

Microbiological Groundwater Quality from Alba County

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Received: 02 October 2009; Accepted: 12 November 2009

Abstract

The paper synthesizes the results of microbiological analysis performed on drinkable water ingathered from wells, in low areas, hills and mountains, all in Alba County. The regulations regarding the procedures of work, interpretation and calculus for drinkable water have been under permanent changing, and thus the paper is structured in two time periods: 2003 – 2005, and 2006 – 2007. Following the microbiological analysis performed on the ingathered samples, within the two time periods, there can be seen that the drinkable water samples (from wells) are microbiologically polluted, regardless of the source, and consequently, life of animals and humans is submitted to hydric decay.

Keywords: potable water, quality, aerobic mesophilic bacteria, coliform bacteria, *E.Coli*, *Enterococcus*, pollution

1. Introduction

Water in nature is never pure; given the interactions with the environment, it contains gases, mineral and organic substances, dissolved in suspension [1]. Quality water (drinking water seen as food) „must be health providing, clean, devoided of microorganisms, parasites or substances which, by number or concentration, can be a potential hazard for human health [2,3].

The voluntary management of private wells is a problem because most health-related pollutants in water are symptomless. As a result, homeowners with private water supplies may be exposed unknowingly to health related pollutants unless they voluntarily have their water tested for the correct water quality parameters [4].

The importance of water to our bodies can be synthesized as follows: vital element with a plastic role, absorption, diffusion and excretion environmental place, contributing to maintain the basic body invariables; transporter for plastic and energetic substances, and the way of removing the residual compounds of metabolism. The assessment of water quality is performed through various parameters determination (physical, chemical, microbiological) with legal limits specification: water thus must not be polluted. Water pollution is though constant [5].

Biological pollution of water occurs by residual waters from households, hospitals, human and veterinary clinics, laboratories, slaughterhouses, factories [6-8].

Polluted water represents a source of infection and parasite infestation not only for animals, but also for humans [9], and water transmitted pathogenic bacteria lead to bacteriosis both in humans and in animals.

E.coli can be transmitted through water consumption and gives gastro-enteritis and sepsis in children and various species of young animals [8]. The incidence of *E. coli* is about 17.5% in well water, surviving in drinkable water from wells for as long as six months, and in sewer water for four months [6]. A severe gastro-enteritis transmitted through water occurred in England in 1980, due to flaws in the sewerage system, which allowed drinkable water contamination [7].

Due to the fact that distinguishing the presence of pathogenic agents involves costly analysis, there have been found some sanitary indicators to allow indirect assessment of epidemiologic hazard [5]. Those microbiologic parameters are: 1) *Aerobic mesophyll germs* [CFU/ml], aerobic bacteria which develop on gellose within 24-48 hours. The bigger number of CFU/ml, the higher the risk of pathogenic agents in

the water (bacteria, viruses, fungi, parasitical agents), 2) *Coliform bacteria* such as: *E.coli*, *Citrobacter*, *Klebsiella*, *Enterobacter*. The importance of distinguishing them resides in the fact that their survival rate in water is close to the non-sporulated pathogenetical germs. *E.coli* presence in the water signifies recent and thus dangerous water pollution. If there are live coliform bacteria in the water, along with those there might also be other pathogenetic microorganisms or parasitical agents, 3) *Enterococcus faecalis* (*Streptococcus*) can be found in feces, less numerous than coliform bacteria, but also more resilient in the environment [5].

Polluted water consumption leads to the state of illness, and imposes more and more obvious, the protection of water quality.

2. Material and methods

Within 2003-2007 interval there have been examined 1127 water samples from wells, from 5 sanitary veterinary districts, and from 57 animal farms in Alba county. The water has been sampled seasonal, 2-4 samples a year in every area. The water has been sampled in different seasons, and from wells more or less well kept.

The microbiological analysis on water samples by 2006 [10,11]:

a. Detection of total number of bacteria growing 37°C (mesophyll). A volume of 1 cm³ from the homogenised sample and (10⁻¹ and 10⁻²) dillutions are introduced into a Petri plate and 10-15 cm³ nutritious gellose (melted and cooled at 45°C) are added; the content is than homogenised and, after the solidification of the gellose the plates are put into the incubator, lid down, and incubated 37±0.5°C, for 48 h. The colonies are counted both those at the surface, and the ones within the gellose, and the plates containing more than 300 colonies are removed.

