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IMPACT OF ENVIRONMENTAL FACTORS ON THE QUALITY OF SPRING WATER FROM ABRUD-CÂMPENI AREA, ALBA COUNTY, ROMANIA

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Abstract

Water is one of the most important natural resource, a social requirement and an important factor in the ecological balance. Water from springs is good for direct consumption if the soil is not contaminated. The population, who is not benefiting from the centralized water, is supplied in different area from springs. Water from springs is good for direct consumption if the area around groundwater is not contaminated. Groundwater pollution, caused by human activities related to the agriculture, industry and population growth, is a current problem with serious consequences to human health. The aim of this paper was to assess the physico-chemical and microbiological qualities of springs water from Abrud-Câmpeni area, Alba County, Roamnia. The water samples were taken in 2018 from 11 functional springs. Of these, only three sources are potable, the rest of the springs are microbiologically contaminated. The average concentration of NO_3^- in the investigated springs from Abrud-Câmpeni area is 6.1 mg/l. Monthly, between January and December 2018, the variation of the nitrate content in the water from the spring Lucia (Sohodol village) was also monitored. Fluctuations were observed with maximum in the cold season (February), 4.37 mg/l, and minimum in the summer (August), 2.14 mg/l. In order to identify the factors that lead to the variation of the NO_3^- content in water, statistical mathematical models were developed. The parameters studied were: atmospheric temperature, the amount of precipitation, respectively the freeze/thaw cycle. The concordance indicators demonstrated a satisfactory correlation between the variation of those factors and the variation of the NO_3^- content in water.

Key words: *spring water, quality water, contaminants, nitrates, statistical analysis.*

INTRODUCTION

Under current conditions, the water resource at planetary level has become increasingly expensive because of human pressure. Therefore, maintaining drinking water quality is increasingly important (Smuleac et al., 2017).

In different areas, that do not have distribution of potable water through public supply systems, the springs and wells that represent sources of groundwater are very important. This water is not treated and is often subjected to chemical and microbiological pollution from anthropogenic sources (Jamshidzadeh and Mirbagheri, 2011; Navarroand Carbonell, 2007).

In the Abrud-Câmpeni area, the springs still represent a very important source of groundwater because it is free. Drinking water from the springs is a new approach that has been generated by the idea that it is healthier than water from public system. Water from springs is generally good for direct consumption if the area around groundwater is not contaminated.

Nitrate has been one of the dominant forms of increased nitrogen loading since the 1970s (Diseand Wright, 1995; Smith et al., 1999; Vitousek et al., 1997). Nitrogen, nutrient of great importance in aquatic ecosystems, enter in several ways and is found in water in many forms: molecular nitrogen, nitrogen oxides, ammonia, ammonium, nitrites and nitrates. Consequently, nitrogen biochemistry involves the consideration of all nitrogen oxidation states, from 5 to 0 and -3 (Lupea, 2003). In ecosystem, nitrogen enters in the biogeochemical cycle, determined by a complex of interactional factors from aquatic

ecosystems. Algae can use the free nitrogen from water and salts ammonia and after their exhaustion even the nitrate (Botnariuc and Vădineanu, 1982).
Previous studies have shown that the quality parameters of underground water may exhibit large variations due to natural conditions and anthropogenic activities (Zereg et al., 2018; Bhurtun et al., 2019). According to Zereg et al., NO_3^- concentration is influenced by rainfall events. Naclerio and the collaborators have been studied the influence of freezing and freeze/thaw cycles on the survival of microorganism in soil, in a climate change perspective. Their results revealed that in a climate change perspective, taking into account also the local trend in air temperature, a different distribution of microbial pollution over time is expected in spring waters, and a higher risk of transmission of infections is expected throughout the year (Naclerio et al., 2012).
The quality of drinking water in Romania, is regulated by Law no. 311/2004 who complete the Law no. 458/2002. These laws transpose the Directive 98/83/EC of drinking water quality. The European community requires that drinking waters should be analysed for several bacteriological parameters as total coliforms/100 ml water, *Streptococus faecal*/100 ml, *E. coli* and *Pseudomonas aeruginosa* (Bushatia et al., 2009).
The present paper seeks ways of testing the quality of drinking water from springs. The study results show the values of physico-chemical and microbiological parameters for water, in the Abrud-Câmpeni area. Microbiological indicators exceed the limits for potable drinking water in most investigated sources. The work also aims to monitor the nitrates content of Lucia spring water in time and to establish the influence of different atmospheric parameters using mathematical models.

