Sydoruk Nadiya O, Zukow Walery. Comparative investigation of immediate effects on neuro-endocrine-immune complex of bioactive water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a. Communication 1. Generic effects. Journal of Education, Health and Sport. 2016;6(8):85-101. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.59762
http://ojs.ukw.edu.pl/index.php/johs/article/view/3734
https://pbn.nauka.gov.pl/sedno-webapp/works/741571

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 755 (23.12.2015).
755 Journal of Education, Health and Sport eISSN 2391-8306 7

Received: 02.07.2016. Revised 25.07.2016. Accepted: 28.07.2016.

COMPARATIVE INVESTIGATION OF IMMEDIATE EFFECTS ON NEURO-ENDOCRINE-IMMUNE COMPLEX OF BIOACTIVE WATER NAFTUSSYA FROM LAYERS TRUSKAVETS', POMYARKY AND SKHIDNYTS'A.
Communication 1. GENERIC EFFECTS

Nadiya O Sydoruk[1], Walery Zukow[2]

[1]JSC "Dnipro-Beskyd", Truskavets', Ukraine dnipro-b@bk.ru
[2]Faculty of Physical Education, Health and Tourism, Kazimierz Wielki University, Bydgoszcz, Poland w.zukow@ukw.edu.pl

Abstracts

Background. Ukraine has a number of fields Bioactive Water Type "Naftussya" focused on the Ukrainian Carpaty: Truskavets', Skhidnytsya, Shklo, Mrazhnytsya, Opaka, Mizun', Selyatyn, Guta etc and Podillya: Sataniv, Husyatyn, Makiv etc. Until now conducted comparative studies of their effects only on clinical symptoms as well as excretion of urine and bile. The **aim** of this study – compare immediate effects of Bioactive Water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a on neuro-endocrine-immune complex. **Methods.** The object of observation were 15 volunteers-men without clinical diagnose but with moderate disfunction of neuroendocrine-immune complex (disadaptation). In basal conditions and after drinking Control (distillated, filtered, well) and Bioactive Waters recorded EEG ("NeuroCom Standard") and HRV ("Cardiolab+VSR"). In blood counted up Leukocytogram, on the basis of which determined Popovych's Adaptation Index and Strain Index. In serum of venous blood determined content of principal adaptation Hormones: Cortisol, Testosterone and Triiodothyronine (ELISA) as well as Na^+ and K^+ (flaming photometry) for evaluation of Mineralocorticoide activity. Immune status evaluated on a set of I and II levels recommended by the WHO. **Results.** Observed cohort characterized disadaptation documented high frequentlyties of Disharmonious General Adaptation Reactions (53,7%) as well as Distress (11,1%), decreased Popovych's Adaptation Index, plasma level of Triiodothyronine, Vagal tone, Completeness of Phagocytose, blood level of "Active", Theophilline resistance and $CD4^+$ T-Lymphocytes, $CD16^+$ NK-Lymphocytes as well as IgA while increased Popovych's Strain Index, plasma level of Cortisol, Mineralocorticoide activity, Bayevskiy's Stress and Activity Regulatory Systems Indexes, Sympathetic tone, blood level of $CD8^+$ T-Lymphocytes as well as IgM. By method od discriminant analysis it is detected 38 variables specific to Basal level and after 1,5 h after drink Control Waters (CW) and Bioactive Waters Naftusssya (BAWN). Discriminant variables divided on four clusters. First cluster reflects caused by BAWN reversion of activating trend after CW for 17 variables (changes in Euklidian Units relative to Basal Level makes -0,14±0,03 and +0,45±0,06 respectively) while second cluster reflects reversion of inhibiting trend for 12 variables (changes relative to Basal Level makes +0,38±0,09 and -0,20±0,07 respectively). Information about these 29 variables condensed in major canonical root (62,6% discriminant properties). Means of its makes for Basal Level (n=54) +0,05±0,13 Mahalanobis' Units, after CW (n=15) -3,10±0,31 while after BAWN in total (n=39) +1,12±0,20, separately for layers Truskavets' (n=12) +1,28±0,28, Pomyarky (n=12) +1,13±0,28 and Skhidnyts'a (n=15) +0,99±0,29. Third cluster reflects attenuation of activating trend for 4 variables (changes relative to Basal Level makes +0,47±0,08 and +0,25±0,02 respectively) while fourth cluster reflects reduction of inhibiting trend for 5 variables (changes relative to Basal Level makes -0,31±0,04 and -0,10±0,03 respectively). Information about these 9 variables condensed in minor canonical root (37,4% discriminant properties). Means of its makes for Basal Level +1,03±0,14, for CW -0,94±0,20, for layers Truskavets' -1,03±0,30, Pomyarky -1,28±0,28 and Skhidnyts'a -0,93±0,30. **Conclusion**. Bioactive Water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a causes approximately equal immediate effects on 29 parameters of neuro-endocrine-immune complex different from effects of Control (distillated, filtered, well) Waters.

Keywords: Bioactive Water Naftussya, EEG, HRV, Cortisol, Testosterone, Triiodothyronine, Leukocytogram, Immunity.

INTRODUCTION

Ukraine has a number of fields Bioactive Water Type "Naftussya" focused on the Ukrainian Carpaty: Truskavets', Skhidnytsya, Shklo, Mrazhnytsya, Opaka, Mizun', Selyatyn, Guta etc and Podillya: Sataniv, Husyatyn, Makiv etc. [11]. Just now commissioned drilled in 1986 on Pomyarky tract in the city Truskavets' bore with Water Naftusya. The reason for including of water in this type is the similarity of physical and chemical indicators, qualitative and quantitative composition of oil-like organic compounds and specific autochtone microflora [11-13,19,20,46,47]. Until now conducted comparative studies of their effects only on clinical symptoms as well as excretion of urine and bile [1,2,19,39,40] based on diuretic-choleretic conception of mechanism their curative effects at patients with chronic urological and gastroenterological diseases [9,10,31,37,46,47]. Long ago known immediate effects of Bioactive Water Naftussya spa Truskavets' as well as course effects of balneotherapeutic complex (drink of Naftussya, application of Ozokerite, Mineral Bathes) spa Truskavets' on diurese, urinary excretion of nitrous metabolites and electrolytes, cholerese and cholekinetics, gastric and pancreatic secretion, hemodynamics as well as inflammation in urinary and digestive systems [1,2,4,8-11,16,37,38,43-47] according to contemporary notions [31,41,42] may be the result of modulation of neuroendocrine-immune complex. IL Popovych [31] advanced conception about stresslimiting adaptogene mechanism of biological and curative activity of Water Naftussya that including participation of nervous, endocrine and immune systems closely interacting in the bounds of neuroendocrine-immune complex [5,7,14,16,22-25,29,30]. The purpose of this study, based on this conception, is compare immediate effects of Bioactive Water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a on neuro-endocrine-immune complex at persons with its disfunction.

MATERIAL AND RESEARCH METHODS

The object of observation were 15 volunteers-men (age 26÷60 yrs, M±SD: 44±12 yrs) without clinical diagnose but with moderate disfunction of neuroendocrine-immune complex (disadaptation).

We recorded firstly for 25 sec EEG a hardware-software complex "NeuroCom Standard" (production KhAI Medica, Kharkiv, Ukraine) 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 of the ears. Among the options considered the average EEG amplitude (μV), modal frequency (Hz), frequency deviation (Hz), index (%), coefficient of asymmetry (%), absolute (μV^2/Hz) and relative (%) power spectrum density (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, calculated Laterality Index (LI) for PSD each Rhythm using formula:

LI, % = Σ [200•(Right – Left)/(Right + Left)]/8.

Then we recorded for 7 min electrocardiogram in II lead to assess the parameters of HRV (hardware-software complex "CardioLab+HRV" "KhAI-MEDICA", Kharkiv). For further analysis the following parameters heart rate variability (HRV) were selected. 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 then 50 ms (pNN_{50}) [6,17]; heart rate (HR), moda (Mo), the amplitude of moda (AMo), variational sweep (MxDMn) [3]. Spectral parameters (Frequency Domain Methods): PSD components 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 ultra low-frequency (ULF, range 0,015÷0,003 Hz) [6,17]. Expectant as classical indexes: LF/HF, LFnu=100%•LF/(LF+HF) and Centralization Index (CI=(VLF+LF)/HF), Bayevskiy's Stress Index (BSI=AMo/2•Mo•MxDMn) as well as Bayevskiy's Activity Regulatory Systems Index (BARSI) [3].

