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STATISTICAL ANALYSIS USED IN EVALUATION OF WATER QUALITY FROM WELLS IN ALBA COUNTY, ROMANIA

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Abstract

Drinking water must be health providing, clean, devoided of pathogenic bacteria, viruses or parasites. Wells water should be checked periodically to determine if it complies with European standards, especially when these waters are used by the population. The aim of this paper is to assess the number of aerobic bacteria, nitrites and ammonium content for 7 location (Alba Iulia, Rosia de Secas, Cenade, Sancel, Spring, Ciugud, Ohaba) from Alba County, Romania. The results show that anthropogenic pollution affecting water quality from wells. The microbial growth varies between 42 CFU in source from Ohaba and 440 CFU in Ciugud. Starting from the nitrogen cycle, for the interpretation of the data a simple linear regression was performed. For all the casese, also a multiple regression was conducted to investigate the relationship between chemical parameters and the bacterial growth. A significant correlation between the microbiological growth and its nitrites and ammonium content was observed.

Key words: *aerobic mesophilic bacteria, ammonium, statistics, nitrites, pollution, quality, wells water.*

INTRODUCTION

Potable water of good quality is a social requirement, and is essential for the maintenance and development of life on our planet. Human activities interfere with natural water cycle. Constantly increasing human population increase demands on exploitation of existing resources including water (Chowdhury, 2013). The water from wells is not treated and is often subjected to chemical and microbiological pollution from anthropogenic sources (Khatri and Tyagi, 2015).

The Romanian Law no. 311/2004 (L.311, 2004) witch modify and revise the Law no. 458/2002 (L.458, 2002) on drinking water quality, it is harmonized with the legislation of the European Union-Directive 98/83/EC (Directive 98/83/EC) on the quality of water intended for human consumption. These provide the following limits: TVC/ml<20. Law 458 no longer specifies limits for wells water, but only for drinking water.

In Romania, the interpretation according to STAS 1342 (STAS 1342, 1991) agrees to the following limits: CFU/ml<300. But, from 2006 the analysis on water samples have been performed according to Water Law, no. 458/2006.

The living organisms, the "dead" organic matter, the mineral and organic compounds dissolved in an aquatic ecosystem there are not biologically and chemically inert. They were in permanent transformation. Thus, between the components of the ecosystem are created complex relationships that ensure the evolutionary stability of these components or the dynamic equilibrium of the system.

Nitrogen is an important nutrient in aquatic ecosystems. It is found in water in many forms: molecular nitrogen, nitrogen oxides, ammonia, nitrates and nitrates.

In the ecosystem, nitrogen enters the biogeochemical cycle, determined by a complex network of interactions of factors in the aquatic ecosystem. Algae can use both free nitrogen from water and ammonium salts (NH_3) and after exhaustion, nitrogen (NO_3^-) (Botnariuc and Vadineanu, 1982).

Bacteria have an important role in nitrogen cycle in the aquatic ecosystem and the nitrogen transformations are reversible. The sense of the processes is mainly dependent on the

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concentration of dissolved oxygen (Berard, 1993).
The purpose of the study is to test in time the quality of drinking water from wells in Alba County, Romania. The study results show that the number of mesophilic aerobic bacteria for water (public sources) have values that exceed the limits for potable drinking water and also for nitrates, nitrites content, but only with some minor exceptions. The aim of the study it is also to determine the correlation between these parameters.

MATERIALS AND METHODS

Study Area and Water Sampling Points
The study monitors for 5 years the chemical and microbiological parameters and also correlates the indicators for the wells water. For the purpose of application, the evolution of nitrates and nitrites content and the number of mesophilic aerobic bacteria for several wells water from Alba County were followed quarterly, in order to compare the results and to estimate the correlations between them.
In Figure 1 is presented the map area of Alba County, Romania and the sampling points.

