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FEATURES OF THE IMMUNOTROPIC EFFECTS OF PARTIAL COMPONENTS OF THE BALNEOTHERAPEUTIC COMPLEX OF SPA TRUSKAVETS'

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Abstract

Background. The arsenal of therapeutic factors of the spa Truskavets' is not limited to bioactive water Naftussya, but also includes ozokerite applications and mineral baths. The purpose of this study is to quantify the partial immunotropic effects of these balneofactors and also the organic substances and microbiota of Naftussya water. **Material and methods.** The object of observation were 41 men and 10 women aged 24-70 years old, who came to the spa Truskavets' for the treatment of chronic pyelonephritis combined with cholecystitis in remission. Immune status evaluated on a set of I and II levels recommended by the WHO. The survey was conducted twice, before and after 7-10-days balneotherapy. 23 patients drinked bioactive water Naftussya (BAWN) by 3,2 mL/kg for 1 hour before meals three times a day, while 7 others volunteers drinked Ozokerite extract by 2 mL dissolved in sweet water (2,5-2,6 mL/kg) one time daily. 8 patients in the third group received BAWN and baths with mineral water (Cl^--SO_4^{2-}-Na^+-Mg^{2+} containing salt concentration 25 g/L) and for the other 13 patients in the balneotherapeutic complex included additionally application of Ozokerite on the lumbar region. **Results.** The most pronounced as stimulating (blood level of total, active and cytolytic T-lymphocytes, Circulating Immune Complexes, IgA and Microbial Count for Staph. aureus) and suppressor (blood level of helper T-lymphocytes and Neutrophils, theirs Killing Index and Bactericydity vs both Staph. aureus and E. coli) action are the organic substances of Ozokerite that contact the surface of the skin, whereas their contact with the mucous of the digestive tract causes less pronounced immunotropic effect. Naftussya water has a stronger effect than the water solution of Ozokerite, apparently due to the additional effects of microbes and organic matter produced by them. In contrast, the bath factors affect the immune parameters of the opposite influences. Microbiota has the most pronounced enhancing effects on the Phagocytose Index of Neutrophils vs. Staph. aureus and blood level of Natural Killers as well as Entropy of Leukocytogram. Instead, organic substances of Ozokerite have the same tangible but opposite effects on these parameters.

Keywords: bioactive water Naftussya, water solution of Ozokerite, application of Ozokerite, baths with mineral water, Immunity.

INTRODUCTION

The arsenal of therapeutic factors of the spa Truskavets' is not limited to bioactive water Naftussya, but also includes ozokerite applications, mineral baths, as well as so-called waters "Mariya" and "Sofiya", which are prepared by diluting brine to mineralization of approximately 5 g/L and 10 g/L respectively [16,31,39].

According to modern ideas, the essence of the healing effect of balneotherapy is the modulation of the neuro-endocrine-immune complex, responsible for the state of adaptive-protective mechanisms [16,17,28-31,41]. In this article, the emphasis will be on the immune system, while the neuro-endocrine system will be the subject of the following article, already prepared for publication.

The lion's share of immunological studies relates to the balneotherapeutic complex [16,19,20,22,29,31,34,35,39]. Separate immunotropic effects of Naftussya water use [2,7,11,13,17,30,33,37,42] and Ozokerite applications [8,9,25,26] are investigated in rat experiments. In the case of mineral baths, data are not available.

Since the listed factors are used on spa Truskavets' as part of the balneotherapeutic complex, information about their partial immunotropic action in humans is limited to Naftussya water, which for certain categories of patients was used in the form of monotherapy [30,37,40,41]. Regarding the partial immunotropic effects of applications of Ozokerite and mineral bulk in the conditions of the resort, direct results can not be obtained because they are not the subject of monotherapy. Therefore, earlier, on the example of endocrine parameters, we proposed our approach for indirect evaluation of the effects of individual components of the balneotherapeutic complex [39].

At present, it is believed that the biological activity of Naftussya water is due to the presence of organic compounds of aquifers related to Ozokerite and Oil (in Greek: Naphta), as well as a specific autochthonous Microflora present in its composition [2,7,13,30,33,43]. The most numerous colony among them is the hydrocarbon-oxidizing microorganisms (from 500 to 60 cells per mL of water), intermediate thionic acid microbes (40-10 cells/mL), and the least sulfate-reducing (7-3 cells/mL) [12]. The Microbiota, on the one hand, transforms a part of organic matter, and on the other hand, interacts with the immune cells of Gut-Associated Lymphoid Tissue (GALT) [14].

We adduce data by OR Dats'ko et al [3] about organic compounds (in mg/L) water Naftussya obtained by Solid Phase Extraction method and mass-spectroscopy [23] by using as Sorbents Tenacle GC 60/80 and Polysorb-2. Paraffins 4,10 and 4,20; monoolefins 1,67 and 1,75; dienes and monocycloolefins 0,84 and 0,85; alkylbenzene 1,55 and 1,54; alkenylbenzene 0,47 and 0,46; esters of aromatic acids 1,32 and 1,33; alkyl phenols 1,14 and 1,14; polyaromatic hydrocarbons 0,077 and 0,059; oxygene-containing connections (acids) 1,12 and 1,14; sulfur-containing connections 0,30 and 0,31; alkylnaphthalenes 0,53 and 0,53; unidentified polyaromatic hydrocarbons 0,19 and 0,19; connections required subsquent identification 0,48 and 0,50 correspondingly. 2/3 are Oil products (alkanes, monoolefita, dienes and monocycloolefins, alkylbenzene, alkenylbenzene, polyaromatic hydrocarbons, alkylnaphthalenes, sulfur-containing connections), a 1/3 are products of processing of oil products Microbas (carboxylic acids, sulfur-containing connections, esters of aromatic acids, alkyl phenols).

Early have been shown that detected in Naftussya phenols (0,5-4,1 μg/L) comed from falled leaves [10].

In laboratory of the scientific company "Verba" using unique nanotechnology stable water solution of the Ozokerite, extracted from the Boryslav's field has been received. It was determined that gross constant of organic carbon is 10800 mg/dm³ or 32 000 mg/100g. The constant of organic nitrogen is 2% from C org. The following classes of organic substances are identified: Oxygen-containing combinations (acids, ketones, aldehydes, ethers); High molecular alcohols; Aromatic and nitrogen-containing connections; Connections with unsaturated bonds. The maintenance of the listed organic substances in a concentrate makes 240 mg/L, and paraffin is 800 mg/L, Carboxylic acids 20 mg/dm³ (formic, oil, acetic, kapron, valerian, palmitic, stearin). Previously, we found that oral administration of this solution simulates a number of immunotropic effects of water Naftussya [27,32].

