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EVALUATION OF THE DEGREE OF MICROBIOLOGICAL CONTAMINATION OF GROUNDWATER IN GORJ COUNTY

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Abstract

Gorj County is located in the south-west of Romania with a predominantly mountainous relief, and the main economic activities are specific to the exploitation of natural resources, agriculture and tourism. As a result of the lack of sewerage systems in most of the villages in Gorj County and the uncontrolled storage of manure resulting from animal husbandry activities, the groundwater has suffered significant microbiological contamination. This paper describes the investigations carried out to determine the degree of microbiological contamination of groundwater in the northern part of Gorj County, in one of the most attractive tourist localities. The investigations consisted of taking 45 water samples from public and private wells in the villages of Gureni and Frâncești, in the commune of Peștișani. The most important microbiological indicators for determining water quality were determined from samples: the number of bacterial colonies, the number of coliform bacteria, the number of Escherichia coli and the number of intestinal enterococci.

Key words*: Evaluation, groundwater, contamination, microbiologic, bacteria.*

INTRODUCTION

Romania, situated in South-Eastern Europe at the crossroads of Central Europe and the Balkans, covers about 238,397 km², ranking as the 12th largest country in Europe. Its landscape features diverse relief - mountains, hills, and plains - and a rich hydrographic network of rivers, lakes, and groundwater.

Gorj County, in southwestern Romania, is known for its mountainous landscapes and cultural heritage. Traversed by the Southern Carpathians and near Retezat National Park, its capital, Târgu Jiu, hosts the iconic works of sculptor Constantin Brâncuși. Rich in forests and minerals, Gorj is a key energy hub, with an economy driven by industry, agriculture, and tourism.

Peștișani commune, located in Gorj County, is a picturesque locality near the city of Târgu Jiu, which stands out for its mountain landscapes and folk traditions. The commune is known for its agricultural activities and local crafts, and among the attractions are the old churches and specific customs, which reflect the culture of the area. The commune of Peștișani is associated with the life and activity of the artist Constantin Brâncuși, having links with the cultural heritage of the county and offering opportunities for rural tourism and nature exploration. The villages that make up the commune of Peştişani are: Boroşteni, Brădiceni, Frâncești, Gureni, Hobiţa, Peştişani and Seuca (Puianu, 2019)

The main source of water in Peștișani commune is groundwater from public and private wells, because not all the inhabitants of the commune have access to the public drinking water network. Given the characteristics of groundwater, it is a very convenient source of drinking water, provided that the reserves are large enough and the quality indicators regulated by the legislation in force are respected. Groundwater composition can be significantly altered by human activities such as improper fertilizer use, household waste disposal, and the infiltration of wastewater or fuels (Begea et al., 2024)

Due to the multiple possibilities of pollution of water sources, it is necessary to establish the sanitary conditions that drinking water must meet. The drinking conditions of the water are the organoleptic characteristics (taste, smell, color), as well as the physical, chemical and biological indicators within the maximum allowed limits and the normal values provided in the national and international legislation in force. The relationship of water with infectious diseases has led to the development of bacteriological

conditions for drinking water. The first bacterial conditions appeared in 1904 and are due to Christiaan Eijkman, being perfected later.

The drinking conditions of the water are divided into four groups:

- organoleptic (taste, smell, coloured);
- (temperature, pH, turbidity, conductivity, radioactivity);
- chemical (fluorine, heavy metals, nitrites, nitrates, ammonia nitrogen);
- microbiological (mesophilic germs, coliform germs, enterococci) (Masciopinto et al., 2021; Cirtină & Căpățână 2017).