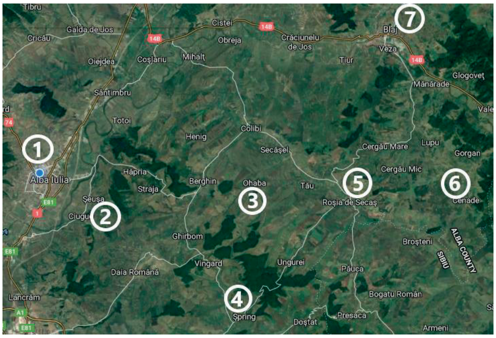

Figure 1. The map of wells water sampling points from Alba County

The locations from Alba County area used for sampling the water are: 1 - Alba Iulia town; 2 - Ciugud village; 3 - Ohaba village; 4 - Spring village; 5 - Rosia de Secas village; 6 - Cenade village; 7 - Sancel village.
The research focused mainly on the areas of county with potential for risk and with a history in terms of water pollution. Water samples were collected from public wells between 2013 and 2017.

Analysis methods
Determination of total number of bacteria growing at 37°C (SR EN ISO 6222/2004; 8199:2008). The method consists in inoculation, by including, of a quantity of 1-2 ml from the sample or decimal dilutions (10-1 and 10-2), into a Petri plate in 10-15 cm^3 nutritive gellose (melted and cooled at 45°C); after the solidification of the gellose the plates are incubated 37 ± 2°C, for 44 ± 4 h. The colonies are counted both those at the surface, and the ones within the gellose.
The methods of rapid spectrophotometric determinations involve the use of the spectrophotometer Spectroquant NOVA 60 (SQ) and SQ specific kits (with reagents and reaction tubes). Following the work pattern from the kit, we read the SQ. The value appears on the screen (Metoda diagnostic medical veterinar, 2004). Ammonium: Kit SQ domain 0.010-2 mg/l NH_4-N or 0.01-2.58 mg/l. It is drop 0.5 ml from the sample in the reaction tube and homogenised. Is added a dose of NH_4-1K, closed, shake, and, after 15 min. readed. In high alkaline solutions, the nitrogen ammonia is present almost totally as ammonia, reacts with hypochlorite ions resulting in monochloramine, which reacts with substitute phenol and forms a blue indocarbolitic derivative. Nitrites: Kit SQ 0.02-1.00 mg/dm^3 NO_2-N or 0.07-3.28 NO_2- NO_2 10 mm tube. 5 ml of sample are drop in the tube. Is added a micro pallet knife of NO_2 -1 reagent and shake until total dissolution. Reaction time: 10 minutes. In acid solution, nitrite ions react with the sulphanilic acid resulting diazonium compound; which then reacts with N-1-naftiletilendiamine dihydro-chloride resulting in a violet red nitro compound.

Statistical analysis
By using the program MATLAB, the experimental data were processed and analysed (Nichici and Cicala, 1996; Vardeman, 1994; Aloman, 1998), obtaining a series of statistic models pointing out the variation of the LogCFU in samples depending on their NH_4^+, respectively NO_2^- content. At first (Iordache et al., 1991), in one variable, a linear function was proposed as follows (1):

$$y = a + b \cdot x \quad (1)$$

respectively a multiple regression (2):

$$y = a + b \cdot x_1 + c \cdot x_2 + d \cdot x_3 \quad (2)$$

where: y - LogCFU/ml;
x_1 - amonium content, mg/l;
x_2 - nitrites content, mg/l
x_3 - time, month.

As indicators of the model adequacy there have been used: the indicator of the precision of the model, R^2 and the correlation coefficient, R (Todinca and Geanta, 1999; Evans, 1996; Gluck, 1977).

RESULTS AND DISCUSSIONS

In the Tables 1, 3, 5, 7, 9, 11 and 13 are presented the results of the tests performed for wells water collected from the four sources of Alba County.

Thus, the experimental results obtained for the well water samples taken quarterly from Alba Iulia, between 2013 and 2017, are presented in Table 1.