The purpose of this study is to quantify the partial immunotropic effects of these balneofactors and also the organic substances and microbiota of Naftussya water.

MATERIAL AND METHODS

The object of observation were 41 men and 10 women aged 24-70 years old, who came to the spa Truskavets' for the treatment of chronic pyelonephritis combined with cholecystitis in remission.

In portion of capillary blood we counted up Leukocytogram and calculated its Entropy by CE Shannon, Adaptation Index as well as Strain Index by IL Popovych [1,16,24].

Immune status evaluated on a set of I and II levels recommended by the WHO. The methods are described in the manual [21]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD16 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Immunoglobulins classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method).

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [4] with moderately modification by MM Kovbasnyuk [11,18]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacterias and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacterias. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". Take into account the following parameters of phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index), intensity (number of microbes absorbed one phagocytes - Microbial Count or Right's Index) and completeness (percentage of dead microbes - Killing Index). Most interesting is the integrated evaluation of phagocytic function of neutrophils by the number of microbes that are able to neutralize neutrophils contained in 1 liter of blood, named as Bactericidity Capacity (BCC) and calculated by formula [16,31,34]:

$$BCC\ (10^9 Bac/L) = Leukocytes\ (10^9/L) \bullet Neutrophils\ (\%) \bullet PhI\ (\%) \bullet MA\ (Bac/Phag) \bullet KI\ (\%)/10^6$$

The survey was conducted twice, before and after 7-10-days balneotherapy.

After first testing 23 patients drinked bioactive water Naftussya (BAWN) by 3,2 mL/kg for 1 hour before meals three times a day, while 7 others volunteers drinked Ozokerite extract by 2 mL dissolved in sweet water (2,5-2,6 mL/kg) one time daily. The calculation demonstrate that daily entering of organic substances (as C organic) with BAWN makes 0,3 mg/kg (bolus of BAWN 3,2 mL/kg•3 time•0,03 mg/mL). The entering equal amount organic substances with Ozokerite extract achieved by usage 2 mL of it (contained 21,6 mg). By the way, in rats experiment infused in stomach aqueous solution of organic substances extracted from BAWN in dose 0,4 mg/kg equally to obtained by drink ad libitum natural BAWN in dose 3,5 mL/kg [30].

8 patients in the third group received BAWN and baths with mineral water (Cl^--SO_4^{2-}-Na^+-Mg^{2+} containing salt concentration 25 g/L, temperature 36-37^{0}C, duration 8-10 minutes, every other day, 5 procedures), and for the other 13 patients in the balneotherapeutic complex included additionally application of Ozokerite on the lumbar region (temperature 45^{0}C, exposure 30 minutes, every other day, 5 procedures).

Results processed using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

In order to evaluate the basal immune status on a single scale according recommendation by IL Popovych [30] immune variables (V) expressed as Z-scores calculated by formula:

Z=(V/N–1)/Cv, where

N is Mean of Normal Variable,

Cv is Coefficient its variation.

If we take a narrow norm of the range: -0,6Z÷+0,6Z, then we can state that the observed contingent of patients is characterized, above all, by a significantly reduced Popovych's Leukocytary Index of Adaptation as an integral marker of the neuroendocrine-immune complex dysfunction (dysadaptosis) [1,6,24]. Immune Dysfunction as an attribute of Dysadaptosis manifests itself in suppressing the parameters of cellular immunity (Fig. 1) in conjunction with the activation of humoral immunity (Fig. 2). It is known that such a state of immunity is characteristic of chronic stress [14], but rather distress [16]. In particular, 40 (78,4%) of our patients revealed dysharmonic general adaptation reactions (rated 0,5; 0,74 and 0,98 points) as a markers of premorbidity; another 6 (11,8%) had a superactivation reaction (0,26 points) as a distress marker, and only 5 (9,8%) had a harmonic general adaptation reactions (rated 1,70 and 1,95 points).

At the same time, a number of other parameters of immunity in these patients are within the normal range (Fig. 3).

