	2,04
Erythrocytes	-0,42	0,54	0,73
Hemoglobin	-0,25	0,37	0,85
P3 PSD Entropy	-0,48	0,78	0,70
P4 PSD Entropy	-0,30	0,82	0,48
O2-θ PSD	-0,27	0,91	0,27
O2 PSD Entropy	-0,35	1,00	0,72
O1-θ PSD	-0,19	1,64	0,21
HRV Entropy	-1,28	1,28	1,09
Eosinophils	1,27	-2,57	-3,05
PhI of Neutrophils	0,83	-0,48	-0,38
Malondyaldehide	0,14	-0,50	-0,92
Bilirubin	-0,01	-0,46	-0,63
Prothrombin Ind	0,07	-0,47	-1,16
MC of Monocytes	0,86	0,10	0,23

MMM Excretion	0,37	0,76	0,64
MC of Neutrophil	0,69	0,26	0,00
Lysozyme	0,71	0,52	-0,12
Amylaseuria	1,62	0,31	-0,44
SDNN	0,45	-0,67	-0,17
HDLP Cholesterol	-0,55	-0,45	-0,17
Leukocyturia	-0,93	-0,38	0,46
Bacteriuria	-1,18	-0,64	0,28
CIC	-0,94	-0,81	-0,20

It was found that both the Hydrogen (H_2) and Bacteria&Fatty acids (Bac&FA) complex affect most parameters in the same direction, with Bac&FA complex being more effective for some, and H_2 for others. However, the effects of the factors on lysozyme activity, amylaseuria, leukocyturia, and bacteriuria are opposite in favor of Hydrogen (Fig. 2).

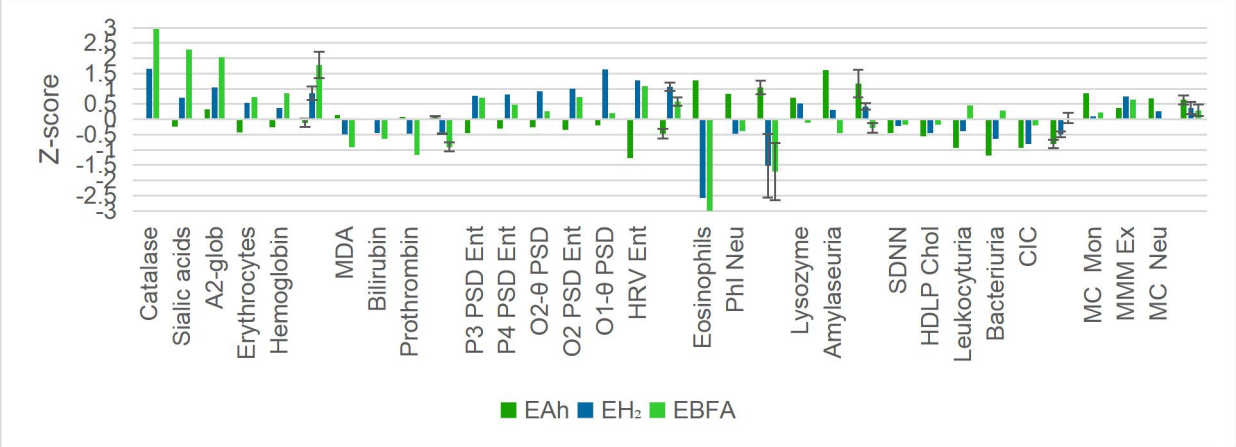


Fig. 2. The patterns of essential effects of the Aryl hydrocarbons (Ah), the Hydrogen (H_2) and Bacteria&Fatty acids (Bac+FA) complex

The use of discriminant analysis allows, firstly, to identify precisely those variables, in the aggregate of which the states of patients before and after different balneotherapy schemes differ significantly; secondly, to visualize each patient in the information space [22].

The forward stepwise program identified 13 discriminant variables (Table 3).