Table 1. Experimental results obtained for the water sampled from Alba Iulia

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	168	0.19	0.21
	124	0.21	0.2
	320	0.37	0.35
	136	0.11	0.1
2014	106	0.04	0.1
	360	0.30	0.50
	302	0.50	0.50
	167	0.29	0.16
2015	201	0.14	0.49
	140	0.10	0.24
	232	0.24	0.50
	154	0.10	0.24
2016	120	0.12	0.14
	149	0.29	0.24
	150	0.45	0.31
	148	0.39	0.27
2017	142	0.09	0.52
	194	0.52	0.11
	175	0.38	0.51
	157	0.34	0.42
*NV**	*100*	*0.50*	*0.50*

NV - Normal values according L. 458/2002*

The microbial load of water-analysed samples exceeds permissible limit value from all investigated source from Alba Iulia. The excesses are recorded every year and regardless of the season. The highest recorded value of TVC is 360 colonies in second quarter of 2014, and the highest value of nitrites and ammonium ions is 0.52 mg/l, in 2017. The equations of statistical models obtained are presented in Table 2. The equation is valid on the studied values range. They show the dependence of the microbiological load (y) on the ammonium content (NH_4^+)-x_1, respectively on the nitrite content (NO_2^-)-x_2, for the source monitored in Alba Iulia.

Table 2. The equations and concordance indicators of the established statistical models for the samples from Alba Iulia

Year	The equations	R^2	R
2013	$y = 1.5871 \cdot x_1 + 1.8981$	0.77	0.88
	$y = 1.4997 \cdot x_2 + 1.9094$	0.77	0.88
2014	$y = 1.0941 \cdot x_1 + 1.9764$	0.93	0.97
	$y = 1.03 \cdot x_2 + 2.0301$	0.63	0.80
2015	$y = 0.6612 \cdot x_1 + 2.0076$	0.92	0.96
	$y = 1.3886 \cdot x_2 + 2.0492$	0.82	0.90
2016	$y = 0.6023 \cdot x_1 + 2.0051$	0.86	0.93
	$y = 0.2924 \cdot x_2 + 2.0583$	0.80	0.90
2017	$y = -0.2328 \cdot x_1 + 2.3105$	0.58	0.76
	$y = 0.3121 \cdot x_2 + 2.116$	0.91	0.95

After calculating the model coefficients, it is necessary to make a comparison between model predictions and data from the actual process. The adequacy indicators show satisfactory correlation between the variables considered.

For the multiple regression analysis of the water samples from Alba Iulia the generated equation is (3):

$$Y_{(x1, x2, x3)}=2.047+0.553 \cdot x_1+0.531 \cdot x_2-0.004 \cdot x_3 \quad (3)$$

with concordance indicators: R^2=0.73, R=0.86 (strong correlation).

For the samples taken quarterly in the period 2013-2017 from Rosia de Secas the experimental results are presented in Table 3.

Table 3. Experimental results obtained for the water sampled from Rosia de Secas

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	142	0.06	0.24
	250	0.3	0.29
	297	0.33	0.5
	96	0.16	0.17
2014	250	0.18	0.38
	157	0.07	0.11
	206	0.15	0.4
	149	0.09	0.29
2015	106	0.12	0.31

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
	286	0.3	0.5
	254	0.25	0.43
	165	0.16	0.34
2016	150	0.16	0.37
	315	0.34	0.5
	240	0.27	0.42
	110	0.23	0.34
2017	150	0.21	0.29
	269	0.5	0.39
	320	0.5	0.5
	186	0.46	0.42

From Table 3 it is notice a considerable increase of mesophilic aerobic bacteria in all periods of year, especially in the second and third quarters of each year. The highest value of 320 colonies is recorded in the third quarter of 2017. Not all values obtained are within the limits allowed by law (100 colonies) for drinking water.

Table 4 presents the equations obtained from the linear regression for the water from Rosia de Secas.

Table 4. The equations and concordance indicators of the established statistical models for the samples from Rosia de Secas

Year	The equations	R^2	R
2013	$y = 1.3818 \cdot x_1 + 1.8368$	0.76	0.87
	$y = 1.4339 \cdot x_2 + 1.9466$	0.64	0.80
2014	$y = 0.5652 \cdot x_1 + 2.1035$	0.51	0.71
	$y = 1.9809 \cdot x_2 + 2.0276$	0.93	0.97
2015	$y = 2.1322 \cdot x_1 + 1.4338$	0.89	0.94
	$y = 2.3119 \cdot x_2 + 1.7963$	0.94	0.97
2016	$y = 2.8245 \cdot x_1 + 1.123$	0.93	0.97
	$y = 2.1045 \cdot x_2 + 1.7479$	0.60	0.78
2017	$y = 1.4264 \cdot x_1 + 1.7745$	0.69	0.83
	$y = 0.89 \cdot x_2 + 1.9735$	0.69	0.83

The correlation coefficients indicate a good correlation of the experimental values and the allure of the proposed equations.

