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**Authors**: Igor L. Popovych, Walery A. Zukow, Vitalii M. Fil, Halyna Y. Kovalchuk, Iryna V. Bryndzia, Olena R. Voloshyn, Iryna Y. Kopko, Oksana M. Lupak, Taras B. Skrobach

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# The interaction of synbiotic of the environment and the endoecosystem as one of the mechanisms of action of balneotherapy

Igor L. Popovych [1,2], Walery A. Zukow [3], Vitalii M. Fil [4], Halyna Y. Kovalchuk [4], Iryna V. Bryndzia [4], Olena R. Voloshyn [4], Iryna Y. Kopko [4], Oksana M. Lupak [4], Taras B. Skrobach [4]

[1] Bohomolets' Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine
[2] Nicolaus Copernicus University, Torun, Poland
[3] Ukrainian Scientific Research Institute of Medicine of Transport, Odesa, Ukraine
[4] Ivan Franko State Pedagogical University, Drohobych, Ukraine

**Corresponding author**: Walery Zukow, e-mail: w.zukow@wp.pl

**ORCID**

IP: https://orcid.org/0000-0002-5664-5591
WZ: https://orcid.org/0000-0002-7675-6117
VF: https://orcid.org/0000-0003-4770-5633
HK: https://orcid.org/0000-0002-5261-8422
IB: https://orcid.org/0000-0002-2873-7712
OV: https://orcid.org/0000-0001-5710-4810
IK: https://orcid.org/0000-0002-8607-7900
OL: https://orcid.org/0000-0002-1969-8643
TS: https://orcid.org/0000-0001-9603-7956

## ABSTRACT

**Introduction and aim**. Drinking mineral waters are one of the environmental factors that affect the condition of the human body. Of particular interest are therapeutic waters of the Naftussya type, which contain autochthonous microbes and organic oil-like substances and can be considered as a kind of ecosystem. On the other hand, gastrointestinal tract also is an ecosystem that associates a resident microbiota and cells of various phenotypes lining the epithelial wall. We assumed that one of the mechanisms of the therapeutic effect of Naftussya water is the interaction of external and internal ecosystems. This article is the first in a series in support of the hypothesis.

**Material 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 objects of study: leukocyturia, bacteriuria, components of microbiota, phagocytosis function of neutrophils, leukocytary adaptation index, plasma and urine electrolytes and nitrogenous metabolites.

**Results**. The weekly use of bioactive Naftussya water from the Opaka deposit causes a favorable normalizing effect on the stool microbiota: it increases the reduced content of *Bifidobacteria* and *Lactobacilli*, instead it reduces the increased content of pathogenic strains of *Escherichia coli*, as well as *Klebsiela* and *Proteus*. Reduction of dysbacteriosis is accompanied by an increase in the reduced bactericidal capacity of blood neutrophils against *E. coli* and *Staphylococcus aureus*, and reduction of bacteriuria and leukocyturia. At the same time, the elevated level of creatinine in the plasma decreases, instead, the decreased levels of sodium and chloride increase. As expected, the daily diuresis and excretion of urea, creatinine, phosphates, calcium, magnesium and chloride increases, but not sodium and uric acid, the concentrations of which in the urine decrease. The described physiologically beneficial effects are interpreted as adaptogenic, which is confirmed by an increase in the reduced leukocyte adaptation index.

**Conclusion**. The healing effect of Naftussya bioactive water is the result of the interaction of external and internal ecosystems. The next article will consider the role of the nervous and endocrine systems in this interaction.

**Keywords**. bacteriuria, metabolites, microbiota, Naftussya water, phagocytosis, pyelonephritis

## Introduction

Drinking mineral water, along with fresh water, is one of the environmental factors that affect the condition of the human body. Back in 1975, with the chemical analysis of over 300 mineral waters of the then USSR, organic matter was discovered in all of them without exception. It is shown that for water of one type, the presence of bitumen, naphthenic acids and phenols is typical, while for other types of humic, carboxylic acids and again phenols are characteristic.[1]

Despite this, it is still assumed that the physiological activity of drinking mineral waters is due to their electrolytes, the concentration of which is from 2 to 30 g/L, as well as the trace elements, while the role of organic substances is ignored, apparently because of their relatively insignificant concentration (5-40 mg/L). And only regarding therapeutic waters of the Naftussya type of Ukrainian Carpathians and Podolia as well as similar to them Berezivs'ka, Kala-Alta, Kurgazak, Volzhanka, Serebryanyi klyuch, Munoc waters, which are not formally mineral, because they contain less than 1 g/L of electrolytes, organic substances are considered as active principles.[2-8]

Another important component of the composition of waters this type is autochthonous microflora. In Naftussya water of the Truskavets’ field 900 bacterial and yeast cultures were found. Identified bacteria are classified as genera: *Pseudomonas, Bacillus, Nocardia, Corinebacterium, Micrococcus, Brevibacterium*. By type of food bacteria are ammonifying, denitrifying, iron bacteria, oligonitrophilic, hydrocarbon oxidizing, sulfate-reducing, thionic acid bacteria.[3] However, only the last three groups are considered specific, which together with aquifer and filtration water are an attribute of the Naftussya "water-forming triad".[9]