In portion of capillary blood counted up Leukocytogram and calculated its Adaptation Index as well as Strain Index by IL Popovych [5,27,31]. About phagocytic function of neutrophils judged by activity (percentage of neutrophils, in which found microbes - Phagocytic Index, PhI), intensity (number of microbes absorbed one phagocyte - Microbial Count, MC) and completeness (percentage of dead microbes - Killing Index, KI) [7,15,18,31] of phagocytose museum culture Staphylococcus aureus (ATCC N 25423 F49) obtained from Laboratory of Hydrogeological Regime-Operational Station spa Truskavets'. Based on these parameters calculated bactericidity of blood neutrophils using formula [31]:

$Bactericidity(10^9 Micr/L)=Leukocytes(10^9/L) \cdot Neutrophils(\%) \cdot PhI(\%) \cdot MC(Micr/Phag) \cdot KI(\%)$

Immune status evaluated on a set of I and II levels recommended by the WHO [18,26]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation [26] and indirect immunofluorescent binding reaction monoclonal antibodies [28] from company "Sorbent" (RF) with visualization under fluorescent microscope. T-cellular immunity assessed by the following parameters: blood levels of a subpopulation of "active", theophilline resistance and sensitive T-lymphocytes and T-lymphocytes phenotype of $CD3^+CD4^+$(helpers/inductors). State of killer link of immunity estimated by the content of $CD3^+CD8^+$-lymphocytes (T-killers) and $CD16^+$-lymphocytes (natural killers). The state of humoral immunity judged by the content of $CD19^+$ B-lymphocytes and concentration in serum of immunoglobulins classes G, A, M (radial immunodiffusion method) and circulating immune complexes (with polyethylene glycol precipitation method), using standardized methods described in manual [26].

In serum of venous blood determined content of principal adaptation Hormones: Cortisol, Testosterone and Triiodothyronine (by the ELISA with the use of analyzer "Tecan", Oesterreich, and corresponding sets of reagents from "Алкор Био", RF) as well as Na and K (by the method of flaming photometry with the use of "СФ-46" ПФМУ 4.2) for evaluation of Mineralocorticoide activity as $(Na/K)^{0,5}$ Ratio [16,31].

After registration Basal Level 5 volunteers consumed some days 30 mL of Control Waters (distillated, filtered, well) and 200 mL of Water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a while 10 volunteers consumed only Water Naftussya from three layers. After 1,5 h all tests was repeated. Results processed using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Observed cohort characterized disadaptation documented high frequentlyties of Disharmonious General Adaptation Reactions and Distress, decreased Popovych's Adaptation

Index, increased Popovych's Strain Index (Table 1), Bayevskiy's Stress and Activity Regulatory Systems Indexes, Mineralocorticoide activity, plasma level of Cortisol while decreased plasma level of Triiodothyronine, increased Index of Sympatho-Vagal Balance (Table 2) resulted increased Sympathetic tone as well as decreased Vagal tone (Table 3).

Disadaptation accompanied decreased Completeness of Phagocytose (Table 4), blood level of "Active", Theophilline resistance and $CD4^+$ T-Lymphocytes, $CD16^+$ NK-Lymphocytes and IgA (Table 5) while increased Activity of Phagocytose (Table 4), blood level of $CD8^+$ T-Lymphocytes and IgM (Table 5). Thus take place immunodisfunction.

Table 1. Comparative characteristics parameters of Leukocytogram and Leukocytary Indexes of General Adaptation Reactions

Variables	Ranges of Variables	Ranges of Frequently, %		
		Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)
Lymphocytes, %	**21÷44**	88,9	86,7	94,9
	<21	11,1	13,3	5,1
	>44	0	0	0
Monocytes, %	**4÷7**	35,2	33,3	43,6
	<4	5,6	0	2,6
	>7	59,2	66,7	53,8
Eosinophiles, %	**1÷6**	94,4	100	97,4
	<1	0	0	0
	>6	5,6	0	2,6
Stub Neutrophiles, %	**1÷6**	81,4	93,3	89,8
	<1	9,3	0	5,1
	>6	9,3	6,7	5,1
Leukocytes, 10^9/L	**4÷8**	81,4	93,3	82,1
	<4	5,6	0	0
	>8	13,0	6,7	17,9
		General Adaptation Reactions Frequently		
	Harmonious, %	35,2	33,3	43,6
	Disharmonious, %	53,7	53,4	53,8
	Distress, %	11,1	13,3	2,6
	Norm	**General Adaptation Reactions Indexes**		
Popovych's Strain Index	0,07±0,01	0,35±0,07 ^{n}	0,19±0,03 ^{nb}	0,27±0,07 ^{n}
Popovych's Adaptation Index	1,46÷1,95	1,03±0,08 ^{n}	1,01±0,18 ^{n}	1,19±0,09 ^{n}

Notice. Significant difference from Norm marked as^{n}, from Basal level as^{b}, from Control Waters as^{c}.

After 1,5 hours of use only 50 mL of waters taken as reference found further reduce low levels of Triiodothyronine and vagal tone markers (Moda, RMSSD, pNN_{50}, HF) in combination with a further increased levels of markers of increased sympathetic tone (Heart Rate, LFnu) as well as Sympatho-Vagal Balance and Bayevskiy's Activity Regulatory Systems Indexes (Tables 2 and 3).

Table 2. Comparative characteristics of principal hormonal and autonomous markers of General Adaptation Reactions

Variables	Normal level (n=30)	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)
Cortisol, nM/L	405±23	542±40 ^{n}	509±61 ^{n}	469±52
$(Na/K)^{0,5}$ as MC Activity, units	5,58±0,04	6,56±0,04 ^{n}	6,59±0,05 ^{n}	6,53±0,03 ^{n}
Triiodothyronine, nM/L	2,20±0,09	1,87±0,03 ^{n}	1,75±0,03 ^{nb}	1,91±0,03 ^{nc}
Testosterone, nM/L	25,2±1,2	26,5±1,1	25,1±2,4	25,2±1,5
Bayevskiy's Stress Index, units	129±2	206±20 ^{n}	265±45 ^{n}	240±24 ^{n}
Bayevskiy's ARS Index, units	0÷3	3,5±0,4 ^{n}	4,6±0,8 ^{n}	4,2±0,4 ^{n}
LF/HF as Sympatho-Vagal Balance	2,8±0,2	4,1±0,4 ^{n}	5,7±0,7 ^{nb}	4,4±0,6 ^{n}

Table 3. Comparative characteristics parameters of Heart Rate Variability

Variables	Normatives (n=54)	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)
Heart Rate, beats/min	69,3±1,0	76,1±1,8 ^{n}	85,4±3,0 ^{nb}	77,1±2,1 ^{nc}
Moda, msec	864±3	790±20 ^{n}	699±26 ^{nb}	778±24 ^{nc}
Amplitude of Moda, %	38±1	51±3 ^{n}	52±3 ^{n}	54±3 ^{n}
Variational Sweep, msec	439±5	198±9 ^{n}	178±16 ^{n}	183±12 ^{n}
SDNN, msec	57±1	41±2 ^{n}	33±4 ^{n}	39±3 ^{n}
RMSSD, msec	32±1	25±2 ^{n}	17±2 ^{nb}	24±2 ^{nc}
pNN_{50}, %	10,6±0,6	6,7±1,7 ^{n}	2,0±0,6 ^{nb}	6,0±2,0 ^{n}
HF, $msec^2$	413±19	329±84	114±20 ^{nb}	313±93 ^{c}
LF, $msec^2$	741±36	722±78	659±136	643±94
VLF, $msec^2$	1495±37	742±91 ^{n}	483±86 ^{nb}	710±172 ^{n}
ULF, $msec^2$	100±10	132±24	126±30	94±33
LFnu, %	64±2	72±2 ^{n}	82±2 ^{nb}	72±3 ^{nc}
Centralization Index, units	5,3±0,4	9,2±0,9 ^{n}	10,9±1,3 ^{n}	9,8±1,5 ^{n}

It seems unlikely that were found significant changes in basal level parameters caused by plain water, so we assume that they are a manifestation of a trend caused by the biorhythms of the autonomic nervous system [32] as well as Triiodothyronine (precisely of Hypothalamo-Pituitary-Thyroide Axis). Further drop in Killing Index (Table 4) due probably to its relationships with LF/HF (r=-0,43) and Bayevskiy's ARS Index (r=-0,39) as well as with Triiodothyronine [5,22].

