Chemical Bulletin of "Politehnica" University of Timisoara, ROMANIA
Series of Chemistry and Environmental Engineering
Chem. Bull. "POLITEHNICA" Univ. (Timisoara) *Volume 58(72), 1, 2013*

Study on the Quality of Spring Waters from Ocna Mures Area, Romania

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Abstract: Seven water samples were collected from different springs located in Ocna Mures area, Romania. The physico-chemical (pH, chlorides, hardness, acidity and alkalinity) and microbiological (aerobic bacteria, coliform bacteria, *Escherichia coli* and *enterococci*) parameters of waters were measured. The quality of water from Banta spring, which was found to be the most polluted source from bacteriological point of view, was monitored for a period of three years. The parameters measured were: total number of germs, *E. coli*, *enterococci*, pH, nitrate, nitrite and ammonium ions. The results obtained surpass the permissible values establish for potable water, especially for microbiological parameters, which indicates a poor hygienic quality of drinking water and allow the identification of possible contamination sources.

Keywords: spring water, microbial and physico-chemical analysis, quality, Ocna Mures area.

1. Introduction

Springs are the places where ground water is discharged at specific locations on the earth and they vary dramatically as to the type of water they discharge. Many of the springs are the result of long cracks or joints in sedimentary rock [1].

The use of spring water is attributed largely to factors such as non availability in urban areas, impression that high quality natural spring water and drinking water offer a healthy, refreshing and great tasting alternative to water provided by public service and also, is free.

Underground waters are an important resource, taking into account that, unlike surface waters, they are usually less polluted or even unpolluted [2]. But water in nature is never pure. Given the interactions with the environment, it contains gases, mineral and organic substances, dissolved in suspension. Drinking water must be health providing, clean, without microorganisms, parasites or substances which, by number or concentration, can be a potential hazard for human and animal health [3].

Today, water quality issues have become a significant concern due to the growth of population, urban expansion and technological development [4]. Erginkaya and Var (1997) in a study on microbial quality of bottled spring waters from Turkey had stated that coliform bacteria were found in 12 of the 130 bottles of spring waters analyzed [5].

Contamination of groundwater by Cl^- is a common occurrence in growing urban areas [6, 7]. Sources of Cl^- are related to human activities include road salt, effluent from industrial facilities, leachate from municipal landfills, effluent from private and municipal septic systems and some agricultural chemicals. Natural sources include rock-water interactions, salt mine, saline seeps and minor atmospheric contributions [8].

The polluted water is a source of infections and parasitic infestations not only for animals but for people too, and the bacterial pathogens that are transmitted through water to humans and animals cause bacterioses. The drinking water quality, is set by Law no. 311/2004 who complete the Law no. 458/2002 (in Romania), these laws transposing the Directive 98/83/EC. This regulation is only for bottled water, the spring or wells waters are not explicitly covered by the legislation. However, in addition, Romanian legislation also provides limits for mineral water at the source.

The present paper seeks ways of testing the quality of drinking water, especially spring waters. The study results show that the organoleptic, physico-chemical and microbiological parameters of spring waters from Ocna Mureş area have values that exceed the limits for potable drinking water.

2. Experimental

In order to determine the spring waters quality from Ocna Mures area, organoleptic, physico-chemical and microbiological parameters of seven public sources were determined. The analyses were conducted in November 2012.

The pH values were obtained using the InoLab pH-meter. The amount of total hardness present in the given sample of water was estimate by EDTA titration method [9]. The acidity and alkalinity (A_T) was determinate using method ASTM D1067 – 11 [10]. SR ISI 9297:2001 method was used for the determination of chloride content.