Table 3. Summary of the analysis of discriminant functions.

Step 13, N of vars in model: 13; Wilks' Λ: 0.0234; approx. $F_{(26.4)}=8,9$; $p<10^{-6}$

Variables currently in the model	**Groups (n)**				**Parameters of Wilks' Statistics**					
	After TW (11)	**Base-line (18)**	**After TW+ H_2(4)**	**After NW (3)**	Wil-ks' Λ	Par-tial Λ	F-re-move (2,21)	p-level	Tole-rancy	Norm Cv
Catalase, μM/L•h	116 -0,17	114 -0,19	210 1,48	285 2,80	0,064	0,364	18,38	10^{-4}	0,689	125 0,458
Leukocyturia, lg L/mL	3,10 0,16	3,69 1,09	2,86 -0,22	3,39 0,62	0,030	0,779	2,985	0,072	0,668	3,00 0,210
Phagocytose Index of Neutrophils, %	66,7 -0,83	57,3 -1,66	61,3 -1,31	62,3 -1,21	0,052	0,452	12,72	10^{-3}	0,256	76,1 0,149
HRV PSD Entropy	0,68 -1,36	0,80 -0,08	0,80 -0,08	0,78 -0,27	0,059	0,398	15,90	10^{-4}	0,345	0,806 0,114
Erythrocytes, 10^{12}/L	3,84 -2,50	3,97 -2,08	4,02 -1,96	4,07 -1,77	0,045	0,518	9,757	0,001	0,031	4,50 0,060
Prothrombin Index, %	93,8 -0,35	92,9 -0,42	88,7 -0,82	81,3 -1,51	0,033	0,713	4,222	0,029	0,534	97,5 0,110

Hemoglobin, g/L	127 -2,43	130 -2,18	132 -2,06	135 -1,58	0,035	0,663	5,326	0,013	0,040	146 0,055
Eosinophils, %	4,00 1,43	2,89 0,16	1,75 -1,14	1,33 -1,62	0,031	0,749	3,524	0,048	0,451	2,75 0,318
Malondyaldehide, µM/L	77,5 0,00	73,9 -0,14	64,3 -0,50	53,3 -0,92	0,028	0,824	2,249	0,130	0,639	77,5 0,339
Bilirubin, µM/L	10,3 -0,33	10,4 -0,32	8,4 -0,79	7,7 -0,96	0,034	0,682	4,887	0,018	0,453	11,7 0,355
MMM Excretion, units/24h	2331 3,43	2197 3,06	2603 4,19	2560 4,07	0,031	0,758	3,361	0,054	0,371	1100 0,326
Amylaseuria, mg/sec•24h	108 3,04	79 1,42	114 3,35	100 2,60	0,029	0,806	2,526	0,104	0,572	53 0,341
Microbial Count of Monocytes, B/Phag	8,6 -1,06	6,2 -1,92	8,9 -0,96	9,2 -0,83	0,026	0,904	1,109	0,349	0,479	11,5 0,240

Note: For each variable, the top row shows the average raw level, the bottom row shows the average Z-score.

Other variables were left out of the discriminant model, apparently due to duplication/redundancy of separating information (Table 4).