When dilutions have not been used, the result is expressed by the number of colonies refered to the vlowme on 1 cm³ submitted to work. If dilutions have been used, the calculus for the total number of bacteria refered to 1 cm³ of water is done with the formula:

$$\text{Total number of mesophyll bacteria} = \frac{\sum (n \cdot d)}{N \cdot V} \quad [\text{CFU} / \text{cm}^3]$$

N-the number of Petri plates taken into consideration; *n*-the number of colonies developed in a single Petri plate; *d*-the revers of the dilution of the sowing sample; *V*-the volume of the water sample in cm³.

b. Determination of the probable number of coliform bacteria (total coliforms); multiple tube method of by distinguishing through the presumption test, sowing water and/or deciman dilutions in a number of vials with liquid enriched medium, the positive reaction being distinguished by a confirmation test on a solid medium at 37±0.5°C, after 24 h. From the number of confirmed positive tubes we calculate, using tables, the probable number of coliform bacteria.

c. Detection of probable number of thermotolerant coliforms (fecal coliforms) going from positive vials in the presumption test for total coliforms through confirmation in selective liquid medium at 44±0.5°C in 24 ore. Taking into consideration the number of positive tubes at 44±0.5°C we calculate, using the tables, the probable number of thermotolerant coliform bacteria (fecal coliforms).

d. Determination of probable number of fecal streptococcus; method of multiple tubes through the presumption test, sowing the sample and/or decimal dilutions in a number of vials with liquid enriched medium at 37±0.5°C, the positive reaction being distinguished through confirmation test in liquid selective medium at 44±0.5°C, for 24 h or on a solid selective medium at 44±0.5°C for de 48 h. Starting from the number of positive confirmed tubes, we calculate, using tables, the possible number of fecal streptococcus.

The interpretation according to STAS 1342 agrees to the following limits: CFU/ml < 300; Total coliforms/100cm³ < 10; Fecal coliforms/100cm³ < 2; Fecal streptococcus /100cm³ < 2.

From 2006 the analysis on water samples have been performed according to Water Law no. 435/2002:

a. Counting of culture microorganisms. Colony counting through sowing in agar medium at 37°C [12]. The method consists in the inoculation, in a specific medium (agar with yeast) in Petri plates, of a quantity of 1-2 ml sample or decimal dilutions. The estimation of colonies forming units (CFU)/ml is performed by direct counting, after incubation at 36±2°C, for 44±4 h [13].

b. Detection and counting of Escherichia coli and coliform bacteria Part 1: Membrane filtration method [14] The water sample is filtered through a membrane, followed by incubation of the membrane on selective medium (TTC) and by the biochemistry characterization of typical lactoso-positive colonies, which leads to the detection and counting of coliform bacteria *E. coli*. Estimation: we count all the colinies

which give negative reaction with the oxidase as being coliform bacteria. We count all the colonies which have a negative reaction with the oxidase and positive reaction with the indole as *E. coli* [13].

c. *Identification and counting of intestinal enterococcus Part 2 Membrane filtration method* [15]. The method is based upon water filtration on a membrane with sodium azide (to inhibit the development of Gram-negative bacteria) and chloride of 2,3,5- trifeniltetrazolium, colourless, which is reduced to red formazan by the intestinal enterococcus. The typical colonies are pink or brown in the middle or all over.

Confirmation: we transfer the membrane with colonies on azide-esculina-bile agar, preheated at 44°C. Intestinal enterococcus hydrolyse the esculine in two hours. The final product, 6,7-dihydroxicumarin,

combines with iron ions to give a bronze to black compound, which spreads into the medium.

Interpretation of the results and calculus: the presence of bronze-black colonies indicates the presence of intestinal enterococcus within the sample; we count all the characteristic colonies and apply the calculus method in SR EN ISO 8199: 2008.

3. Results and discussion

During the 2003-2005 period there have been analysed 775 water samples. From the total of the analysed samples 503 have proven positive reactions for the investigated parameters. The samples with positive results have been grouped in categories after the place of origin. That showed that in 22 from 54 places/animal farms, water is microbiologically polluted having at least one outrun parameter.