The method use to determine the total number of bacteria growing at 37°C and 22°C (mesophyll) [11,12] consists in inoculation of 1-2 mL sample in 10-15 cm^3 nutritive gellose (melted and cooled at 45°C). The cultures were realised in Petri dishes. After solidification of the gellose, the plates were incubated 37±2°C, for 44±4 h. The colonies were 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 [13]: 100 mL of water sample were filtered through a membrane settled on lactose TTC agar and incubated at 36±2°C, 21±3 h. The colonies developing on yellow medium were counted as lactoso-positive bacteria. The confirmation: a) Oxidase test: 10 characteristic colonies were passed on nonselective agar (TSA) and incubated at 36±2°C, 21±3h. Then, from each Petri plates it was taken one colony and put it on filtering paper impregnated with oxidase reagent. The occurence of purple blue coloration in 30 sec was considered as a positive reaction. b) Indole test: simultaneous inseminations in tryptophane broth ware made and incubated at 44°C, 21±3h. After that, 0.2-0.3 mL of Kovac's reagent was added. The occurrence of a purple red ring was considered a positive reaction. The colonies with negative reaction to oxidase were counted as being *coliform bacteria*. The colonies with negative reaction to the oxidase and positive reaction with the indol were counted as being *E. coli*.

Identification and counting of intestinal *enterococci*. Part 2. Membrane filtration method [14, 15]: 100 mL or 10 mL from the sample and/or dilutions were filtered through the membrane placed on Slanetz Bartley medium and incubated at 36±2°C, 44±4h. The typical colonies are pink or brown, in the middle or totally. They were transfered on bile-esculin-azide agar, pre-heated at 44°C and incubated at 44°C, for 2h. The bronze-black colonies were conted and the calculus method from SR EN ISO 8199: 2008 was applyed.

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, the SQ was read [16].

Ammonium: Kit SQ domain 0.010-2 mg/ dm^3 NH_4-N or 0.01-2.58 mg/ dm^3. 0.5 ml from the sample was droped in the reaction tube and homogenised. A dose of NH_4-1K was added. The tube was closed, shake, and after 15 min. the value was 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 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 sample was putted in the tube. 1 ml NO3-1K was added. The tube was shaked 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 were droped in the tube. A micro pallete knife of NO_2 -1 reagent was 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.

3. Results and Discussion

The organoleptic test results of spring waters (method EN 1622/2007) show that the samples are characterized as a clear liquid, colorless, without particles and without sediment impurities. The samples don't have foreign taste or smell, except samples taken from Banta spring, which were liquids slightly opalescent with suspended impurities (solids).

The values of physico-chemical and microbiological parameters of the seven spring waters samples collected from the Ocna Mures area are presented in tables 1 and 2.

The pH values of the seven sources analyzed lies within the range stipulated by law: greater than or equal to 6.5, respectively less than or equal to 9.5.

All analyzed samples have a permanent hardness greater than 5 German degrees (°dH), the lowest value being for the water coming from Uioara spring with 10°dH permanent hardness and 29.6°dH total water hardness (calculated as a sum of the molar concentrations of Ca^{2+} and Mg^{2+}). The highest hardness was identified in the Caprioara source water with 39.94 °dH (permanent hardness).

In terms of chloride content, the concentrations are relatively high in some water sources. The values obtained exceeded the limit recommended for drinking waters - mg/L.

Spring water samples from Ocna Mures area were negative for the presence of microorganisms, except spring Banta and Cisteiu of Mures, where was identified a microbiological growth exceeding the maximum allowed by law. The faecal strepotococci (enterococcus) and coliforms were present only in spring water taken from source Banta (the values obtained were compared with the limits for bottled drinking water). The total number of germs that grow at 22 °C, respectively 37 °C exceeded the limits stipulated in Romanian Law no. 458/2002 with amendments, in the waters of the two springs.

For spring Banta a microbial growth of $3.5x10^2$, respectively $1.5x10^2$ colony forming units was obtain and for the spring Cisteiu of Mures: $1.20x10^2$ and 23 colonies respectively.

