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https://pbn.nauka.gov.pl/sedno-webapp/works/896700

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ROLE OF ORGANIC CARBON AND NITROGEN OF MINERAL WATERS IN THEIR METABOLIC EFFECTS AT FEMALE RATS

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Abstract

Background. Organic substances, despite their presence in all drinking mineral waters, are still considered to be active only in the water of Naftussya type (which, due to mineralization less than 1 g/L, are not formally mineral), whereas the physiological activity of true mineral waters (which mineralization is greater than 1-2 g/L) are associated with electrolytes and trace elements. The purpose of this study was to clarify the role of organic carbon and nitrogen of mineral waters in their effects on certain parameters of metabolism. **Material and Methods.** Experiment was performed on 48 healthy female Wistar rats 240-290 g divided into 5 groups. Animals of the first group for 6 days administered a single tap water through the probe at a dose of 1,5 mL/100 g of body mass. In the second group we administered the water Naftussya from the Truskavets' layer, in the third group the water Sophiya of the Truskavets' field. The rats of the fourth group received the native water from the Gertsa (Bucovyna) field, and the last group its artificial salt analogue. The day after the completion of the drinking course in all rats some metabolic parameters were registered. **Results.** On the basis of the correlation analysis with step-by-step exclusion, four metabolites of blood as well as five metabolites of urine are included in the regressive model for organic carbon (R=0,697; R^2=0,486; Adjusted R^2=0,347; $F_{(10,4)}$=3,5;

p=0,0025). Organic nitrogen of mineral waters affects five metabolic parameters of urine only, but with approximately the same force ($R=0,621$; $R^2=0,385$; Adjusted $R^2=0,312$; $F_{(5,4)}=5,3$; $p=0,0008$). **Conclusion.** Organic substances of mineral waters play an essential role in their effects on the parameters of metabolism.

Key words. Mineral waters, organic carbon and nitrogen, female rats, metabolic parameters.

INTRODUCTION

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 [11]. 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 for Naftussya and Berezovs'ka waters, which are not formally mineral, because they contain less than 1 g/L of electrolytes, organic substances are considered as active principles [1,7,9,10,12,13].

We adduce data by OR Dats’ko et al [2] about organic compounds (in mg/L) water Naftussya 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; 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 respectively. Early have been shown that detected in Naftussya phenols (0,5-4,1 μg/L) [8].

As such a complete analysis is extremely labor intensive and expensive, the organic component of water is usually judged by the gross organic carbon and nitrogen content.

Previously, we have shown that drinking water with different contents of electrolytes, trace elements and organic substances has both general [15,16] and different effects on the parameters of metabolism [5] and the neuroendocrine-immune complex [6] in rats, which they have been drinking for 6 days.

The purpose of this study was to clarify the role of organic carbon and nitrogen of mineral waters in their effects on certain parameters of metabolism.

MATERIAL AND METHODS

Experiment was performed on 48 healthy female Wistar rats 240-290 g divided into 5 groups. Rats of the first (control) group for 6 days administered a single tap water through the probe at a dose of 1,5 mL/100 g of body mass. In the second group (reference for the organic component) was given daily drinking of animals with water Naftussya from the Truskavets' layer, in the third group (reference to the salt component) the rats were louded with the water Sophiya of the Truskavets' field. The rats of the fourth group received the native water from the Gertsa field, and the second control group its artificial salt analogue. The chemical composition of the applied waters (according to Truskavetsian Hydrogeological Regime-operational Station data) is given in Table 1.

Table 1. The chemical composition of the applied mineral waters

	Daily Water	Sofiya	Gertsa	Salt analog	Naftussya
Electrolytes, mM/L					
Na^+	0,5	156	196,7	196,7	0,6
Cl^-	3,4	142	205	205	1,0
HCO_3^-	2,9	7,5	5,6	5,6	8,2
Ca^{2+}	3,4	5,3	3,40	3,40	2,9
Mg^{2+}	0,5	4,3	3,44	3,44	2,3
K^+	0,4	0,3	0,4	0,4	0,3
SO_4^{2-}	1,2	13,1	0,1	0,1	1,0
Trace elementes, mg/L					
H_2SiO_3	5	4,43	9,88	0	9,5
H_3BO_3	0,25	8,39	42,76	0	0,200
Br	8,3	6,7	21,17	0	0,034
J	0,025	1,29	6,62	0	0,004
F	0,95	0,52	0,57	0	0,160
Organic substances, mg/L					
C org	5,0	5,5	34	0	12,8
N org	0,02	0,8	0,14	0	0,33

The day after the completion of the drinking course we determined the plasma levels of the electrolytes: calcium, magnesium, phosphates, chloride, sodium and potassium (both in plasma and in erythrocytes); nitric metabolites: creatinine, urea, uric acid, bilirubin; lipid peroxidation products: diene conjugates and malonic dialdehyde, antioxidant enzymes: superoxide dismutase erythrocytes and catalase plasma, as well as amylase, medium molecular polypeptides and glucose. Most of the listed parameters of metabolism were also determined in daily urine.