MATERIALS AND METHODS

Study Area and Water Sampling Points

The Abrud-Câmpeni area is situated in a depression, with an altitude of 600 m, surrounded by mountains (Anghel and Anghel, 2006). In the area there are meadows, coniferous and deciduous forests. The main rivers crossing the area are the Aries River and Abrudel River. The climate is moderate continental, with milder shades in small intramontane depressions. Annual average temperatures are between 2-6°C. Winters are generally frosty and long, with the average cold season being within the isotherm of -3 and -6°C. Frosty days are between October and April and range from 100 to 150 days. Annual average precipitation ranges from 900-1100 mm/year. The amount of precipitation in the form of snow is significant and the duration of it is about 4 months a year.
In Figure 1 is presented the map of Abrud-Câmpeni area, Alba County, Romania and the springs used for sampling the water.

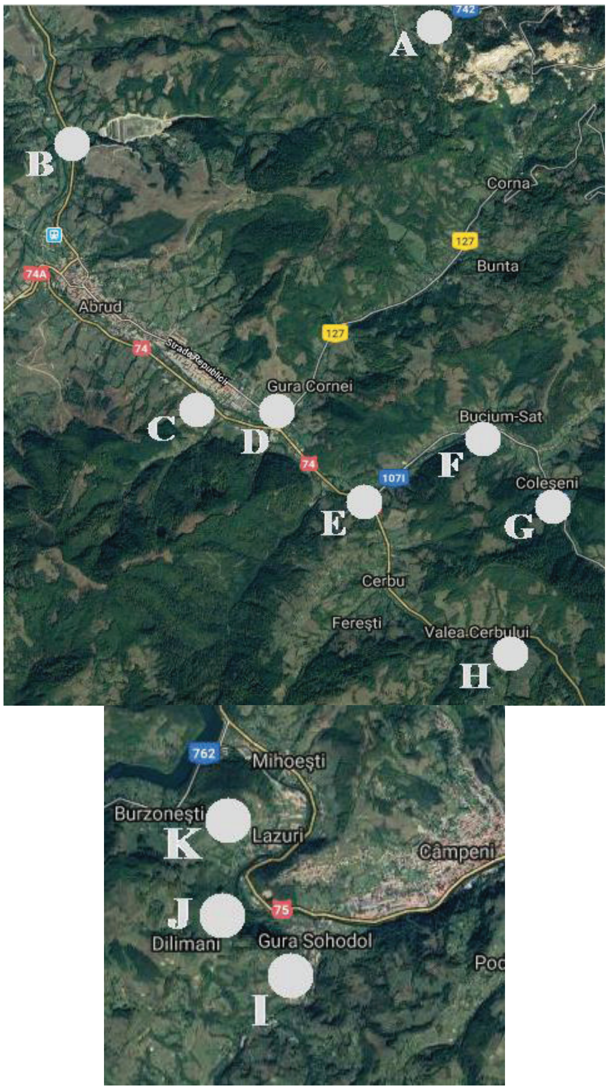


Figure 1. The map of sampling -Abrud-Câmpeni area, Alba County

In Figure 1, the springs from Abrud area used for sampling the water were: A – Roşia

Montana, B - Valea Săliştei, C - Bâdea, D - Gura Cornei, E - Ciuta, F - Bucium, G - Coleşeni, H - Dealu Mare. The springs from Câmpeniare were: I - Lucia-Sododol, K - Toplite, J - Detunata.