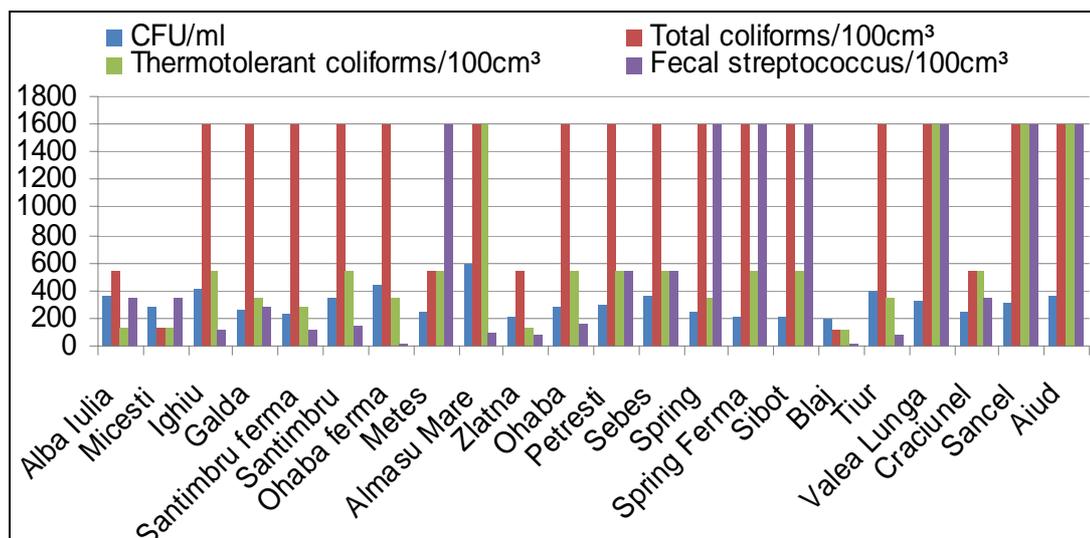


Figure 1. The weight of microbiological parameters for the studied samples between 2003-2005

Figure 1 present the results of the analysis performed. Thus one can see outrun limits in the water samples analysed as follows: UFC/ml up to 600 in Almasu Mare – mountain area, as well as in Alba Iulia, Ighiu, Sântimbru, Ohaba, Sebeș, Tiur, Valea Lungă, Sâncel, Aiud.

Total coliforms/100cm³ and Thermotolerant coliforms/100cm³ have values within 109 and 1609. The highest values are registered in Ighiu, Galda, Sântimbru, Ohaba, Almasu Mare, Petrești, Sebeș, Spring, Șibot, Tiur, Valea Lungă, Sâncel and Aiud.

Following the analysis for *Fecal streptococcus*/100cm³ values between 23 and 1609 are registered, meaning that the allowed values have been outrun in 65% of analysed waters.

The second work stage is the one between 2006-2007. The work methodology interpretation of results and the calculus impose the compliance of regulations in Water Law no. 458/2002 supplemented with Law no.311/2004. The results, according to the new regulations, are expressed just by reporting CFU/ml, *E. coli* and *Enterococcus*/100ml.

The indicator total coliform bacteria, which importance has been undelined, is no longer used, although durind the test stages for *E.coli* all the steps of analysis must be covered.



Figure 2. Coliform bacteria; multiple tube method - positive reaction

Law 458 no longer states the limits for well water, but for drinkable water, and the maximum allowed limit for CFU este „lowered” from 300 to 20 colonies, with the same work method as in STAS 3001. If we refer this interpretation to the past years, than the

percentage of positive samples, CFU/ml would have been higher during 2003-2005.

352 water samples have been examined under the aspect of their microbiological parameters, and 1760 analysis have been performed. From the total of analysed samples, 224 have proven positive (to one or more indicators) The results of the higher values are presented in figure 4.