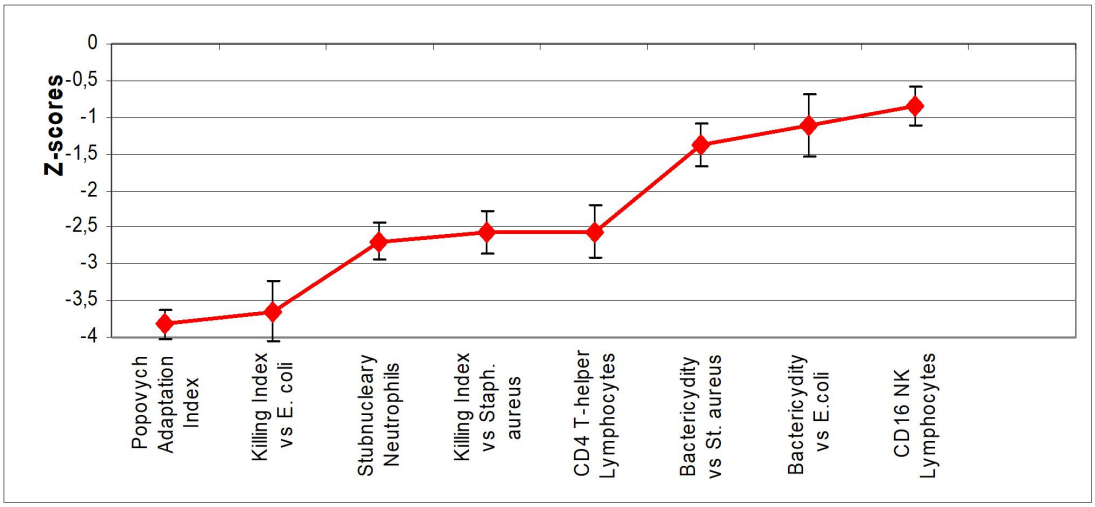


Fig. 1. Ranked profile of initially suppressed immune parameters

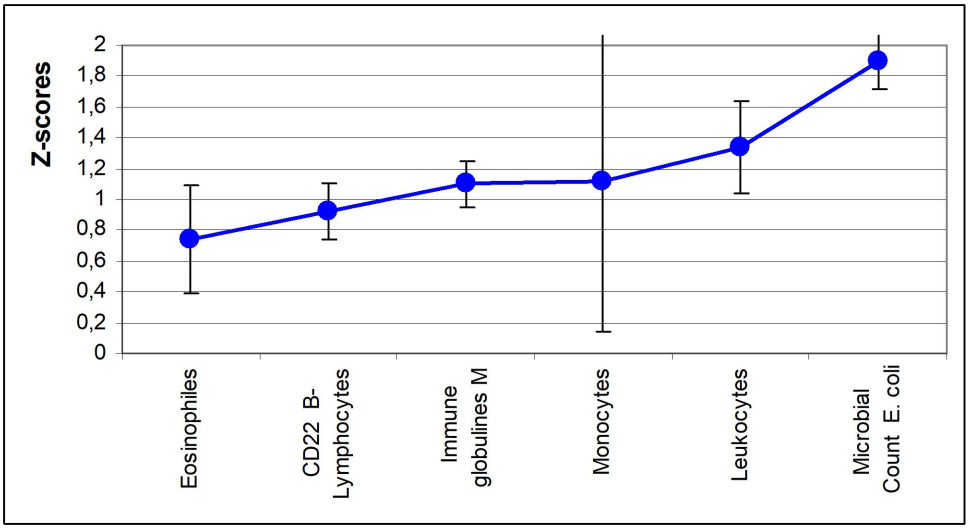


Fig. 2. Ranked profile of initially activated immune parameters

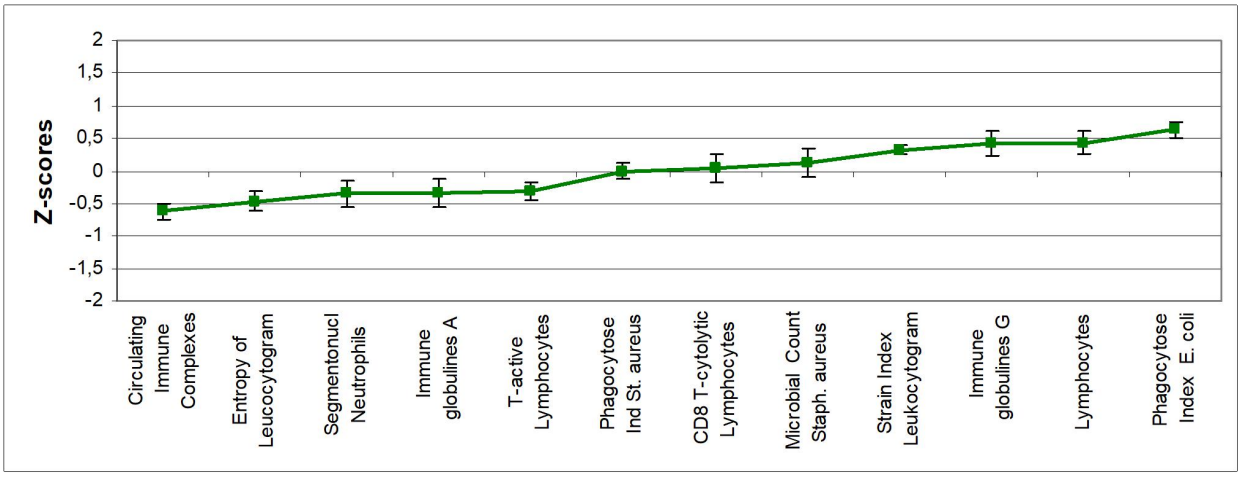


Fig. 3. The ranked profile is initially quasi-normal immunity parameters

About immunotropic effects, we judged the direct differences between the end and the initial individual values of immunity parameters.

As a tried approach to detecting the **features** of immune responses to different balneofactors we used a discriminant analysis [15]. The program included 18 variables in the model (among them 7 refer to **Phagocytosis**, 2 to **Humoral immunity,** 4 to **Cellular immunity** as well as 5 to **Leukocytogram**. Instead, other variables were out of the model (Tables 1 and 2).

Table 1. Summary of Stepwise Analysis of partial immunotropic effects of balneofactors

Variables currently in the model	F to enter	p-level	Lam-bda	F-va-lue	p-level
T-active Lymphocytes, %	5,01	,004	,758	5,0	,004
$CD4^+$ T-helper Lymphocytes, %	4,07	,012	,599	4,5	10^{-3}
Microbial Count for Staph. aureus	4,57	,007	,459	4,6	10^{-4}
Phagocytosis Index vs Staph. aur., %	2,33	,087	,396	4,1	10^{-4}
$CD8^+$ T-cytolytic Lymphocytes, %	3,15	,035	,325	4,0	10^{-5}
Circulating Immune Complexes, units	2,40	,081	,277	3,8	10^{-4}
Immunoglobulines A, g/L	1,83	,157	,244	3,6	10^{-4}
$CD16^+$Natural Killer Lymphocytes, %	1,70	,182	,186	3,3	10^{-4}
Killing Index vs E. coli, %	2,00	,130	,160	3,2	10^{-4}
Lymphocytes total, %	1,84	,157	,139	3,2	10^{-4}
Bactericydity vs E.coli, 10^9 Bacteres/L	2,03	,127	,119	3,1	10^{-4}
Phagocytosis Index vs E. coli, %	1,85	,156	,103	3,1	10^{-4}
Killing Index vs Staph. aureus, %	1,84	,159	,089	3,1	10^{-4}
Strain Index of Leukocytogram	1,50	,232	,078	3,0	10^{-4}
Stubnucleary Neutrophils, %	1,07	,375	,071	2,9	10^{-4}
Bactericydity vs St. aur, 10^9 Bacteres/L	1,09	,369	,064	2,8	10^{-4}
Segmentonucleary Neutrophils, %	1,04	,389	,058	2,7	10^{-4}
Entropy of Leukocytogram	1,62	,206	,050	2,7	10^{-4}

The dividing information contained in 18 variables is condensed in 3 canonical discriminant roots (Table 3). At the same time, the first root contains 51% of discriminative opportunities, the second is 31% the third only 18%.

The calculation of the discriminant root values for each patient as the sum of the products of raw coefficients (Table 3) to the individual values of discriminant variables together with the constant enables the visualization of each patient in the information space of the roots (Fig. 4- 5).

