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Comparative study of the attributive effects of fresh and canned Naftussya water

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Background. There are several patented methods of preservation of medicinal waters of the Naftussya type. The authors of the patents as evidence of the correctness of the methods cite the facts of preservation of the ability of canned water to enhance motility rat's v. portae in vitro and/or increase diuresis. However, such tests are not attributive. The most characteristic manifestation of the biological activity of Naftussya water is the stimulation of phagocytosis. Another "Method of preservation of hydrogen sulfide mineral waters" was recently patented. Based on the above, we will use attributive tests for its verification. **Materials and Methods**. The object of clinical-physiological observation were residents of the city of Truskavets' (21 men aged 24-67 years and 8 women 33-76 years) with chronic pyelonephritis in remission. The subject of the study were the phagocytic function of neutrophils, excretory function of kidneys as well as leukocyturia and bacteriuria levels and components of microbiota of feces. **Results**. Weekly use of fresh Naftussya water causes an increase in the reduced content of probiotics in the microbiota and a decrease in the increased content of conditionally pathogenic microflora, which is accompanied by an increase in reduced bactericidal capacity of neutrophils against both E. coli and Staph. aureus, normal diuresis, phosphaturia, magnesiumuria, ureauria while reduction of bacteriuria and leveling of leukocyturia as well as decrease in concentration of uric acid in urine and creatinine and cholesterol in plasma. The beneficial effect on the listed parameters of canned Naftussya water is less pronounced, but the differences are statistically insignificant. **Conclusion**. The obtained results confirm the effectiveness of the Dovzhenko© method for Naftusya water conservation and provide grounds for continuing research into the effects of canned Naftusya water on the body of urological patients.

Keywords: Naftussya bioactive water, Dovzhenko© conservation method, chronic pyelonephritis, urinary syndrome, microbiota, phagocytosis, diuresis, saluresis.

INRODUCTION

There are several patented methods of preservation of medicinal waters of the Naftussya type. The authors of the patents as evidence of the correctness of the methods cite the facts of preservation of the ability of canned water to enhance motility rat's v. portae in vitro and/or increase diuresis (even without recording urinary excretion of electrolytes and nitrogenous metabolites!?) [14,21]. However, it is well known that even fresh Naftussya water affects such tests ambiguously [10,23,24], so they do not reflect the inherent properties of Naftussya water, that is, they are not attributive. The Truskavetsian Scientific School has shown that the most characteristic manifestation of the biological activity of Naftussya water is the stimulation of phagocytosis in both rats and humans [10,12,13,17,18] caused by its organic substances, in particular, polyphenols [3,9] as Ah receptor ligands [19].

Another "Method of preservation of hydrogen sulfide mineral waters" [15] was recently patented. Based on the above, we will use attributive tests for its verification.

MATERIALS AND METHODS

The object of clinical-physiological observation were residents of the city of Truskavets' (21 men aged 24-67 years and 8 women 33-76 years) with chronic pyelonephritis in remission. The day before, samples of morning urine and feces was collected, in which was determined the leukocyturia and bacteriuria levels and components of microbiota respectively. Unified methods are applied. Urinary syndrome was assessed by quantitative and qualitative levels of bacteriuria and leukocyturia [6,17]. The inclusion criteria were the presence of pronounced urinary syndrome (bacteriuria: 0,285÷0,715 points; leukocyturia: 0,1÷0,5 points) with preservation of functional renal reserve (>10%), previously assessed by the Gozhenko AI method [5].

In daily urine was determined the concentration of electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (flamming photometry); nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method). The same metabolic parameters were determined in plasma as well as total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack). The analysis carried out according to instructions [4] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) with corresponding sets of reagents, and flamming photometer "СФ-47".

Parameters of phagocytic function of neutrophils estimated as described by Kovbasnyuk MM [13,18]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Take into account the following parameters of Phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index PhI), intensity (number of microbes absorbed one phagocytes - Microbial Count MC or Right's Index) and completeness (percentage of dead microbes - Killing Index KI).