The most important bacteriological condition of potability is the total lack of pathogenic germs in the water:

- **Mesophilic germs** bacteria that grow at 37°C, typically originating from humans and warm-blooded animals; higher counts indicate a greater presence of potentially pathogenic microorganisms.
- **Coliform germs** (relatively heterogeneous group, found in large numbers in the feces of humans and warm-blooded animals)
- **Enteric virus** (germs that are found in feces, but in smaller numbers than coliforms, which makes them more difficult to determine; they are more resistant in water than fecal coliforms and do not suffer from the phenomenon of microbial variability; they have types characteristic for humans and animals, which allows differentiation of the type of water pollution) (Cheong et al., 2009)

The presence and intensity of fecal pollution is an important factor in assessing water quality and the infectious risk posed to human health. Examination of water samples for the presence of *Escherichia coli*, which normally populates the intestines of humans and other warm-blooded animals, provides an indication of water pollution (Kampouris et al., 2022).

Analyzing the latest statistical information provided by the National Institute of Statistics in 2022, the population connected to the public water supply system was 14,277,262 people, representing 74.9% of Romania's resident population. At the level of the development regions, the lowest degree of connection to the public water supply system was recorded in the North-East region (50.7%), followed by the South-West Oltenia region (63.4%), a region to which Gorj county, the area taken in the study belongs (Figure 1).

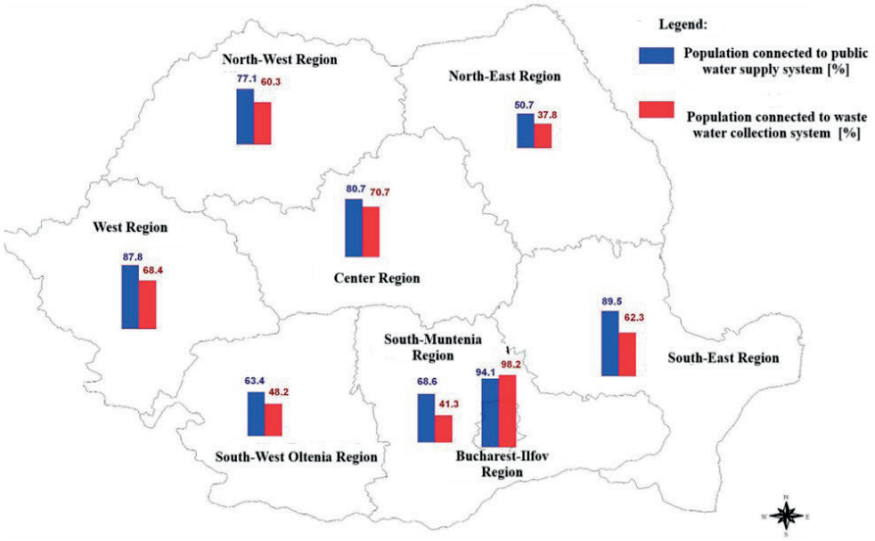

Figure 1. Romania's population connected to the public drinking water supply and sanitation system, by development regions, in 2022 (National Institute of Statistics of Romania, 2022)

Regarding wastewater treatment, 11,062,432 people - 58.1% of Romania's resident population - were connected to sewerage systems with treatment plants. The lowest

connection rates were in the North-East (37.8%), South-Muntenia (41.3%), and South-West Oltenia (48.2%) regions.

MATERIALS AND METHODS

To assess groundwater microbiological contamination in Gorj County, a case study was conducted in the Gureni and Frâncești villages of Peștișani commune.

A total of 45 water samples were collected from public and private wells - 19 from Gureni village and 26 from Frâncești village - as shown in Figure 2.

Figure 2. Distribution of the sampling points of the 45 water samples in the area of Gureni and Frâncești villages

The microbiological quality indicators determined from the collected groundwater samples, as well as the methods of analysis were:

- *The number of colonies at 22^{0}C*, by counting the cultures formed in a nutrient agar culture medium, after incubation in aerobiosis at a temperature of 22^{0}C, in accordance with the international technical provisions referred to in SR EN ISO 6222:2004.
- *Number of colonies at 37^{0}C*, by counting cultures formed in a nutrient agar culture medium, after incubation in aerobiosis at a temperature of 37 ^{0}C, in accordance with the international technical provisions referred to in SR EN ISO 6222:2004.
- *Coliform bacteria*, by filtering the analysis water sample through the membrane, followed by chromogenic agar culture and counting the coliform bacteria in the sample, in accordance with the international technical provisions mentioned in SR EN ISO 9308:2015.
- *Escherichia coli,* by filtering the analysis water sample through the membrane, followed by chromogenic agar culture and counting the coliform bacteria in the sample, in accordance with the international technical provisions referred to in SR EN ISO 9308:2015.
- *Intestinal enterococci,* by filtering the analysis water sample through the membrane, followed by chromogenic agar culture and counting the coliform bacteria in the sample, in accordance with the international technical provisions mentioned in SR EN ISO 9308:2015.