The extremal right-side localization along the axis of the first root of clusters of patients who received BAWN and Baths reflects the maximum **suppression** of 4 parameters that correlate with this root **inversely** as well as maximum **enhancing** of 3 parameters that correlate with this root **directly** (Table 4). Instead, the leftmost zone of the axis occupies at once two clusters whose members are mixed, which reflects an almost identical degree of enhancing/supression of the mentioned parameters caused by drink both BAWN and water solution of Ozokerite. The intermediate position is occupied by patients who received all three balneofactors.

In contrast, the last cluster occupies the highest position along the second root axis while the lowest localized patients received BAWN and Baths. Such a disposition reflects the opposite changes of 8 parameters caused by these therapeutic complexes.

Finally, along the third root axis, the highest localized patients who drank the water solution of Ozokerite, which caused a decrease in the Activity of phagocytosis and the blood level of Natural Killer as well as the Entropy of Leukocytogram, while other balneofactors had an opposite or less effect on these parameters.

Table 2. Discriminant Function Analysis Summary of partial immunotropic effects of balneofactors

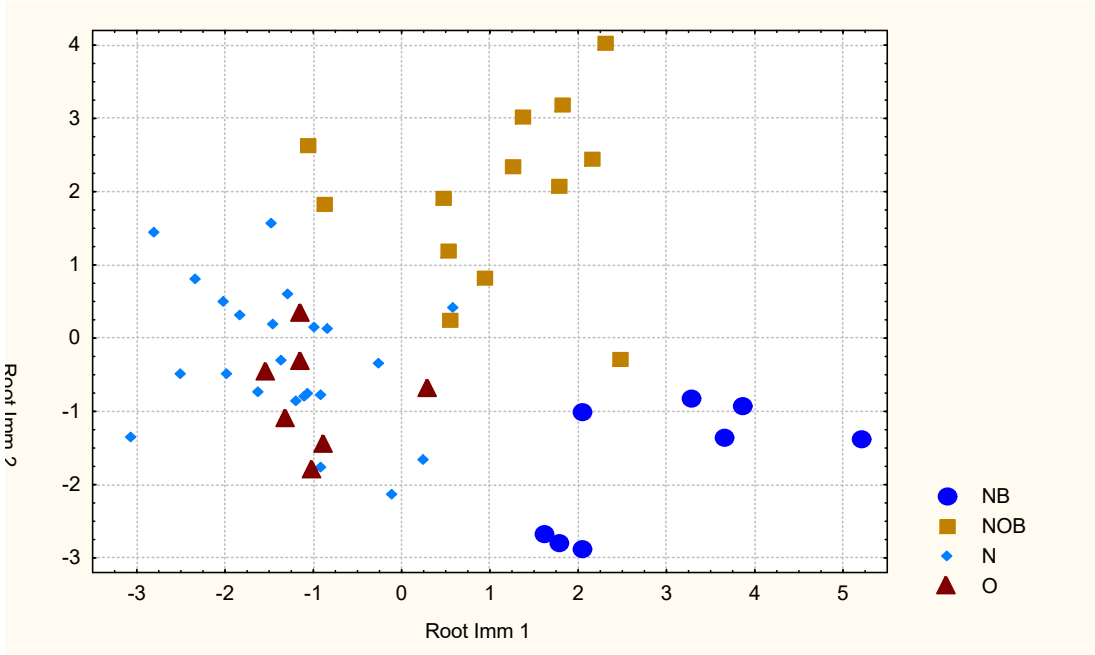
Step 18, N of vars in model: 18; Grouping: 4 grps

Wilks' Lambda: 0,0497; approx. $F_{(58)}$=2,66; $p<10^{-5}$

Variables currently in the model	Norm	Cv	Basal level (51)	Change after course of				Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
				N (23)	O dr (7)	NBO ap (13)	NB (8)					
T-active, %	30,0	0,167	28,5	+2,2	+2,3	-1,1	-4,4	,066	,751	3,21	,038	,621
T-cytolytic, %	23,5	0,138	23,6	+2,0	-1,9	-0,5	-3,1	,061	,821	2,11	,121	,284
MC St. aur	61,6	0,080	62,2	+4,2	+2,8	-3,1	-3,9	,060	,823	2,08	,125	,359
CIC, units	45	0,389	34	+6	+12	-3	-1	,072	,692	4,30	,013	,497
T-helper, %	39,5	0,082	31,2	+0,4	-0,3	-0,2	+6,1	,079	,630	5,67	,004	,235
KI St. aur, %	58,9	0,071	48,2	+1,3	-1,7	+8,3	+9,2	,069	,718	3,79	,021	,106
Segm Neutr, %	55,0	0,100	53,1	-0,1	+1,3	+0,4	+3,5	,059	,836	1,90	,152	,006
IgA, g/L	1,875	0,167	1,77	-0,07	-0,15	+0,14	-0,30	,065	,768	2,92	,051	,617
KI E. coli,%	62,0	0,078	44,3	+1,6	+6,6	+10,9	+6,3	,062	,796	2,48	,081	,123
Lymphocyt, %	32,0	0,174	34,4	+0,3	-0,1	+1,1	-3,1	,061	,820	2,12	,120	,008
Stub Neutr, %	4,25	0,147	2,57	+0,4	-0,3	-0,25	+0,8	,056	,887	1,23	,318	,330
PhI E. coli, %	98,3	0,012	99,0	-0,1	+0,5	-0,5	-0,05	,062	,796	2,48	,081	,258
BCEC, 10^9 B/L	99	0,100	88	+13	+11	+0,1	+16	,055	,896	1,13	,355	,120
BCSA, 10^9 B/L	106	0,100	91	+16	+7	+1	+14	,059	,846	1,77	,176	,116
Strain of LCG	0		0,32	-0,16	0,00	-0,19	+0,02	,069	,724	3,68	,023	,053
Entropy LCG	0,96	0,059	0,93	0,00	-0,02	-0,01	-0,01	,058	,856	1,62	,206	,017
PhI St. aur, %	98,3	0,018	98,3	+0,5	-0,6	-0,2	+0,2	,069	,723	3,70	,023	,557
NK Lymph, %	17,0	0,172	14,5	+1,6	-0,7	+1,1	+1,2	,069	,719	3,78	,021	,271
Variables currently not in the model								Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
IgM, g/L	1,15	0,239	1,45	-0,06	-0,05	+0,08	+0,13	,047	,945	,54	,659	,458
Adaptation Ind	1,70	0,147	0,74	+0,08	+0,21	+0,24	+0,42	,047	,956	,43	,731	,614
IgG, g/L	12,75	0,206	13,8	+0,51	+0,21	+2,58	+1,60	,047	,956	,43	,733	,462
Eosinophils, %	2,75	0,318	3,40	-0,39	-0,29	+0,19	-0,43	,049	,979	,20	,894	,154
Monocytes, %	6,0	0,083	6,56	-0,18	-0,57	-1,47	-0,52	,047	,937	,62	,606	,182
MC E. coli	54,7	0,097	64,8	+2,4	-2,7	-8,2	+0,8	,049	,983	,16	,923	,097
B-Lymph, %	20,0	0,175	23,2	+0,2	+3,0	+1,5	+1,9	,050	,999	,01	,998	,584
Leukocytes, G/l	5,0	0,100	5,67	+0,61	+0,49	-1,25	-0,59	,048	,950	,10	,900	,505