We adduce data by Dats'ko et al., 2008[10] about organic compounds (in mg/L) Naftussya water obtained by Solid Phase Extraction method and mass-spectroscopy 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; sulfur-containing connections 0.30 and 0.31; alkylnaphthalenes 0.53 and 0.53; carboxylic (fatty) acids 1.12 and 1.14; unidentified polycyclic aromatic hydrocarbons 0.19 and 0.19; connections required subsquent identification 0.48 and 0.50 respectively. The authors note that approximately 2/3 of the mass of organic substances is leached from the aquifer, and 1/3 are the products of their biotransformation by autochthonous microbes, mainly hydrocarbon oxidizing (60÷500 cells/mL). Sulfate-reducing microbes in the process of vital activity transform sulfate into hydrogen sulfide, which, in turn, is assimilated by thion microbes, transforming again into sulfate. It is interesting that an additional source of polyphenols is fallen leaves, from where they seep through the soil to the aquifer.[11] Therefore, Naftussya water is a unique ecosystem and is quite rightly nominated as "Living Water".[12]

On the other hand, the gastrointestinal tract also is a complex ecosystem that associates a resident microbiota and cells of various phenotypes lining the epithelial wall expressing complex metabolic activities. The resident microbiota in the digestive tract is a heterogeneous microbial ecosystem containing up to $10^{14}$ colony-forming units (CFUs) of bacteria. The intestinal microbiota plays an important role in normal gut function and maintaining host health.[13-24]

## Aim

Based on the above, we assumed that one of the mechanisms of the therapeutic effect of Naftussya water in chronic diseases accompanied by dysbacteriosis, in particular pyelonephritis, is the interaction of external and internal ecosystems. This article is the first in a series in support of the hypothesis.

## Material and methods

### *Ethics approval*

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. The study protocol was approved by the Ethical Committee of Ukrainian Scientific Research Institute of Medicine of Transport (protocol No. 35, 05.10.2022). During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

### *Participants*

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.

### *Study design and procedure*

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. Then daily urine was collected, in which 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); as well as 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. The analysis carried out according to instructions Goryachkovskiy, 1998 with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents.[25]

Urinary syndrome was assessed by quantitative and quantitative-qualitative levels of bacteriuria and leukocyturia. To qualitatively assess the manifestations of pyelonephritis, a single-point Popovych's[26,27] scale, built on the basis Harrington's desirability function,[28] was used. In particular, bacteriuria over $10^6$ CFU/mL is quantified at 0.9 points (strongly expressed), within $(0.3 \div 1.0) \bullet 10^6$ CFU/mL – 0.715 p (more than average, but not strong), $10^5$ CFU/mL – 0.5 p (moderately expressed), $(0.2 \div 0.5) \bullet 10^5$ CFU/mL – 0.285 p (weakly expressed), $(0.01 \div 0.1) \bullet 10^5$ CFU/mL – 0.1 p (very weak), less than $0.01 \bullet 10^5$ CFU/mL - 0 p (absent). Leukocyturia over $60 \bullet 10^3$/mL – 0.715 p, within $(20 \div 60) \bullet 10^3$/mL – 0.5 p, $(4 \div 20) \bullet 10^3$/mL – 0.285 p, $(2 \div 4) \bullet 10^3$/mL – 0.1 p, less than $2 \bullet 10^3$/mL – 0 p.

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's method.[29]

In portion of capillary blood counted up leukocytogram (lcg) (eosinophils, stub and segmentonucleary neutrophils, lymphocytes and monocytes) and calculated its adaptation index (Table 1) as well as strain index by Popovych.[30] The second version of the indices, built on the basis of the specified parameters of the authors of the concept of anti-stressor general adaptive reactions of the body, was applied.[31,32]

**Table 1**. Quantification of general adaptive reaction of the body, second version[30]

| Leukocytogram Lymphocytes level, % | General adaptive reaction of body | Eosinophils: 1–4.5 %; stub neutrophils: 3–5.5 %; monocytes: 5–7 %; leukocytes: 4–6 G/l | Eosinophils: <1;>4.5% stub neutrophils: <3; >5.5; monocytes: <5; >7; leukocytes: <4; >6 G/l |
|---|---|---|---|

| | | | |
|---|---|---|---|
| <21 | Stress | 1.22 | 0.02 |
| 21–27 | Training | 1.46 | 0.74 |
| 28–33 | Quiet Activation | 1.95 | 0.98 |
| 34–43.5 | Heightened Activation | 1.70 | 0.50 |
| ≥44 | Overactivation | 0.26 | |

Strain index-2 = $[(Eo/2.75-1)^2 + (SN/4.25-1)^2 + (Mon/6-1)^2 + (Leu/5-1)^2]/4$.

Parameters of phagocytic function of neutrophils estimated as described by Kovbasnyuk.[33] 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 – was calculated by the formula Popovych et al, 2003:[26]

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

After the initial testing, the patients received a one-week course of balneotherapy by Naftussya water (3 ml/kg 1 hour before meals three times a day) from the Opaka deposit, which is located next to the Truskavets’ and Skhidnytsya deposits. On the second day after the end of the course, re-testing was conducted.

### *Statistical analysis*

Statistical processing was performed using a software package “Microsoft Excell” and “Statistica 6.4 StatSoft Inc” (Tulsa, OK, USA).

