QUALITY ASSESSMENT AND HEALTH SAFETY OF NATURAL SPRING WATER

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ABSTRACT:
In this paper the natural spring water was investigated. The sample of spring water was taken at the beginning of July 2019 in Tuzla, Bosnia and Hercegovina (BIH), which is consumed by the local population. The source of investigated spring water is located above salt mine. All parameters of health safety were analysed by the methods of laboratory tests regulated by the State regulation on natural mineral and natural spring waters "Official Gazette BiH" No. 26/10 and 32/12, i.e. physical-chemical and microbiological analysis was performed. The results of the study showed that water is not for drinking because it contains higher concentrations of ammonium ion (NH_4^+) which is 0.89 mg/L, nitrite (NO_2^-) 2.20 mg/L and sulphate (SO_4^{2-}) 398.34 mg/L, and coliform bacteria are isolated. The pH value of 9.07 indicate that this spring water is alkaline. The concentrations of iron (Fe^{2+}) 0.007 mg/L and manganese (Mn^{2+}) 0.0144 mg/L are within the allowed limit values while the concentration of lead (Pb^{2+}) 0.0088 is at the upper maximum allowable value. The results of this research indicate that water in this untreated state is not for drinking.

KEYWORDS: spring water, ammonium, nitrites, sulphates, coliform bacteria.

INTRODUCTION

In recent years with the rapidly growing industries and population, water pollution has emerged as major challenge for scientific community that demands an intense and real-world solution. The problem with plastics contaminats present in water, as well as other organic compounds such as polycyclic aromatic hydrocarbons (PAHs), endocrine disrupting chemicals (EDCs), pesticides and herbicides, dyes and its photocatalytic degradation has been studied recently by Bratovcic [1-3]. According to the United Nation World Water Development report, around 748 million people around the world do not have provision for pure drinking water [4].

The civilization has managed to pollute the water supplies to the point where water for drinking has to be purified before using. The shortage of affordable pure water forces an estimated 1.2 billion people to drink unclean water. As a result, water related diseases kill 5 million people a year, mostly children, around the world. The UN estimates that 2.7 billion people will face water shortages by 2025. Only 0.06% is easily accessible from 3% of total freshwater available to us. This is reflected by the fact that over 80 countries now have water deficits. It is clear that water is a scarce and valuable commodity and we need to sustain its quality and use it judiciously, i.e. assure water sustainability. To achieve sustainability, we must ensure that as we meet our needs, we do not compromise the requirements of future generations [5].

Drinking water comes largely from rivers, lakes, wells, and natural springs. These sources are exposed to a variety of conditions that can contaminate water. The failure of safety measures relating to the production, utilization, and disposal of thousands of inorganic and organic compounds causes pollution of our water supplies [6].

Water is essential for life. Potable water is any water suitable for human consumption. It is an indispensable resource for the economy and also plays a fundamental role in the climate regulation cycle. Among the EU countries, Croatia, recorded the highest freshwater resources (with a long-term average of 27330 m^3 per inhabitant) followed by Finland and Sweden with around 20000 m^3 each [7]. Although there is an abundance of water resources, the water quality in Bosnia and Herzegovina is lacking. In most countries, the principal risks to human health associated with the consumption of polluted water are microbiological in nature, although the importance of chemical contamination should not be underestimated. Contaminated water and poor sanitation are linked to transmission of diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio. Inadequate management of urban, industrial, and agricultural wastewater means the drinking-water of hundreds of millions of people is dangerously contaminated or chemically polluted. Some 829000 people are estimated to die each year from diarrhoea as a result of unsafe drinking-water, sanitation, and hand hygiene. Yet diarrhoea is largely preventable, and the deaths of 297000 children aged under 5 years could be avoided each year if these risk factors were addressed [8].