On the basis of the registered partial parameters of phagocytosis, taking into account the content of neutrophils (N) in 1 L of blood, the integral parameter - the bactericidal capacity of neutrophils (BCCN) - was calculated by the formula [17]:

$BCCN\ (10^9\ Bact/L) = N\ (10^9/L) \bullet PhI\ (\%) \bullet MC\ (Bact/Phag) \bullet KI\ (\%) \bullet 10^{-4}$.

Every day, 6 patients were examined, who were divided into two groups, approximately equal in terms of gender, age and severity of urinary syndrome.

After the initial testing, the members of the main group received a weekly course of balneotherapy [22] with fresh Naftussya water (taken directly from the field), instead, for the experimental group, Naftussya water, previously preserved by Dovzhenko© method [15], was used. On the second day after the end of the drinking course, repeated testing was carried out.

Normal (reference) values of variables are taken from the database of the Truskavetsian Scientific School of Balneology [19].

For statistical analysis used the software package "Statistica 6.4".

RESULTS AND DISCUSSION

Both groups were equal in terms of gender (4 women each), age (M±SD: 52±15 and 50±10 years), body mass index (26,8±4,2 and 27,8±3,3 kg/m^2) as well as of the initial symptoms of urinary syndrome and components of the microbiota of feces (Table 1), parameters of phagocytosis and metabolism (Table 2).

A fragment of the obtained results was published earlier [16], but in order to preserve the integrity of the picture, we consider it necessary to repeat it.

Screening of changes in the registered parameters revealed a significant decrease in some parameters, instead of an increase in others.

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

$Z = 4•(V – N)/(Max – Min) = (V – N)/SD = (V/N – 1)/Cv$, where

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

Table 1. Comparative characteristics of the microbiota of feces and urine

		Naftussya water used for balneotherapy		Student's Statistics		Reference value	
Bacteria		Fresh (n=15)	Canned (n=14)	t	p	Mean	Cv/ SD
Lactobacilli, lg CFU/g	Before	5,36±0,36***	5,41±0,36***	0,10	>0,5	8,10	0,179
	After	6,85±0,35**	6,37±0,35***	0,98	>0,5		
	Change	+1,49±0,54**	+0,96±0,47*	0,75	>0,5		
Bifidobacteria, lg CFU/g	Before	4,76±0,32***	4,91±0,29***	0,35	>0,5	6,94	0,164
	After	6,02±0,29**	5,64±0,27***	0,96	>0,5		
	Change	+1,26±0,45**	+0,73±0,38	0,90	>0,5		
Escherichia coli common, lg CFU/g	Before	8,18±0,07***	8,15±0,07***	0,31	>0,5	8,66	0,045
	After	8,31±0,07***	8,26±0,07***	0,47	>0,5		
	Change	+0,12±0,09	+0,11±0,10	0,12	>0,5		
Escherichia coli hemolytic, %	Before	29±11**	39±12**	0,56	>0,5	0	25
	After	8±7	14±7*	0,62	>0,5		
	Change	-21±15	-25±15	0,16	>0,5		
Escherichia coli attenuated, %	Before	77±5***	74±5***	0,42	>0,5	17	1,0
	After	50±7***	65±6***	1,63	>0,05		
	Change	-27±8**	-9±7	1,70	>0,05		
Klebsiela& Proteus, %	Before	19±4***	19±6***	0,03	>0,5	0	11
	After	10±3**	11±3**	0,16	>0,5		
	Change	-9±6	-8±6	0,06	>0,5		
Bacteriuria actual, lg CFU/mL	Before	2,20±0,12***	2,01±0,11***	1,14	>0,2	0	0,98
	After	1,10±0,24***	1,22±0,25***	0,35	>0,5		
	Change	-1,10±0,22***	-0,79±0,20***	1,04	>0,5		
Leukocyturia actual, lg L/mL	Before	3,61±0,14***	3,51±0,18***	0,43	>0,5	3,00	0,21
	After	3,14±0,14	3,35±0,13*	1,04	>0,5		
	Change	-0,46±0,20*	-0,16±0,17	1,15	>0,2		

Note. Significant deviations from the norm as well as changes are indicated by stars (p<0,05*, <0,01**, <0,001***).