The analyzes were carried out according to the instructions described in the manual [3]. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a flamming spectrophotometer "СФ-47".

Digital material is statistically processed on a computer using the software package "Statistica 5.5".

Abstracts are published in the conference materials [17].

RESULTS AND DISCUSSION

Calculations by the formula:

$$|r| \geq \{exp[2t/(n-1,5)^{0,5}] - 1\}/\{exp[2t/(n-1,5)^{0,5}] + 1\},$$

showed that for a sample of 48 animals, the critical value of the modulus of the correlation coefficient |r| for p<0,05 (t>2,0) is **0,28**, for p<0,01 (t>2,7) is **0,38**, for p<0,001 (t>3,5) is **0,48**.

First of all it was found that there is no correlation between the content of organic carbon and nitrogen in liquids (r=-0,12).

It was found (Table 2) that the content in organic carbon fluids is significantly negatively correlated with the concentration in daily urine of diene conjugates and excretion (but not concentration) of magnesium. Instead, the content in organic nitrogen fluids correlates with the excretion (and concentration) of magnesium positively, as well as with the excretion and concentration of uric acid.

Table 2. Matrix of correlations between the content of organic carbon and nitrogen in liquids and metabolic parameters of daily urine after weekly water-salt loads

Concentration	**Corg**	**Norg**
Diene conjugates Urine, E[232]/mL	**-,46**	,03
Magnesium Urine, mM/L	-,24	**,47**
Uric Acid Urine, mM/L	,18	**-,29**
Creatinine Urine, mM/L	-,13	,26
Urea Urine, mM/L	,18	,00
Amylase Urine, mg/h•mL	,22	,08
Malonic Dialdehyde Urine, µM/L	,06	,25
Catalase Urine, nM/h•mL	-,11	-,13
Excretion		
Magnesium Excretion, µM/24h•100 g	**-,33**	**,30**
Uric Acid Excretion, µM/24h•100 g	,02	**-,40**
Creatinine Excretion, µM/24h•100 g	-,18	,03
Urea Excretion, µM/24h•100 g	,10	-,13
Phosphates Excretion, µM/24h•100 g	-,02	-,16

Among the metabolic parameters of the blood (Table 3), only phosphate and diene conjugates are significantly correlated with the content of organic nitrogen in the liquids; the boundary values for malonic dialdehyde, potassium, amylase, chloride and uric acid, as well as sodium red blood cells, are worthy of significance . Instead, no metabolic parameter of blood is significantly correlated with the content of organic carbon in liquids, but it is still worth paying attention to phosphate and diene conjugates.

Table 3. Matrix of correlations between the content of organic carbon and nitrogen in liquids and metabolic parameters of blood after weekly water-salt loads

Level of	**Corg**	**Norg**
Phosphate Plasma, mM/L	-,22	**,29**
Diene conjugates Plasma, E[232]/mL	-,21	**-,28**
Malonic Dialdehyde Plasma, µM/L	,15	-,26
Potassium Plasma, mM/L	-,12	-,26
Amylase Plasma, mg/h•mL	,13	,25
Chloride Plasma, mM/L	-,07	-,22
Sodium Erythrocytes, mM/L	-,10	-,21
Uric Acid Plasma, µM/L	,20	-,20
Calcium Plasma, mM/L	-,18	,02

Magnesium Plasma, mM/L	-,13	-,06
Potassium Erythrocytes, mM/L	-,19	,02
Sodium Plasma, mM/L	-,01	-,18
Creatinine Plasma, µM/L	-,15	,16
Urea Plasma, mM/L	-,07	,16
Glucose Plasma, mM/L	-,19	-,10
Middle Mass Molecules Plasma, units	-,09	-,15
Cholesterol Plasma, mM/L	,14	,10
Superoxide Dismutase Erythrocytes, un/mL	,09	,17

Despite such a structure of the correlation matrix, based on the result of regressive analysis with step-by-step exclusion, some metabolic parameters were included in the model with very small modules of their correlation coefficients with the content of organic carbon (Table 4) and nitrogen (Table 5).