In Bosnia and Herzegovina only about 65 percent of the country’s population has a connection to municipal or public water utilities – the average of European Union countries is 90 percent. Only large urban centres have a satisfactory supply of water, both in terms of quality and quantity. Unfortunately, the poorest and most vulnerable of Bosnia and Herzegovina’s population live in rural areas [9]. There are several types of mineral, thermal and thermomineral waters known in the territory of Tuzla Canton (TC). From mineral waters in the area of TC, salt and carbonated waters are registered. Drinking groundwater on the territory of the TC is located in the coastal areas of the river and in the areas of the northern and southern parts of the Canton. In the watercourses on the river Spreca exists sources of Banovici, Tuzla, Zivinice and Kalesija. The total amount of water used for the water supply of Tuzla Canton is 3683032 m^3/year, and the total number of inhabitants serving water is 243900. From the total amount of the taken water from the sources, households are supplied with about 24% of the volume, the industry is 31%, and even 45% of the water makes up the losses.

The water supply of the Tuzla and surrounding places are operated through the inter-municipal water supply system Tuzla-Zivinice-Lukavac and make it the following sources:

groundwater source Sprecko polje, 120-200 l/sec, source Stupari, 220-300 l/sec, source Toplice, 140-230 l/sec.

Tuzla is the third-largest city of Bosnia and Herzegovina, located in the northeastern part of Bosnia and very well known by deposit of the rock salt (halite) and abundant coal deposits in the region around Tuzla. The salt deposits around Tuzla are younger geological age, compared to well-known deposits in Europe and the world, formed in the Miocene. The rock salt deposit “Tetima” is located about 8 km in the northeast of Tuzla. The construction of the salt mine was started in 1986, and it was a substitute capacity for the production of the saturated salty water (brine), from the Hukalo and Trnovac, whose uncontrolled exploitation provoked the ground surface settlement of the urban area, thus endangering the undisturbed development of life and existence of people in these city quarters. It is estimated that the balance reserves on this area is up to 342 million tons, out balance 33 million tons, while 54.7 million tons for exploitation. The life span of the mine with an annual production of 2.6 milion m^3 per annum is 62 years. The exploitation area is 406.38 hectares [10].

Water losses are estimated at about 37% and water supply reconstruction is expected in the forthcoming period. Safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production or recreational purposes. Improved water supply and sanitation, and better management of water resources, can boost countries’ economic growth and can contribute greatly to poverty reduction.

Groundwater is the water that lies beneath the surface of the earth and is formed by rainfall, from watercourses and condensation of water vapour in the earth [11]. Groundwater dissolves various minerals from the rocks through which they pass. In the groundwater solution there is CO_2 which is partly derived from the atmosphere and the second part is formed by the decomposition of plant material. Carbon dioxide affects the mild acidity of the water, and if such water comes into contact with limestone, carbonates dissolves and bicarbonates are formed and then a mild alkaline environment occurs. Ground waters can be soft or hard, depending on soil composition and dissolution of magnesium and calcium carbonates. In addition, this water dissolves all the minerals and organic materials present in the aqueous layer. Water depths up to 80 m are softer, with higher concentrations of ammonia, organic materials, iron and manganese, have a low sodium concentration and a slightly increased content of arsenic and chloride. The waters over 80 m deep have been trapped in underground tanks for tens of thousands of years are exceptionally soft, they have increased sodium content, organic matter, ammonia, arsenic and orthophosphate ion (HPO_4^{2-}), and the dominant anion in these waters is bicarbonate [12].

In this paper the quality and health safety of the spring water was studied. Extended physical-chemical analysis will be performed and it will include beside the determination of temperature, pH, electrical conductivity, alkalinity, organic matter, chlorine, hardness, total hardness, sulfate, sulfide and minerals such as iron, manganese and lead. The microbiological analysis that would be performed will covered the determination of the *Escherichia coli*, *Coliform bacteria*, *Enterococci*, the total number of microorganisms at 22°C and 37 °C and *Clostridium perfringens*.