## Results

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.

Further, profiles (Fig. 1) of normalized parameters were created, the levels of which differ significantly before and after treatment, as well as several parameters which according to the following discriminant analysis were still recognizable, despite the insignificant value of Student's t criterion.

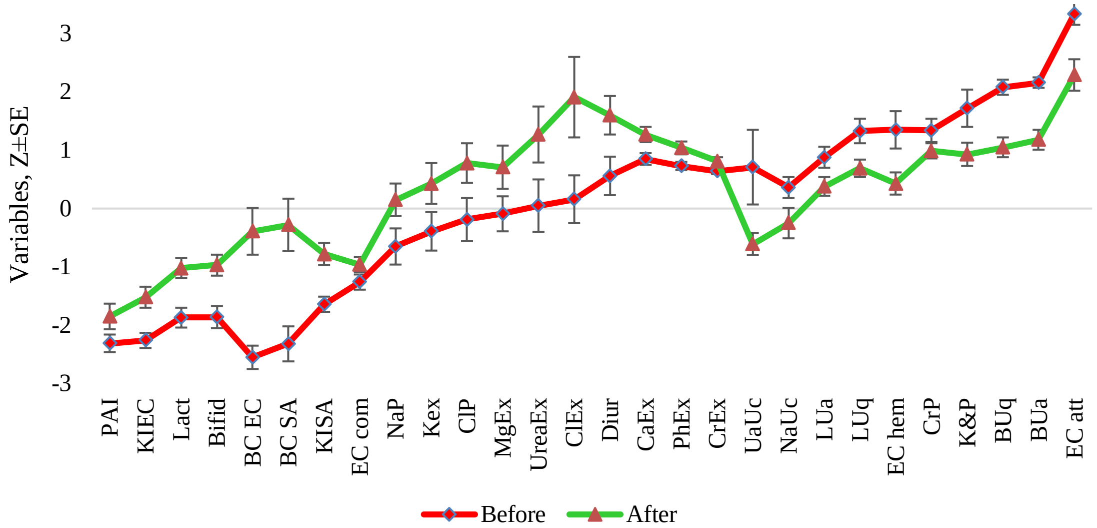

**Fig. 1.** Profiles of variables whose normalized levels (Z±SE) are changing under the influence of the Naftussya water

Another approach to quantifying effects is to calculate the direct differences between the final and initial parameters levels of each patient (Fig. 2).

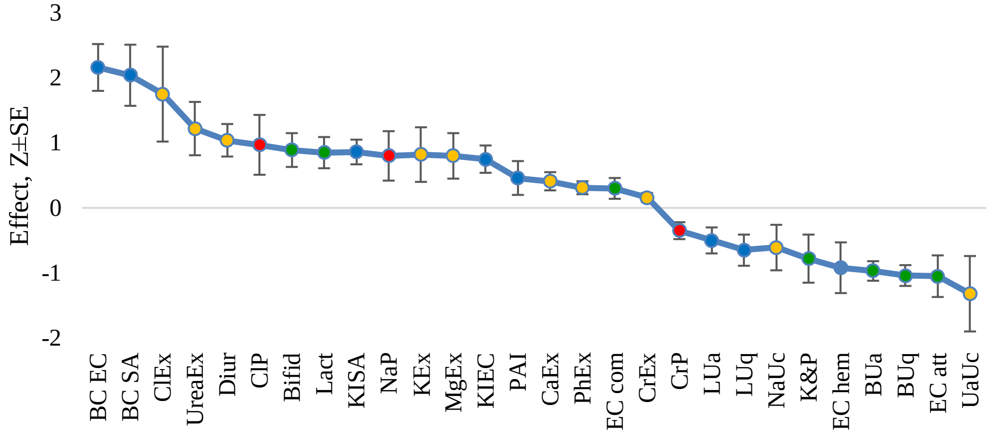

**Fig. 2.** The effects of the Naftussya water as direct differences of normalized variables (Z±SE)

As you can see, the weekly use of bioactive Naftussya water from the Opaka deposit causes a favorable normalizing effect on the microbiota: it increases the reduced content of Bifidobacteria and Lactobacilli, instead it reduces the increased content of Klebsiela and Proteus as well as pathogenic strains of E. coli (hemolytic and with attenuated enzymatic capacity), while the content of E. coli common increases.

Reduction of dysbacteriosis is accompanied by an increase in the reduced bactericidal capacity of blood neutrophils against E. coli and Staphylococcus aureus, and reduction of bacteriuria and leukocyturia.

At the same time, the elevated level of creatinine in the plasma decreases, instead, the decreased levels of sodium and chloride increase.

As expected, the daily diuresis and excretion of urea, creatinine, phosphates, calcium, magnesium and chloride increases, but not sodium and uric acid, the concentrations of which in the urine decrease.

Thus, the reduced levels of the parameters increase, and the increased ones decrease, as a rule, to normal. That is, there is a normalizing (ambivalence-equilibratory) effect of the Naftussya water as one of the attributes of adaptogensaccording to the good old "law of initial level".[34-36] At the same time, normal levels of some parameters also increase or decrease, albeit slightly. The described physiologically beneficial effects are interpreted as adaptogenic, which is confirmed by an increase in the reduced Popovych's adaptation index.[20] The dynamics of the adaptation index reflects an increase in the share of harmonious (normal) adaptation reactions from 6.9% to 24.2%, instead of a decrease in the share of disharmonious (premorbid) adaptation reactions from 82.8% to 65.5%, however, in 3 patients (10.3%) the pathological reaction of overactivation still remained.