Table 3. Standardized and Raw Coefficients and Constants for Discriminant Variables as well as Chi-Square Tests with Successive Roots Removed

Coefficients	Standardized			Raw		
Variables currently in the model	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
T-active Lymphocytes, %	-,737	-,054	-,018	-,162	-,012	-,004
T-helper Lymphocytes, %	1,274	-,766	-,145	,279	-,168	-,032
Microbial Count for St. aureus	-,614	,514	-,319	-,073	,061	-,038
Phagocytosis Index vs St. aur, %	,003	-,112	-,993	,003	-,106	-,935
T-cytolytic Lymphocytes, %	,844	,093	-,452	,161	,018	-,086
Circulating Immune Compl, units	-,886	-,159	,234	-,045	-,008	,012
Immunoglobulines A, g/L	-,279	,710	-,056	-,663	1,690	-,133
Natural Killer Lymphocytes, %	-1,128	,237	-,364	-,344	,072	-,111
Killing Index vs E. coli, %	1,240	-,078	1,033	,089	-,006	,074
Lymphocytes total, %	-2,828	-5,083	-,369	-,458	-,822	-,060
Bactericydity vs E.coli, 10^9 B/L	-,757	-,436	-,811	-,023	-,014	-,025
Phagocytosis Index vs E. coli, %	,102	,684	,994	,059	,398	,579
Killing Index vs Staph. aur, %	,368	1,711	-1,194	,036	,166	-,115
Strain Index of Leukocytogram	1,535	2,343	,239	3,951	6,033	,615
Stub Neutrophils, %	,659	,122	-,167	,464	,086	-,117
Bactericydity vs St. aur, 10^9 B/L	-,525	-1,181	,707	-,017	-,038	,023
Segmented Neutrophils, %	-2,380	-6,153	-,387	-,391	-1,010	-,064
Entropy of Leukocytogram	-1,469	-3,282	-,521	-28,06	-62,70	-9,955
		Constants		,097	,079	,443
Eigenvalues	2,757	1,699	,985			
Canonical R	0,857	0,793	0,704			
Wilks' Λ	0,050	0,187	0,504			
χ^2	116	65	26			
Degree of Freedom	57	36	17			
p-level	$<10^{-5}$	0,002	0,068			



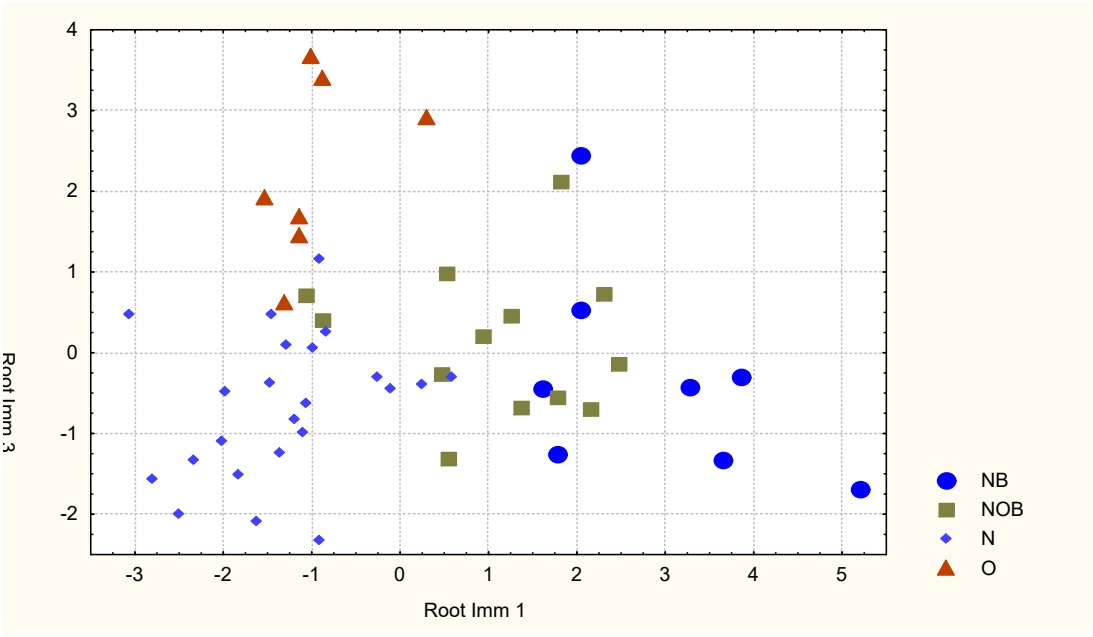
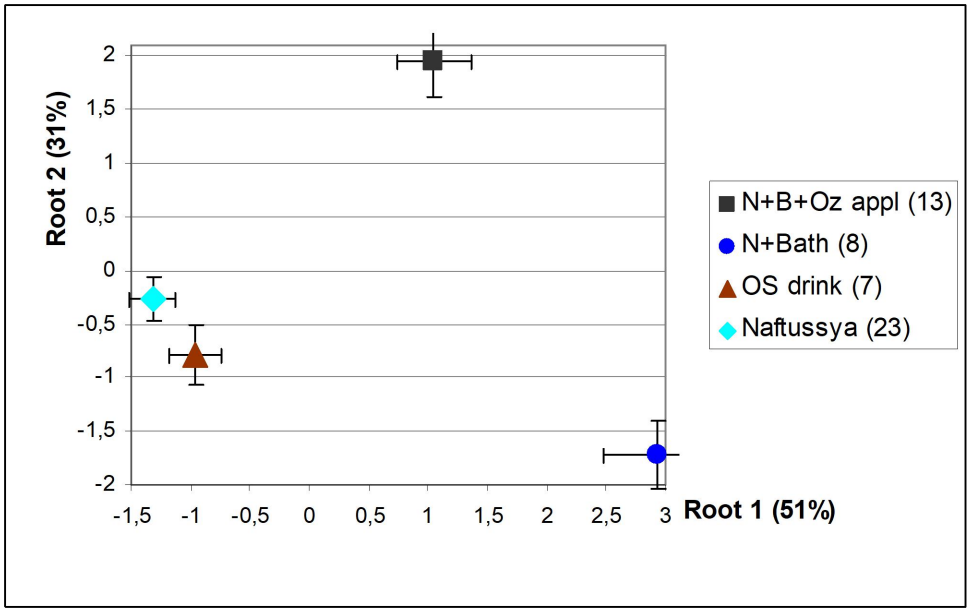