In 2018, eleven samples of spring water from the Abrud-Câmpeni area, Alba County, were analyzed. All samples were taken during the same period of the year - March. Previously, in 2018, the water from Lucia spring, Sohodol commune, was tracked monthly for NO_3^- (mg/l) content.

Analysis methods

The pH values were obtained using the InoLab pH-meter. Water samples were analyzed as such, while soil samples were dried for 7 days at 40°C, sieved to less than 2 mm in a plastic sieve and ground to a fine powder using an agate ball mill. Soil samples, treated as describe above, were mixed with potassium chloride solution 1 mol·L^{-1} in a 1:5 (g:ml) ratio and shacked for 15 min before measuring (SR ISO 10390,1999).

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 in 2 seconds (IDSA, 2004).

Ammonium: Kit SQ, domain 0.010-2 mg/l NH_4-N or 0.01-2.58 mg/l. 0.5 ml from the sample is drop in the reaction tube and homogenised. A dose of NH_4-1K is added, closed, shake, and, after 15 min read. In high alkaline solutions, the nitrogen ammonia is present almost totally as ammonia, reacts with hypochlorite ions resulting in monochloramine, which reacts with substitute fenol and forms a blue indocarbolitic derivative.

Nitrates: Kit SQ, domain 1.0-50.0 mg/dm^3 NO_3-N or 2.2 - 79.7 mg/dm^3 NO_3^-. 0.5 ml of test is putted in the tube. 1 ml NO_3-1K is added, shacked for a minute and let aside for 10 minutes. In sulphuric acid solution nitrate ions react with a derivative of benzoic acid to form a red nitro compound.

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. A micro palette knife of NO_2 -1 reagent is added 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.

Determination of total number of bacteria growing at 37°C, 22°C (mesophyll) (SR EN ISO 6222:2004). 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.

Detection and counting of *Escherichia coli* and coliform bacteria Part 1: Membrane filtration method (SR EN ISO 9308-1, 2004, SR EN ISO 7889-2,2002). 100 ml from the water sample are filtered through a membrane settled on lactose TTC agar: the incubation is made at 36±2°C, 21±3 h. It is count as lactoso-positive bacteria the colonies developing on yellow medium. The confirmation: a) *Oxidase test*: 10 characteristic colonies are passed on nonselective agar (TSA) and incubate at 36±2°C, 21±3h; then, from each Petri plates it is take one colony and put it on filtering paper impregnated with oxidase reagent. Positive reaction: the occurence of purple blue coloration in 30 sec. b) *Indole test*: is make simultaneous inseminations in tryptophane broth, which incubates at 44°C, 21±3 h, then is added 0.2-0.3 ml of Kovac's reagent. Positive reaction: the occurrence of a purple red ring. The colonies with negative reaction to oxidase are counted as being *coliform bacteria*. The colonies with negative reaction to the oxidase and positive reaction with the indol are counted as being *E. coli*.

Identification and counting of intestinal enterococci Part 2: Membrane filtration method (SR ISO 21528-1/2, 2004; SR EN ISO 6887-1, 2002). 100 ml or 10 ml from the sample and/or dilutions is filtered through the membrane placed on Slanetz Bartley medium and incubated at 36±2°C, 44±4 h. The typical colonies are pink or brown, in the middle or totally. We transfer them on bile-esculin-azide agar, pre-heated at 44°C. The incubation conditions are: 44°C, for 2 h. The characteristic colonies which are bronze-black are counted, and the calculus method from SR EN ISO 8199:2008 is applied. Note: The preparation of culture medium is performed according to *The Guide of Preparation and Obtaining the culture medium.*

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 in 2 seconds (IDSA, 2004).