The previously selected variables were further subjected to discriminant analysis[37] with the aim not so much to discover which of them are formally characteristic, but to visualize the integral state of each patient. The forward stepwise program included only 10 variables in the discriminant model (Tables 2 and 3), including those subject to non-significant (t<2,02) effects according to the Student's criterion, while other variables were outside the model, despite significant (*) changes (Tables 4 and 5). On the face of it, the Wilks' and Student's statistics do not match completely.

**Table 2.** Discriminant function analysis summary[a]

| **Variables currently in the model** | **State (n) and Mean±SE** | | | **Parameters of Wilks' Statistics** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Before (29)** | **After (29)** | **Effect (29)** | Wilks' Λ | Partial Λ | F-remove (1,47) | p | Tolerancy | Refer Cv **SD** |
| **Bacteriuria actual, lg CFU/mL** | 2.11 | 1.16 | -0.95 | 0.345 | 0.999 | 0.03 | 0.860 | 0.159 | 0 |
| | 0.08 | 0.17 | 0.15* | | | | | | 0.98 |
| **Bacteriuria qualitative, points** | 0.5 | 0.25 | -0.25 | 0.369 | 0.934 | 3.30 | 0.076 | 0.148 | 0 |
| | 0.03 | 0.04 | 0.04* | | | | | | 0.24 |
| **Leukocyturia qualitative, point** | 0.2 | 0.1 | -0.1 | 0.371 | 0.928 | 3.64 | 0.062 | 0.341 | 0 |
| | 0.03 | 0.02 | 0.04* | | | | | | 0.15 |
| ***Klebsiela* and *Proteus faeces*, %** | 18.9 | 10.3 | -8.6 | 0.358 | 0.962 | 1.85 | 0.180 | 0.145 | 0 |
| | 3.5 | 2.2 | 4.1* | | | | | | 11 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***E. coli* common, lg CFU/g** | 8.17<br>0.05 | 8.29<br>0.05 | +0.12<br>0.06* | 0.389 | 0.886 | 6.06 | 0.018 | 0.169 | 8.66<br>0.045 |
| **Killing index vs *S aureus*, %** | 45.2<br>1.1 | 52.4<br>1.6 | +7.2<br>1.6* | 0.388 | 0.887 | 5.97 | 0.018 | 0.717 | 58.9<br>0.142 |
| **Popovych's adaptation index-2** | 0.74<br>0.06 | 0.93<br>0.09 | +0.19<br>0.11 | 0.355 | 0.971 | 1.43 | 0.238 | 0.820 | 1.705<br>0.245 |
| **Chloride plasma, mM/L** | 100.9<br>1.2 | 104<br>1.1 | +3.2<br>1.5* | 0.368 | 0.937 | 3.18 | 0.081 | 0.870 | 101.5<br>0.032 |
| **Chloride excretion, mM/24 h** | 172<br>12 | 223<br>20 | +50<br>21* | 0.379 | 0.909 | 4.73 | 0.035 | 0.768 | 167.5<br>0.172 |
| **Sodium Urine concentration, mM/L** | 118<br>4 | 104<br>6 | -14<br>8 | 0.398 | 0.865 | 7.32 | 0.009 | 0.792 | 110<br>0.211 |

[a] Step 10, N of vars in model: 10; Grouping: 2 grps; Wilks' Lambda: 0,3446; approx. $F_{(11)}$=8,9; $p<10^{-6}$; in each column, the first line is the average, the second – SE; in norm column – the average and Cv or SD. The "Effect" and "Norm" columns are not the result of discriminant analysis

**Table 3.** Summary of stepwise analysis of discriminant variables ranked by criterion Λ

| **Variables currently in the model** | F to enter | p-level | **Λ** | F-value | p |
|---|---|---|---|---|---|
| **Bacteriuria actual, lg CFU/mL** | 25 | $10^{-5}$ | 0.692 | 25 | $10^{-5}$ |
| **Killing index vs *S. aureus*, %** | 12.8 | 0.001 | 0.561 | 21.6 | $10^{-6}$ |
| **Sodium urine concentration, mM/L** | 4.52 | 0.038 | 0.517 | 16.8 | $10^{-6}$ |
| **Leukocyturia qualitative, point** | 3.45 | 0.069 | 0.486 | 14 | $10^{-6}$ |
| **Popovych's adaptation index-2** | 3.36 | 0.073 | 0.456 | 12.4 | $10^{-6}$ |
| **Chloride excretion, mM/24 h** | 4.06 | 0.049 | 0.423 | 11.6 | $10^{-6}$ |
| **Bacteriuria qualitative, points** | 2.12 | 0.151 | 0.405 | 10.5 | $10^{-6}$ |
| ***E. coli* common, lg CFU/g** | 3.14 | 0.083 | 0.381 | 9.96 | $10^{-6}$ |
| **Chloride plasma, mM/L** | 3.04 | 0.087 | 0.358 | 9.56 | $10^{-6}$ |
| ***Klebsiela&Proteus faeces*, %** | 1.85 | 0.18 | 0.345 | 8.94 | $10^{-6}$ |