Table 2. Comparative characteristics of the immune and metabolic parameters

		Naftussya water used for balneotherapy		Student's Statistics		Reference value	
Parameters		Fresh (n=15)	Canned (n=14)	t	p	Mean	SD
Killing Index vs Staph. aur., %	Before After Change	45,1±1,8*** 54,5±2,0* +9,5±2,5***	45,4±1,2*** 50,1±2,3** +4,8±2,1*	0,14 1,44 1,44	>0,5 >0,2 >0,2	58,9	0,142
Killing Index vs E. coli, %	Before After Change	39,3±1,7*** 49,9±2,7*** +10,5±2,9**	40,9±1,8*** 44,5±1,9*** +3,6±2,3	0,63 1,59 1,86	>0,5 >0,2 >0,2	62,0	0,156
Bactericidity vs E. coli, 10^9 B/L	Before After Change	73±3*** 96±6 +24±6***	75±3*** 93±6 +19±4***	0,56 0,38 0,72	>0,5 >0,5 >0,5	99	0,100
Bactericidity vs St. aur., 10^9 B/L	Before After Change	80±5*** 105±5 +26±7***	83±4*** 100±8 +17±8*	0,46 0,57 0,84	>0,5 >0,5 >0,5	106	0,100
Diuresis, L/24 h	Before After Change	1,56±0,17 1,95±0,18** +0,39±0,12**	1,67±0,19 2,08±0,19** +0,40±0,17*	0,45 0,49 0,05	>0,5 >0,5 >0,5	1,40	0,274
Urea Excretion, mM/24 h	Before After Change	464±59 559±63 +96±49	461±49 573±55* +113±55*	0,04 0,17 0,23	>0,5 >0,5 >0,5	458	0,186
Phosphate Excretion, mM/24h	Before After Change	17,9±1,8*** 27,6±4,1 +9,7±3,5**	18,7±3,0* 24,7±3,4 +5,9±3,3	0,23 0,56 0,79	>0,5 >0,5 >0,5	25,2	0,294
Magnesium Excretion, mM/24 h	Before After Change	3,86±0,45 4,77±0,50 +0,91±0,38*	4,16±0,44 4,92±0,61 +0,76±0,59	0,48 0,19 0,22	>0,5 >0,5 >0,5	4,10	0,256
Uric Acid Urine Concen., mM/L	Before After Change	2,26±0,22 1,96±0,16 -0,30±0,24	2,15±0,19 1,66±0,12 -0,49±0,19*	0,36 1,15 0,43	>0,5 >0,5 >0,5	2,14	0,250
Creatinine Plasma, µM/L	Before After Change	88,7±4,3*** 82,2±2,9*** -6,6±3,1*	86,4±4,0*** 83,0±2,3*** -3,4±2,5	0,40 0,22 0,81	>0,5 >0,5 >0,5	77,0	0,167
Cholesterol Plasma, mM/L	Before After Change	5,60±0,28 5,05±0,25 -0,55±0,32	5,40±0,33 5,31±0,33 -0,09±0,32	0,45 0,62 1,02	>0,5 >0,5 >0,5	5,44	0,193

Figs. 1-4 visualizes how a weekly intake of fresh Naftussya water causes an increase in the significantly reduced content in the microbiota of feces/gut of classical probiotics [2,8,20] to the lower normal zone. The beneficial effect is less noticeable in relation to the total content of Escherichia coli. This is accompanied by a significant increase (but without normalization) of the reduced bactericidal activity of neutrophils/microphages against both gram-positive and gram-negative bacteria; at the same time, the bactericidal capacity of blood neutrophils is completely restored, due to an increase in their content. Instead, the marginally increased content of the E. coli strain capable of hemolysis is almost completely normalized; the less elevated content of conditionally pathogenic Klebsiela&Proteus decreases to the upper normal zone; the extremely high content of the E. coli strain with weakened enzymatic activity remains in the pessimistic zone. Obviously, this is a manifestation of antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens [20].