Table 4. Variables currently not in the model

Variables	**Groups (n)**				**Parameters of Wilks' Statistics**					
	After TW (11)	**Base-line (18)**	**After TW+ H_2(4)**	**After NW (3)**	Wilks' Λ	Par-tial Λ	F to enter	p-level	Tole-rancy	Norm Cv
SDDN HRV, msec	36 -0,69	23 -1,14	17 -1,36	18 -1,31	0,023	0,969	0,318	0,731	0,677	56 0,516
O1-θ PSD, %	7,0 -0,24	8,0 -0,05	14,9 1,40	8,1 -0,03	0,022	0,927	0,793	0,466	0,539	8,2 0,584
Microbial Count of Neutrophils, B/Phag	7,0 -0,53	5,7 -1,22	7,5 -0,27	7,0 -0,53	0,022	0,944	0,589	0,564	0,547	8,0 0,234
O2 PSD Entropy	0,65 -0,90	0,70 -0,55	0,79 0,10	0,75 -0,18	0,023	0,970	0,305	0,740	0,158	0,776 0,178
Bacteriuria, lg CFU/mL	0,88 0,90	2,04 2,08	0,25 0,26	1,16 1,18	0,022	0,946	0,568	0,575	0,331	0 0,98
O2-θ PSD, %	5,1 -0,50	6,2 -0,23	8,7 0,41	6,2 -0,23	0,023	0,996	0,040	0,960	0,244	7,1 0,554
Lysozyme, nM/L	157 -2,27	132 -2,98	175 -1,75	153 -2,39	0,023	0,979	0,215	0,809	0,544	236 0,148
HD LP Cholesterol, mM/L	1,46 0,33	1,68 0,88	1,28 -0,12	1,40 0,16	0,023	0,970	0,304	0,741	0,283	1,35 0,300
P4 PSD Entropy	0,79 -0,13	0,83 0,17	0,89 0,69	0,85 0,35	0,023	0,973	0,278	0,760	0,725	0,810 0,147
Sialic acids, units	0,161 0,88	0,167 1,11	0,170 1,58	0,217 3,16	0,022	0,953	0,496	0,616	0,659	0,140 0,175
A_2-globulines, g/L	8,22 1,23	7,78 0,90	9,58 2,27	10,9 3,27	0,023	0,982	0,186	0,832	0,463	6,6 0,199
P3 PSD Entropy	0,79 -0,11	0,85 0,34	0,89 0,67	0,88 0,59	0,022	0,949	0,533	0,595	0,723	0,802 0,167
CIC, units	125 3,13	146 4,07	106 2,32	120 2,93	0,023	0,976	0,246	0,785	0,758	54 0,417

The identifying information contained in the 13 discriminant variables is condensed into two roots. The major root contains 57.3% of discriminatory opportunities (r^*=0.930; Wilks' Λ=0.0234; $\chi^2_{(26)}$=101; $p<10^{-6}$), while minor root 42.7% (r^*=0.909; Wilks' Λ=0,1734; $\chi^2_{(12)}$=47; $p<10^{-5}$).

Calculating the values of discriminant roots for each patient by coefficients and constants given in Table 5 allows visualization of each patient in the information space of roots (Fig. 3).

Table 5. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Catalase, µM/L•h	0,519	0,914	0,012	0,021
Leukocyturia, lg L/mL	0,231	-0,587	1,385	-3,519
Phagocytose Index of Neutrophils, %	-0,921	1,305	-0,101	0,143
HRV PSD Entropy	1,345	-0,471	12,05	-4,221
Erythrocytes, 10^{12}/L	3,643	-2,260	14,88	-9,231
Prothrombin Index, %	-0,558	-0,569	-0,068	-0,069
Hemoglobin, g/L	-2,417	1,996	-0,233	0,192
Eosinophils, %	0,190	-0,798	0,087	-0,365
Malondyaldehide, µM/L	-0,563	0,043	-0,023	0,002
Bilirubin, µM/L	-0,845	-0,319	-0,246	-0,093
MMM Excretion, units/24h	0,868	-0,049	0,0013	-0,0001
Amylaseuria, mg/sec•24h	-0,439	0,457	-0,011	0,012
Microbial Count of Monocytes, B/Phag	-0,403	-0,267	-0,132	-0,088
		Constants	-24,68	11,55
		Eigenvalues	6,405	4,767
		Cumulative Proportion	0,573	1