Fig. 4. Individual sizes of canonical discriminatory roots of changes in immunity caused by balneofactors



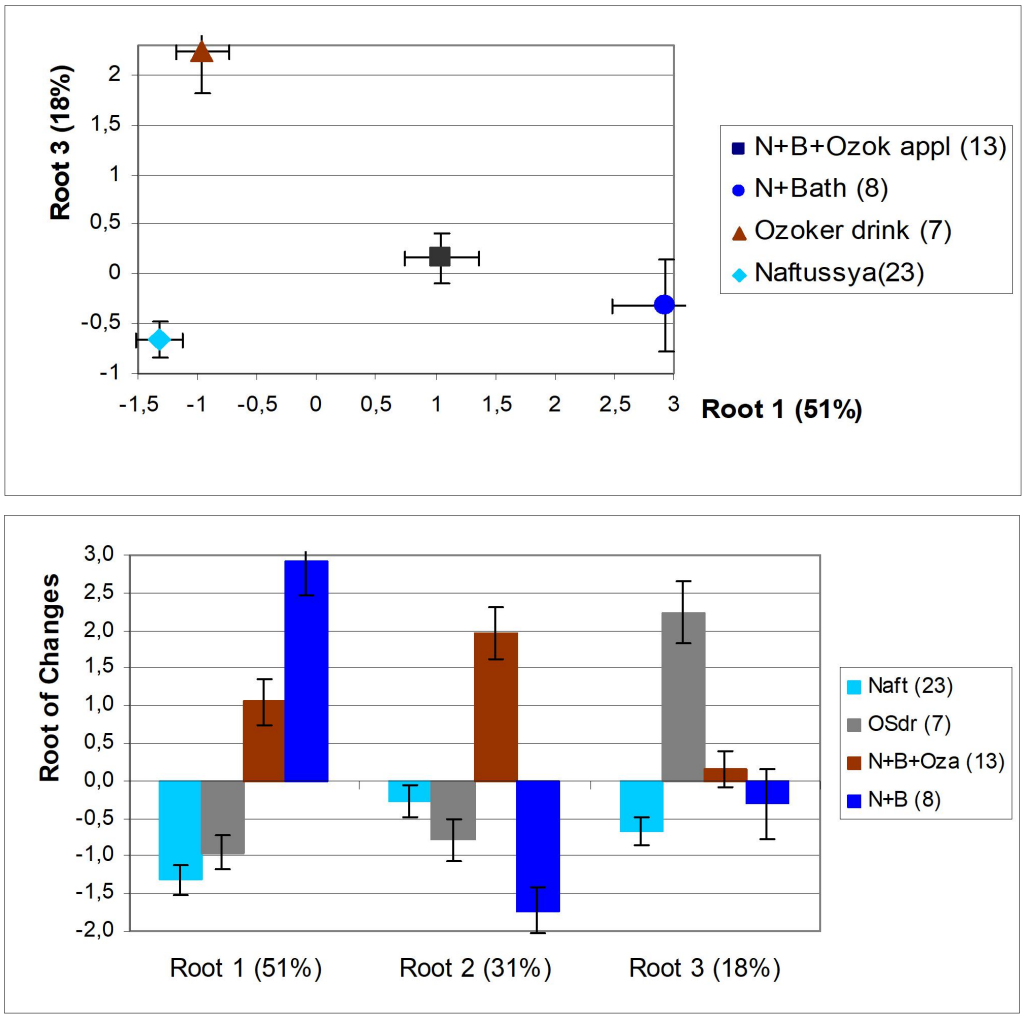


Fig. 5. Means of canonical discriminatory roots of changes in immunity caused by balneofactors

Table 4. Factor Structure Matrix (Correlations Variables-Canonical Roots), Roots Means, Z-scores of Basal levev and Changes in Variables

Variables currently in the model	Root 1	Root 2	Root 3	Naftu-ssya	Ozok. drink	N+B+ Oz ap	Naft+ Baths	Basal level (n=51)
Root 1 (51%)				-1,32	-0,96	+1,05	**+2,93**	
T-active Lymphocytes	**-,339**	,017	,050	+0,44	+0,46	-0,21	**-0,87**	-0,31±0,14
Microbial Count for Staph. aureus	**-,256**	-,098	-,039	+0,84	+0,58	-0,62	**-0,80**	+0,12±0,21
T-cytolytic Lymphocytes	**-,188**	,069	-,218	+0,62	-0,57	-0,14	**-0,96**	+0,03±0,22
Circulating Immune Complexes	**-,130**	-,106	,097	+0,34	+0,66	-0,18	-0,07	-0,62±0,12
Killing Index vs Staph. aureus	**,226**	,103	-,108	+0,30	-0,41	+1,97	**+2,27**	-2,57±0,28
T-helper Lymphocytes	**,223**	-,249	-,120	+0,11	-0,09	-0,07	**+1,89**	-2,56±0,35
Segmented Neutrophils	**,104**	-,093	,048	-0,02	+0,23	+0,07	**+0,64**	-0,35±0,20
Root 2 (31%)				-0,27	-0,79	**+1,96**	**-1,72**	
Imunoglobulines A	-,039	**,265**	-,013	-0,21	-0,47	**+0,44**	**-0,96**	-0,34±0,22
Lymphocytes total	-,079	**,139**	,013	+0,04	-0,03	**+0,19**	**-0,55**	+0,43±0,18
Killing Index vs E. coli	,112	**,129**	,133	+0,34	+1,37	**+2,25**	+1,31	-3,65±0,41
Stub Neutrophils	,027	**-,162**	-,199	+0,64	-0,46	-0,40	**+1,28**	-2,69±0,25
Bactericydity vs E.coli	-,021	**-,141**	-,044	+1,32	+1,12	**+0,01**	**+1,64**	-1,12±0,42
Bactericydity vs Staph. aureus	-,046	**-,126**	-,113	+1,50	+0,68	**+0,08**	+1,35	-1,37±0,29
Phagocytosis Index vs E. coli	-,042	**-,108**	,093	-0,05	+0,40	**-0,44**	**-0,04**	+0,63±0,13
Strain Index of Leukocytogram	,053	**-,129**	,108	-0,16	0,00	**-0,19**	**+0,02**	0,32±0,07
Root 3 (18%)				-0,66	**+2,24**	+0,16	-0,31	
Phagocytosis Index vs St. aureus	-,081	-,082	**-,362**	**+0,30**	**-0,32**	-0,10	+0,09	0,00±0,12
Natural Killer Lymphocytes	-,003	,020	**-,238**	**+0,54**	**-0,24**	+0,37	+0,40	-0,85±0,26
Entropy of Leukocytogram	-,045	-,010	**-,113**	**0,00**	**-0,38**	-0,25	-0,24	-0,47±0,15
Var-s currently not in the model								
Popovych's Adaptation Index				+0,34	+0,83	+0,97	**+1,68**	-3,82±0,20
Imunoglobulines M				-0,22	-0,19	+0,28	**+0,45**	+1,10±0,15
Imunoglobulines G				+0,19	+0,08	**+0,98**	+0,61	+0,42±0,20
Eosinophils				-0,44	-0,33	**+0,21**	**-0,49**	+0,74±0,35
Monocytes				-0,35	-1,15	**-2,95**	-1,05	+1,12±0,98
Microbial Count for E. coli				+0,45	-0,51	**-1,55**	+0,15	+1,90±0,19
Leukocytes total				+0,61	+0,49	**-1,25**	-0,59	+1,34±0,30
B-Lymphocytes				**+0,05**	**+0,86**	+0,42	+0,54	+0,92±0,18