For isolation and counting of *Pseudomonas aeruginosa* in water samples, 250 ml of sample was filtered. The membrane was introduced on selective growth medium consisting of acetate as a source of carbon and ammonium sulfate as a source of nitrogen and incubated at 37°C for 48 hours (SR EN 12780, 2003; SR EN ISO 8199, 2008).

RESULTS AND DISCUSSIONS

The results of physico-chemical and microbiological analyzes of the samples taken from the 11 springs located in Abrud-Câmpeni area, Alba County, Romania are presented in Tables 1 and 2. These are compared to the maximum admissible values provided by the national and European legislation in force. The quality of drinking water is regulated by Law no. 458/2002 (transposing the Directive 98/83/EC on the quality of drinking water), respectively by H.G. no. 1020/2005 regarding the Technical Norms for exploitation and commercialization of natural mineral waters (HG 1020, 2005).

Table 1. Result of physico-chemical and microbiological analyzes of the samples taken from spring waters located in the Abrud area

Source of water / Parameter	A	B	C	D	E	F	G	H
pH	7.07	6.67	6.77	7.63	7.39	7.19	6.93	6.55
NO_2^- [mg/l]	<0.02	<0.02	< 0.02	<0.02	0.05	<0.02	<0.02	<0.02
NO_3^- [mg/l]	1.5	1.5	9.0	2.8	21.0	0.4	22.0	0.4
NH_4^+ [mg/l]	<0.025	<0.025	0.2	<0.025	0.37	<0.025	0.15	<0.05
UFC/ml at 22°C	3	87	1	Absent	**120**	1	**160**	**190**
UFC/ml at 37°C	**23**	**23**	abs	4	8	**22**	**25**	**22**
Total coliforms/100 ml	absent	absent	absent	Absent	absent	absent	absent	absent
E. coli/100 ml	absent	absent	absent	Absent	absent	absent	absent	absent
Enterococi/100 ml	**4**	**4**	absent	**2**	absent	absent	absent	absent
P. aeruginosa	absent	absent	absent	Absent	absent	absent	absent	absent

Table 2. Result of physico-chemical and microbiological analyzes of the samples taken from spring waters located in the Câmpeni area

Source of water / Parameter	I	K	J	Maximum allowed values - according to law 458/2002 for bottled water	Maximum allowed values for mineral waters according to government decision 1020/2005 - at source
pH	7.14	7.06	7.21	6.5÷9.5	according to the natural state of the water
NO_2^- [mg/l]	<0.02	<0.02	<0.02	0.50 mg/l	0.1 mg/l
NO_3^- [mg/l]	3.34	3	2.14	50 mg/l	50 mg/l
NH_4^+ [mg/l]	<0.025	<0.025	<0.025	0.50 mg/l	0.50 mg/l*
UFC/ml at 22°C	absent	absent	absent	100	20
UFC/ml at 37°C	absent	4	2	20	5
Total coliforms/100 ml	absent	**70**	absent	absent	absent
E. coli/100 ml	absent	**50**	absent	absent	absent
Enterococi/100 ml	absent	absent	absent	absent	absent
P. aeruginosa	absent	absent	absent	absent	absent

*Valid value considering ammonium as an indicator of water pollution from surface sources. As long as the endogenous origin of ammonium is scientifically demonstrated, values up to 5 mg/l can be accepted.

It has been found that in the mountain area, the spring water located in different areas – Abrud and Câmpeni - have different but close values. Data obtained from the investigation of the quality of spring waters are aimed at detecting specific impurifiers, areas and sources of pollution.

The physico-chemical parameters of the spring waters are within the limits of the potability. However, exceedances of the admissible limits for microbiological parameters are observed in the analyzed water samples. Thus, only the Bâdea, Lucia - Sohodol and Detunata springs are drinkable.

In order to test the factors that can lead to variation in the NO_3^- content of spring waters, the Lucia Spring from the Sohodol commune was selected. The sampling was carried out on a regular basis, so the monitoring of water quality from Lucia - Sohodol source was done monthly in 2018, and the nitrate content was measured. The results are shown in Table 3.