**Table 4.** Microbiota and Phagocytosis variables currently not in the discriminant model

| **Variables currently not in the model** | **State (n) and Mean±SE** | | | **Parameters of Wilks' Statistics** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Before (29)** | **After (29)** | **Effect (29)** | Wilks' Λ | Partial Λ | F to enter | p-level | Tolerancy | Refer Cv SD |
| **Leukocyturia actual, lg L/mL** | 3.56<br>0.11 | 3.24<br>0.1 | -0.32<br>0.13* | 0.344 | 0.998 | 0.11 | 0.748 | 0.135 | 3<br>0.21 |
| ***Bifidobacteria faeces*, lg CFU/g** | 4.83<br>0.21 | 5.84<br>0.2 | +1.01<br>0.29* | 0.344 | 0.997 | 0.16 | 0.690 | 0.406 | 6.94<br>0.164 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***Lactobacilli faeces*, lg CFU/g** | 5.38 | 6.62 | +1.24 | 0.344 | 0.997 | 0.14 | 0.707 | 0.417 | 8.1 |
| | 0.25 | 0.25 | 0.35* | | | | | | 0.179 |
| ***E. coli* attenuated faeces, %** | 75.6 | 57.2 | -18.4 | 0.339 | 0.984 | 0.76 | 0.388 | 0.384 | 17.4 |
| | 3.4 | 4.8 | 5.6* | | | | | | 1 |
| ***E. coli* hemolytic faeces, %** | 33.8 | 10.8 | -23.0 | 0.344 | 0.997 | 0.15 | 0.704 | 0.618 | 0 |
| | 8.1 | 4.7 | 9.9* | | | | | | **25** |
| **Killing index vs *E. coli*, %** | 40.1 | 47.3 | +7.2 | 0.341 | 0.989 | 0.51 | 0.477 | 0.378 | 62 |
| | 1.2 | 1.7 | 2.0* | | | | | | 0.156 |
| **Bactericidity vs *S. aureus*,** | 81 | 103 | +22 | 0.345 | 1 | 0.00 | 0.983 | 0.404 | 106 |
| **$10^9$ bacteria/L** | 3 | 5 | 5 | | | | | | 0.1 |
| **Bactericidity vs *E. coli*,** | 74 | 95 | +21 | 0.343 | 0.995 | 0.23 | 0.633 | 0.080 | 99 |
| **$10^9$ Bacteria/L** | 2 | 4 | 4 | | | | | | 0.1 |

**Table 5.** Metabolic variables currently not in the discriminant model

| | **State (n) and Mean±SE** | | | **Parameters of Wilks' Statistics** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variables currently not in the model** | **Before (29)** | **After (29)** | **Effect (29)** | Wilks' Λ | Partial Λ | F to enter | p-level | Tolerancy | Refer Cv |
| **Diuresis, L/24h** | 1.61 | 2.01 | +0.40 | 0.344 | 0.998 | 0.11 | 0.740 | 0.398 | 1.4 |
| | 0.13 | 0.13 | 0.10* | | | | | | 0.274 |
| **Uric acid urine concentration,** | 2.52 | 1.82 | -0.70 | 0.339 | 0.984 | 0.72 | 0.399 | 0.669 | 2.14 |
| **mM/L** | 0.34 | 0.1 | 0.31* | | | | | | 0.25 |
| **Urea excretion, mM/24 h** | 462 | 566 | +104 | 0.344 | 0.999 | 0.07 | 0.798 | 0.411 | 458 |
| | 38 | 41 | 35* | | | | | | 0.186 |
| **Creatinine excretion, mM/24 h** | 7.06 | 8.87 | +1.81 | 0.344 | 0.997 | 0.13 | 0.717 | 0.776 | 11 |
| | 0.6 | 0.8 | 0.82* | | | | | | 0.3 |
| **Calcium excretion, mM/24 h** | 3.74 | 5.54 | +1.80 | 0.343 | 0.996 | 0.2 | 0.658 | 0.441 | 4.38 |
| | 0.42 | 0.57 | 0.62* | | | | | | 0.214 |
| **Phosphate excretion, mM/24 h** | 18.3 | 26.2 | +7.9 | 0.343 | 0.996 | 0.18 | 0.672 | 0.612 | 25.2 |
| | 1.7 | 2.7 | 2.4* | | | | | | 0.294 |
| **Magnesium excretion, mM/24 h** | 4.00 | 4.84 | +0.84 | 0.343 | 0.996 | 0.19 | 0.664 | 0.553 | 4.1 |
| | 0.31 | 0.39 | 0.36* | | | | | | 0.256 |
| **Potassium excretion, mM/24 h** | 58.3 | 72.5 | +14.2 | 0.343 | 0.996 | 0.19 | 0.669 | 0.766 | 65 |
| | 5.7 | 6.0 | 7.4 | | | | | | 0.269 |
| **Creatinine plasma, µM/L** | 87.6 | 82.6 | -5.0 | 0.342 | 0.993 | 0.34 | 0.564 | 0.77 | 77 |
| | 2.9 | 1.8 | 1.9* | | | | | | 0.17 |
| **Sodium plasma, mM/L** | 141.8 | 145.8 | +4.0 | 0.344 | 0.999 | 0.05 | 0.821 | 0.426 | 145 |
| | 1.5 | 1.4 | 1.9* | | | | | | 0.034 |

On the basis of the raw coefficients and constant (Table 6), the individual values of the canonical discriminant roots were calculated with the following visualization in Fig. 3.