MATERIAL AND METHODS

In order to determine health safety of the spring water, the sample was analyzed on organoleptic, microbiological and physical-chemical properties. The source of spring water is located in Tuzla in Bosnia and Herzegovina and more precisely in Tusanj near salt mine. A sample of water for physical-chemical analysis was taken in a clean plastic flask of 1.5 liter previously washed three times with water to be analyzed. A sample of water for microbiological analysis was taken in a sterile glass flask of 1 liter. The temperature of spring water at the place of sampling was 15 °C. The rate of water flow and the capacity of this source of spring water is 52704 m^3/day. This information is about the natural wealth that this source of natural water abounds in. It would be a great pity that due to the health deficiency, this water source remains unused, given the fact that there is a downward trend in clean spring water, as well as the final price of water. The sample was taken at the beginning of July 2019. The analysis of organoleptic properties, physical-chemical and microbiological analysis have been done.

PHYSICAL-CHEMICAL ANALYSIS

Physical – chemical analysis of the water has been done by Regulations on Natural mineral and natural spring waters [13]. Organoleptic properties were determined by sensory analysis and they included the determination of color, odor and taste. The turbidity of the tested water has been determined by standard method BAS EN ISO 7027-1:2017.

The extended physical-chemical analysis involves the determination of temperature, pH, electrical conductivity, alkalinity, organic matter, chlorine, hardness, total hardness, iron, manganese, lead, sulfate, and sulfide.

The spring water temperature is measured at the sampling site, by mercury thermometer. The pH value has been determined by BAS EN ISO 10523:2013. The electrical conductivity implies measuring electrical conductivity of water by conductometer following the standard method BAS EN 27888:2002 at 20 and 25 °C.

Organic matter analysis by using potassium permanganate ($KMnO_4$) can be used as an indicator of the content of organic matter in water. The potassium permanganate solution is heated in an acidic medium, oxidation of the organic substance occur and a certain amount of $KMnO_4$ is consumed, depending on the amount and the chemical structure of the organic substance present in the water. This indicator can only be conditional use as a measure of the content of organic substances because potassium permanganate is also reduced in the presence of some other substances in the sample of water [14].

Dry residue by gravimetric method was determined.

The concentration of ammonium ion (NH_4^+), nitrites (NO_2^-) and sulphates by spectrophotometry were determined. The instrument used was UV-VIS spectrophotometer Lambda 25.

Water hardness is the result of the presence of ions such as calcium, iron, manganese and magnesium dissolved in water. In natural waters, magnesium and calcium ions are more common. Thus, the sum of calcium and magnesium ions is expressed as hardness of water. The total hardness of water, alkalinity, calcium and magnesium were determined by a volumetric method.

Residual chlorine was determined by colorimetry.

Nitrates (NO_3^-) by Standard methods for examination of water and wastewater, 4500 NO_3^-B 2005 were determined. Sulfides were determined by standard iodometric method Standard methods 45000 S^{2-} F (Iodometric method, APHA-AWWA-WEF 2012).

Iron and Manganese were determined spectrophotometrically by Atomic spectroscopy Standard Methods 3111 (B), APHA-AWWA-WEF 2012.

Lead has been determined by BAS ISO 8288:2002 – Method of flame atomic absorption spectroscopy.

Microbial analysis implies determination of pathogenic and other microorganisms.

In this paper the Escherichia coli and Coliform bacteria were determined by standard method BAS ISO 9308-1:2015; BAS EN ISO 9308-2:2015. Enterococci were determined by BAS ISO 7899-2:2003 standard method, while Pseudomonas aeruginosa by BAS EN ISO 16266:2009 method. The total number of microorganisms at 22 °C and 37 °C by standard method BAS EN ISO 6222:2003 were determined, and Clostridium perfringens by BAS EN 26461-1:2003; BAS EN ISO 26461-2:2003.

MICROBIOLOGICAL ANALYSIS

To determine the total number of coliform bacteria such as *Escherichia coli* and *Enterobacter*, the membrane filtration firstly has to be done. Under sterile conditions, using a vacuum pump on the membrane filtration device, 100 ml of the sample is passed through a filter paper having a pore size of 0.45 μm. Thereafter, the filter paper is transferred to a sterile Petri dish with agar medium for the total number of microorganisms, and after 24 hours at 37 °C and after 72 hours at 22 °C, the total number of microorganisms were determined. The presence of coliform bacteria after filtration, were determined by growing them on Endo agar in the thermostated incubator for 24 h-48 hours at 37 °C.

