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Original Scientific Paper

MICROBIOLOGICAL SAFETY OF WATER IN PRIMARY PRODUCTION OF FOOD

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Abstract
Health-safe water is the basis of a healthy life and is one of the priorities in primary health care. Water safety involves microbiological, physicochemical and radiologically clean water, a sufficient amount of water and continuous delivery.
Due to the great epidemiological importance of water, whose impact is immediate and through which various infectious diseases can be transmitted harmful and dangerous chemicals can be introduced, it is necessary, in order to protect human health, to control the safety of drinking water.
The aim of this study was to determine the microbiological safety of water used for watering animals, as well as in the food industry.
Based on data collected from year 2015 to 2017, 26.20% water samples did not meet requirements defined by Regulation on hygienic quality of drinking water. The greatest number of samples was unsatisfactory due to an increased number of microorganisms at the temperatures of 22°C (63.40%) and 37°C (54.90%), and when it comes to pathogenic microorganisms due to the presence of intestinal enterococci (58.80%).
The lowest risk of the presence of coliforms and *E. coli* is in the water from the water supplying network, while it's much higher presence is in wells and in the water from the wellspring. The presence of intestinal enterococci is significant in all three types of drinking water, while the presence of sulphite-reducing clostridia was observed only in the wellspring water.
Keywords: drinking water, water supply, microbiological safety

INTRODUCTION

Construction of the first plumbing systems is mentioned 3000 years BC. Systems were built for the planned and rational use of drinking water and water used for other necessities. Wells more than 200 meters in the depth, were dug in ancient Egypt and water was taken out by buckets and distributed by ceramic, wooden or lead pipes. The most famous ancient water supplying networks are the Iranian canals and Roman aqueducts. In Greece and

Rome, greater and centralized water supply systems existed. At the end of the 19th century, the first water supplying networks were built in our country (Đukić and Ristanović, 2005).

Water is one of the basic conditions for life on our planet, since it is essential for maintaining of all vital processes in the biosphere. Its role in metabolism, maintaining of general and personal hygiene, food production and providing numerous needs in nature, agriculture and industry is irreplaceable. However, water is also a vector for transmission not only infectious diseases, but also harmful chemical substances, carcinogenic, radioactive and other substances. Thus, it is understandable why many countries, as well as international community, tend to protect water, primary drinking water, against any kind of pollution (Kalaba et al., 2015; Kalaba, 2014).

Water must be health-safe if used as drinking water, water for personal and general hygiene, food preparation and processing or as water in animal nutrition. Nowadays, the pollution of water sources is common, and getting a hygienically safe drinking water is big issue for modern man (Panić, 2009; Rajković, 2010)

Enhancements in engineering and technology, as well as industrialization and urbanization, have contributed to improving the quality of human life, but also contributed to the great degradation of nature i.e. contamination of water, air and soil, destruction of biocenosis and ozone layer, which are basic perquisites for human survival on earth (Gavrilović and Lješević, 1999). Therefore, water is considered to be a resource that will mark the 21st century, in an even more dramatic way than oil marked the 20th century (Todd, 1970).

Due to the great epidemiological importance of water, whose impact is immediate and through which various infectious diseases can be transmitted or harmful and dangerous chemicals can be introduced, it is necessary, in order to protect human and animal health, to control health based safety of drinking water (Gržetić, 1999).

In modern technology, food is washed before processing, in order to remove soil and dust from surface, and measures are taken, during production, to prevent dust contamination.

Natural water has its own microflora, which can be divided into two groups. The first group inlcludes microorganisms for which water is a natural habitat, and these are various saprophytes. Second group inlcudes drinking water contaminants. These are various microorganisms from the air and the earth, from plants, animals and humans. In addition to saprophytic species, patogenic species can also enter the water from the ground. If the soil is contaminated with human and animal feces, then non-pathogenic and pathogenic intestinal microorganisms can enter the water from soil. Feces contamination of drinking water is the greatest danger to human health, and therefore has the greatest medical significance (Kalaba et al., 2015).

In the food industry, plenty of water is used for washing work rooms and surfaces, machines, devices and utensils for maintaining personal hygiene of workers, then for washing raw materials, for blanching and for cooling heated products. Water must be hygienically safe, because it comes into direct or indirect contact with food (Kalaba, 2014).