The permissible limits for microbiological indicators in groundwater taken from wells are regulated by Ordinance no. 7/2023 on the quality of water intended for human consumption, which transposes Directive (EU) 2020/2.184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption, published in the Official Journal of the European Union, series L, no. 435 of 23 December 2020 and which provides that for the indicators: number of colonies at 22 ^{0}C, Number of colonies at 37^{0}C, coliform bacteria, *Escherichia coli* and intestinal *Enterococci,* the permissible value is 0 Cfu/ml.

Thus, for the evaluation of the level of microbiological pollution, the values obtained

were compared with the allowed value 0 Cfu/ml and graphically represented. (Popa et al., 2022)

RESULTS AND DISCUSSIONS

The evolution of colony counts at 22°C for water samples from Gureni and Frâncești villages is presented in Figures 3 and 4.

According to Figure 3, 17 out of 19 water samples from Gureni village exceeded the permitted limit, representing 89.47%. Similarly, Figure 4 shows that 19 out of 26 samples from Frâncești village were above the allowed limit, accounting for 73.07% (Șchiopu, 2023). The evolution of colony counts at 37°C for samples from both villages is shown in Figures 5 and 6.

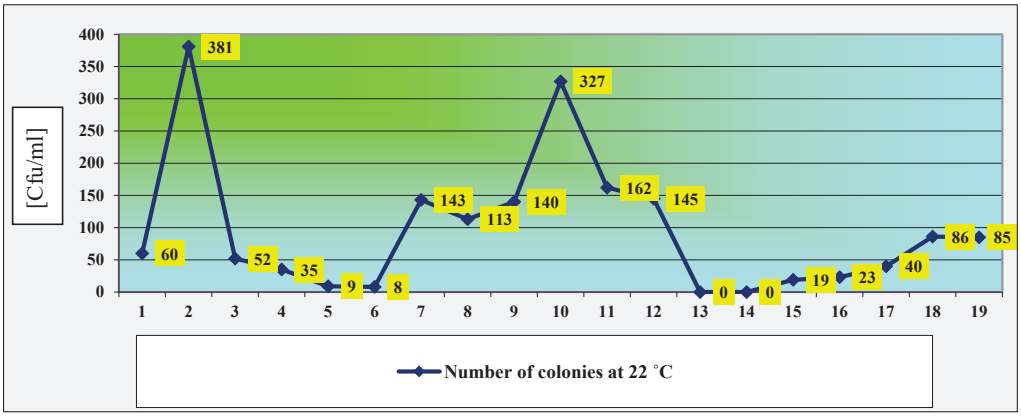

Figure 3. Number of colonies at 22^{0}C from groundwater samples taken on 22.02.2023 from Gureni village, Gorj county, South-West region Romania