On the whole, in the information space of the three discriminating roots, all four clusters are clearly delineated, that is, they differ from each other by constellation of 18 parameters of Immunity. This distinction is documented by calculating the squared Mahalanobis distances between them (Table 5).

Table 5. Squared Mahalanobis Distances (over diagonal), F-values (under diagonal) and p-levels (in brackets) between immunotropic effects of balneofactors

Groups	Naftussya+Baths	N+Baths+Ozok appl	Naftussya	Ozokerite drink
Naftussya+Baths	0	18,8	22,0	24,4
N+Baths+Ozok appl	2,7 (0,008)	0,0	12,2	17,3
Naftussya	3,8 ($<10^{-3}$)	3,1 (0,003)	0,0	9,6
Ozokerite drink	2,6 (0,011)	2,2 (0,024)	1,5 (0,172)	0,0

The application of the classifying functions (Table 6) enables the retrospective identification immunotropic effect of BAWN+Baths complex unmistakable and other balneofactors with a single or two errors (Table 7).

Table 6. Coefficients and Constants for Classification Functions

Variables currently in the model	Naf-tussya	Ozokerite drink	Naftussya + Baths	N+B+ Oz app
	p=,451	p=,137	p=,157	p=,255
T-active Lymphocytes, %	,295	,231	-,378	-,119
T-helper Lymphocytes, %	-,283	-,188	1,136	-,023
Microbial Count for Staph. aureus	,137	-,031	-,275	,069
Phagocytosis Index vs St. aur, %	1,117	-1,543	,949	,122
T-cytolytic Lymphocytes, %	-,201	-,402	,427	,150
Circulating Immune Compl, units	,059	,082	-,117	-,056
Immunoglobulines A, g/L	,219	-1,275	-5,098	2,317
Natural Killer Lymphocytes, %	,636	,153	-,968	-,108
Killing Index vs E. coli, %	-,169	,082	,245	,091
Lymphocytes total, %	,970	1,056	,197	-2,001
Bactericydity vs E.coli, 10^9 B/L	,070	-,004	-,019	-,036
Phagocytosis Index vs E. coli, %	-1,142	,356	-1,261	,364
Killing Index vs Staph. aur, %	,018	-,390	-,112	,378
Strain Index of Leukocytogram	-8,140	-8,035	,129	15,21
Stubnucleary Neutrophils, %	-,456	-,674	1,349	,740
Bactericydity vs St. aur, 10^9 B/L	,027	,106	,019	-,079
Segmentonucleary Neutrophils, %	,912	1,107	,693	-2,324
Entropy of Leukocytogram	59,77	53,02	27,82	-155,0
Constants	-2,830	-4,889	-8,122	-3,994

Table 7. Classification Matrix

Rows: Observed classifications, Columns: Predicted classifications

Groups	Percent correct	Naftussya	Ozok dr	N+Baths	N+B+O
		p=,451	p=,137	p=,157	p=,255
Naftussya	91,3	**21**	1	0	1
Ozokerite drink	85,7	1	**6**	0	0
Naftussya+Baths	100	0	0	**8**	0
N+Bath+Ozok appl	84,6	1	0	1	**11**
Total	90,2	23	7	9	12

If we assume that the biological activity of Naftussya water in the first approximation is determined by the presence in its composition of autochthonous microbes and Organic Substances related to such Ozokerite [2,7,33,43], then the immunotropic effect of Microbiota (microbes and organic matter transformed by them) can be quantified by the difference between the effects of drinking Naftussya water and water solution of Ozokerite (OS drinking). Applying a similar algorithm, the virtual partial immunotropic action of Organic Substances at applications of Ozokerite as such (OS application) can be calculated from the difference between the effects of the Naftussya+Baths+OS app and Naftussya+Baths complexes. Finally, the virtual partial immune effect of Baths is estimated by the difference between Naftussya+Baths and Naftussya only effects.

The results of such calculations are visualized in Figures 6-8. As you can see, the parameters of immunity, the information on which is condensed in the first root (Fig. 6), the most sensitive as stimulating (blood level of active and cytolytic T-lymphocytes, Circulating Immune

Complexes and Microbial Count for Staph. aureus) and suppressor (Killing Index vs both Staph. aureus and E. coli as well as blood level of helper T-lymphocytes and Segmentonucleary Neutrophils) action are the organic substances of Ozokerite that contact the surface of the skin, whereas their contact with the mucous of the digestive tract causes less pronounced immunotropic effect. Naftussya water has a stronger effect than the water solution of Ozokerite, apparently due to the additional effects of microbes and organic matter produced by them. In contrast, the bath factors affect the immune parameters of the opposite effects.