With regard to the cohort of patients with chronic pyelonephritis observed by us, the data that **pyelonephritogenic** Escherichia coli was highly suppressed by Lactobacillus rhamnosus and both Bifidobacteria strains [8] are of particular interest. This gives us reason to assume that inhibition of the growth of **pyelonephritogenic** Escherichia coli in the intestine by probiotics reduces its translocation to the kidneys via lymph and/or blood. In addition,

circulating bacteria are destroyed by neutrophils, whose bactericidal capacity increases significantly. The result is a decrease in bacteriuria, as well as leukocyturia as a marker of pyelonephritis.

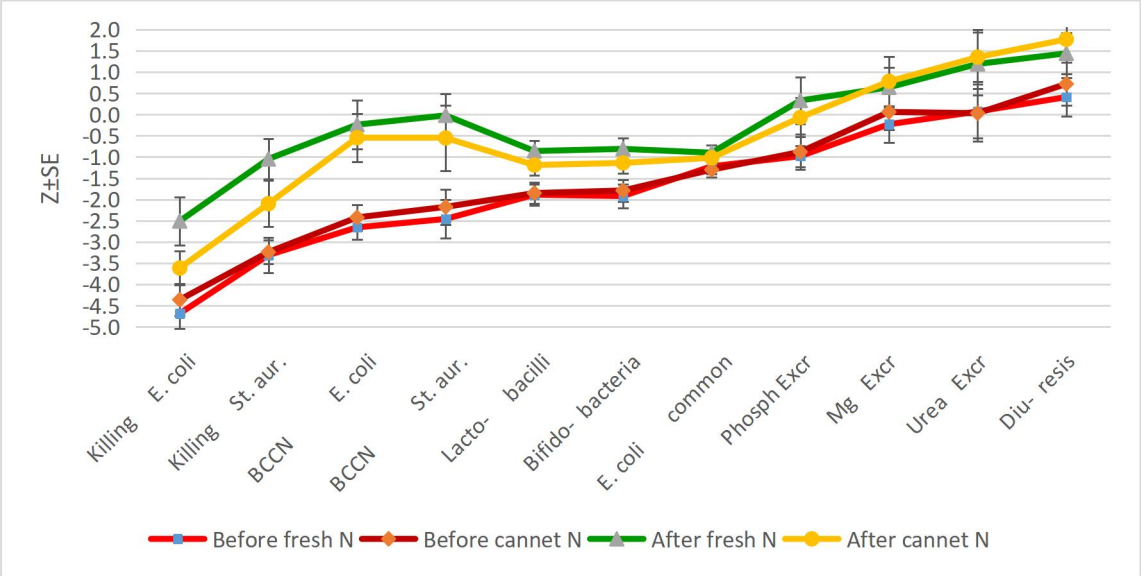

Fig. 1. Profiles of normalized parameters in patients with chronic pyelonephritis which are increasing after a course of balneotherapy with fresh and canned Naftussya water