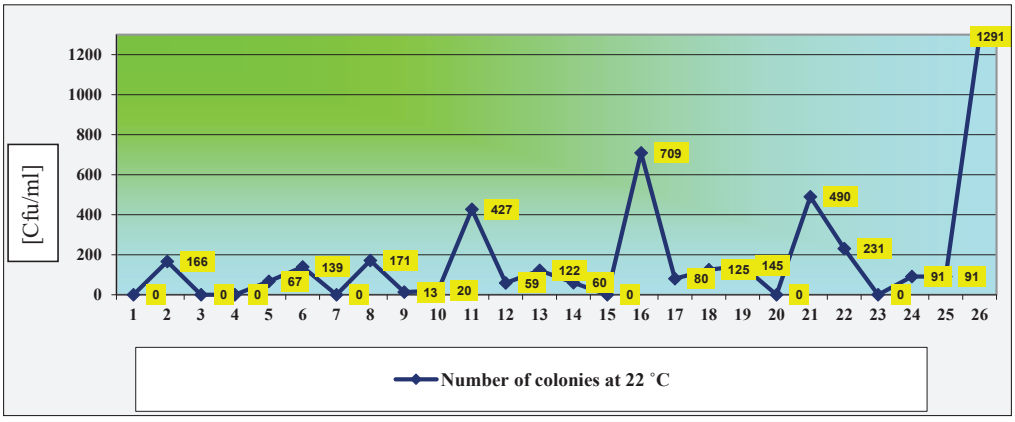

Figure 4. Number of colonies at 22^{0}C from groundwater samples taken on 22.02.2023 from Frâncești village, Gorj County, South-West region Romania

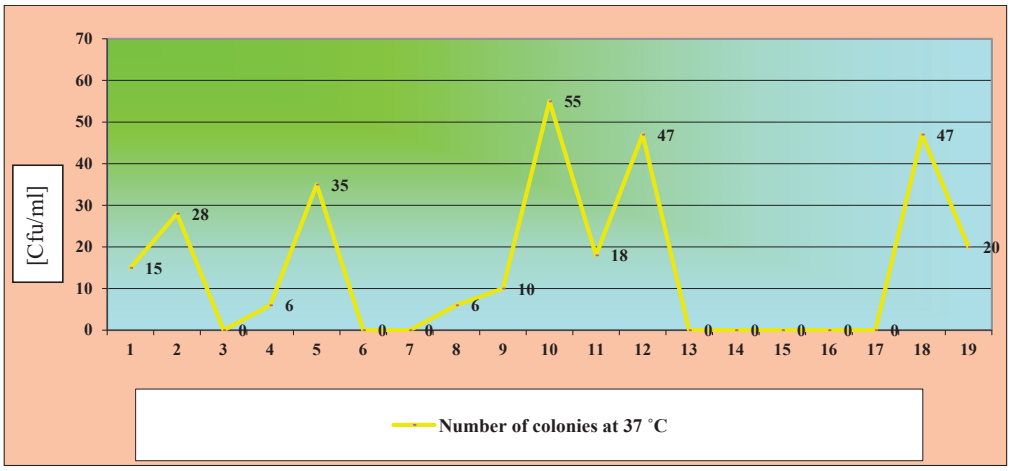

Figure 5. Number of colonies at 37^{0}C in groundwater samples taken on 22.02.2023 from Gureni village, Gorj County, South-West region Romania

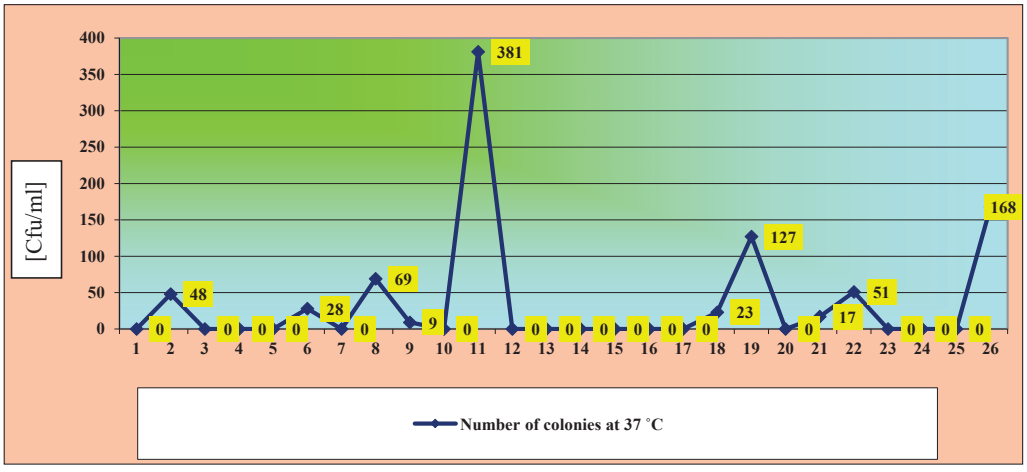

Figure 6. Number of colonies at 37^{0}C from groundwater samples taken on 22.02.2023 from Frâncești village, Gorj County, South-West region Romania