For the multiple regression analysis for the parameters of the water samples from Rosia de Secas the generated equation is (4):

$$y_{(x1,x2,x3)}=1.926+0.867 \cdot x_1+0.637 \cdot x_2-0.004 \cdot x_3 \quad (4)$$

with concordance indicators: R^2=0.70, R=0.84 (strong correlation).

The water from Cenade area is less polluted. The results obtained for the chemical and micro-biological parameters for the period 2013-2017 are presented in Table 5.

Table 5. Experimental results obtained for the water sampled from Cenade

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	89	0.09	0.34
	93	0.12	0.21
	250	0.31	0.46
	156	0.17	0.38
2014	146	0.08	0.25
	261	0.19	0.47
	292	0.24	0.71
	298	0.25	1.8
2015	104	0.31	0.54
	293	0.38	5.4
	204	0.29	1.6
	96	0.19	0.29
2016	146	0.32	0.35
	260	0.63	0.45
	126	0.25	0.26
	53	0.17	1.2
2017	94	0.35	0.51
	262	0.27	3.71
	140	0.34	0.26
	89	0.42	0.34

Table 6 presents the equations obtained from the linear regression for the water source from Cenade.

Table 6. The equations and concordance indicators of the established statistical models for the samples from Cenade

Year	The equations	R^2	R
2013	$y = 1.7064 \cdot x_1 + 1.5342$	0.71	0.84
	$y = 2.1094 \cdot x_2 + 1.7634$	0.94	0.97
2014	$y = 0.136 \cdot x_1 + 2.2703$	0.41	0.64
	$y = 1.8485 \cdot x_2 + 2.0289$	0.97	0.99
2015	$y = 0.0896 \cdot x_1 + 2.0186$	0.82	0.91
	$y = 2.2837 \cdot x_2 + 1.526$	0.59	0.77
2016	$y = -0.5196 \cdot x_1 + 2.3946$	0.61	0.78
	$y = 1.2668 \cdot x_2 + 1.6671$	0.80	0.89
2017	$y = 0.1157 \cdot x_1 + 1.9823$	0.80	0.90
	$y = -3.1888 \cdot x_2 + 3.2219$	0.82	0.91

For the multiple regression analysis for the parameters of the water samples from Cenade the generated equation is (5):

$$y_{(x1,x2,x3)}=2.053+0.075 \cdot x_1+0.972 \cdot x_2-0.007 \cdot x_3 \quad (5)$$

with concordance indicators: R^2=0.48, R=0.69 (moderate positive correlation).

The microbiological and chemical results obtained for the water samples taken quarterly from Sancel, from 2013-2017, are presented in Table 7.

Table 7. Experimental results obtained for the water sampled from Sancel

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	88	0.09	0.33
	93	0.11	0.21
	251	0.31	0.47
	155	0.17	0.38
2014	146	0.08	0.25
	262	0.19	0.47
	292	0.25	0.70
	298	0.25	1.80
2015	104	0.31	0.54
	293	0.38	5.4
	203	0.29	1.60
	96	0.19	0.29
2016	145	0.32	0.35
	261	0.63	0.46
	126	0.25	0.26
	54	0.17	1.20
2017	94	0.35	0.51
	261	0.27	3.71
	140	0.34	0.26
	90	0.41	0.33

Table 8 presents the equations obtained from the linear regression for the water source from Sancel.

Table 8. The equations and concordance indicators of the established statistical models for the samples from Sancel

Year	The equations	R^2	R
2013	$y = 2.476 \cdot x_1 + 1.5144$	0.57	0.76
	$y = 1.1526 \cdot x_2 + 1.6234$	0.74	0.86
2014	$y = 1.6555 \cdot x_1 + 1.605$	0.98	0.99
	$y = 20.249 \cdot x_2 - 3.4066$	0.57	0.76
2015	$y = 0.7672 \cdot x_1 + 2.1343$	0.67	0.82
	$y = 0.7332 \cdot x_2 + 2.2707$	0.84	0.92
2016	$y = 1.2209 \cdot x_1 + 1.763$	0.63	0.79
	$y = 1.12 \cdot x_2 + 1.8764$	0.65	0.81
2017	$y = 1.1545 \cdot x_1 + 1.7141$	0.90	0.95
	$y = 0.9346 \cdot x_2 + 1.8038$	0.81	0.90

The concordance indicators prove a satisfactory correlation between the variables considered.