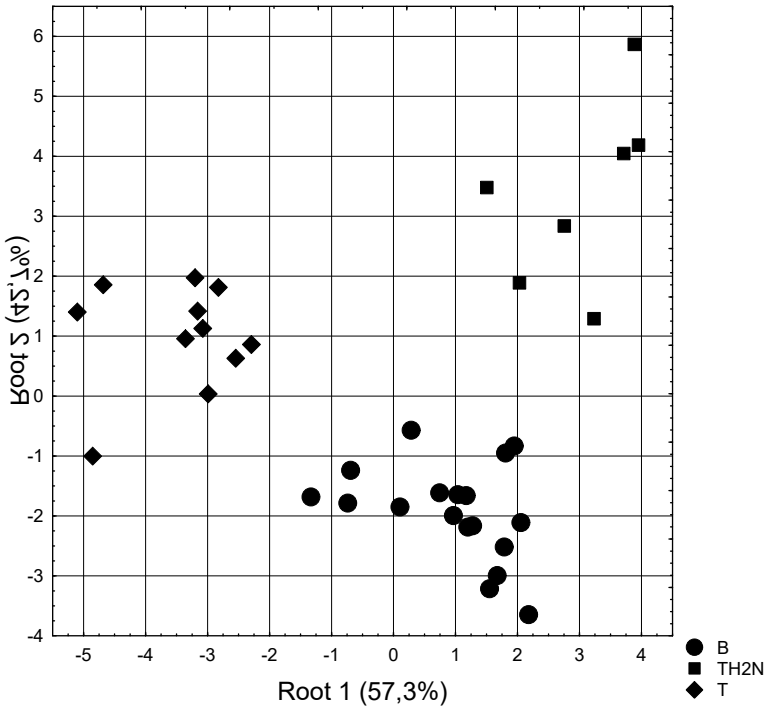


Fig. 3. Scattering of individual values of the first and second discriminant roots of patients before **(circles)** and after the course of drinking of the "Truskavetska" Water **(rhombuses)**, the "Truskavetska" Water plus Hydrogen and Naftussya Water **(squares)**

The shift along the axis of the first root of the patients drinking the "Truskavetska" Water to the left relative to its initial localization reflects both a decrease in the parameters that are positively correlated with the root (Table 6), and an increase in the parameters associated with it inversely. Instead, the opposite shift of the patients who received both the "Truskavetska" Water plus Hydrogen and Naftussya Water reflects its opposite effects on these parameters.

An additional delimitation of clusters occurs along the axis of the second root. The top position of the patients who received both the "Truskavetska" Water plus Hydrogen and Naftussya Water reflects their maximal for sample levels of the parameters that are positively correlated with the root, and minimal levels of the parameters associated with it inversely (as well as on those not included in the model, but presented in Table 6).

The calculation of the root centroids visualizes the contribution of hydrogen in approximating the effects of the "Truskavetska" Water to those of the Naftussya Water (Figs. 4-6).

Table 6. Correlations between variables and roots, centroids of clusters and Z-scores of clusters