TABLE 1. Physico-chemical parameters of spring waters considered

Parameter / Spring source	pH	Cl^-(mg/L)	Hardness, °dH			AT (mg/L)	Acidity (mg/L)
			Temporary	Permanent	Total		
Razboieni	7.4	114.25	9.24	22.76	32.3	6	3.3
Noslac	7.1	55.30	18.2	21.5	39.7	14.5	6.5
Uioara	7	252.96	19.6	10	29.6	10.5	7
Unirea	7.1	202.25	10.64	24.36	35	12.4	3.8
Banta	7.2	175.10	13.72	14.98	28.7	5	4.9
Caprioara	6.9	53.88	7.56	39.94	47.5	11	2.7
Cisteiu de Mures	7	256.20	5.04	20.08	25.13	7	1.8

TABLE 2. Microbiological parameters of spring waters

Parameter / Spring source	TNG/mL		Total coliforms /100 mL	E.coli/ 100 mL	Enterococci /100 mL
	22^0C	37^0C			
Razboieni	4	abs	abs	abs	abs
Noslac	22	abs	abs	abs	abs
Uioara	1	abs	abs	abs	abs
Unirea	20	abs	abs	abs	abs
Banta	**$3.5x10^2$**	**$1.5x10^2$**	**12**	abs	**4**
Caprioara	25	1	abs	abs	abs
Cisteiu de Mures	**$1.20x10^2$**	**23**	abs	abs	**2**
Normal values	**100/ml**	**20/ml**	**abs**	**abs**	**abs**

Statistical analysis. Chloride (Cl^-) parameter, which describes the level of groundwater salinity from Ocna Mures area, is impossible to be calculated directly from experimental determinations made all around Ocna Mures. Therefore, that parameter can be approximated on the basis of isolated experimental measurements. Given that the test results are not identical, it is possible to calculate the confidence interval (probability), chosen according to the desired accuracy.

Thus, having a number of seven determinations for chlorides parameter in springs from Ocna Mures area, it is possible to calculate the confidence interval which included the actual media content of Cl^-. The threshold value was chosen to be $\alpha = 0.050$.

Following the calculation, the arithmetic average value $\bar{x} = 165.948$ and the standard deviation $s = 95.576$ was obtained.

For $\nu=7-1=6$ degree of freedom and for $\alpha = 0.050$, the critical value of t-distributions found in tables is $t_{0.05}=2.447$. Thus, we will have:

$$\left(165.948 - 2.447\frac{95.576}{7};\quad 165.948 + 2.447\frac{95.576}{7}\right)$$

Therefore, the average content of chlorine ions in spring waters from Ocna Mures area is in the range [132.538, 199.358]. The chosen 95% value of confidence interval confirms these values.

Time evolution of the physico-chemical and microbiological parameters of water from the spring Banta. In figures 1 – 7 the evolution in time of physico-chemical and microbiological parameters of spring water samples collected from the Banta source (near the town Ocna Mures) are presented.

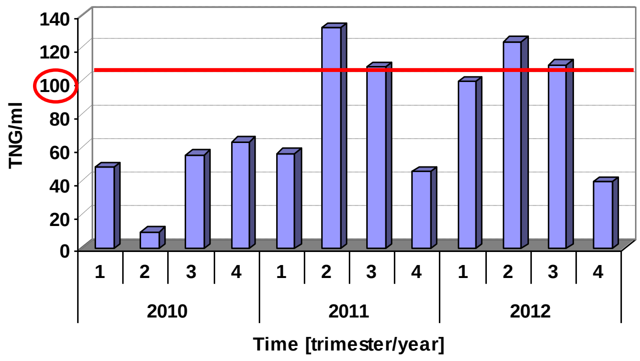

Figure 1. The variation of total number of germs for the period 2010-2012 in the Banta spring water