RESULTS AND DISCUSSION

The results include determination of organoleptic properties, extended physical – chemical properties and microbacterial analysis. Physical characteristics of water include temperature, colour, taste, odour, turbidity and total dissolved solids. Temperature is the single most important physical characteristic affecting the pH of water.

Table 1. Results of physical-chemical analysis

Parameter	Unit	Determined value	Allowed values
Colour	-	without	acceptable for the consumer
Odour	-	without	acceptable for the consumer
Taste	-	-	acceptable for the consumer
Turbidity	NTU	0.61	acceptable for the consumer
pH value (25°C)	pH	9.07	6.5-9.5
Consumption $KMnO_4$ (oxidation)	mg/l O_2	1.36	up to 5
Dry residue	mg/l	942	-
Electrical conductivity (25°C)	µS/cm	1537	-
Electrical conductivity (20°C)	µS/cm	1377	2500
Ammonium (NH_4^+)	mg/l	0.89	0.50
Residual chlorine	mg/l	0.00	0.50
Chloride (Cl^-)	mg/l	11.00	250
Nitrites (NO_2^-)	mg/l	2.20	0.50
Nitrates (NO_3^-)	mg/l	3.94	50.0
Iron	mg/l	0.007	0.2
Manganese	mg/l	0.0144	0.05
Total hardness	°nj	0.56	-
Alkalinity	°nj	27.44	-
Sulphates	mg/l	398.34	250
Sulphides	mg/l	0.0	0.1
Calcium	mg/l	12.01	-
Magnesium	mg/l	1.70	-
Lead (Pb)	mg/l	0.0088	0.01

In table 1 there are results of organoleptic properties which include the determination of colour, odour and taste by sensory analysis and extended physical – chemical parameters such as pH, turbidity, organic matter, dry residue, electrical conductivity, residual chlorine, the most relevant anions and cations, total hardness.

The results of the study showed that water is not for drinking because it contains higher concentrations of ammonium (NH_4^+) which is 0.89 mg/L, nitrite (NO_2^-) 2.20 mg/L and sulphate (SO_4^{2-}) 398.34 mg/L, and coliform bacteria are isolated. The pH of water as a measure of the acid and base equilibrium, and in most natural waters, is controlled by the carbon dioxide, bicarbonate and carbonate equilibrium system [15]. The pH value of 9.07 indicates that this spring water is alkaline and according to previous discussion suggests the lower concentration of carbon dioxide concentration and presence of carbonate or hydroxide ions. The pH level of water can affect, and in some cases be affected by, the physical, chemical and microbiological characteristics of the water.

The concentrations of iron (Fe^{2+}) 0.007 mg/L and manganese (Mn^{2+}) are 0.0144 mg/L a within the allowed limit values while the concentration of lead (Pb) 0.0088 is at the upper maximum allowable value. The maximum allowed concentrations (MAC) regulated by the State regulation on natural mineral and natural spring waters for (NH_4^+) is 0.50, for NO_2^- is 0.50 mg/L, (SO_4^{2-}) is 250 mg/L, (Fe^{2+}) is 0.2 mg/L, (Mn^{2+}) 0.05 mg/L and (Pb) 0.01.

In Figure 1 the concentrations of ammonia and nitrite are presented.