Table 4. Comparative characteristics parameters of Phagocytose

Variables	Normal level (n=30)	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)
Total Leukocytes, 10^9/L	5,78±0,33	6,22±0,21	6,19±0,31	6,57±0,29
Neutrophiles, %	56,5±1,8	59,2±0,9	58,0±1,7	58,7±1,1
Phagocytose Index, %	80,0±0,5	86,8±0,2 ^{n}	86,4±0,4 ^{n}	86,7±0,3 ^{n}
Microbial Count, Micr./Phag.	14,4±0,8	14,3±0,4	14,3±0,8	14,2±0,5
Killing Index, %	40±2	35±2	26±3 ^{nb}	36±2^{c}
Bactericidity, 10^9 Microbes/L	15,0±0,9	15,4±0,9	11,1±1,3 ^{nb}	16,3±1,2^{c}

Table 5. Comparative characteristics parameters of Immunity

Variables	Normal level (n=30)	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)
Total Lymphocytes, 10^9/L	1,96±0,04	2,26±0,07 ^{n}	2,09±0,09	2,32±0,07 ^{n}
"Active" T-Lymphocytes, %	29,6±0,8	26,1±0,5 ^{n}	25,1±0,9 ^{n}	25,8±0,6 ^{n}
Theophilline resistance T-Lymph., %	33,2±1,2	22,8±0,9 ^{n}	22,1±1,4 ^{n}	23,8±1,0 ^{n}
Theophilline sensitive T-Lymph., %	20,9±0,4	19,0±0,8	18,9±1,4	18,3±1,1 ^{n}
$CD4^{+}CD3^{+}$ T-Lymphocytes, %	29,1±1,0	24,1±0,5 ^{n}	22,8±0,9 ^{n}	23,9±0,6 ^{n}
$CD8^{+}CD3^{+}$ T-Lymphocytes, %	24,8±0,5	27,3±0,4 ^{n}	27,6±0,7 ^{n}	27,2±0,7 ^{n}
$CD16^{+}$ NK-Lymphocytes, %	16,4±0,8	11,5±0,4 ^{n}	12,8±0,9 ^{n}	11,3±0,5 ^{n}
$CD19^{+}$ B-Lymphocytes, %	21,7±0,8	22,0±0,6	21,9±0,7	20,9±0,9
IgM, g/L	1,15±0,05	1,43±0,07 ^{n}	1,32±0,13	1,57±0,08 ^{n}
IgG, g/L	11,5±0,4	13,0±0,9	10,1±1,4	13,0±1,1
IgA, g/L	1,90±0,06	1,31±0,04 ^{n}	1,26±0,07 ^{n}	1,37±0,10 ^{n}
Circulating Immune Complexes, un.	54±5	52±10	40±5	41±6

None of the registered Immunity parameters did not change after the use of a Control Waters and Waters Naftussya (Table 5).

However, the use of Waters Naftussya distracted or decrease trends of Triiodothyronine, Sympatho-Vagal Balance and Bayevskiy's Activity Regulatory Systems Indexes as well as Killing Index and Bactericidity of Blood Neutrophils, due to biorhythms.

So it seems that Waters Naftussya per se are able to activate Triiodothyronine release and Bactericidity of Blood Neutrophils as well as increase vagal and decrease sympathetic tone. This assumption is not entirely consistent with previously obtained data on multivariate nature vegetotropic and thyreotropic effects of Waters Naftussya [16,22,24,25,34,37].

Previously, we found relationships between parameters of HRV and EEG [35,36], so very interesting to follow the influence on the latest drinking Control Waters and Waters Naftussya.

Since the number of registered EEG parameter exceeds 150, it is advisable to limit only those that are different for basal level and after drinking tested waters.

By method of discriminant analysis (forward stepwise [21]) it is detected 38 parameters (variables, including **six already mentioned**) specific to Basal level and after 1,5 h after drink Control Waters (CW) and Bioactive Waters Naftusssya (BAWN) (Table 6).

Table 6. Discriminant Function Analysis Summary

Step 38, N of variables s in model: 38; Grouping: 3 groups

Wilks' Lambda: 0,168; approx. $F_{(76)}$=2,58; $p<10^{-6}$

Variables currently in model	Parameters of Wilks' Statistics					Standardized Coefficients		Raw Coefficients	
	F to enter	p-level	Λ	F-value	p-level	Root 1	Root 2	Root 1	Root 2
PSD F3-α, %	7,2	,001	,879	7,21	,001	-,009	-1,585	-,001	-,095
PSD P4-α, %	4,2	,018	,814	5,64	10^{-3}	1,534	-,284	,079	-,015
CD19 B-Lymphocytes, %	3,3	,039	,764	4,94	10^{-4}	-,496	,223	-,104	,047
PSD P3-δ, μV²/Hz	3,4	,037	,717	4,62	10^{-4}	-1,774	,0004	-,021	$2•10^{-6}$
Frequency of δ-Rhythm, Hz	3,0	,054	,676	4,36	10^{-4}	,808	,064	2,737	,216
PSD T3-θ, μV²/Hz	2,5	,083	,644	4,11	10^{-5}	,128	-,232	,007	-,012
PSD T5-θ, %	2,6	,078	,611	3,95	10^{-5}	-,550	-,335	-,108	-,066
PSD C3-α, %	3,3	,041	,573	3,94	10^{-5}	-1,130	1,452	-,070	,090
PSD F3-θ, %	2,1	,128	,549	3,77	10^{-5}	-,712	,962	-,145	,196
PSD O2-α, μV²/Hz	2,2	,115	,525	3,65	10^{-5}	,853	,174	,0014	,0003
PSD Fp1-α, %	2,2	,118	,502	3,56	10^{-5}	-1,148	,527	-,061	,028
PSD F7-θ, %	2,1	,128	,480	3,47	10^{-5}	,795	-,285	,178	-,064
Bayevskiy's ARS Index	2,0	,140	,460	3,39	10^{-5}	-,105	-1,269	-,037	-,446
LF/HF Ratio HRV	1,8	,165	,443	3,31	10^{-5}	,459	-,631	,138	-,189
Popovych's Strain Index	1,9	,157	,425	3,24	10^{-5}	,525	,592	1,107	1,248
PSD O2-θ, μV²/Hz	1,8	,175	,409	3,17	10^{-5}	-,230	-,625	-,008	-,022
PSD P4-δ, μV²/Hz	1,8	,176	,393	3,11	10^{-5}	1,396	,763	,020	,011
Deviation of β-Rhythm, Hz	2,1	,123	,375	3,10	10^{-6}	,274	-,254	,360	-,333
PSD P4-β, μV²/Hz	1,7	,180	,360	3,05	10^{-6}	,812	-,349	,015	-,006
PSD P3-θ, %	2,4	,099	,341	3,06	10^{-6}	,914	1,007	,208	,229
Killing Ind. of Neutroph., %	1,9	,159	,327	3,03	10^{-6}	,552	,233	,042	,018
PSD T5-θ, μV²/Hz	2,0	,143	,312	3,02	10^{-6}	,625	,218	,032	,011
PSD T6-α, %	1,8	,165	,299	2,99	10^{-6}	,878	1,579	,047	,085
PSD F8-θ, μV²/Hz	2,1	,129	,284	2,99	10^{-6}	,165	,590	,013	,045
PSD T6-α, μV²/Hz	1,4	,247	,275	2,94	10^{-6}	-,817	-,410	-,009	-,004
PSD Fp1-θ, μV²/Hz	1,5	,230	,265	2,90	10^{-6}	-1,069	-,601	-,030	-,017
PSD T4-α, μV²/Hz	1,6	,210	,255	2,87	10^{-6}	-1,036	,892	-,012	,010
PSD O2-θ, %	1,5	,235	,245	2,84	10^{-6}	-,413	-,278	-,124	-,083
PSD Fp1-α, μV²/Hz	1,7	,184	,235	2,83	10^{-6}	,247	1,836	,002	,016
PSD Fp2-θ, %	1,5	,227	,226	2,80	10^{-6}	,132	-,708	,031	-,169
PSD F4-β, %	1,5	,238	,217	2,77	10^{-6}	-,140	,744	-,009	,048
Frequency of θ-Rhythm, Hz	1,8	,176	,207	2,77	10^{-6}	,343	-,223	,278	-,180
PSD C4-α, μV²/Hz	1,3	,289	,200	2,73	10^{-6}	-,406	-2,186	-,003	-,016
PSD O1-θ, μV²/Hz	1,6	,201	,192	2,72	10^{-6}	,439	,782	,014	,024
IgM of Serum, g/L	1,3	,273	,185	2,69	10^{-6}	,302	-,504	,625	-1,043
Deviation of δ-Rhythm, Hz	1,0	,366	,180	2,65	10^{-6}	-,208	,298	-,665	,955
PSD P4-α, μV²/Hz	1,2	,305	,173	2,61	10^{-5}	,138	-1,731	,0005	-,0065
PSD Fp2-θ, μV²/Hz	1,2	,320	,168	2,58	10^{-5}	,750	-,029	,017	-,0006
Chi-Square Tests with Successive Roots Removed							**Constants**	-6,65	-1,76