Variables	**Correlations Variables-Roots**		**After TW (11)**	**Base-line (18)**	**After TW+ H_2(4)**	**After NW (3)**
Root 1 (57,3 %)	**Root 1**	**Root 2**	**-3,46**	**0,94**	**3,46**	
Catalase	**0,282**	0,436	-0,17	**-0,19**	**1,48**	**2,80**
Sialic acids			**0,88**	**1,11**	**1,58**	**3,16**
Erythrocytes	**0,123**	0,007	**-2,50**	**-2,08**	**-1,96**	**-1,77**
Hemoglobin	**0,078**	0,034	**-2,43**	**-2,18**	**-2,06**	**-1,58**
P3 PSD Entropy			**-0,11**	**0,34**	**0,67**	**0,59**
P4 PSD Entropy			**-0,13**	**0,17**	**0,69**	**0,35**
O2-θ PSD			**-0,50**	**-0,23**	**0,41**	**-0,23**
O2 PSD Entropy			**-0,90**	**-0,55**	**0,10**	**-0,18**
O1-θ PSD			**-0,24**	**-0,05**	**1,40**	**-0,03**
HRV Entropy	**0,182**	-0,084	**-1,36**	**-0,08**	-0,08	-0,27
Eosinophils	**-0,152**	-0,055	**1,43**	**0,16**	**-1,14**	**-1,62**
PhI of Neutrophils	**-0,137**	0,144	**-0,83**	**-1,66**	**-1,31**	**-1,21**
Malondyaldehide	**-0,086**	-0,079	**0,00**	**-0,14**	**-0,50**	**-0,92**
Bilirubin	**-0,064**	-0,101	**-0,33**	**-0,32**	**-0,79**	**-0,96**
Prothrombin Ind	**-0,107**	-0,127	**-0,35**	**-0,42**	**-0,82**	**-1,51**
Root 2 (42,7 %)	**Root 1**	**Root 2**	**1,01**	**-1,93**	**3,37**	
A_2-globulines			**1,23**	**0,90**	**2,27**	**3,27**
MC of Monocytes	-0,049	**0,194**	**-1,06**	**-1,92**	**-0,96**	**-0,83**
MC of Neutrophil			**-0,53**	**-1,22**	**-0,27**	**-0,53**
MMM Excretion	0,025	**0,116**	**3,43**	**3,06**	**4,19**	**4,07**
Lysozyme			**-2,27**	**-2,98**	**-1,75**	**-2,39**
Amylaseuria	-0,061	**0,166**	**3,04**	**1,42**	**3,35**	**2,60**
Leukocyturia	0,091	**-0,269**	**0,16**	**1,09**	**-0,22**	**0,62**
Bacteriuria			**0,90**	**2,08**	**0,26**	**1,18**
CIC			**3,13**	**4,07**	**2,32**	**2,93**
HDLP Cholesterol			**0,33**	**0,88**	**-0,12**	**0,16**
SDNN			**-0,69**	**-1,14**	**-1,36**	**-1,36**

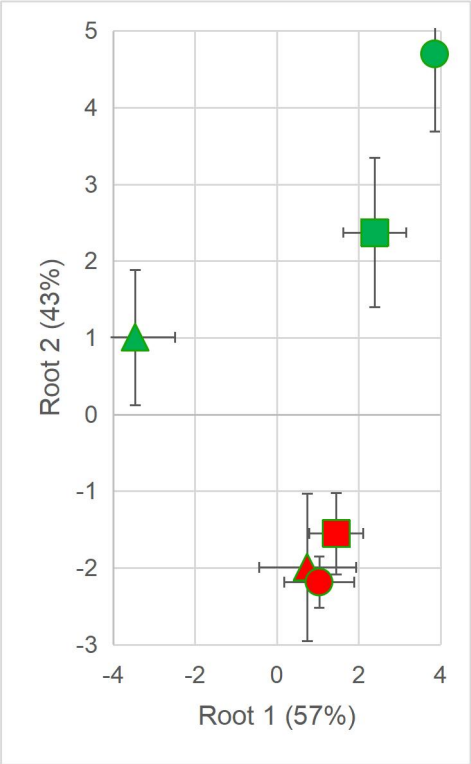


Fig. 4. Scattering of average values (M±SD) of the first and second discriminant roots of patients **before** and after the course of drinking of the "Truskavetska" Water **(rhombuses)**, the "Truskavetska" Water plus Hydrogen **(squares)** and Naftussya Water **(circles)**