**Table 6.** Standardized and raw coefficients and constant for discriminant variables

| | **Coefficients** | |
|---|---|---|
| **Variables** | **Standardized** | **Raw** |
| **Bacteriuria actual, lg CFU/mL** | 0.08 | 0.11 |
| **Killing index vs *S. aureus*, %** | -0.49 | -0.069 |
| **Sodium urine, mM/L** | 0.51 | 0.018 |
| **Leukocyturia qualitative, points** | 0.567 | 3.859 |
| **Popovych's adaptation index-2** | -0.234 | -0.561 |
| **Chloride excretion, mM/24 h** | -0.426 | -0.005 |
| **Bacteriuria qualitative, points** | 0.823 | 4.272 |
| ***E. coli* com., lg CFU/g** | 1.015 | 3.839 |
| **Chloride plasma, mM/L** | -0.333 | -0.053 |
| ***Klebsiela&Proteus faeces*, %** | 0.633 | 0.04 |
| | **Constant** | -26.29 |
| | **Eigenvalue** | 1.90 |
| **Squared Mahalanobis Distance=7.35; $F_{(11)}$=8.9; $p<10^{-6}$** | | |
| **Canonical R=0.810; Wilks' Λ=0.3446; $\chi^2_{(10)}$=54; $p<10^{-6}$** | | |

The level of the root after the course of using the Naftussya water in almost everyone patients, with two exceptions, is lower than the initial level to one degree or another. The variability of the body's responses to adaptogens and stressors is due to individual reactivity.[38-40] This reflects both increasing levels of variables represented in the root inversely and decreasing levels of variables that are positively correlated with the root (Table 7).

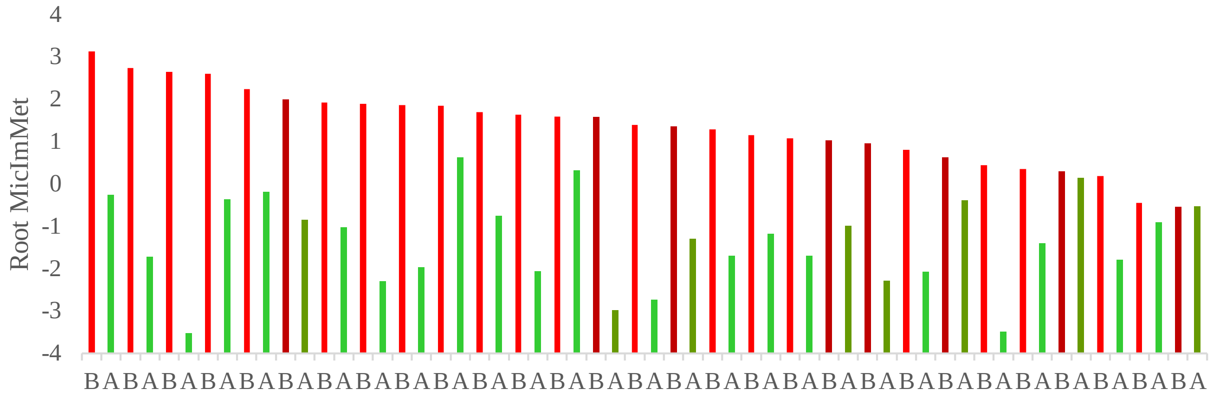

**Fig. 3.** Individual values (M±SE) of discriminant Root before (B) and after (A) course of intake of the Naftussya water. Women are highlighted in shades of colors