Figure 5 shows that 11 of the 19 water samples from Gureni village exceeded the maximum limit, representing 57.89%. In Figures 6-10 of the 26 samples from Frâncești village also surpassed the limit, accounting for 38.46%. The evolution of coliform bacteria in samples from Gureni and Frâncești villages is illustrated in Figures 7 and 8, respectively.

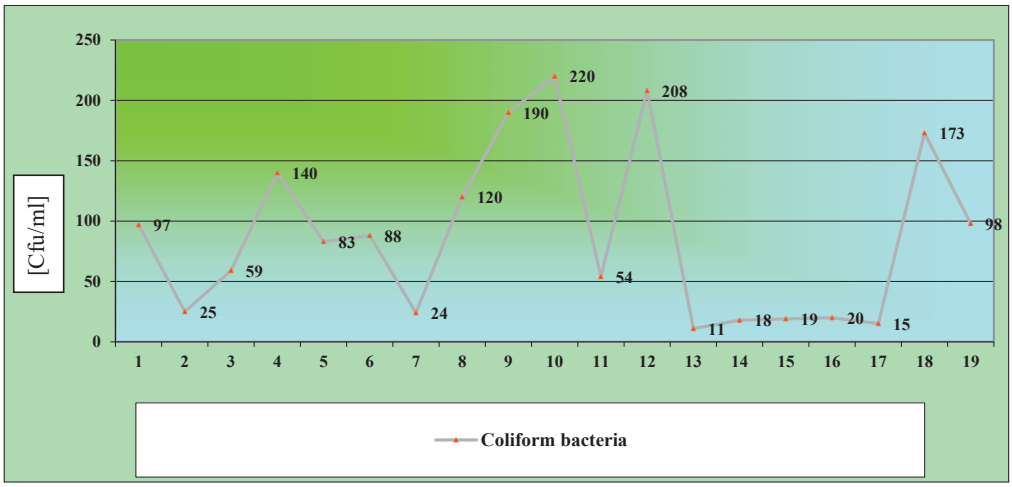

Figure 7. Concentration of coliform bacteria in groundwater samples taken on 22.02.2023 from Gureni village, Gorj County, South-West region Romania

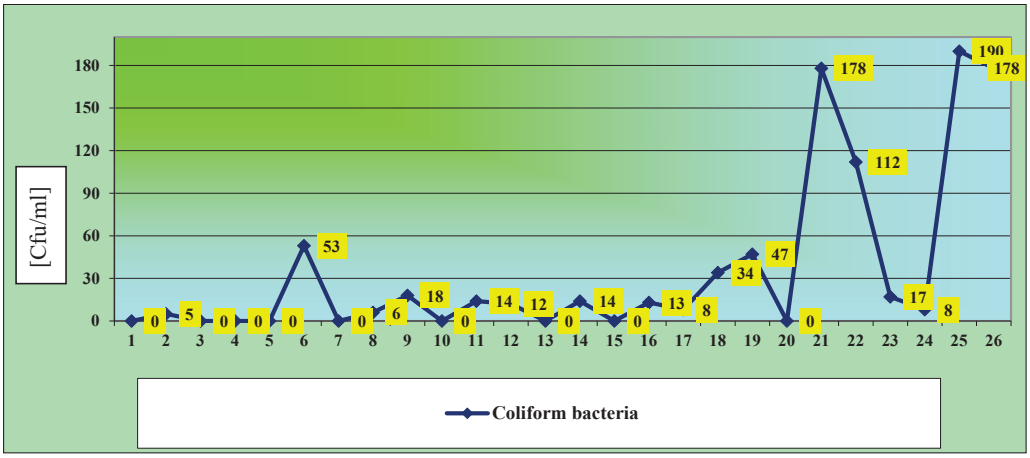

Figure 8. Concentration of coliform bacteria in groundwater samples taken on 22.02.2023 from Gureni village, Gorj County, South-West region Romania

As shown in Figure 7, all water samples from Gureni village exceeded the maximum limit for coliform bacteria, representing 100%. Figure 8 indicates that 17 of the 26 samples from Frâncești village also surpassed the limit, accounting for 65.38%. Figures 9 and 10 present the evolution of *Escherichia coli* levels in water samples from Gureni and Frâncești villages, respectively.