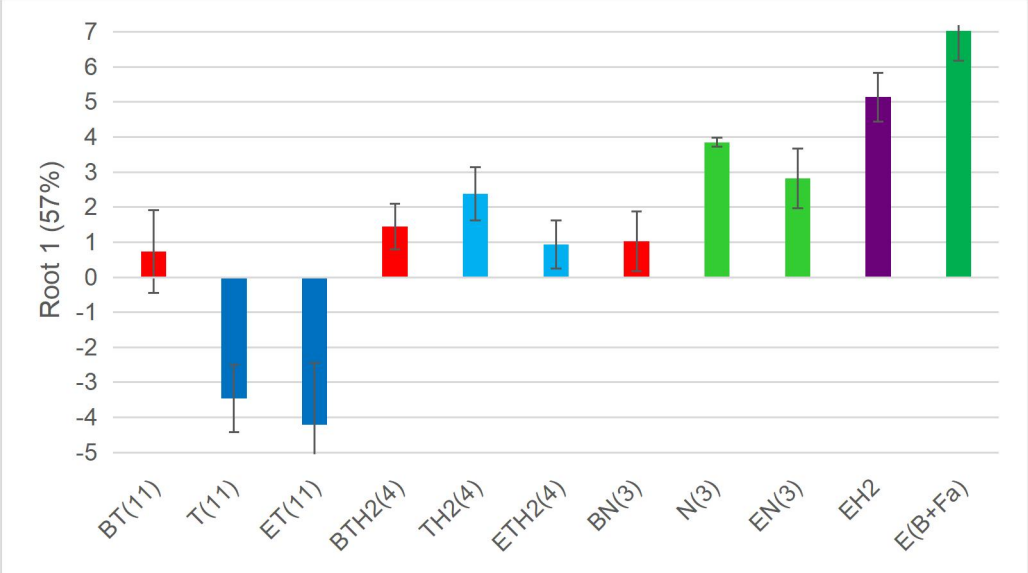


Fig. 5. The average values (M±SD) of the first discriminant root of patients **before** and after the course of drinking of the **"Truskavetska" Water** (T), the **"Truskavetska" Water plus Hydrogen** (TWH_2)**,** and **Naftussya Water** (N) as well as their changes as effects (E) of Waters and essential effects of H_2 and Bacteria&Fatty acids (B+Fa) complex

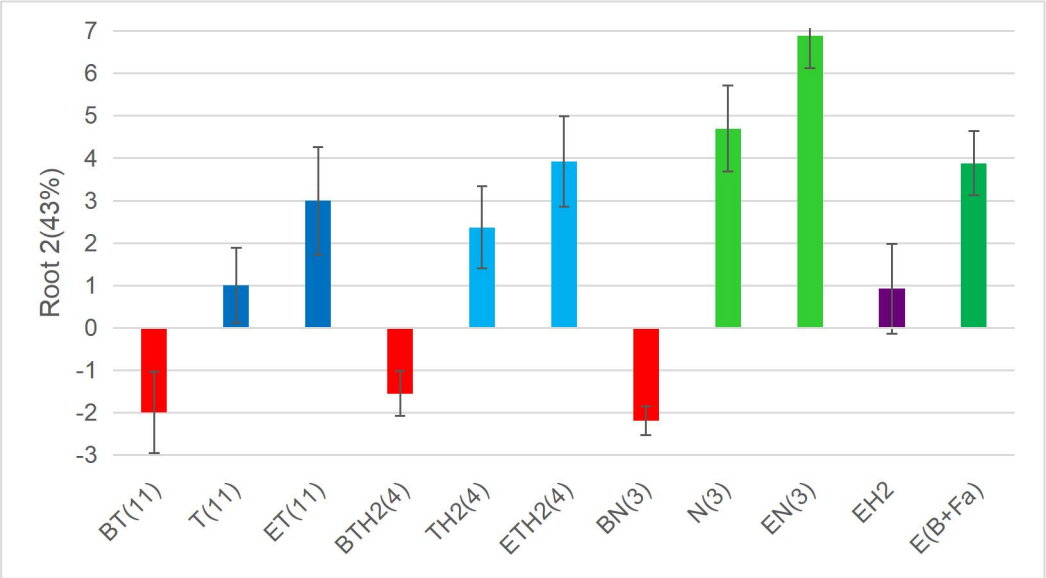


Fig. 6. The average values (M±SD) of the second discriminant root of patients **before** and after the course of drinking of the **"Truskavetska" Water** (T), the **"Truskavetska" Water plus Hydrogen** (TWH_2)**,** and **Naftussya Water** (N) as well as their changes as effects (E) of Waters and essential effects of H_2 and Bacteria&Fatty acids (B+Fa) complex