In the mean time, in order to identify the meteorological factors (monthly during 2018) that may lead to the variation of the NO_3^- content of water, the average monthly values of those factors for Romania, Abrud-Câmpeni area were used (average temperature, amount of precipitation, frosty days, sunny days) (Meteoblue, 2019).

Table 3. Content of nitrate in the Lucia - Sohodol spring water and the meteorological parameters of the area

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
NO_3^- [mg/l]	3.68	4.37	3.16	3.34	3.15	3.15	2.71	2.14	3.21	2.83	3.34	3.52
Average temperature [°C]	0	4	13	21	23	26	30	29	23	15	8	1
Amount of precipitation [mm]	53	50	60	87	116	124	100	88	74	59	56	64
Frosty days	25.6	22.3	16.3	3	0.1	0	0	0	0	3	11.1	23.5
Sunny days	4.2	3.3	4.4	4	3.8	3.9	7.4	8.7	6.9	9.5	6.4	5.4

The value of NO_3^- content during one year has not exceeded the value of 44 mg/l, recommended by the World Health Organization standard or the 50 mg/l limit set by European and national legislation.

Further, the experimental results were subjected to a multiple correlation analysis to compare the effect of the studied parameters on the nitrate content of the spring waters.

The investigated parameters were: monthly average temperature, monthly amount of precipitation, frosty days, sunny days.

Using Matlab, statistical models were generated. The equations of the models are as follows:

$$y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2$$

where:

a_0, a_1 and a_2 - model coefficients;

y - NO_3^- content [mg/l];

x_1 - time [months];

x_2 - a) monthly average temperature [°C];

b) monthly amount of precipitation [mm];

c) monthly frosty days [days];

d) monthly sunny days [days].

By performing the statistical correlation, the equations from Table 4 were obtained:

Table 4. Equations of mathematical statistical models

Type of x_2 variable	Equation
a)	$y = 4.1083 – 0.0495 \cdot x_1 – 0.0354 \cdot x_2$
b)	$y = 4.3779 - 0.0612 \cdot x_1 - 0.0098 \cdot x_2$
c)	$y = 3.1387 - 0.0335 \cdot x_1 + 0.0339 \cdot x_2$
d)	$y = 4.2736 + 0.0187 \cdot x_1 - 0.2083 \cdot x_2$

The equations above are valid on the studied values range. After calculating the model coefficients, it is necessary to make a comparison between model predictions and data from the actual process.

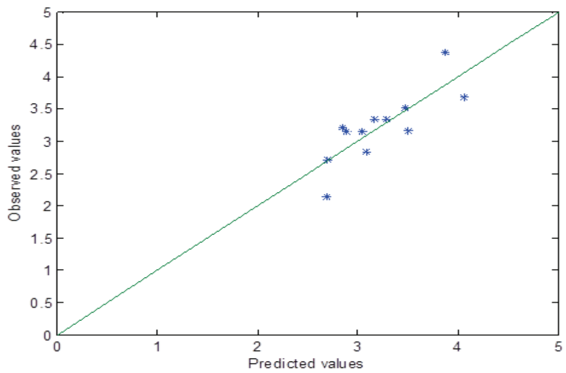
The values of adequacy indicators are presented in Table 5.