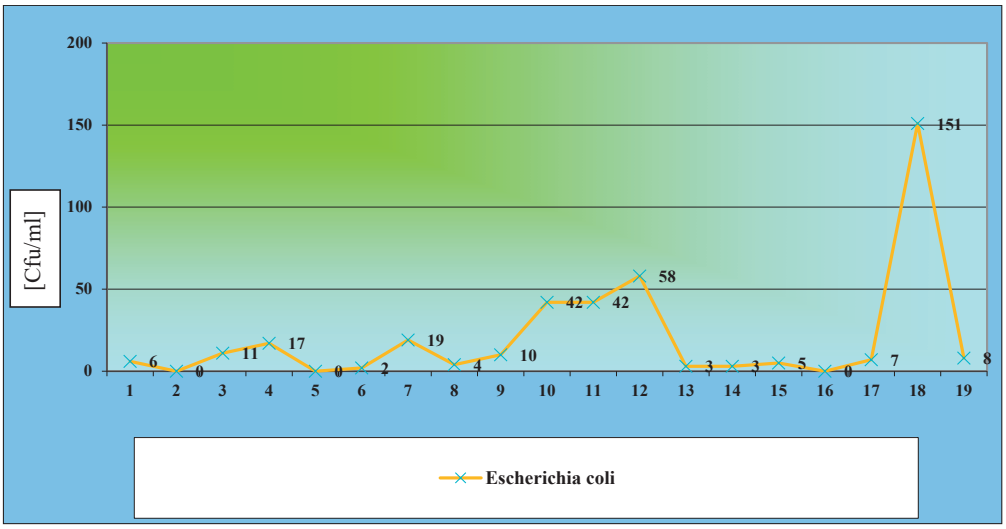

Figure 9. The number of *Escherichia coli* in groundwater samples taken on 22.02.2023 from Gureni village, Gorj County, South-West region Romania

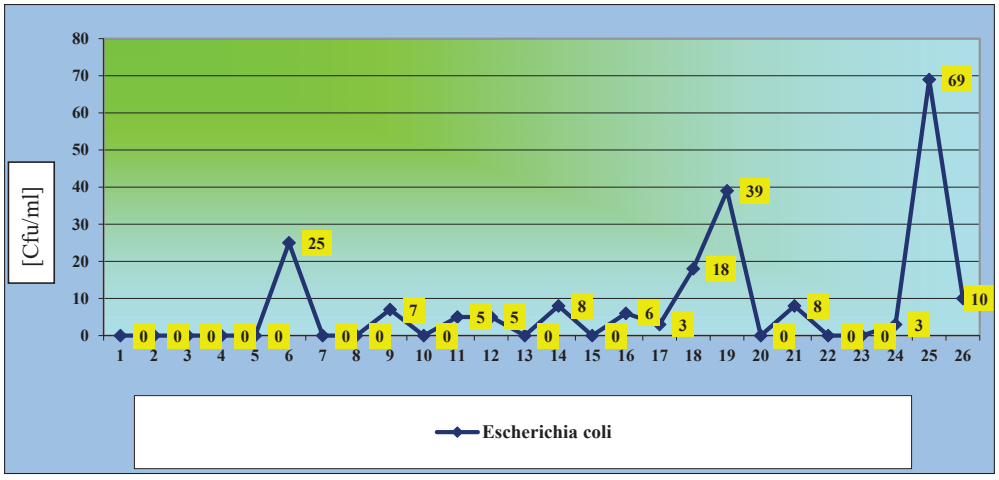

Figure 10. The number of *Escherichia coli* in groundwater samples taken on 22.02.2023 from Frâncești village, Gorj County, South-West region Romania

Figure 9 shows that 16 of the 19 water samples from Gureni village exceeded the maximum limit for *Escherichia coli*, representing 84.21%. According to Figure 10, 13 of the 26 samples from Frâncești village also exceeded the limit, accounting for 50%.