Eigen-value	Canonical R	Wilks' Λ	χ^2	df	p-level	**Discriminant Properties, %**	62,6	37,4
						Squared Mahalanobis Distances		
1,84	0,805	0,168	154	76	10^{-6}	**B-W: 14,2 (F=2,7; $p<10^{-3}$**		
1,10	0,724	0,476	64	37	,004	**B-N: 5,7 (F=2,2; p=0,003**		
						N-W: 18,3 (F=3,2; $p<10^{-4}$		

Discriminant variables divided on six clusters by followed consolidation to four. Thus the purpose of integrated assessment of all parameters they are transferred in Euklidian units (d) by the formula [31,32]:

d = (Postprandial level/Basal level – 1)/Coefficient of Variation.

First cluster reflects caused by BAWN **reversion** or **reduction** of **expressed** activating trend after CW for 12 variables EEG, concerning PSD of α-Rhythm as well as Frequency of θ-Rhythm (Table 7).

Table 7. Caused by Waters Naftussya reversion or reduction of expressed activating trend

Variables currently in model	**Means and Standard Errors**			**Parameters of Wilks' Statistics**				
	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)	Wilks' Λ	Partial Λ	F-remove (2,7)	p-level	Tolerancy
PSD F3-**α**, % Cv=0,478	36±2 0	49±4 +0,76	30±3 -0,35	,175	,958	1,5	,234	,032
PSD C3-**α**, % Cv=0,469	35±2 0	49±4 +0,85	31±2 -0,24	,179	,935	2,3	,103	,033
PSD Fp1-**α**, % Cv=0,479	38±2 0	49±5 +0,60	30±3 -0,44	,182	,924	2,8	,067	,076
PSD T4-**α**, $\mu V^2/Hz$ Cv=0,937	96±12 0	150±33 +0,60	81±11 -0,17	,175	,957	1,5	,225	,039
PSD C4-**α**, $\mu V^2/Hz$ Cv=0,922	138±17 0	222±49 +0,66	131±19 -0,06	,179	,935	2,4	,102	,025
PSD T6-**α**, % Cv=0,553	34±2 0	39±5 +0,27	28±3 -0,32	,194	,864	5,3	,007	,075
PSD Fp1-**α**, $\mu V^2/Hz$ Cv=1,057	113±16 0	160±36 +0,39	94±17 -0,16	,185	,905	3,6	,033	,053
PSD P4-**α**, $\mu V^2/Hz$ Cv=1,142	220±34 0	339±94 +0,47	203±38 -0,07	,174	,966	1,2	,312	,021
PSD T6-**α**, $\mu V^2/Hz$ Cv=1,006	79±11 0	124±31 +0,57	77±16 -0,03	,177	,945	2,0	,148	,105
PSD P4-**α**, % Cv=0,468	42±3 0	49±5 +0,36	40±3 -0,10	,184	,911	3,3	,043	,057
PSD O2-**α**, $\mu V^2/Hz$ Cv=1,353	392±72 0	640±200 +0,47	386±104 -0,01	,174	,962	1,3	,267	,078
Frequency of **θ**-Rhythm, Hz Cv=0,197	6,4±0,2 0	7,0±0,2 +0,48	6,3±0,2 -0,08	,176	,954	1,6	,203	,448
Change relative to Basal Level (n=12)	0	+0,54 ±0,05	-0,17 ±0,04					
Change relative to Control Waters (n=12)		0	-0,71 ±0,07					

Second cluster reflects caused by BAWN **reduction** of **moderately** activating trend after CW for 3 variables EEG, concerning PSD and Deviation of δ-Rhythm as well as LF/HF Ratio HRV and tends to decrease normal level of B-Lymphocytes (Table 8). This trend is due to a moderate relationships between level of B-Lymphocytes and PSD of α-(r=-0,52÷-0,28) as well as δ-(r=-0,38) Rhythms.

Table 8. Caused by Waters Naftussya reduction of moderately activating trend

Variables currently in model	Means and Standard Errors			Parameters of Wilks' Statistics				
	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)	Wilks' Λ	Partial Λ	F-remove (2,7)	p-level	Tolerancy
PSD P3-δ, $\mu V^2/Hz$ Cv=1,090	81±12 0	114±31 +0,37	72±10 -0,10	,215	,781	9,5	,000	,107
PSD P4-δ, $\mu V^2/Hz$ Cv=1,012	72±10 0	82±18 +0,14	68±9 -0,05	,194	,863	5,4	,007	,087
Deviation of δ-Rhythm, Hz Cv=0,409	0,74±0,04 0	0,77±0,08 +0,10	0,73±0,05 -0,03	,175	,959	1,5	,241	,550
LF/HF Ratio HRV Cv=0,776	4,1±0,4 0	5,7±0,7 +0,50	4,4±0,6 +0,09	,188	,894	4,0	,022	,308
CD19 B-Lymphocytes, % Cv=0,209	22,0±0,6 0	21,9±0,7 -0,02	20,9±0,9 -0,24	,179	,939	2,2	,118	,328
Change relative to Basal Level (n=5)	0	+0,22 ±0,09	-0,07 ±0,05					
Change relative to Control Waters (n=5)		0	-0,28 ±0,07					

Algebraic sum changes parameters after drinking BAWN and CW reflects the effect BAWN (balneoeffect) per se. Integrated balneoeffects in respect parameters of both clusters are shown in Fig.1.

Caused by BAWN integrated changes 17 variables relative to Basal Level makes -0,14±0,03 Euklidian Units, but with view the activating trend (+0,45±0,06 EU) balneoeffect revealed significantly inhibitory (-0,58±0,07 EU).

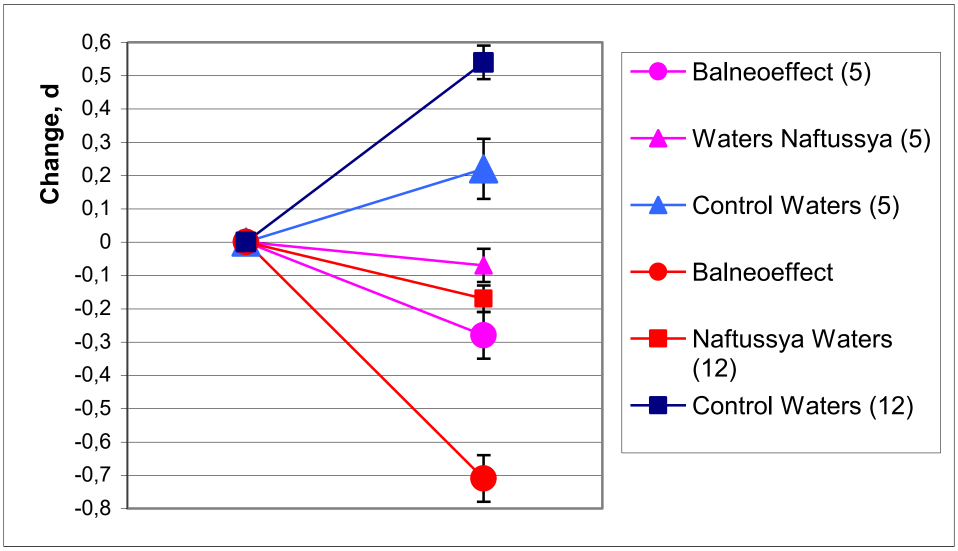

Fig. 1. Reversion or reduction of activating trends after drink Waters Naftussya

Third cluster reflects caused by BAWN **reversion of inhibiting** trend for 4 parameters and **initiation of activation** for 3 parameters of θ-Rhythm (Table 9).