For the multiple regression's analysis for the parameters of the water samples from Sancel the generated equation is (6):

$$y_{(x1,x2,x3)}=1.675+1.406 \cdot x_1+0.353 \cdot x_2-0.002 \cdot x_3 \quad (6)$$

with concordance indicators: $R^2=0.68$, $R=0.82$ (strong correlation).

The microbiological and chemical results obtained for the water samples taken quarterly from Spring in the period 2013-2017 are presented in Table 9.

Table 9. Experimental results obtained for the water sampled from Sancel

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	189	0.04	0.2
	204	0.02	0.5
	196	0.04	0.32
	185	0.09	0.15
2014	161	0.15	0.49
	290	0.19	1.2
	143	0.02	0.3
	145	0.08	0.28
2015	237	0.23	0.5
	298	0.3	2.17
	159	0.15	0.5
	98	0.09	0.32
2016	124	0.31	0.37
	230	0.55	1.7
	128	0.34	0.28
	167	0.38	0.34
2017	206	0.09	0.45
	242	0.04	0.5
	198	0.1	0.54
	162	0.15	0.24

Table 10 presents the equations obtained from the linear regression for the water source from Spring.

Table 10. The equations and concordance indicators of the established statistical models for the samples from Spring

Year	The equations	R^2	R
2013	$y = 0.1189 \cdot x_1 + 2.2516$	0.98	0.99
	$y = -0.5329 \cdot x_2 + 2.3117$	0.73	0.85
2014	$y = 0.3364 \cdot x_1 + 2.0556$	0.99	0.99
	$y = 1.5555 \cdot x_2 + 2.0754$	0.65	0.80
2015	$y = 0.1789 \cdot x_1 + 2.1043$	0.54	0.73
	$y = 2.2699 \cdot x_2 + 1.8234$	0.97	0.98
2016	$y = 0.161 \cdot x_1 + 2.088$	0.78	0.89
	$y = 1.1272 \cdot x_2 + 1.751$	0.94	0.97
2017	$y = 0.4294 \cdot x_1 + 2.1153$	0.64	0.80
	$y = -1.5857 \cdot x_2 + 2.4516$	0.99	0.99

The concordance indicators show an acceptable correlation between the variables considered.
For the multiple regression analysis of the water samples from Spring the generated equation is (7):

$$y_{(x1, x2, x3)}=2.202+0.194\cdot x_1-0.290\cdot x_2-0.0002\cdot x_3 \quad (7)$$

With concordance indicators: $R^2=0.49$, $R=0.70$ (moderate positive correlation).
The results obtained for the water samples taken quarterly from Ciugud during the period 2013-2017 are presented in Table 11.

Table 11. Experimental results obtained for the water sampled from Ciugud

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	248	0.19	0.46
	210	0.24	0.42
	208	0.28	0.41
	167	0.29	0.29
2014	158	0.3	0.24
	252	0.29	0.43
	268	0.28	0.5
	242	0.29	0.48
2015	196	0.24	0.34
	158	0.17	0.27
	244	0.3	0.47
	109	0.11	0.16
2016	204	0.08	0.41
	430	0.5	0.62
	301	0.42	0.53
	224	0.09	0.21
2017	315	0.34	0.59
	440	0.5	0.55
	210	0.31	0.6
	97	0.2	0.13

Nitrites are found due to the pollution of water with organic matter, or by partial oxidation of the amino radical or by reducing nitrate. Their presence indicates a more back pollution of water, but with higher concentrations of ammonia (maximum 0.62 mg/l in second quarter of 2016) shows that the pollution is continuous.
Table 12 presents the equations obtained from the linear regression for the water source from Ciugud.