Figures 11 and 12 illustrate the evolution of intestinal Enterococci levels in water samples from Gureni and Frâncești villages, respectively.

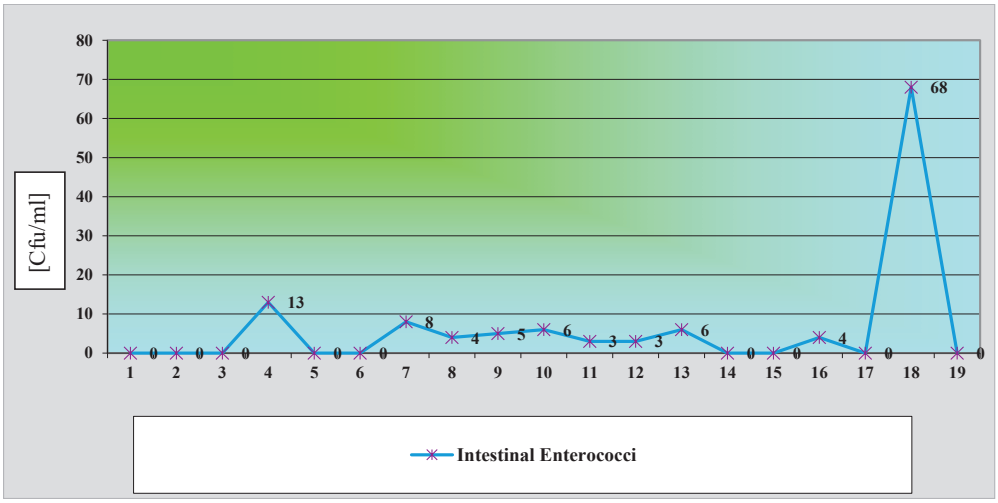

Figure 11. The number of intestinal *Enterococci* in groundwater samples taken on 22.02.2023 from Gureni village, Gorj County, South-West region Romania

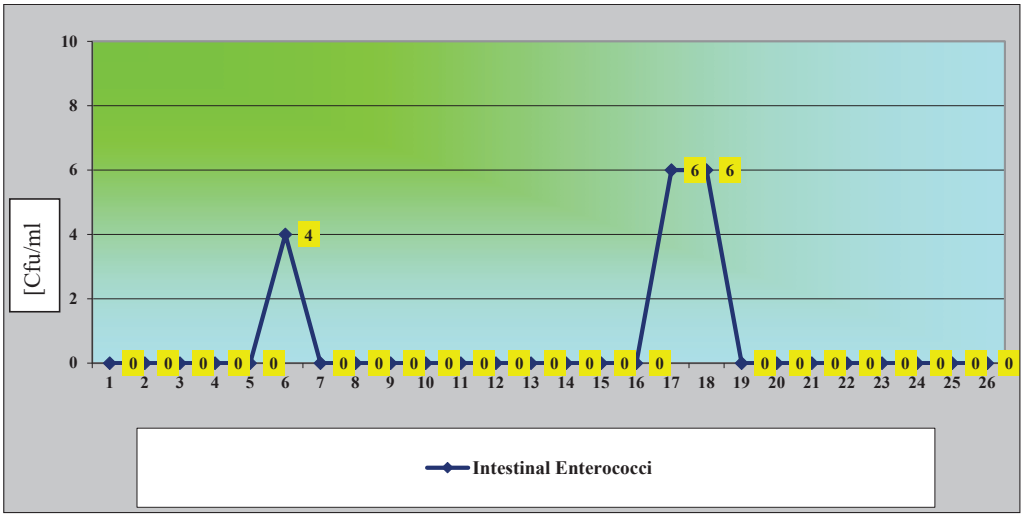

Figure 12. The number of intestinal *Enterococci* in groundwater samples taken on 22.02.2023 from Gureni village, Gorj County, South-West region Romania

Figure 11 shows that 10 of the 19 water samples from Gureni village exceeded the maximum limit for intestinal Enterococci, representing 52.63%. In Figure 12, only 3 of the 26 samples from Frâncești exceeded the limit, accounting for 11.53%.