Judging by the coefficients of the multiple correlation, both carbon and nitrogen organic in the composition of liquids have significant effects on the parameters of metabolism of animals loaded by them.

Table 4. Summary of regression analysis with stepwise exclusion of metabolic parameters regarding the content of organic carbon in liquids

Carbon Organic (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(37)}$	p-level
Dependent Variables	r		Intercpt	45,22	24,44	1,85	,072
Diene conjugates Urine, E232/mL	**-,46**	-,302	,122	-8,675	3,497	-2,48	,018
Magnesium Excretion, µM/24h•100 g	**-,33**	,861	,443	3,653	1,879	1,94	,059
Magnesium Urine, mM/L	-,25	-,944	,391	-6,849	2,838	-2,41	,021
Potassium Erythrocytes, mM/L	-,19	-,287	,135	-,575	,270	-2,13	,040
Creatinine Excretion, µM/24h•100 g	-,18	-,618	,263	-1,595	,679	-2,35	,024
Middle Mass Molecules Plasma, units	-,19	-,210	,140	-36,18	24,08	-1,50	,141
Amylase Urine, mg/h•mL	,22	,407	,172	,149	,063	2,37	,023
Uric Acid Plasma, µM/L	,20	,225	,140	,006	,004	1,61	,116
Urea Urine, mM/L	,18	,353	,144	,091	,037	2,46	,019
Amylase Plasma, mg/h•mL	,18	,227	,156	,084	,058	1,45	,154
	R=0,697; R^2=0,486; Adjusted R^2=0,347; $F_{(10,4)}$=3,50; p=0,0025						

Table 5. Summary of regression analysis with stepwise exclusion of metabolic parameters regarding the content of organic nitrogen in liquids

Nitrogen Organic (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(42)}$	p-level
Dependent Variables	r		Intercpt	-,3492	,2666	-1,31	,197
Magnesium Urine, mM/L	**,47**	,345	,134	,0600	,0232	2,59	,013
Creatinine Urine, mM/L	,26	,236	,134	,0431	,0246	1,75	,087
Malonic Dialdehyde Urine, µM/L	,28	,225	,136	,0024	,0015	1,65	,106
Uric Acid Excretion, µM/24h•100 g	**-,40**	-,341	,142	-,0381	,0158	-2,41	,020
Phosphates Excretion, µM/24h•100 g	-,16	,218	,150	,013	,009	-1,46	,152
	R=0,621; R^2=0,385; Adjusted R^2=0,312; $F_{(5,4)}$=5,3; p=0,0008						

At the final stage, the canonical correlation between the content of organic carbon and nitrogen in liquids taken as a factor (argument), and the parameters of the metabolism, taken as a productive feature (function) is analyzed.

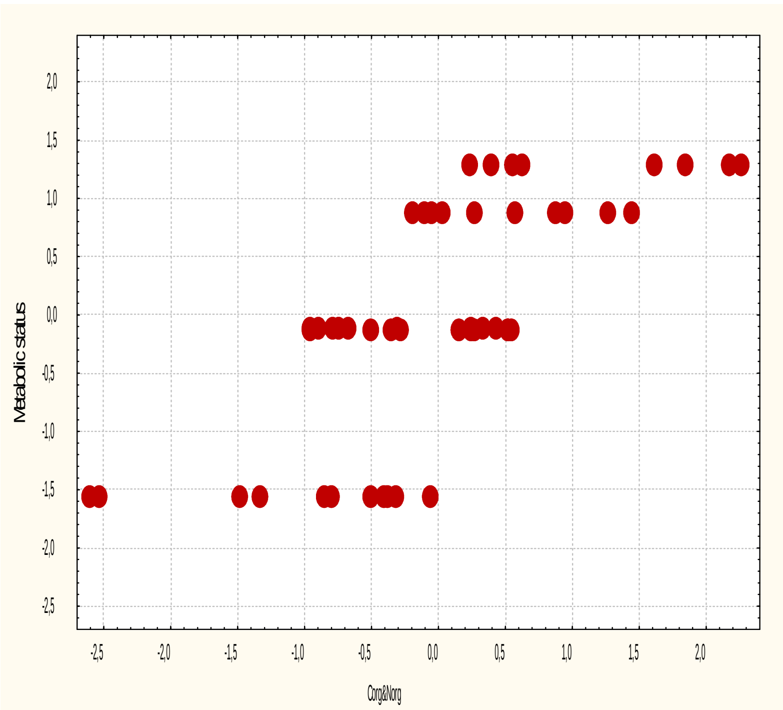
Two equal pairs of canonical roots were found. In this case, the causal canonical root of the first pair, judging by the factor load, represents mainly organic carbon, while the factor load on it from the organic nitrogen is much weaker, but with the same sign (Table 6).