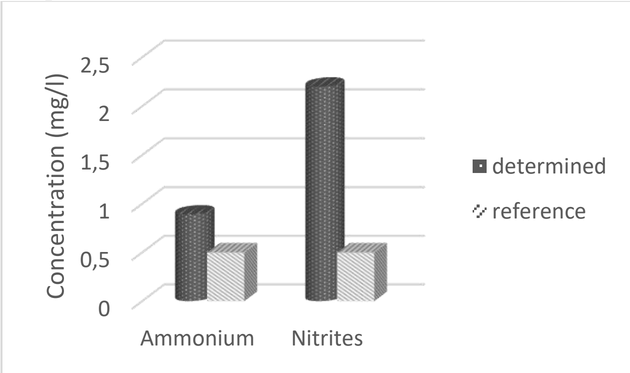

Figure 1. The concentrations of ammonia and nitrite in spring water

From Figure 1 is noticeable that the concentrations of ammonium and nitrites exscide the MAC. The concentration of ammonim is 1.8 times higher (0.89 mg/l) than MAC (0.5 mg/l), while the concentration of nitrites (2.2 mg/l) is 4.4 times higher than it is maximum allowed (0.5 mg/l).

The environmental cycling of nitrogen relies mainly on nitrate, followed by ammonia and the ammonium cation, which predominates. The ammonium cation is less mobile in soil and water than ammonia and is involved in the biological processes of nitrogen fixation, mineralization, and nitrification [16]. Since that ammonia is used in fertilizer and animal feed production we assume that this high concentration of ammonium ion may originate from these sources. The local population where the water source is located is engaged in agriculture and livestock breeding. Nitrate can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilizers and manures), from wastewater treatment and from oxidation of nitrogenous waste products in human and animal excreta, including septic tanks [17].

Although the concentration of nitrate in the tested sample of water is within the permitted limits (3.94 mg/l) and maximum allowed is 50 mg/l, it is assumed that due to the presence of microorganisms nitrates are reduced to nitrite.

The nitrate concentration in surface water is normally low (0–18 mg/l) but can reach high levels as a result of agricultural runoff, refuse dump runoff or contamination with human or animal wastes. Concentrations of nitrate in rainwater of up to 5 mg/l have been observed in industrial areas [18]. In rural areas, concentrations are somewhat lower. Since that Tuzla is an industrial city and in our sample the concentration of nitrate is 3.94 mg/l which is in line with a previous results related to rainwater.

In Figure 2 the concentrations of sulphate is presented.

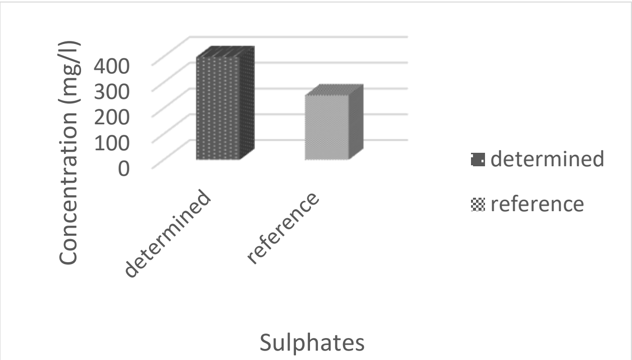

Figure 2. The concentrations of sulphate in spring water.

Sulphates occur naturally in numerous minerals which dissolved contribute to the mineral content of many drinking-waters. Sodium, potassium and magnesium sulphates are all highly soluble in water, whereas calcium and barium sulphates and many heavy metal sulfates are less soluble. Levels of sulphate in rainwater and surface water correlate with emissions of sulphur dioxide from anthropogenic sources [19].

Since the concentration of H_2S was not detected in the analyzed water sample, the relation between sulphate and sulphide concentrations is absent. The concentration of sulphate in analyzed sample was 398.34 mg/l and MAC is 250 mg/l. This indicates 1.6 times higher concentration upper maximum allowed limit which may originate from the sulphur containing detergents used for personal care.

The results of the physical-chemical analysis of the examined spring water indicate that the water does not meet the conditions of the *Regulation on natural mineral and natural spring waters” Official Gazette BiH” No. 26/10 and 32/12* because the tested water contains a higher content of ammonia, nitrite and sulfate.