Overall, analysis of all microbiological indicators confirmed that all 19 samples from public and private wells in Gureni exceeded at least one microbiological parameter, rendering them unfit for human consumption. Specifically, 7 samples exceeded the limit for all five indicators, 3 samples for four indicators, 8 for three indicators, and 1 sample for two indicators. The primary cause of contamination is the proximity of animal shelters and manure storage areas to the wells.

The sample with exceedances for only two of the five indicators came from a public well located at a relatively greater distance from known pollution sources. In Frâncești village, 6 of the 26 analyzed wells (codes: 1, 3, 4, 7, 15, and 20)

were free of exceedances for all five microbiological indicators and are considered safe for drinking. These samples were collected from public wells near roads or institutions (e.g., schools, cultural centers, and stores) where no animal waste was stored and septic tanks are regularly emptied.

Among the remaining 20 samples, 3 exceeded all five indicators, 5 exceeded four, 8 exceeded three, and 4 exceeded only one. The primary cause of microbiological contamination in both villages is the absence of a sewerage system and the uncontrolled storage of animal manure.

For consumption, contaminated water from wells must be applied by one of the following methods:

1. Treatment with chlorine solution, going through the steps mentioned in Order 119/2014 for the approval of Rules of hygiene and public health regarding the living environment of the population, applicable on the territory of Romania, namely:

Calculating the volume of water in the well:

$$V = 3.14r\ x\ rH$$

where:

V = volume of water (m^3);

r = 1/2 of the diameter of the well (m);

H = the height of the water column in the well (m);

Choosing a substance with a chlorine concentration of 15 – 25%;

Calculation of the quantity of chlorine substance according to the concentration of free residual chlorine to be obtained, according to the following example:

0.5 mg Residual Cl ..... 1 l water

X mg Residual Cl ..... 1,000 l water

X = 0.5 g residual chlorine/m^3 water

100 g chlorine ..... 25 g active chlorine

X ..... 0.5 g active chlorine

X = 2 g chlorine/m^3 water

For a water column height of 7 m and a well tube diameter of 1 m, the volume of water is determined as follows:

$$V = 3.14 \cdot 0.5\ x\ 0.5 \cdot 7$$

$$V = 11.02\ m^3 \text{ water}$$

The amount of chlorine required to disinfect the volume of water is calculated as follows:

2 g chlorine/m^3 water x 11.02 m^3 = 22.04 g chlorine of concentration 25%

2. Boil water before consumption for at least 1-3 minutes, to destroy pathogenic microorganisms.

CONCLUSIONS

Human activities, such as: uncontrolled and improper use of fertilizers, disposal of household waste, infiltration of wastewater or fuels, carried out in the South-West area of Romania, Gorj County, Peștișani commune are sources of groundwater pollution, and it is necessary to establish the sanitary conditions that must meet the drinking water.

The bacteriological condition of potability of the water in the wells is the total absence of pathogenic germs (mesophils, coliforms and enterococci). In this regard, this study provided for the collection of groundwater samples and microbiological analysis, according to international standards, of five microbiological indicators of drinking water quality (Number of colonies at 22^0C, Number of colonies at 37^0C, coliform bacteria, *Escherichia coli* and intestinal *Enterococci).*

Following the analysis of the 45 water samples collected from public and private wells and the comparison of the results obtained with the admitted value 0 Cf/ml, it was shown that for most of them there were exceedances of the analyzed microbiological indicators, the main cause being the lack of a centralized sewerage system and the uncontrolled storage of animal manure.

It is recommended that drinking water extracted from wells be treated with chlorine solution or boiled.

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