Table 6. The factor structure of the canonical correlation between the first pair of roots that represent the content of organic carbon and nitrogen in liquids and the parameters of the metabolism

Right set	Root 1
Carbon Organic (mg/L)	**-,932**
Nitrogen Organic (mg/L)	**-,245**
Left set	Root 1
Diene conjugates Urine, E^{232}/mL	**,353**
Middle Mass Molecules Plasma, units	**,351**
Creatinine Excretion, μM/24h•100 g	**,296**
Magnesium Excretion, μM/24h•100 g	**,288**
Potassium Erythrocytes, mM/L	**,250**
Uric Acid Excretion, μM/24h•100 g	**,169**
Phosphates Excretion, μM/24h•100 g	**,115**
Magnesium Urine, mM/L	**,094**
Creatinine Urine, mM/L	**,048**
Amylase Plasma, mg/h•mL	**-,390**
Amylase Urine, mg/h•mL	**-,309**
Urea Urine, mM/L	**-,236**
Malonic Dialdehyde Urine, μM/L	**-,234**
Uric Acid Plasma, μM/L	**-,174**

Both organic components of the chemical composition of the loading liquids have a **negative** effect on the level of nine metabolic parameters (concentration in the urine of diene conjugates, magnesium and creatinine, excretion of creatinine, magnesium, uric acid and phosphates, the level of middle mass molecules in plasma and potassium in erythrocytes). Instead, the other five parameters (the activity of amylase in plasma and urine, concentration in urine of urea and malonic dialdehyde, plasma level of uric acid), the influence of organic components is **positive**.

Together, the organic components of the chemical composition of loading fluids determine their effect on the listed parameters of metabolism by 53% (Fig. 1).



R=0,728; R^2=0,530; $\chi^2_{(28)}$=57; p=0,0009; Λ Prime=0,225

Fig. 1. Canonical correlation between the first pair of roots representing the content of organic carbon and nitrogen (X axis) and metabolic parameters (Y axis)

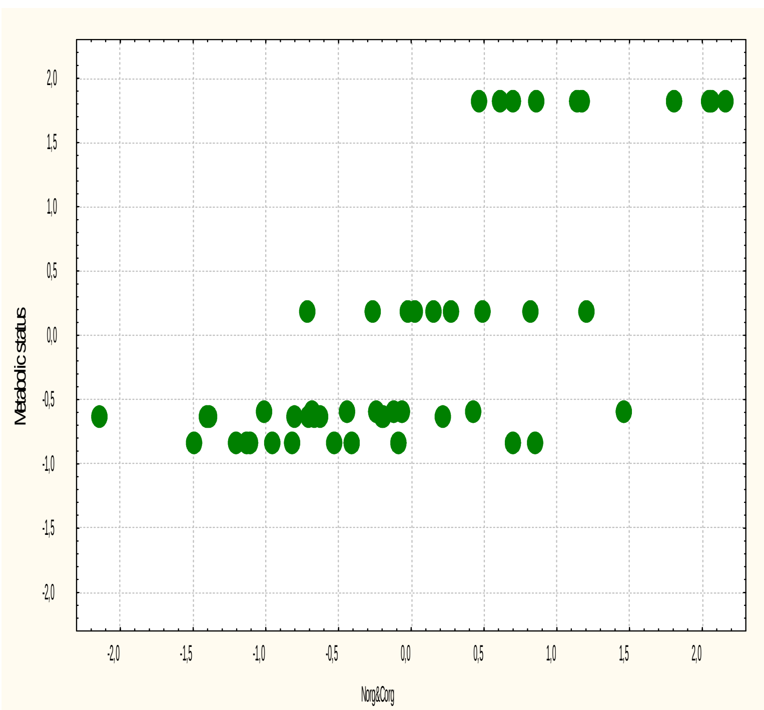
Instead, in the second pair, the causal canonical root is mainly represented by organic nitrogen, and the factor load on it from organic carbon is not only much weaker, but also with the opposite sign (Table 7).