Table 5. Indicators of adequacy of statistical models determined

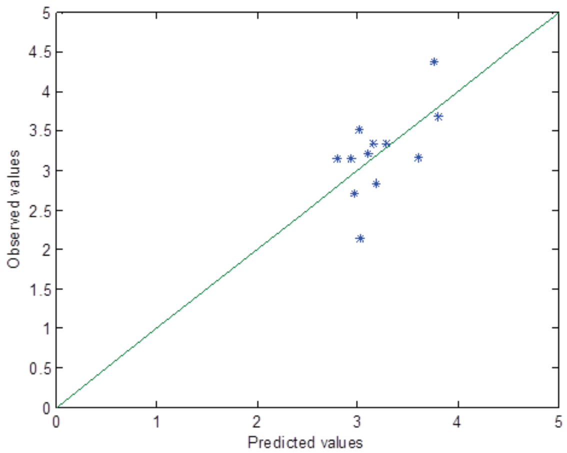
Situation	Variance, σ^2	Standard deviation, σ	Correlation coefficient, R
a)	0.1015	0.3186	0.8099
b)	0.1848	0.4299	0.6111
c)	0.1318	0.3631	0.7438
d)	0.1408	0.3753	0.7229

The values of adequacy indicators show satisfactory correlation between the experimental values and those generated by the first-order polynomial model.

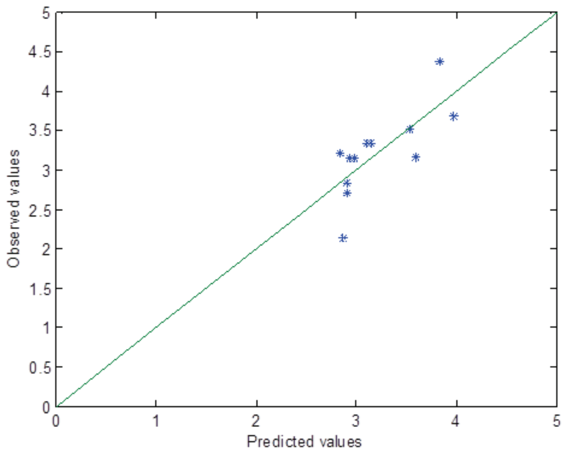
The experimental results versus the theoretical ones are presented in Figure 2.



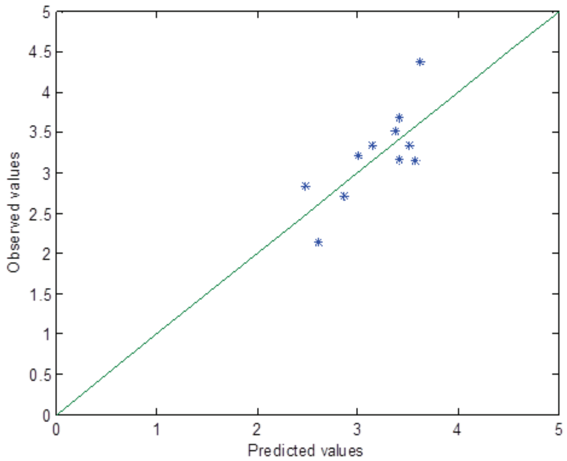
a) monthly average temperature



b) monthly amount of precipitation



c) monthly frosty days



d) monthly sunny days

Figure 2. Correlation between the experimental data and computed values

The disposal of the experimental results and of those obtained based on the statistical mathematical models (y=x), correlations presented in Figure 2, confirm the satisfactory precision of the generated models.

CONCLUSIONS

The chemical parameters of waters from the investigated springs are in the legal limits, but the microbiological indicators in some of the spring waters sources are higher than the maximum allowed limits. These results indicate a poor hygienic quality of certain spring water sources and the need for permanent monitoring in order to identify possible contamination sources.

Variations of NO_3^- content at Lucia spring source were observed during time – monthly, for a year. Thus, although the current nitrate levels is safe for the moment, in the future, the nitrate concentration could exceed the drinking limit due to anthropogenic pollution. In addition, the equations of statistical models can approximate the nitrates content in the water from Lucia spring knowing the month of the year when the sample was collected and the weather conditions (monthly average temperature, monthly amount of precipitation, monthly frosty days, monthly sunny days). Correlation parameters show a satisfactory prediction capacity of the mathematical statistical models. Also, the model predictions can constitute a control criterion for assessing the quality of water from springs.

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