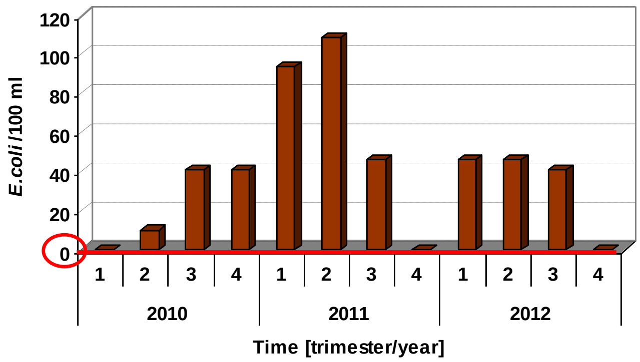

Figure 2. The variation of *E. coli* in samples taken in the period 2010-2012 from the Banta spring water

From the graphical representation of aerobic mesophilic bacteria evolution in sample taken from Banta spring, it can be notice an increase of number of colonies in time (during years). The two and third quarter of the year (summer – autumn), come with a significant increase of microorganisms in spring waters that exceed the limit of 100 CFU/mL allowed by law for drinking water. The maximum value is 132 colonies recorded in the second quarter of year 2011.

Except three samples, all the other were positive for the presence of the microorganism - *Escherichia coli*, which indicate the contamination of source due to their poor planning. Springs should be constructed in a way that protects against contamination with surface waters and against entering of rodents and insects. Also the soil around the spring must be sloped so that rain water cannot enter in the spring.

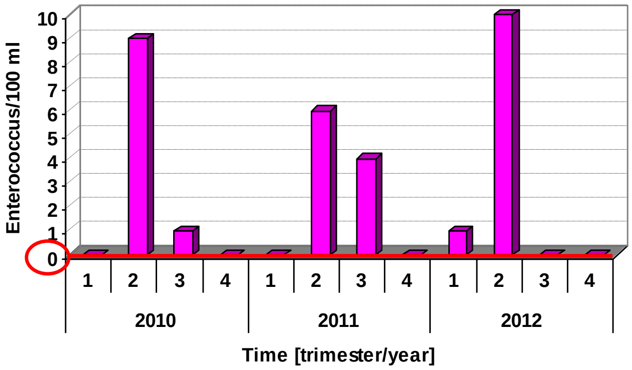

Figure 3. The variation of *enterococci* in samples taken in the period 2010-2012 from the Banta spring water

Enterococci were identified in the spring Banţa. Measured values, exceeding the limits stipulated in Law no. 458/2002, were found in the two and third quarter of the studied period (2010-2012). However, some intestinal *enterococci* isolated from the water may occasionally also originate from other habitats, including soil, in the absence of faecal pollution.

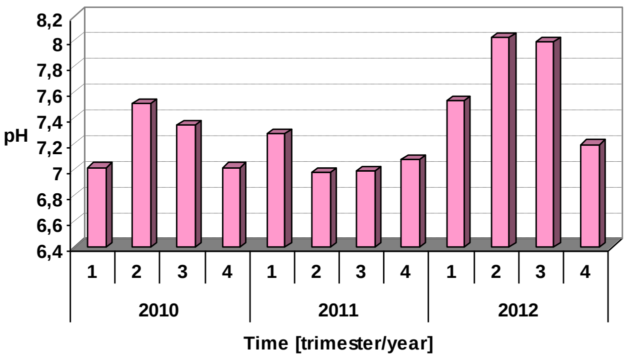

Figure 4. The variation of pH values in samples collected in the period 2010-2012 from Banta spring water

The pH values were between 6.98 and 8.03 and falled within the permitted ones (6.5-9.5) for drinking water.

Nitrogen is found in water in many forms: molecular nitrogen, nitrogen oxides, ammonia, ammonium, nitrites and nitrates. So nitrogen biochemistry involves the consideration of all its oxidation states, from 5 to 0 and -3 [17].

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 (NH_3) and after their exhaustion even the nitrate (NO_3^-) [18].