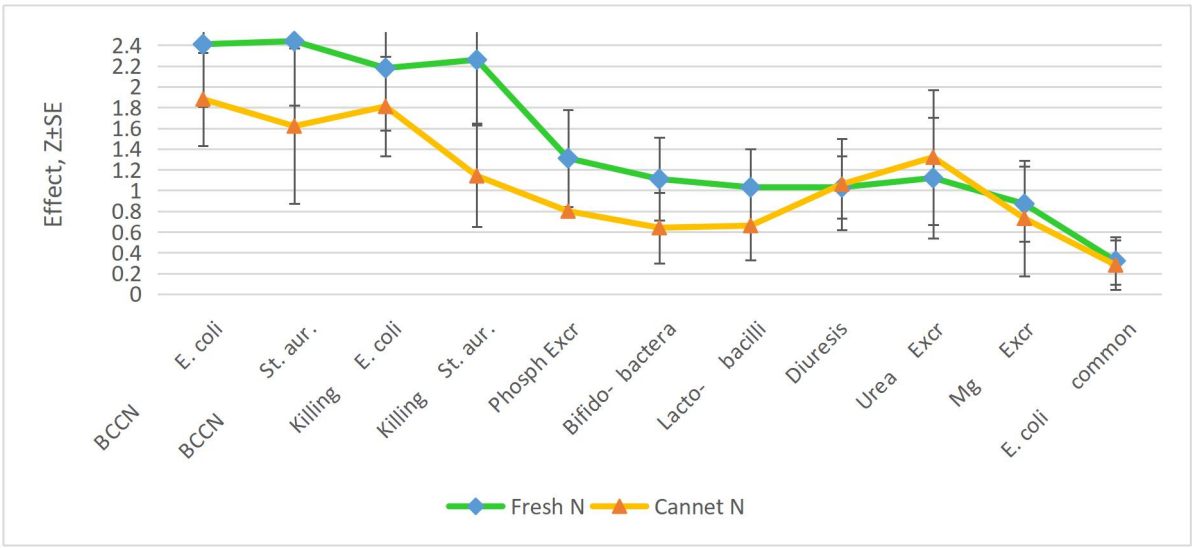

Fig. 2. Profiles of upregulating effects on parameters in patients with chronic pyelonephritis caused by course of balneotherapy with fresh and canned Naftussya water

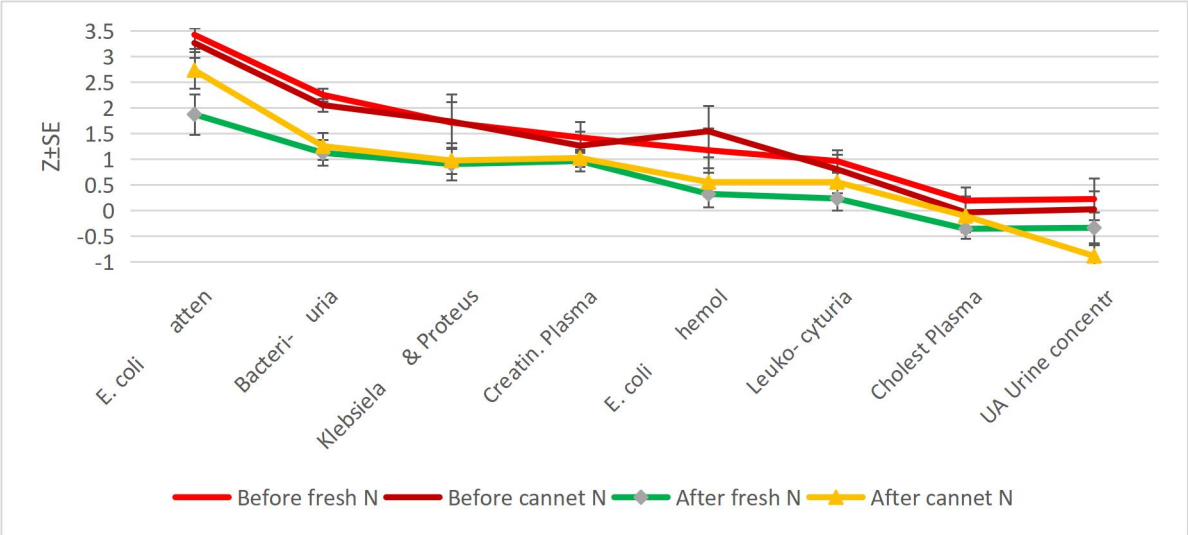

Fig. 3. Profiles of normalized parameters in patients with chronic pyelonephritis which are decreasing after a course of balneotherapy with fresh and canned Naftussya water