It seems that the Hydrogen in the "Truskavetska" Water compensates for the effects of Bacteria&Fatty acids complex of Naftussya Water. This is consistent with the data of Uyar B et al. [46], which demonstrated Hydrogen production by the anaerobic bacteria (*R. sphaeroides* O.U. 001 DSM 586) from volatile Fatty acids present in dark fermentation effluents. This fact gives grounds for assuming that Hydrogen production is also carried out by bacteria of Naftussya Water in gut of patients from volatile Fatty acids produced by their microbiome. Interestingly, the opposite process is also known: production of medium chain Fatty acids from H_2 and CO_2 in a hollow-fiber membrane biofilm reactor by mixed microbial culture [49].

Since the most important effect of balneotherapy in this situation is the reduction of bacteriuria and leukocyturia, let us consider in more detail the connections of these markers of pyelonephritis with other parameters. If the revealed negative connections with serum lysozyme and the intensity of phagocytosis of blood macrophages and macrophages are quite expected (Table 7), then the presence of α2-globulines in this set requires interpretation. It is known that this fraction includes α2-macroglobulin, which defends the host organism against attacks by external toxins and other virulence factors during infection and envenomation. In parallel, it participates in several other biological functions by modifying the activity of cytokines and regulating hormones, growth factors, lipid factors and other proteins, which has a great impact on physiology [52].

Table 7. Matrix of correlations between immune factors and pyelonephritis markers

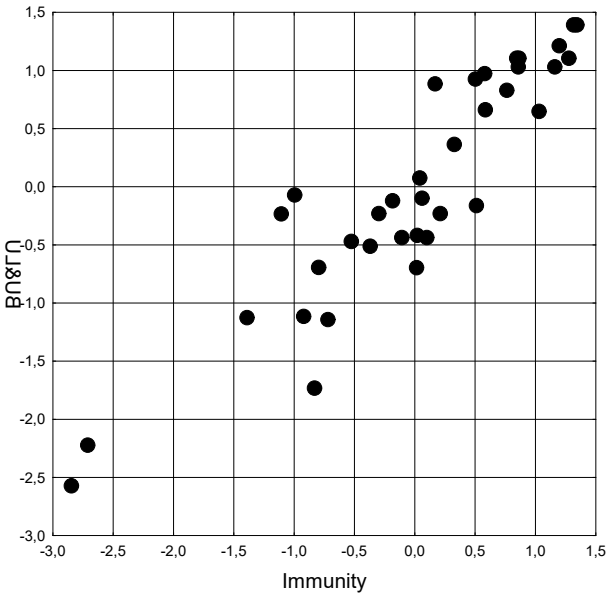
Variable	Leukocyturia, lg Leu/mL	Bacteriuria, lg CFU/mL
Lysozyme, nM/L	-0,859	-0,881
α_2-globulines, g/L	-0,254	-0,327
MC Neutrophils, Bac/Ph	-0,197	-0,251
MC Monocytes, B/Phag	-0,168	-0,217

Taken together, these immune factors determine a reduction in pyelonephritis markers by 84% (Table 8 and Fig. 7).

Table 8. Factor structure of immune and pyelonephritis canonical Roots

Left set	R
Lysozyme, nM/L	**0,980**

α_2-globulines, g/L	**0,338**
MC Neutrophils, Bac/Ph	**0,260**
MC Monocytes, B/Phag	**0,224**
Right set	R
Bacteriuria, lg CFU/mL	**-0,985**
Leukocyturia, lg Leu/mL	**-0,950**



R=0,916; R^2=0,839; $\chi^2_{(8)}$=58; p<10^{-6}; Λ Prime=0,157

Fig. 7. Scatterplot of canonical correlation between the immune parameters (X-line) and Bacteriuria&Leukocyturia levels (Y-line)

In addition, pyelonephritis markers were found to be negatively associated with urinary amylase excretion, serum catalase activity and P4 PSD Entropy, but positively associated with HD LP Cholesterol and Bilirubin levels (Table 8).