Table 9. Caused by Waters Naftussya reversion of inhibiting trend or initiation of activation parameters of θ-Rhythm

Variables currently in model	Means and Standard Errors			Parameters of Wilks' Statistics				
	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)	Wilks' Λ	Partial Λ	F-remove (2,7)	p-level	Tolerancy
PSD Fp1-θ, $\mu V^2/Hz$ Cv=0,764	22±2 0	18±3 -0,24	36±9 +0,83	,178	,940	2,2	,120	,065
PSD F7-θ, % Cv=0,440	8,6±0,5 0	7,5±0,8 -0,29	9,7±0,9 +0,29	,189	,887	4,3	,017	,249
PSD Fp2-θ, % Cv=0,517	8,3±0,6 0	7,1±0,7 -0,28	8,7±0,7 +0,09	,180	,932	2,5	,090	,250
PSD Fp2-θ, $\mu V^2/Hz$ Cv=0,720	20±2 0	18±3 -0,14	35±11 +1,04	,173	,967	1,2	,320	,090
PSD O2-θ, % Cv=0,561	5,2±0,4 0	5,2±0,8 0,00	6,6±0,6 +0,47	,175	,961	1,4	,256	,260
PSD F8-θ, $\mu V^2/Hz$ Cv=0,740	11,3±1,1 0	11,5±1,7 +0,02	15,2±3,0 +0,47	,177	,948	1,9	,165	,259
PSD T5-θ, $\mu V^2/Hz$ Cv=0,907	20±2 0	24±4 +0,22	25±4 +0,29	,178	,940	2,2	,123	,216
Change relative to Basal Level (n=7)	0	-0,10 ±0,07	+0,50 ±0,13					
Change relative to Control Waters (n=7)		0	+0,60 ±0,15					

Fours cluster reflects caused by BAWN **reversion of inhibiting** trend for parameters of EEG and Immunity (Table 10).

Table 10. Caused by Waters Naftussya reversion of inhibiting trend parameters of EEG and Immunity

Variables currently in model	Means and Standard Errors			Parameters of Wilks' Statistics				
	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)	Wilks' Λ	Partial Λ	F-remove (2,7)	p-level	Tolerancy
Frequency of δ-Rhythm, Hz Cv=0,189	1,11±0,03 0	1,00±0,00 -0,52	1,19±0,07 +0,38	,202	,830	7,0	,002	,400
Deviation of β-Rhythm, Hz Cv=0,569	1,24±0,10 0	1,17±0,22 -0,10	1,44±0,13 +0,28	,177	,948	1,9	,163	,631
PSD P4-β, $\mu V^2/Hz$ Cv=0,676	94±9 0	81±11 -0,20	98±6 +0,06	,189	,887	4,3	,017	,231
Killing Ind. of Neutrophils, % Cv=0,390	35±2 0	26±3 -0,66	36±2 +0,06	,176	,951	1,7	,182	,216
IgM of Serum, g/L Cv=0,334	1,43±0,07 0	1,32±0,13 -0,23	1,57±0,08 +0,29	,175	,958	1,5	,233	,219
Change relative to Basal Level (n=5)	0	-0,34 ±0,11	+0,21 ±0,07					
Change relative to Control Waters (n=5)		0	+0,56 ±0,12					

Changes in Killing Index of Neutrophils are due to a moderate relationships with Popovych's Strain Index (r=-0,49), LF/HF Ratio (r=-0,43) and Bayevskiy's Activity Regulatory Systems Index (r=-0,39). Serum level of IgM has relationships with Bayevskiy's Activity Regulatory

Systems Index (r=0,39), LF/HF Ratio (r=-0,36) as well as PSD of α-(r=-0,62÷-0,46) and θ-(r=0,37÷0,43) Rhythms.

Integrated balneoeffects in respect parameters of both clusters are shown in Fig.2.

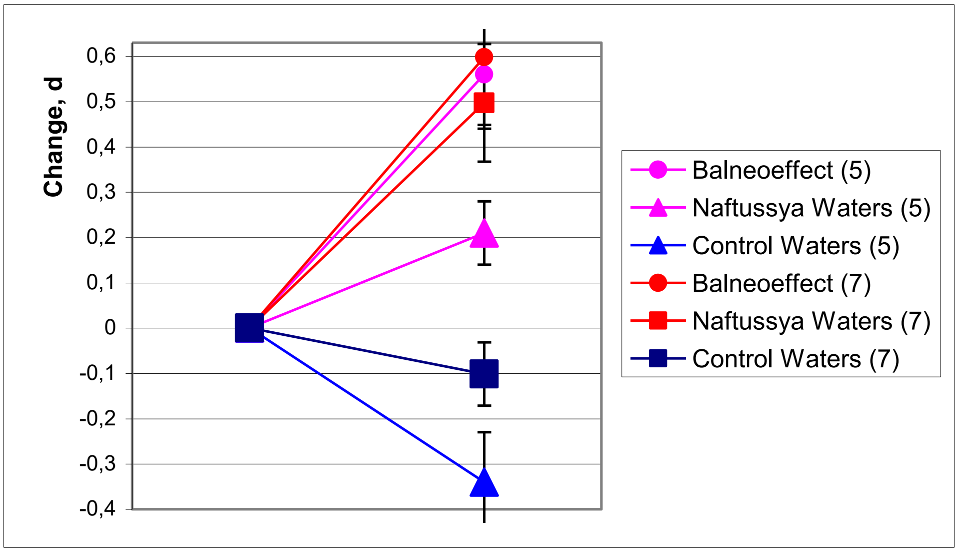

Fig. 2. Reversion of inhibiting trend and initiation of activation after drink Waters Naftussya

Caused by BAWN integrated changes relative to Basal Level makes +0,38±0,09 EU, but with view the inhibiting trend (-0,20±0,07 EU) balneoeffect revealed significantly activating (+0,58±0,10 EU).

Information about these 29 variables condensed in major canonical root (62,6% discriminant properties). The calculation of personal Root 1 values based on Raw Coefficients for discriminant variables and Constant (Table 6) allows to visualize the status of each volunteer before and after drinking various waters (Fig. 3 and 4).

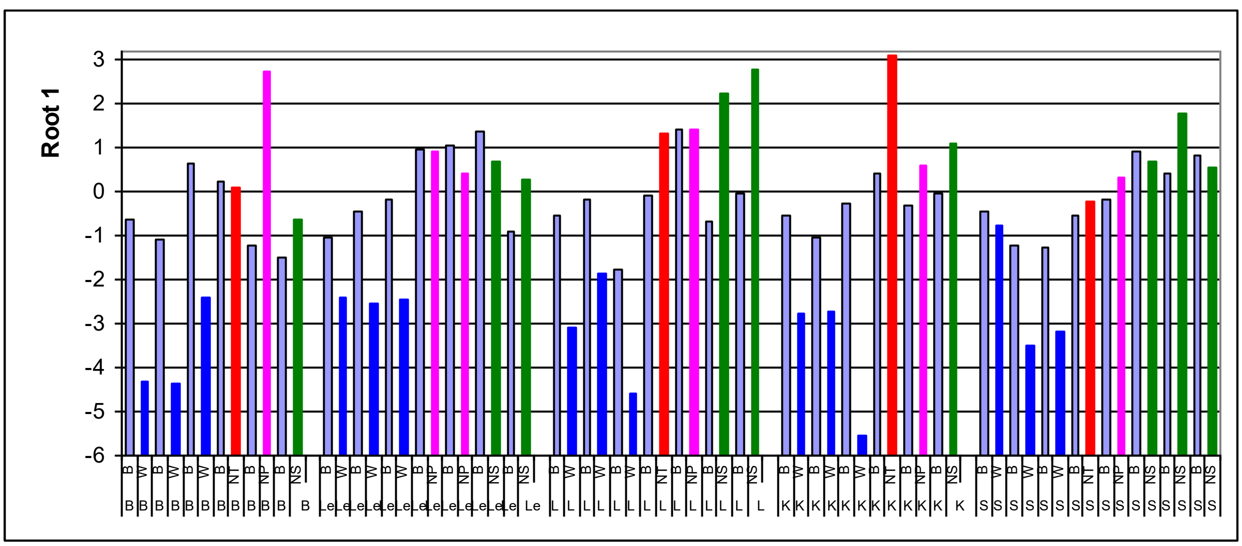

Fig. 3. Personal Root 1 values for 5 volunteers (B, Le, L, K, S) before (B) and after drinking Control Waters (W) as well as Water Naftussya from Truskavets' (NT), Pomyarky (NP) and Skhidnytsya (NS) layers