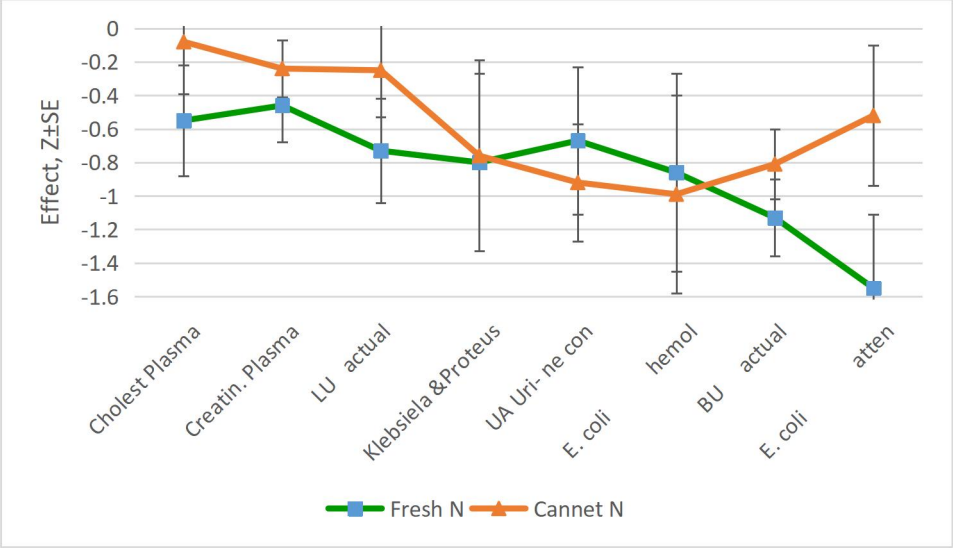

Fig. 4. Profiles of downregulating effects on parameters in patients with chronic pyelonephritis caused by course of balneotherapy with fresh and canned Naftussya water

As expected [7,10,19,23,24], the daily diuresis and excretion of urea, phosphates, and magnesium increases, but not uric acid, the concentrations of which in the urine decrease. At the same time, the elevated plasma level of creatinine as well as normal level of cholesterol decreases, which is clearly interpreted as beneficial effects.

It can be seen that the effect of canned Naftussya water on the 7 parameter are almost the same, and on the 12 parameters are somewhat weaker, but the differences are statistically insignificant.

The obtained results confirm the effectiveness of the Dovzhenko© method [15] used for Naftusya water conservation and provide grounds for continuing research into the effects of canned Naftusya water on the body of urological patients [1].

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We express sincere gratitude to administration “Truskavets’ SPA” and clinical sanatorium “Moldova” for help in conducting this investigation.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, 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.