Table 8. Matrix of correlations between metabolic-neural factors and pyelonephritis markers

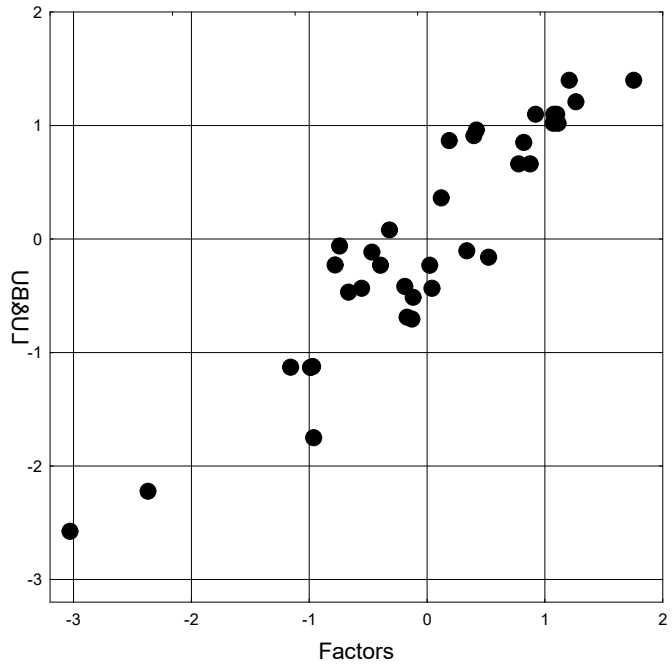
Variable	Leukocyturia, lg Leu/mL	Bacteriuria, lg CFU/mL
Amylaseuria, mg/sec•24h	-0,310	-0,333
Catalase, µM/L•h	-0,154	-0,271
P4 PSD Entropy	-0,247	-0,251
HD LP Cholesterol, mM/L	0,326	0,378
Bilirubin, µM/L	0,269	0,285

This immune-metabolic-neural constellation determines levels of pyelonephritis markers by 84% (Table 9 and Fig. 7).

Table 9. Factor structure of immune-metabolic-neural and pyelonephritis canonical Roots

Left set	R
Lysozyme, nM/L	**0,966**
Amylaseuria, mg/sec•24h	**0,359**
α_2-globulines, g/L	**0,332**
P4 PSD Entropy	**0,276**
MC Neutrophils, Bac/Ph	**0,256**
Catalase, µM/L•h	**0,252**
HD LP Cholesterol, mM/L	**-0,398**
Bilirubin, µM/L	**-0,309**
Right set	R
Bacteriuria, lg CFU/mL	**-0,984**

Leukocyturia, lg Leu/mL **-0,953**



R=0,929; R^2=0,862; $\chi^2_{(16)}$=61; $p<10^{-6}$; Λ Prime=0,128

Fig. 8. Scatterplot of canonical correlation between the immune-metabolic-neural parameters (X-line) and Bacteriuria&Leukocyturia levels (Y-line)

CONCLUSION

Enriching the low-activity "Truskavetska" bottled water with hydrogen significantly increases its physiological/therapeutic activity to a level comparable to those of therapeutic Naftussya water, which is manifested in a favorable effect on phagocytosis, metabolism, erythron, as well as leukocyturia and bacteriuria in patients with chronic pyelonephritis.

The obtained data open up the prospect of amelioration the quality of both "Truskavetska" bottled water and other low-activity waters [21,44].

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are carried out conducted in accordance with positions of Helsinki Declaration 1975 and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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