The concordance indicators prove a good correlation between the variables considered. For the multiple regression's analysis for the parameters of the water samples from Ciugud the generated equation is (8):

$$y_{(x1,x2,x3)}=1.939+0.795\cdot x_1+0.307\cdot x_2-0.0002\cdot x_3 \quad (8)$$

with concordance indicators: $R^2=0.76$, $R=0.87$ (strong correlation).

Table 12. The equations and concordance indicators of the established statistical models for the samples from Ciugud

Year	The equations	R^2	R
2013	$y = 0.9367\cdot x_1 + 1.9444$	0.95	0.97
	$y = -1.3611\cdot x_2 + 2.6546$	0.77	0.88
2014	$y = 0.8545\cdot x_1 + 2.0005$	0.94	0.97
	$y = -11.474\cdot x_2 + 5.6804$	0.80	0.89
2015	$y = 1.1302\cdot x_1 + 1.8786$	0.97	0.98
	$y = 1.7816\cdot x_2 + 1.8637$	0.97	0.98
2016	$y = 0.6663\cdot x_1 + 2.1481$	0.65	0.81
	$y = 0.6337\cdot x_2 + 2.2703$	0.90	0.95
2017	$y = 1.0523\cdot x_1 + 1.8708$	0.71	0.84
	$y = 2.1436\cdot x_2 + 1.6392$	0.88	0.94

The results obtained for the water samples taken quarterly from Ohaba, from 2013-2017, are presented in Table 13.

Table 13. Experimental results obtained for the water sampled from Ohaba

Year	Microbiological	Chemical	
	CFU/ml	NO_2^-, mg/l	NH_4^+, mg/l
2013	42	0.08	0.24
	190	0.22	0.29
	256	0.28	0.42
	136	0.24	0.36
2014	240	0.34	0.42
	280	0.42	1.4
	210	0.29	0.5
	167	0.24	0.34
2015	201	0.29	0.46
	295	1.14	2.27
	269	0.5	0.75
	201	0.22	0.37
2016	196	0.02	0.49
	410	0.49	1.9
	237	0.5	0.54
	259	0.28	0.42
2017	240	0.5	0.38
	320	1.4	1.02
	157	0.42	0.35
	116	0.34	0.24

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The microbiological parameter in correlation with chemical value registered in Ohaba village show that the risk due to consumption of groundwater is significant. The water source exceed the limits allowed by legislation for the number of mesophilic aerobic bacteria - in particular.
Table 14 presents the equations obtained from the linear regression for the water source from Ohaba.

Table 14. The equations and concordance indicators of the established statistical models for the samples from Ohaba

Year	The equations	R^2	R
2013	$y = 3.46 \cdot x_1 + 0.9778$	0.63	0.79
	$y = 3.8135 \cdot x_2 + 1.3292$	0.93	0.96
2014	$y = 0.15 \cdot x_1 + 2.2433$	0.61	0.78
	$y = 1.2111 \cdot x_2 + 1.9525$	0.95	0. 98
2015	$y = 0.081 \cdot x_1 + 2.2985$	0.69	0.83
	$y = 0.1817 \cdot x_2 + 2.2788$	0.76	0.88
2016	$y = 0.1755 \cdot x_1 + 2.2763$	0.84	0.92
	$y = 0.4133 \cdot x_2 + 2.29$	0.47	0.68
2017	$y = 0.4635 \cdot x_1 + 2.0558$	0.71	0.84
	$y = 0.3268 \cdot x_2 + 2.0691$	0.69	0.83

Overall, the concordance indicators show a satisfactory correlation between the variables considered.
For the multiple regressions analysis for the parameters of the water samples from Ohaba the generated equation is (9):

$$y_{(x1,x2,x3)}=2.095+0.173 \cdot x_1+0.073 \cdot x_2-0.002 \cdot x_3 \quad (9)$$

with concordance indicators: $R^2=0.39$, $R=0.62$ (weak correlation).

CONCLUSIONS

The results obtained evidence that anthropogenic pollution affect water quality from wells in the Alba County, Romania. All activities taking place on the surface have an impact on groundwater quality.
The equations of statistical models can approximate microbiological growth in wells water knowing its nitrites content, or ammonium ion ant the time of the year when the sample was collected. Correlation parameters calculated based each on other arguments a satisfactory capacity of prediction for the statistical models. Also, the model predictions can constitute a control criterion for assessing groundwater quality.

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