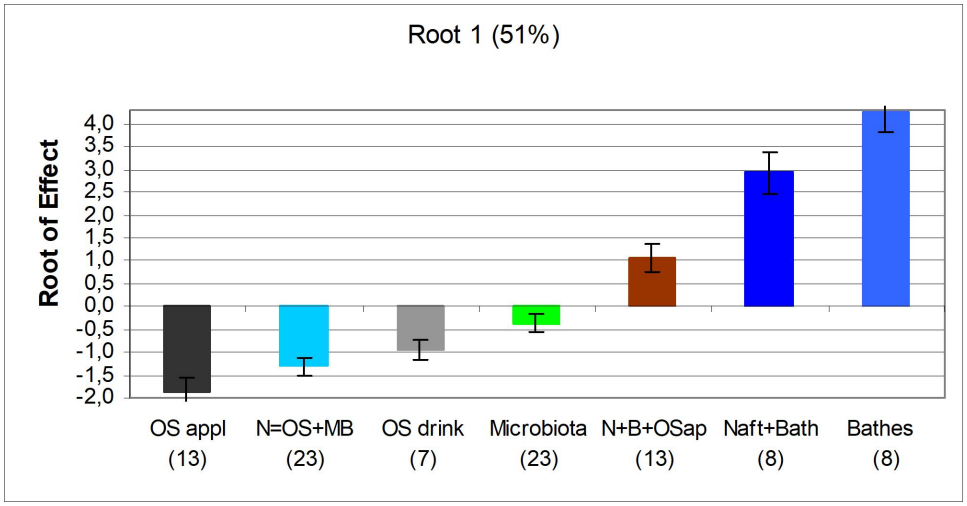


Fig. 6. Actual and calculated means of root 1 of changes in immunity caused by balneofactors

The organic substances of Ozokerite through contact with the Skin-Associated Lymphoid Tissue (SALT) [14] significantly decreased Strain Index of Leukocytogram, blood level of Stubnucleary Neutrophils, Phagocytose Index of Neutrophils vs E. coli as well as their Bactericydity vs both E. coli and Staph. aureus while increased blood level of total Lymphocytes and IgA, whereas contact with GALT causes the opposite immunotropic effects which are neutralized by a microbiote, so that Naftussya water is ineffective in relation to the listed parameters. Even more pronounced the opposite immunotropic effects cause the factors of the bath (Fig. 7).

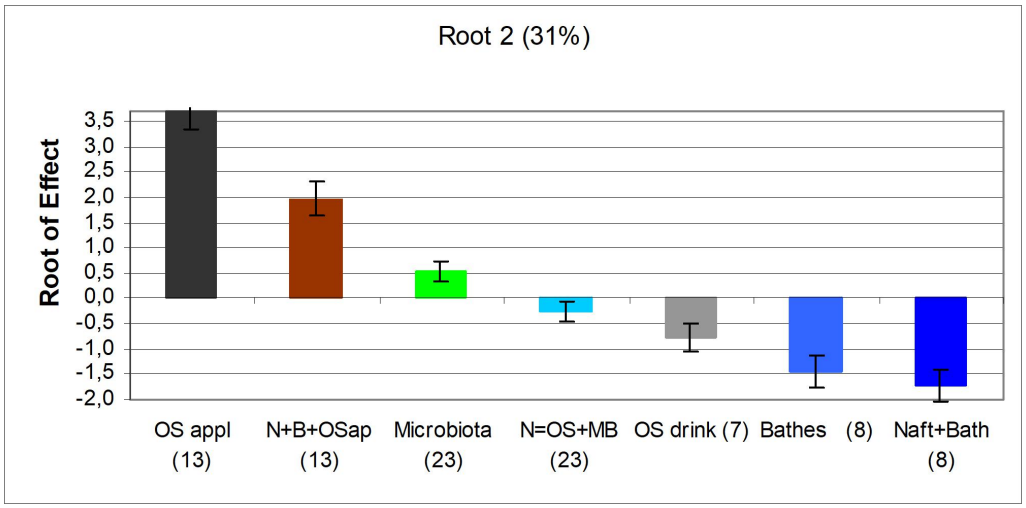


Fig. 7. Actual and calculated means of root 2 of changes in immunity caused by balneofactors

Microbiota has the most pronounced enhancing effects on the Phagocytose Index of Neutrophils vs. Staph. aureus and blood level of Natural Killers as well as Entropy of Leukocytogram. Instead, organic substances of Ozokerite have the same tangible but opposite effects on these parameters.

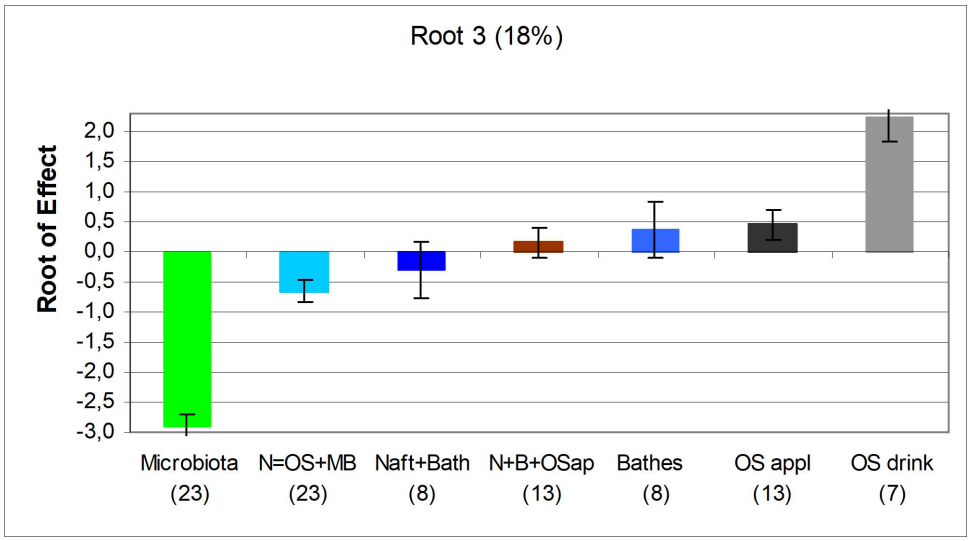


Fig. 8. Actual and calculated means of root 3 of changes in immunity caused by balneofactors

For more on this, let us dwell on the next article, which will outline the effects of the balneofactors on the parameters of HRV and EEG.

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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.

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