Figure 3. Positive reaction - coliform bacteria

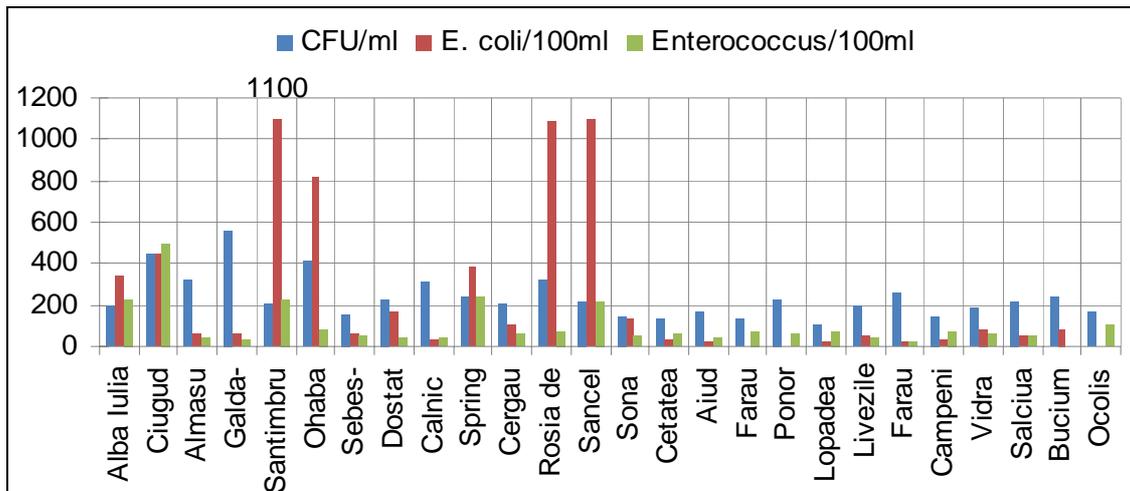


Figure 4. The weight of microbiological indicators for the studied samples between 2006-2007

From the total of samples taken into account within this period, 69% have reacted positively to one or more of the determined parameters (values over the limits allowed by the regulations).

The classification on places of origin highlights that 24 units, from the total of 54 taken into account, give inadequate results, as follows:

- UFC/ml has values between 137 and 560 (20 colonies are allowed),
- *E. coli*/100ml values between 23 -1100 (0 colonies are allowed),
- *Enterococcus*/100ml between 23 and 500 (no colony is allowed).



Figure 5. Positive *Escherichia coli*

The results show that within the same place, water from wells in various areas (upstream, downstream and the center) had increased microbial charge, close in value though (example: in Şona and Blaj).

The data gathered following the investigation of phreatic water table quality had as a major purpose to find the specific impurifiers, the areas and sources of pollution. Thus, raised CFU/ml indicates the risk of presence in the water of pathogenic agents (bacteria, viruses, fungae, parasitic agents).



Figure 6. *Enterococcus* – positive reaction

Fecal coliforms, including *E. coli*, are bacteria that originate from the intestines of warm-blooded animals [16]. These bacteria usually have a strong association with fecal contamination that originates from warm-blooded animals [17].

As a result of outrunning the values for the microbiological parameters, we can conclude that well waters are polluted due to infiltrations from animal waste or residual waters.

It is noticeable also a diffuse microbiological contamination (Şona and Blaj) as a result of uncontrolled fertilization of farming land, or chaotic deposit of household and animal waste.

For the majority of places studied in Alba County the phreatic water table is used in private households and farms, due to their accessibility, even if they have large variations of the flow and microbiological and physico-chemical properties, and thus, the importance of protecting the wells from outside contamination is of the greatest importance (including protection from internal pollution).

The protection also became established by building a paved perimeter, with the slope radiating in the outside of the well, to prevent undesirable contamination.

4. Conclusion

Following the analysis performed we can see that one or all of the assessed parameters, for the majority of water

samples, are far over the maximum limit allowed by the law.

A number of 503 (out of 775) and 224 (out of 325) samples have had a positive reaction to one or all the parameters that have been determined, which represents a percentage over the average, within 65% and 69%.

From the 54 places, 22-24 (43%) have in their wells microbiologically polluted water.

The water samples are microbiologically polluted, regardless of the work method, the way the well is built, the season or the place of origin so the quality of water is not given by its depth, type of well construction and exploitation.

It results that the incidence of well water pollution is bigger and the health of humans and animals is exposed to hydric decay.

There is also a diffuse microbiological pollution in some of these places which might be explained by the pollution of phreatic waters and thus the natural purification phenomena have been suffering transformations – nature does not succeed, on its own, to accomplish its purifying role.

The acquired results involve a higher attention on the water we drink. Supplying with well water has a relative security, the inconsistency of the flow being taken into consideration.

Heavy arrangements are necessary to the wells (paved perimeter, covering), also the periodical decontamination of the wells and sustained water checking through laboratory analysis.

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