Bacterial analysis included:

- *Escherichia coli* in 250 ml of water
- *Coliform bacteria* in 250 ml of water
- *Enterococci (Enterococcus faecalis)* in 250 ml of water
- *Pseudomonas aeruginosa* in 250 ml of water
- *Clostridium perfringens* in 100 ml of water
- Total number of microorganisms at 37 °C in 1 ml of water
- Total number of microorganisms at 22 °C in 1 ml of water

Table 2. Results of microbiological analysis

Parameter	Maximum allowed concentration (MAC) according to the Regulations (cfu)**	Results
Escherichia coli	0/250 ml	not isolated
Coliform bacteria	0/250 ml	are isolated
Enterococci	0/250 ml	3
Pseudomonas aeruginosa	0/250 ml	not isolated
Total number of microorganisms at 22 °C	100/1ml	50
Total number of microorganisms at 37 °C	20/1ml	20
Clostridium perfringens	0/100 ml	not isolated

** cfu – colony forming units

In Figure 3 the microbial analysis results are shown.

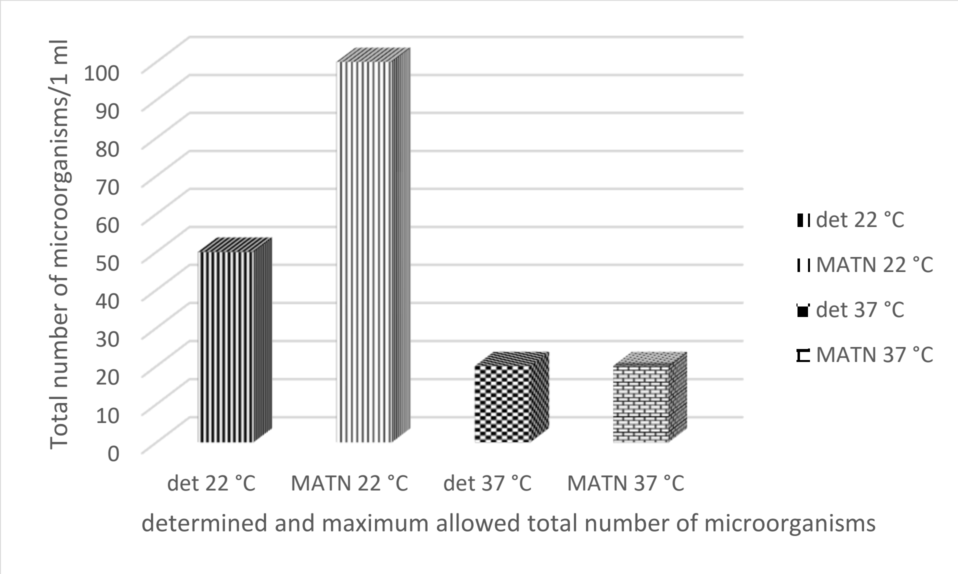

Figure 3. The microbial analysis of tested spring water

According to the Regulations, the total number of bacteria at the spring must not exceed 100 in 1 ml at 22 °C for 72 hours of incubation on nutritious agar and 20 in 1 ml at 37 °C for 24 hours of incubation on nutritious agar. In our sample were found 50 bacteria in 1 ml at 22 °C i.e. 50 % from maximum allowed value and 20 in 1 ml at 37 °C i.e. 100% of maximum allowed value.

The results of the microbiological analysis of the examined spring water indicate that water does not meet the conditions of the *Regulations on natural mineral and natural spring waters "Official Gazette BiH" No. 26/10 and 32/12*.

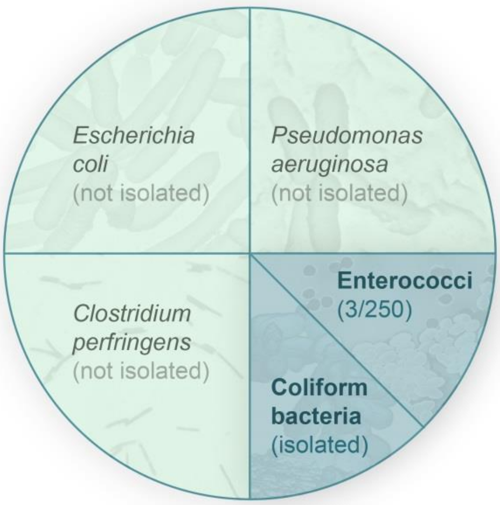

Figure 4. Results of microbial analysis of tested spring water.