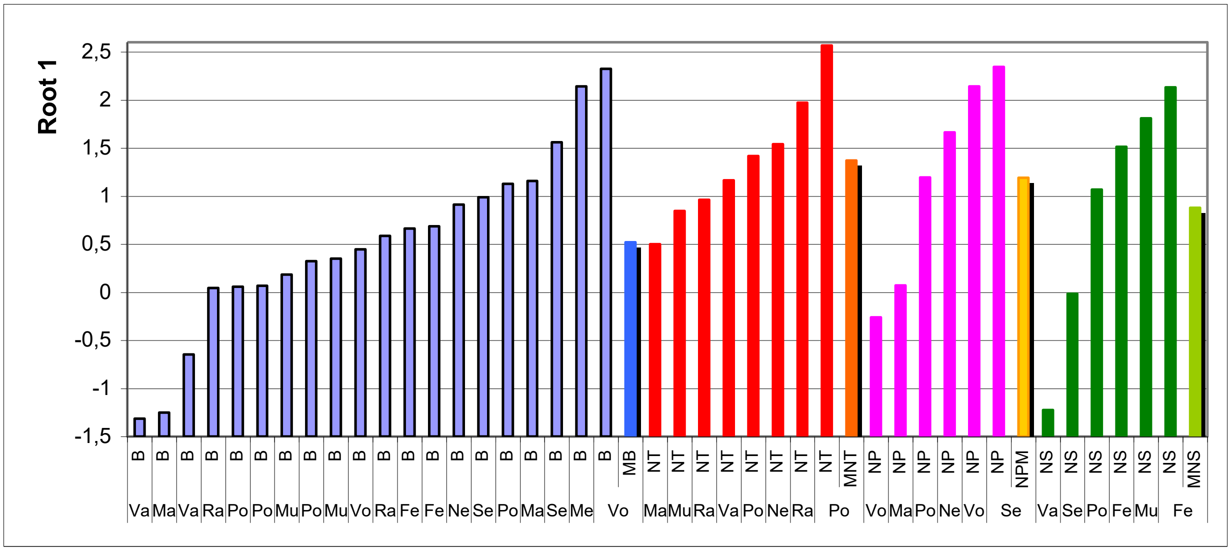

Fig. 4. Personal Root 1 values and means (M) for 10 volunteers before (B) and after drinking Water Naftussya from Truskavets' (NT), Pomyarky (NP) and Skhidnytsya (NS) layers

Means of Root 1 makes for Basal Level (n=54) +0,05±0,13 Mahalanobis' Units, after CW (n=15):-3,10±0,31 while after BAWN in total (n=39) +1,12±0,20, separately for layers Truskavets' (n=12) +1,28±0,28, Pomyarky (n=12) +1,13±0,28 and Skhidnyts'a (n=15) +0,99±0,29.

Other 9 discriminant variables associated with minor canonical root (37,4% discriminant properties). We divided them into two clusters. The first of these reflects **attenuation (weakening) of activating** trend for PSD of θ-Rhythm as well as Bayevskiy's Activity Regulatory Systems Index (Table 11) while second cluster reflects **reduction of inhibiting** trend for PSD of θ-Rhythm in other loci as well as Popovych's Strain Index (Table 12).

Table 11. Caused by Waters Naftussya attenuation of activating trend

Variables currently in model	Means and Standard Errors			Parameters of Wilks' Statistics				
	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)	Wilks' Λ	Partial Λ	F-remove (2,7)	p-level	Tolerancy
PSD O2-**θ**, $\mu V^2/Hz$ Cv=0,961	25±3 0	42±11 +0,71	30±4 +0,21	,172	,976	0,8	,438	,100
PSD T5-**θ**, % Cv=0,559	7,8±0,6 0	9,7±1,5 +0,44	9,1±0,9 +0,30	,180	,934	2,4	,099	,258
PSD O1-**θ**, $\mu V^2/Hz$ Cv=1,112	26±4 0	36±8 +0,35	33±6 +0,24	,177	,947	1,9	,157	,119
Bayevskiy's ARS Index Cv=0,831	3,5±0,4 0	4,6±0,8 +0,38	4,2±0,4 +0,24	,205	,820	7,5	,001	,212
Change relative to Basal Level (n=4)	0	+0,47 ±0,08	+0,25 ±0,02					
Change relative to Control Waters (n=4)		0	-0,22± 0,09					

Table 12. Caused by Waters Naftussya reduction of inhibiting trend

Variables currently in model	Means and Standard Errors			Parameters of Wilks' Statistics				
	Basal level (n=54)	After Control Waters (n=15)	After BAW Naftussya (n=39)	Wilks' Λ	Partial Λ	F-remove (2,7)	p-level	Tolerancy
PSD T3-θ, $\mu V^2/Hz$ Cv=0,893	25±3 0	19±3 -0,26	25±3 -0,01	,169	,993	0,2	,790	,178
PSD F3-θ, % Cv=0,458	11,6±0,7 0	10,2±1,1 -0,26	10,7±0,7 -0,17	,186	,901	3,7	,029	,122
PSD P3-θ, % Cv=0,524	9,2±0,7 0	7,9±1,0 -0,27	8,6±0,6 -0,12	,202	,829	7,0	,002	,159
PSD F4-β, % Cv=0,543	30±2 0	23±3 -0,45	29±2 -0,06	,181	,928	2,6	,079	,238
Strain Index of Leukocytogram Cv=1,564	0,35±0,07 0	0,19±0,03 -0,29	0,27±0,07 -0,15	,189	,887	4,3	,017	,313
Change relative to Basal Level (n=5)	0	-0,31 ±0,04	-0,10 ±0,03					
Change relative to Control Waters (n=5)		0	+0,20 ±0,05					

Integrated balneoeffects in respect parameters of both clusters are shown in Fig. 5.

Caused by BAWN integrated changes 4 variables relative to Basal Level makes +0,25±0,02 EU, but with view the activating trend (+0,47±0,06 EU) balneoeffect revealed moderate inhibitory (-0,22±0,09 EU). For the other 5 parameters BAWN has practically no influence (change: -0,10±0,03 EU), but the account of the inhibitory trend (-0,31±0,04 EU) effect of its turns activating (+0,20±0,05 EU).

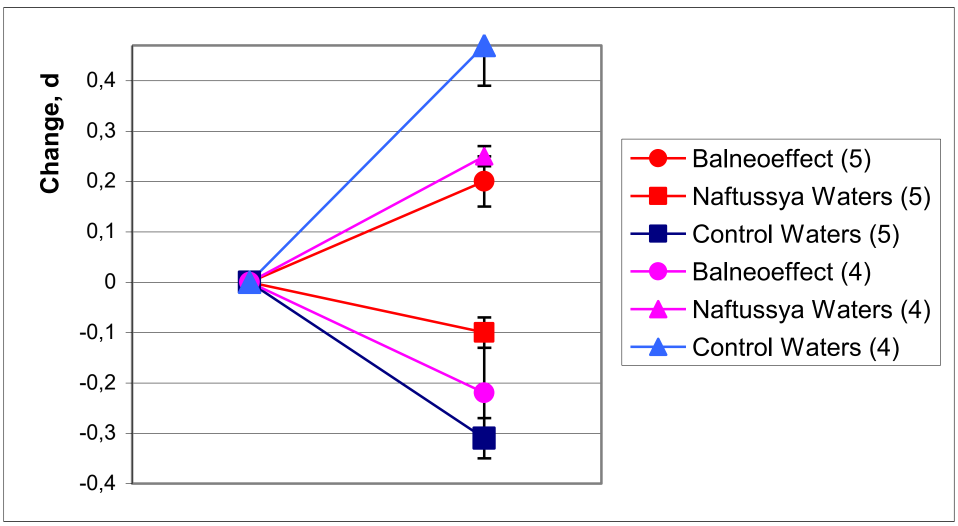

Fig. 5. Attenuation of activating and reduction of inhibiting trends after drink Waters Naftussya

Thus, BAWN causes substantial (-0,58±0,07) and moderately (-0,22±0,09) inhibiting effects on 17 and 4 variables respectively as well as substantial (+0,58±0,10) and moderately (+0,20±0,05) activating effects on 12 and 5 variables respectively (Fig. 6).