**Table 7.** Effects of Naftussya water as differences between levels (Z±SE) after and before treatment

| **Clusters and Variables** | **R** | **Before (29)** | **After (29)** | **Effect (29)** |
|---|---|---|---|---|
| **Bacteriuria actual** | 0.484 | +2.16±0.09 | +1.18±0.17 | -0.97±0.15* |
| **Bacteriuria qualitative** | 0.476 | +2.08±0.13 | +1.05±0.17 | -1.04±0.16* |
| **Leukocyturia qualitative** | 0.245 | +1.33±0.21 | +0.69±0.15 | -0.65±0.24* |
| **Leukocyturia actual** | | +0.88±0.18 | +0.38±0.16 | -0.50±0.20* |
| ***Klebsiela&Proteus faeces*** | 0.201 | +1.72±0.32 | +0.93±0.20 | -0.78±0.37* |
| ***E. coli* hemolytic faeces** | | +1.35±0.32 | +0.43±0.19 | -0.92±0.39* |
| ***E. coli* attenuated faeces** | | +3.34±0.19 | +2.29±0.27 | -1.05±0.32* |
| **Sodium urine concentration** | 0.185 | +0.36±0.18 | -0.25±0.26 | -0.61±0.35 |
| **Uric acid urine concentration** | | +0.71±0.64 | -0.61±0.19 | -1.32±0.58* |
| **Creatinine Plasma** | | +1.34±0.20 | +0.99±0.13 | -0.35±0.13* |
| **Killing index vs *S. aureus*** | -0.373 | -1.64±0.13 | -0.78±0.19 | +0.86±0.19* |
| **Killing index vs *E. coli*** | | -2.27±0.13 | -1.52±0.18 | +0.75±0.21* |
| **Bactericidity vs *S. aureus*** | | -2.32±0.30 | -0.28±0.45 | +2.04±0.47 |
| **Bactericidity vs *E. coli*** | | -2.55±0.20 | -0.39±0.40 | +2.16±0.36 |
| ***Bifidobacteria faeces*** | | -1.86±0.19 | -0.97±0.18 | +0.89±0.26* |
| ***Lactobacilli faeces*** | | -1.87±0.17 | -1.02±0.17 | +0.85±0.24* |
| ***E. coli* common** | -0.163 | -1.26±0.13 | -0.96±0.13 | +0.30±0.16 |
| **Chloride excretion** | -0.212 | +0.16±0.41 | +1.91±0.69 | +1.75±0.73* |
| **Chloride plasma** | -0.187 | -0.19±0.37 | +0.78±0.34 | +0.97±0.46* |
| **Sodium plasma** | | -0.65±0.31 | +0.15±0.28 | +0.80±0.38* |
| **Urea excretion** | | +0.05±0.45 | +1.27±0.48 | +1.22±0.41* |
| **Diuresis** | | +0.56±0.33 | +1.60±0.33 | +1.04±0.25* |
| **Potassium excretion** | | -0.39±0.33 | +0.43±0.35 | +0.82±0.42 |
| **Magnesium excretion** | | -0.09±0.30 | +0.71±0.37 | +0.80±0.35* |

| | | | | |
|---|---|---|---|---|
| **Calcium excretion** | | +0.85±0.10 | +1.27±0.13 | +0.41±0.14* |
| **Phosphate excretion** | | +0.73±0.07 | +1.04±0.11 | +0.31±0.1* |
| **Creatinine excretion** | | +0.64±0.05 | +0.81±0.07 | +0.16±0.07* |
| **Popovych's adaptation index-2** | -0.169 | -2.31±0.15 | -1.85±0.22 | +0.46±0.26 |

The accuracy of the retrospective classification of effects by calculating individual classification functions based on its coefficients and constants (Table 8) is 91,4% (two and three mistakes before and after treatment responsibility) (Table 9).

**Table 8.** Coefficients and constants of classification functions

| **Clusters** | **Before** | **After** |
|---|---|---|
| **Variables** | **0.500** | **0.500** |
| **Bacteriuria actual, lg CFU/mL** | -8.630 | -8.928 |
| **Killing index vs *S. aureus*, %** | -1.761 | -1.575 |
| **Sodium urine concentration, mM/L** | 0.870 | 0.821 |
| **Leukocyturia qualitative, point** | -14.88 | -25.34 |
| **Popovych's adaptation index-2** | 51.64 | 53.16 |
| **Chloride excretion, mM/24 h** | -0.116 | -0.103 |
| **Bacteriuria qualitative, points** | 229.9 | 218.3 |
| **Escherichia coli com., lg CFU/g** | 676.6 | 666.2 |
| **Chloride plasma, mM/L** | -0.018 | 0.127 |
| ***Klebsiela&Proteus faeces*, %** | 9.923 | 9.815 |
| **Constants** | -2925 | -2853 |

**Table 9.** Classification matrix

| | **Rows: Observed classifications Columns: Predicted classifications** | | |
|---|---|---|---|
| **Group** | Percent correct | B p=0.50000 | A p=0.50000 |
| **Before** | 93.1 | 27 | 2 |
| **After** | 89.7 | 3 | 26 |
| **Total** | 91.4 | 30 | 28 |

## Discussion

It seems to us that the primary effect of Naftussya bioactive water is a significant increase (though without normalization) of a significantly reduced content in the microbiota of *Bifidobacteria* and *Lactobacilli*. These beneficial bacteria and the cells lining the gastrointestinal epithelium are two partners that properly and/or synergistically function to promote an efficient host system of defence. One of the basic physiological functions of the resident microbiota is that it functions as a microbial barrier against microbial pathogens. The mechanisms by which the species of the microbiota exert this barrier effect are as follows: bacterial interference, acid and pH effects, antimicrobial substances, immunomodulation.[13,14,16]

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 are of particular interest.[15] This gives us reason to assume that inhibition of the growth of pyelonephritogenic *E. 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.

The mechanism of the increase in the bactericidal capacity of neutrophils that we discovered requires a separate discussion. We know that feeding of mice with *Bifidobacteria* or *Lactobacilli* strains resulted in significant increase in the phagocytic activity of peripheral blood leukocytes and peritoneal macrophages compared to that in control mice. Administration of *Lactobacillus rhamnosus* to healthy volunteers is followed by a relative increased proportion of polymorphonuclear cells showing phagocytic activity. The phagocytic capacity of polymorphonuclear and mononuclear phagocytes in elderly subjects was also elevated after *Bifidobacter lactis* consumption.[14]

Thus, Naftussya bioactive water increases the bactericidal ability of neutrophils through the mediation of gut probiotics, that is, it manifests itself as a prebiotic.