REFERENCES

1. Androssova SO. Sanatorium treatment of persons with kidney diseases. In: Nephrology. A guide for doctors. Volume 2 (edited by IE Tareeva) [in Russian]. Moskva. Meditsina; 1995: 393-396.
2. Bamigbade GB, Subhash AJ, Kamal-Eldin A, Nyström L, Ayyash M. An Updated Review on Prebiotics: Insights on Potentials of Food Seeds Waste as Source of Potential Prebiotics. Molecules. 2022; 27(18): 5947.
3. Dats'ko OR, Bubnyak AB, Ivassivka SV. The organic part in mineral water Naftussya. Development of knowledges about its composition and origination [in Ukrainian]. Medical Hydrology and Rehabilitation. 2008; 6(1): 168-174.
4. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa: Astroprint; 1998: 608.
5. Gozhenko AI, Kravchuk AV, Nykytenko OP, Moskalenko OM, Sirman VM. Funktsional'nyi nyrkovyi rezerv. Functional renal reserve [in Ukrainian]. Odesa. Feniks; 2015: 182.
6. Gozhenko AI, Kushneruk AV, Zukow W, Popovych IL. Comparative study of electrolyte exchange in patients with varying severity of urinary syndrome of chronic pyelonephritis. Journal of Education, Health and Sport. 2015; 5(1): 305-310.
7. Gozhenko AI, Sydoruk NO, Babelyuk VYe, Dubkowa GI, Flyunt VR, Hubyts'kyi VYo, Zukow W, Barylyak LG, Popovych IL. Modulating effects of bioactive water Naftussya from layers Truskavets' and Pomyarky on some metabolic and biophysic parameters at humans with dysfunction of neuro-endocrine-immune complex. Journal of Education, Health and Sport. 2016; 6(12): 826-842.
8. Hütt P, Shchepetova J, Lõivukene K, Kullisaar T, Mikelsaar M. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J Appl Microbiol. 2006; 100(6) :1324-1332.
9. Ivassivka SV, Bubnyak AB, Kovbasnyuk MM, Popovych IL. Genesis and role of phenols in waters from Naftussya layer [in Ukrainian]. In: Problems of pathology in experiment and clinic. Scientific works of Drohobych Medical Institute. Vol XV. Drohobych. 1994: 6-11.
10. Ivassivka SV, Popovych IL, Aksentiychuk BI, Bilas VR. The Nature of Naftussya Water Balneofactors and the Essence of its Therapeutic and Preventive Action [in Ukrainian]. Truskavets'. Truskavets'kurort; 1999: 125.
11. Kaur AP, Bhardwaj S, Dhanjal DS, et al. Plant Prebiotics and Their Role in the Amelioration of Diseases. Biomolecules. 2021; 11(3): 440.
12. Kul'chyns'kyi AB**,** Gozhenko AI, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite**.** Communication 3. Correlations between parameters EEG, HRV and Immunogram. Journal of Education, Health and Sport. 2017; 7(3): 53-71.
13. Kul'chyns'kyi AB, Kovbasnyuk MM, Korolyshyn TA, Kyjenko VM, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 2. Correlations between parameters EEG, HRV and Phagocytosis. Journal of Education, Health and Sport. 2016; 6(10): 377-401.
14. Moiseev AYu. Features of Chemical Conposition and Balneal Use of Mineral Waters [in Russian]. Kyïv. Publish Hous "KIM"; 2017: 464.
15. Patent UA 148377 U. The method of preservation of hydrogen sulfide mineral waters. Dovzhenko OM. Bull. N30. 28.07.2021.
16. Popovych AI, Ruzhylo SV, Zakalyak NR, Popovych DV, Żukow X. Comparative study of the effects on the microbiota and leukocyturia of fresh and canned Naftussya water. Quality in Sport. 2022; 8(4): 67-72.

17. Popovych IL, Flyunt IS, Alyeksyeyev OI, Hrytsak LYa, Hrinchenko BV, Barylyak LG et al. Sanogenetic Bases of Rehabilitation on Spa Truskavets' Urological Patients from Chornobylian Contingent [in Ukrainian]. Kyïv. Computerpress; 2003: 192.
18. Popovych IL, Kul'chyns'kyi AB**,** Gozhenko AI, Zukow W, Kovbasnyuk MM, Korolyshyn TA**.** Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018; 8(2): 135-156.
19. Popovych IL, Gozhenko AI, Korda MM, Klishch IM, Popovych DV, Zukow W (editors). Mineral Waters, Metabolism, Neuro-Endocrine-Immune Complex. Odesa. Feniks; 2022: 252.
20. Serven AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiology Reviews. 2004; 28: 405-440.
21. Shestopalov VM, Moiseeva NP, Ishchenko AP, Kondratiuk YeI, Usov VYu, Moiseev AYu, et al. "Naftusia" Medicinal Waters of Ukrainian Carpathians and Podolia [in Russian]. Chernivtsi. Bukrek; 2013: 600.
22. Truskavetsian mineral waters and methods of their internal use. Guidelines. Truskavets'; 1998: 39.
23. Yaremenko MS, Ivassivka SV, Popovych IL, Bilas VR, Yassevych HP, Zahorodnyuk VP et al. Physiological Bases of Curative Effect of Water Naftussya [in Russian]. Kyïv. Naukova dumka; 1989: 144.
24. Yessypenko BYe. Physiological action of "Naftussya" mineral water [in Russian]. Kyïv. Naukova dumka; 1981: 216.