Enterococci have been adopted as indicators of human fecal pollution in water. In drinking water, coliforms, including total and fecal coliforms (and *Escherichia coli* in particular) are the primary method of assessing contamination. In the European Union (EU), enterococci are used as indicators of drinking water contamination. In the EU, enterococci are not permitted in a 100 mL sample of tested drinking water that flows from a tap, and they are not permitted in a 250 mL sample of bottled water [20]. In our sample there are 3 of them in 250 ml of water. In Figure 4. The schematic presentation of the results of microbial analysis are presented.

Over the last ten years, about 10 residential houses have been built near the spring source water, and as a consequence it is reasonable to assume that the source of pollution is coming from the waste water used by these households. Namely, high concentrations of nitrite and ammonia as well as isolated coliform bacteria allude to fecal matter contamination, while high concentration of sulfate can be originated from the use of detergents to maintain personal hygiene.

One of the most important steps to make sure drinking water is safe is regular testing for coliform bacteria. Their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the faeces of humans or animals. Total coliform bacteria are common in the environment (soil or vegetation) and are generally harmless.

Considering that total coliform bacteria in examined spring water were detected, it is important to find and resolve the source of the contamination. It is assumed that the source is probably by faecal contaminated due to a large number of houses built in the surrounding area and inadequate sewage disposal. *Faecal coliform* bacteria are a subgroup of total coliform bacteria. They exist in the intestines and faeces of people and animals. *E. coli* is a subgroup of the faecal coliform group. Most *E. coli* bacteria are harmless and exist in the intestines of people and warm-blooded animals. However, some strains can cause illness. The presence of *E. coli* in a drinking water sample usually indicates recent faecal contamination. That means there is a greater risk that pathogens are present. In this study the *E. coli* were not detected.

The next step should be in finding out how the contamination got into the water. If the assessment identifies the cause of the contamination, the water system can usually correct the problem with repairs, treatment, or improved operation and maintenance practices. Since that this study has confirmed the presence of total coliform bacteria in spring drinking water, it is necessary to inform users as soon as possible. It is important to explain what is needed to do to correct the problem, when the problem will likely be resolved, and what users may need to do until then [21].

Ideally, drinking-water should not contain any microorganisms known to be pathogenic—capable of causing disease—or any bacteria indicative of faecal pollution. The detection of *Escherichia coli* provides definite evidence of faecal pollution; in practice, the detection of thermotolerant (faecal) coliform bacteria is an acceptable alternative.

When a guideline value is exceeded, the cause should be investigated and corrective action taken. Terminal disinfection is essential for surface waters after treatment and for protected groundwater sources when *E. coli* or thermotolerant (faecal) coliforms are detected. Chlorine in one form or another is the most commonly used disinfectant worldwide.

For terminal chlorination, there should be a free chlorine residual of at least 0.5 mg/litre after a minimum contact time of 30 minutes at a pH of less than 8.0, as for inactivation of enteric viruses. When chlorine is used as a disinfectant in a piped distribution system, it is desirable to maintain a free chlorine residual of 0.2–0.5 mg/litre throughout, to reduce the risk of microbial regrowth and the health risk of recontamination. In emergencies, e.g. in refugee camps, during outbreaks of potentially waterborne disease, or when faecal contamination of a water supply is detected, the concentration of free chlorine should be increased to greater than 0.5 mg/litre throughout the system [22].

CONCLUSION

The physical-chemical and microbiological analyzes of the investigated spring water clearly indicate that water in this untreated state is not for drinking. Due to the existence of a grounded suspicion that water could be contaminated due to inadequate disposal of municipal wastewater from the surrounding housing, it is urgent to inform the local population not to use water without prior cleaning or boiling water for health reasons. The results of this research clearly show the necessity of continuous monitoring of the quality of spring water consumed by the local population.

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