Table 7. The factor structure of the canonical correlation between the second pair of roots that represent the content of organic carbon and nitrogen in liquids and the parameters of the metabolism

Right set	Root 2

Nitrogen Organic (mg/L)	**,970**
Carbon Organic (mg/L)	**-,363**
Left set	Root 2
Magnesium Urine, mM/L	**,690**
Magnesium Excretion, µM/24h•100 g	**,506**
Creatinine Urine, mM/L	**,380**
Malonic Dialdehyde Urine, µM/L	**,340**
Amylase Plasma, mg/h•mL	**,326**
Diene conjugates Urine, E^{232}/mL	**,106**
Potassium Erythrocytes, mM/L	**,085**
Amylase Urine, mg/h•mL	**,072**
Creatinine Excretion, µM/24h•100 g	**,072**
Uric Acid Excretion, µM/24h•100 g	**-,528**
Uric Acid Plasma, µM/L	**-,328**
Phosphates Excretion, µM/24h•100 g	**-,206**
Middle Mass Molecules Plasma, units	**-,188**
Urea Urine, mM/L	**-,063**

Organic components of the chemical composition have opposite effects on individual metabolic parameters (see Tables 4 and 5), and in aggregate, organic matter determines the effect of loading liquids on metabolism by 52% (Fig. 2).



$R=0,722$; $R^2=0,521$; $\chi^2_{(13)}=28$; $p=0,0081$; Λ Prime=0,479

Fig. 2. Canonical correlation between the second pair of roots representing the content of organic carbon and nitrogen (X axis) and metabolic parameters (Y axis)

Consequently, organic substances of mineral waters play an essential role in their effects on the parameters of metabolism. It is obvious that the mediators of the effects are nervous and endocrine systems, which are activated by chemoreceptors of gut mucosa. In its turn, metabolic changes become the cause of other neuroendocrine reactions, that is, the results obtained fit into AI Gozhenko's concept of functional-metabolic continuum [4].

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The carrying out of experiments was approved by the Ethics Committee of the Horbachevskyi Ternopil' State Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

REFERENCES

1. 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.
2. 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.
3. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa: Astroprint; 1998: 608 p.
4. Gozhenko AI. Functional-metabolic continuum [in Russian]. J of NAMS of Ukraine. 2016; 22 (1): 3-8.
5. Gozhenko AI, Zavidnyuk YV, Sydliaruk NI, Mysula IR, Klishch IM, Zukow W, Popovych IL, Korda MM. Features of metabolic reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018; 8(4): 496-518.
6. Gozhenko OA, Zavidnyuk YV, Korda MM, Mysula IR, Klishch IM, Zukow W, Popovych IL. Features of neuro-endocrine and immune reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018; 8(9): 11-31.
7. Ivassivka SV. Biological Active Substances of Water Naftussya, their Genesis and Mechanisms of Physiological Effects [in Ukrainian]. Kyiv: Naukova dumka; 1997: 110 p.
8. 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.
9. Ivassivka SV, Popovych IL, Aksentijchuk BI, Bilas VR. Nature of Balneofactors of Water Naftussya and Essence its Curative and Prophilactive Effects [in Ukrainian]. Truskavets': Truskavets'kurort; 1999: 125 p.
10. 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.
11. Tsarfis PG, Danilova YuYe. Basic Principles of Treatment for Patients at the Resorts of the USSR [n Russian]. Moskva: Meditsina; 1975: 312 p.
12. 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.
13. Yaremenko MS, Yasevich AP, Moiseeva NP. Studying of the nature and forms of migration of nitrogen-containing connections [in Russian]. Mat. 27 Vsesoyuz. Hidrokhim. Soveshch. (Leningrad, May of 1984). Leningrad: Hidrometizdat. 1987: 195.

14. Zavidnyuk YV. Features of metabolic reactions to various water-salt loads in female rats. Experimental and Clinical Physiology and Biochemistry. 2018; 2(82): 21-30.

15. Zavidnyuk YV, Mysula IR, Klishch IM, Zukow W, Popovych IL, Korda MM. General non-specific metabolic, neuroendocrine and immune reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018; 8(3): 513-524.

16. Zavidnyuk YV, Mysula IR, Klishch IM, Zukow W, Popovych IL, Korda MM. Non-specific metabolic, neuroendocrine and immune reactions to various water-salt loads in female rats. In: XVII reading the name of VV Podvysotsky. Bulletin of Sci. Conf. (May 24-25, 2018). Odesa: UkrSRI of Transport Medicine, 2018: 34-36.

17. Zavidnyuk YV, Mysula IR, Korda MM, Klishch IM, Popovych IL. Role of organic carbon and nitrogen of mineral waters in their metabolic effects at female rats. In: Proceedings XI Scientific Conference "Issues of pathology in conditions of extreme factors action on the body" (Ternopil', 4-5 October 2018). Ternopil', 2018: 50-51.