At the same time, direct effects on phagocytes (as well as others immunocytes) through their aryl hydrocarbon receptors (AhR) are quite real.[41,42]

This assumption is based on the fact that cells within the immune system, such as macrophages, dendritic cells, granulocytes, natural killer cells and lymphocytes (T cells and B cells), express AhR. The expression of the AhR in a majority of immune cell types and the expression of multiple xenobiotic- or dioxin-responsive elements (XREs/DREs) in the promoter region of many genes that regulate the immune response demonstrates the importance of this receptor in immunological processes.[43-47] Researchers have discovered a wide range of AhR ligands, both natural and synthetic, including environmental contaminants, dietary compounds, microbial byproducts, and endogenous mediators. Typically components of environmental pollutants: polycyclic aromatic hydrocarbons such as benzo(a)pyrene, anthracene, and 3-methylcholanthrene as well as halogenated aromatic hydrocarbons such as polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls.[48]

On the other hand, among the organic substances of Naftussya water probable AhR ligands are present (alkylbenzene, alkenylbenzene, esters of aromatic acids, alkyl phenols, alkylnaphthalenes, polycyclic aromatic hydrocarbons). By the way, probiotics themselves are capable of producing an endogenous ligand of AhR 6-Formylindolo[3,2-b] Carbazole (FICZ).[49]

Ubiquitous AhRs are also expressed by neurons, so a neuro-immune mechanism of phagocytosis activation is also possible, the analysis of which will be the topic of the next article.[50,51] So let us limit ourselves to the announcement: Naftussya bioactive water has a modulating effect on the autonomic and central nervous systems, which, in turn, activate phagocytosis, as well as modulate cellular and humoral immunity.[33,52-59]

## Conclusion

The healing effect of Naftussya bioactive water is the result of the interaction of external and internal ecosystems. Naftussya bioactive water increases the bactericidal ability of neutrophils through the mediation of gut probiotics, that is, it manifests itself as a prebiotic. At the same time, direct effects on phagocytes (as well as others immunocytes) through their aryl hydrocarbon receptors are quite real. The next our article will consider the role of the nervous and endocrine systems in this interaction.

In conclusion, we want to put forward the concept of Naftussya bioactive water as synbiotic.

It is known that prebiotics are a group of biological nutrients that are capable of being degraded by microflora in the gastrointestinal tract, primarily *Lactobacilli* and *Bifidobacteria*. When prebiotics are ingested, either as a food additive or as a supplement, the colonic microflora degrade them, producing short-chain fatty acids (SCFA), which are simultaneously released in the colon and absorbed into the blood circulatory system. The two major groups of prebiotics that have been extensively studied in relation to human health are fructo-oligosaccharides (FOS) and galactooligosaccharides (GOS). The candidature of a compound to be regarded as a prebiotic is a function of how much of dietary fiber it contains.[60] Prebiotics are either natural or synthetic non-digestible (non-) carbohydrate substances that boost the proliferation of gut microbes. Undigested FOS in the large intestine are utilised by the beneficial microorganisms for the synthesis of short-chain fatty acids for their own growth.[61] Generally, non-digestible carbohydrates are considered prebiotic. However, our data indicate the ability of another group of substances to boost the proliferation of gut microbes.

In May 2019, the International Scientific Association for Probiotics and Prebiotics (ISAPP) convened a panel of nutritionists, physiologists and microbiologists to review the definition and scope of synbiotics. The panel updated the definition of a synbiotic to “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host”. The panel concluded that defining synbiotics as simply a mixture of probiotics and prebiotics could suppress the innovation of synbiotics that are designed to function cooperatively. Requiring that each component must meet the evidence and dose requirements for probiotics and prebiotics individually could also present an obstacle. Rather, the panel clarified that a complementary synbiotic, which has not been designed so that its component parts function cooperatively, must be composed of a probiotic plus a prebiotic, whereas a synergistic synbiotic does not need to be so. A synergistic synbiotic is a synbiotic for which the substrate is designed to be selectively utilized by the co-administered microorganisms. This Consensus Statement further explores the levels of evidence (existing and required), safety, effects upon targets and implications for stakeholders of the synbiotic concept.[62]

As follows from the above, Naftussya bioactive water fully meets the requirements for synbiotics.

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### *Author contributions*

Conceptualization, I.P.; Methodology, I.P.; Software, I.P. and W.Z.; Validation, I.P. and W.Z.; Formal Analysis, I.P. and W.Z.; Investigation, V.F., H.K., I.B., O.V., I.K., O.L. and T.S.; Resources, W.Z.; Data Curation, I.P. and W.Z.; Writing – Original Draft Preparation, I.P. and W.Z.; Writing – Review & Editing, I.P. and W.Z.; Visualization, I.P. and W.Z.; Supervision, I.P. and W.Z.; Project Administration, I.P. and W.Z.; Funding Acquisition, V.F., H.K., I.B., O.V., I.K., O.L. and T.S.

### *Conflicts of interest*

The authors declare no competing interests.

### *Data availability*

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

### *Ethics approval*

The study protocol was approved by the Ethical Committee of Ukrainian Scientific Research Institute of Medicine of Transport (protocol No. 35, 05.10.2022).

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