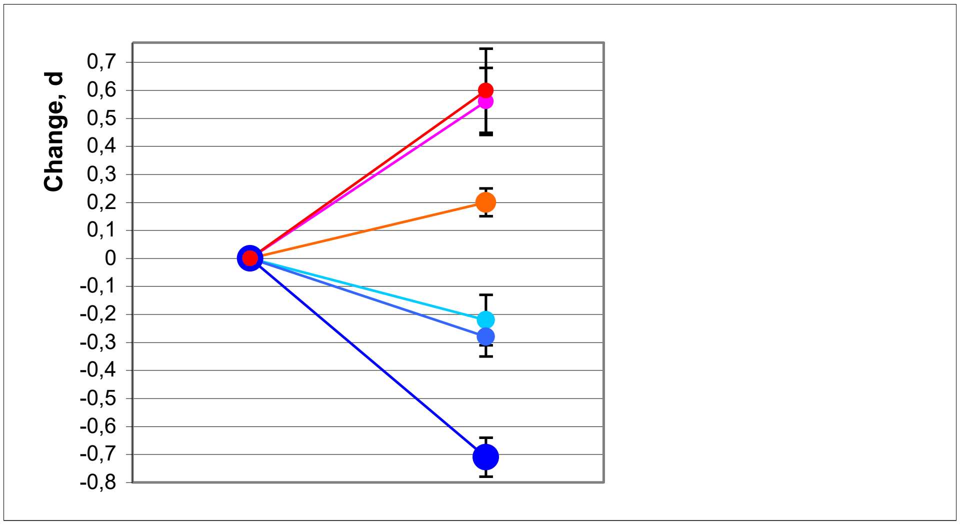

Fig. 6. Variantes of inhibiting and activating changes after drink Waters Naftussya

Means of minor Root 2 which contains information about these 9 variables makes for Basal Level +1,03±0,14, for CW -0,94±0,20, for layers Truskavets' -1,03±0,30, Pomyarky: -1,28±0,28 and Skhidnyts'a -0,93±0,30, that influence them both various Control Waters and Waters Naftussya from various layers were not significantly different (Fig.7 and 8).

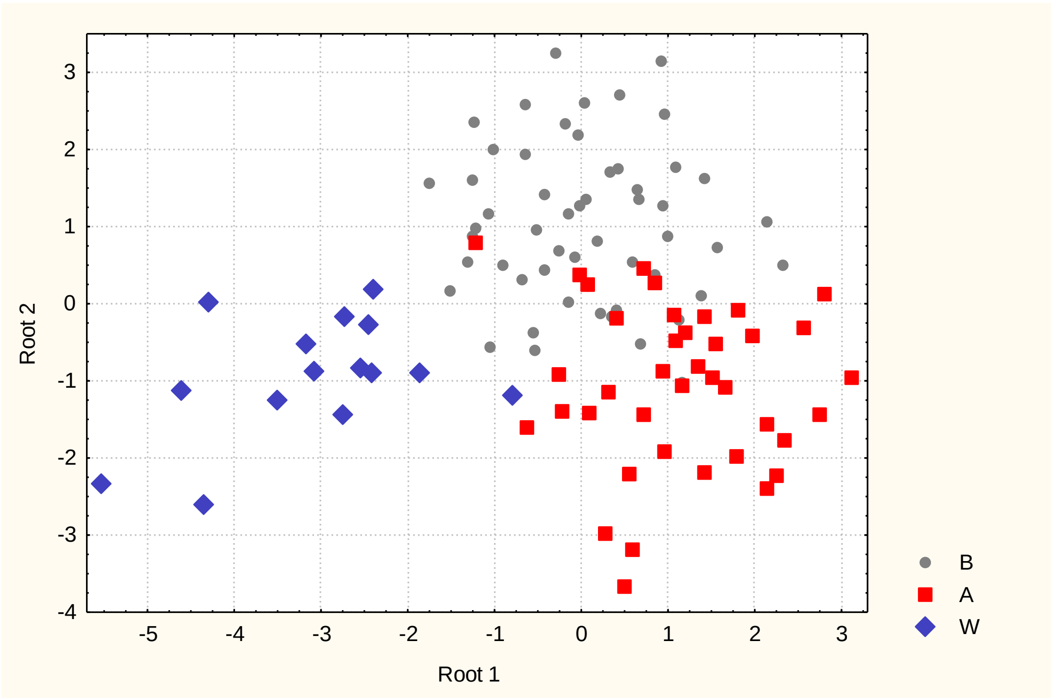

Fig. 7. Personal values for Root 1 and Root 2 before (B) and after drinking Control Waters (W) as well as Waters Naftussya (A) from various layers

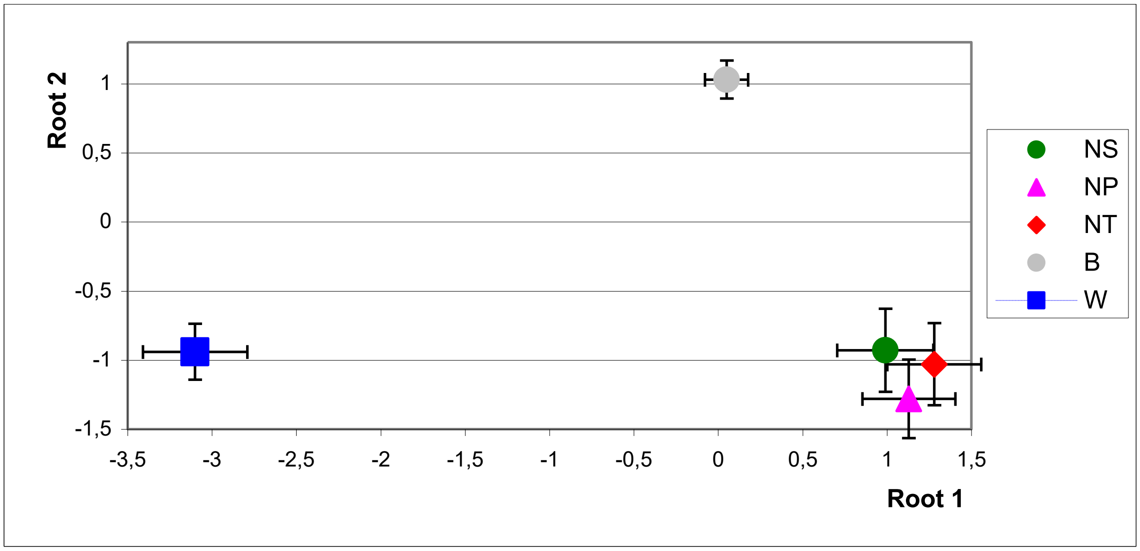

Fig. 8. Means for Root 1 and Root 2 before (B) and after drinking Control Waters (W) as well as Waters Naftussya from Truskavets' (NT), Pomyarky (NP) and Skhidnytsya (NS) layers

Conclusion. Bioactive Water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a causes approximately equal immediate effects on 29 parameters of neuro-endocrine-immune complex different from effects of Control (distillated, filtered, well) Waters.