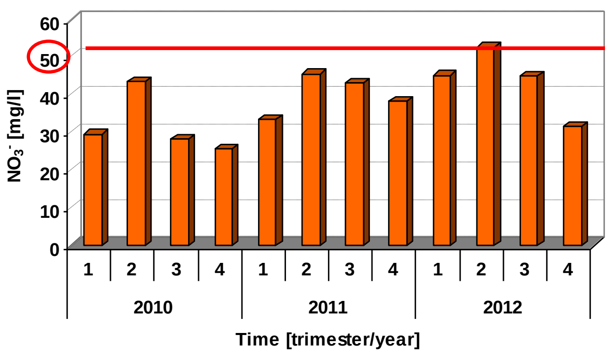

Figure 5. The variation of nitrates in samples taken in the period 2010-2012 from the Banta spring water

In the studied period, the nitrates content doesn't exceed the legal value for drinking water of 50 mg/L. Exception is the sample collected in the second quarter of 2012, for which the value is 52.76 mg/L.

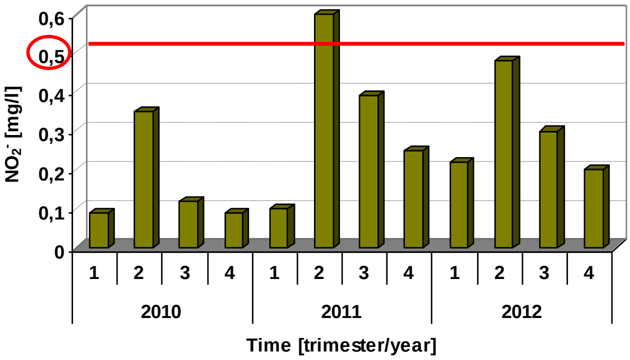

Figure 6. The variation of nitrites in samples taken in the period 2010-2012 from the Banta spring water

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 an old pollution of water and, together with higher concentrations of ammonia, shows that the pollution is continuous.

In terms of nitrites content, in the second quarter of 2011, an amount that exceeds the maximum limit allowed by law was recorded. Otherwise, the samples analyzed had values under the 0.5 mg/L.

Ammonia occurs after water pollution by organic substances that undergo decomposition, the first step of nitrogenous substances degradation. It's presence indicates recent pollution.

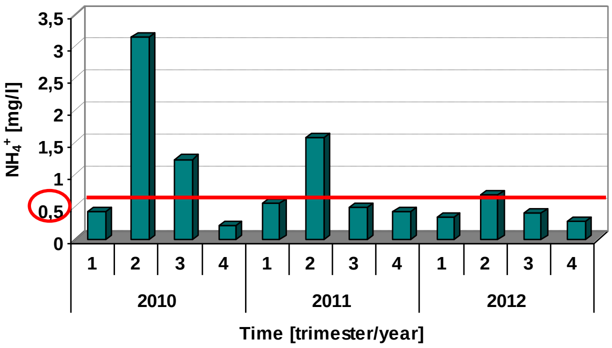

Figure 7. The variation of ammonium ions in samples taken in the period 2010-2012 for the Banta spring water

The values of ammonium ions in the spring water taken from Banta source exceed the permissible limit in quarters II and III of the studied period. Overall, the values correspond to the values established for drinking water in Directive 98/83/EC (Law no. 458/2002 completed by Law no. 311/2004)

4. Conclusions

The main source of environmental pollution in the Ocna Mures area is the brine. Underground mining of salt in the past and lack of maintenance for the old galleries can lead to surface and groundwater contamination. The dispersion range that fits chloride content in spring waters from investigated area is [132.538; 199.358] with a probability of 95 %.

The test results of the spring water samples collected from seven points of Ocna Mures area indicate that one or more parameters are above the maximum allowed by law. For most spring waters the values of measured parameters are within the limits of potability.

The results of laboratory tests prove that anthropogenic pollution affects spring water quality from Ocna Mures area. There is a particular major contamination at Banta Cisteiu Mures spring waters. All activities take place at the surface have an impact on groundwater quality.

The study shows that the risk due to consumption of spring water from Ocna Mures area is reduced, but not completely. There are water sources whose parameters exceed the limits allowed by law, especially for microbiological parameters.

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Received: 16 January 2013
Accepted: 15 February 2013