References

1. Alyeksyeyev OI, , Orlov OB, Matroshylin OG, Fil' VM. Pearl of Prykarpattya [in Ukrainian]. Drohobych: Posvit. 2013. 216 p.
2. Alyeksyeyev OI, Shymonko IT, Orlov OB. Treatment and Rehabilitation on Spa Truskavets' and Skhidnytsya. [in Russian]. Kyiv: Zdorovya. 1994. 176 p.
3. Bayevskiy RM, Ivanov GG. Heart Rate Variability: theoretical aspects and possibilities of clinical application [in Russian]. Ultrazvukovaya i funktsionalnaya diagnostika. 2001; 3: 106-127.
4. Balanovs'kyi VP, Popovych IL, Karpynets' SV. About ambivalence-equilibratory character of influence of curative water Naftussya on organism of human [in Ukrainian]. Dopovidi ANU. Mat., pryr., tekhn. Nauky. 1993; 3: 154-158.
5. Barylyak LG., Malyuchkova RV, Tolstanov OB, Tymochko OB, Hryvnak RF, Uhryn MR. Comparative estimation of informativeness of leucocytary index of adaptation by Garkavi and by Popovych. Medical Hydrology and Rehabilitation. 2013; 11(1): 5-20.
6. Berntson GG, Bigger JT jr, Eckberg DL, Grossman P, Kaufman PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, Van der Molen MW. Heart Rate Variability: Origines, methods, and interpretive caveats. Psychophysiology. 1997: 34; 623-648.
7. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(1): 68-102.
8. Chebanenko OI, Chebanenko LO, Popovych IL. Variety Balneoeffects of Factors Spa Truskavets' and their Forecast [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2012. 496 p.
9. Chebanenko OI, Flyunt IS, Popovych IL, Balanovs'kyi VP, Lakhin PV. Water Naftussya and Hydro-Mineral Exchange [in Ukrainian]. Kyiv: Naukova dumka. 1997. 141 p.
10. Chebanenko OI, Popovych IL, Bul'ba AYa, Ruzhylo SV, Perchenko VP. Cholekinetic Action of Water Naftussya [in Ukrainian]. Kyiv: Computerpress. 1997. 103 p.
11. Curative Mineral Waters Type "Naftussya" / Ed. Shestopalov VM [in Russian]. Kyiv: Naukova dumka. 1986. 192 p.

12. 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.
13. Dats'ko OR, Bubnyak AB, Polyuzhyn IP. Oxidation-reduction potential of mineral waters from Truskavets' and Skhidnytsya deposits [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(4): 72-79.
14. Dranovskyi AL, Babelyuk VYe, Popovych AI,. Krayevyi VO, Flyunt VR. Immediate effects of bioactive water Naftussya on plasma level of testosterone at healthy men and concomitant changes of some physiologycal parameters [in Ukrainian]. Health of Man. 2013; 4(47): 161-163.
15. Douglas SD, Quie PG. Investigation of Phagocytes in Disease. Churchil. 1981. 110 p.
16. Gumega MD, Levyts'kyi AB, Popovych IL. Balneogastroenterology [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2011. 243 p.
17. Heart Rate Variability. Standards of Measurement, Physiological Interpretation, and Clinical Use. Task Force of ESC and NASPE. Circulation. 1996; 93(5): 1043-1065.
18. Immunological Methods / Ed. Frimel G [Transl. from German in Russian]. Moskwa: Meditsina. 1987. 472 p.
19. Ivassivka SV. Biological Active Substances of Water Naftussya, their Genesis and Mechanisms of Physiological Effects [in Ukrainian]. Kyiv: Naukova dumka. 1997. 110 p.
20. 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.
21. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskwa: Finansy i Statistika. 1989: 78-138.
22. Kozyavkina NV, Gozhenko AI, Barylyak LG., Korolyshyn TA, Popovych IL. Variety immediate thyrotropic effects of bioactive water Naftussya, their neuroendocrine-immune accompaniment and possibility of forecast [in Ukrainian]. Medical Hydrology and Rehabilitation. 2013; 11(4): 27-54.
23. Kozyavkina O.V., Barylyak L.G. Ambivalent vegetotropic effects of bioactive water Naftussya and opportunity of their forecasting at rats[in Ukrainian]. Medical Hydrology and Rehabilitation. 2008; 6(3): 123-127.
24. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG., Popovych IL. Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2015. 349 p.
25. Kozyavkina OV, Popovych IL, Zukow W. Immediate vegetotropic effects of bioactive water Naftussya and their neuro-endocrine-immune accompaniment in healthy men. Journal of Health Sciences. 2013; 3(5): 391-408.
26. Lapovets' LYe, Lutsyk BD. Handbook of Laboratory Immunology [in Ukrainian]. L'viv. 2002. 173 p.
27. Petsyukh SV, Petsyukh MS, Kovbasnyuk MM, Barylyak LG, Zukow W. Relationships between Popovych's Adaptation Index and parameters of ongoiging HRV and EEG in patients with chronic pyelonephrite and cholecystite in remission. Journal of Education, Health and Sport. 2016; 6(2): 99-110.
28. Pinchuk VG, Gluzman DF. Immunocytochemistry and Monoclonal Antibodies in Oncohematology [in Russian]. Kyiv: Naukova dumka. 1990. 230 p.
29. Popovych IL. Functional relationships between parameters of neuro-endocrine-immune complex at male rats [in Ukrainian]. Achivements of Clinical and Experimental Medicine. 2008; 2(9): 80-87.
30. Popovych IL. Influence drinking in course of bioactive water Naftussya on vegetative regulation iin rats in basal and poststressor periods [in Ukrainian]. Medical Hydrology and Rehabilitation. 2008; 6(2): 79-83.
31. Popovych IL. Stresslimiting Adaptogene Mechanism of Biological and Curative Activity of Water Naftussya [in Ukrainian]. Kyiv: Computerpress. 2011. 300 p.
32. Popovych IL, Babelyuk VYe, Gozhenko AI, Zukow W, Zubow PG, Dubkova GI, Korolyshyn TA, Nesterova LF, Barylyak LG. Holy water changes EEG and HRV because of autosuggestion and his information (God Grace, World Mind, Cosmic Energy etc). Journal of Education, Health and Sport. 2015; 5(1): 203-220.
33. Popovych IL, Ivassivka SV. Role of organic substances of water Naftussya in its physiological activity [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(2): 6-26.
34. Popovych IL, Kozyavkina OV. Term vegetotropic effects bioactive water Naftussya and their neuro-endocrine-immune accompaniment in apparently healthy men [in Ukrainian]. Medical Hydrology and Rehabilitation. 2012; 10(3): 32-65.
35. Popovych IL, Kozyavkina OV, Kozyavkina NV, Korolyshyn TA, Lukovych YuS, Barylyak LG. Correlation between Indices of the Heart Rate Variability and Parameters of Ongoing EEG in Patients Suffering from Chronic Renal Pathology. Neurophysiology. 2014; 46(2): 139-148.
36. Popovych IL, Lukovych YuS, Korolyshyn TA, Barylyak LG, Kovalska LB, Zukow W. Relationship between the parameters heart rate variability and background EEG activity in healthy men. Journal of Health Sciences.

2013; 3(4): 217-240.
37. Popovych IL, Ruzhylo SV, Ivassivka SV, Aksentiychuk BI (editors). Balneocardioangiology [in Ukrainian]. Kyiv: Computerpress. 2005. 229 p.
38. Popovych IL, Vis'tak HI, Gumega MD, Ruzhylo SV. Vegetotropic Effects of Bioactive Water Naftussya and their Endocrine-Immune, Metabolic and Hemodynamic Accompaniments [in Ukrainian]. Kyiv: UNESCO-SOCIO. 2014. 163 p.
39. Shymonko IT. Truskavets' and Skhidnytsya. Spas of Karpaty Foothills [in Ukrainian]. Drohobych: Surma. 2009. 260 p.
40. Spa Truskavets' /Ed. Shymonko IT. 3-th ed. [in Russian]. Kyiv: Zdorovya. 1987. 144 p.
41. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific response to pathogens. Nat Rev Immunol. 2006; 6(4): 318-328.
42. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.
43. Vis'tak H.I., Kozyavkina O.V., Popovych I.L., Zukow W. Vegetotropic effects of bioactive water Naftussya spa Truskavets' and their thyroide, metabolic and haemodynamic accompaniments at the women. Journal of Health Sciences. 2013; 3(10): 557-582.
44. Vis'tak HI, Popovych IL. Vegetotropic effects of bioactive water Naftussya and their endocrine and immune support in female rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011; 9(2): 39-57.
45. Vovchyna YuV, Lukovych YuR, Matiyishyn GYo, Hryvnak RF, Tymochko OO, Andrusiv OV, Burkovs'ka MM. Hemodynamic variants term effects of bioactive water Naftussya on blood pressure, their electrolyte accompaniment and the ability to forecast [in Ukrainian]. Medical Hydrology and Rehabilitation. 2013; 11(3): 50-59.
46. Yaremenko MS, Ivassivka SV, Popovych IL, Bilas VR et al. Physiological Bases of Curative Effect of Water Naftussya [in Russian]. Kyiv: Naukova dumka. 1989. 144 p.
47. Yessypenko BYe. Physiological Bases Action of Mineral Water "Naftussya" [in Russian]. Kyiv: Naukova dumka. 1981. 216 p.