The aim of this study is to determinne the microbiological safety of drinking water, as well as water used for food industry and watering animals.

MATERIALS AND METHODS

Material for testing were drinking water samples originated from farms and food industry plants, submitted within self-control and official control during the period from year 2015 to 2017. A total of 584 samples were analysed, 515 within self-control and 69 within official control.

Microbiological testing and interpretation of test results were performed in the Public Institution Veterinary Institute of the Republic of Srpska „Dr Vaso Butozan“ Banja Luka, according to the requirements of the Regulation (2010) and the Regulation (2015).

Water was sampled in aseptic conditions, in 1000 mL sterile bottles, and delivered to the laboratory in the appropriate time period and transport conditions.

In order to determine the microbiological safety of water, the method of membrane filtration (*Escherichia coli*, coliforms, intestinal enterococci, sulfite-reducing anaerobs) and method of cultivation on a solid nutrient by the pour-plate technique (figure 1. and 2.) were used, in accordance with ISO standards requirements, as follows:

1. Detection and enumeration of *Escherichia coli* and coliform bacteria (ISBIH, 2015)
2. Detection and enumeration of intestinal enterococci (ISBIH, 2003a)
3. Detection and enumeration of sulfite-reducing anaerobes (clostridia) spores (ISBIH, 2003b)
4. Enumeration of culturable microorganisms – determination of colony count by inoculation in a nutrient agar culture medium (ISBIH, 2003c)

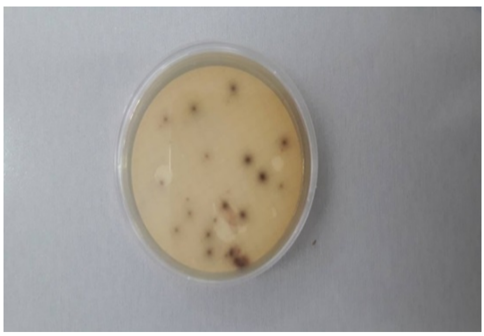

Figure 1. Membrane filtration system
Figure 2. Detection of intestinal enterococci growth on Slanetz Bartley agar

RESULTS AND DISCUSSION

The number and percentage of analyzed water samples for period from 2015-2017 are presented in table 1. and figure 3.

Table 1. Number of analyzed water samples per year

Year	Number of samples
2015	191
2016	165
2017	228
Total:	584

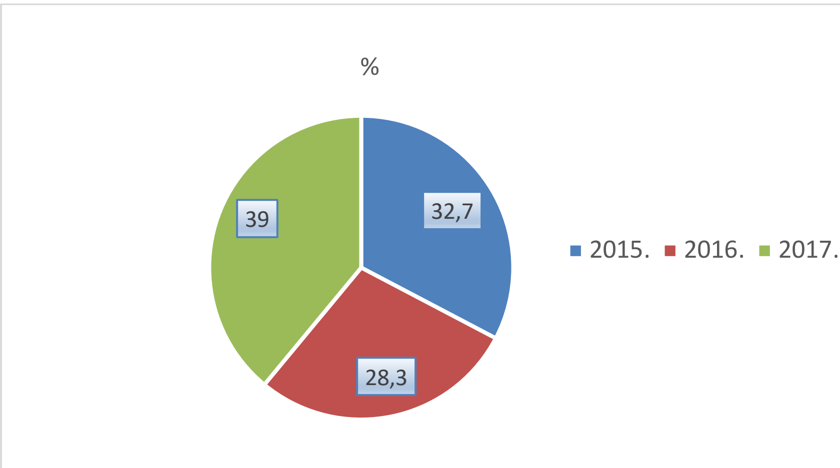

Figure 3. Percentage of analyzed samples for period from 2015 to 2017

A total of 584 samples were analyzed for period from 2015 to 2017. For the same time period, percentage of analyzed samples was 28.30-39%, compared to total number of samples.
Table 2. and figure 4. show the analyzed water samples in relation to the control method.

Table 2. Water samples in relation to the control method

Year	%	
	Self-control	Official control
2015	88	12
2016	84.80	15.20
2017	90.80	9.20

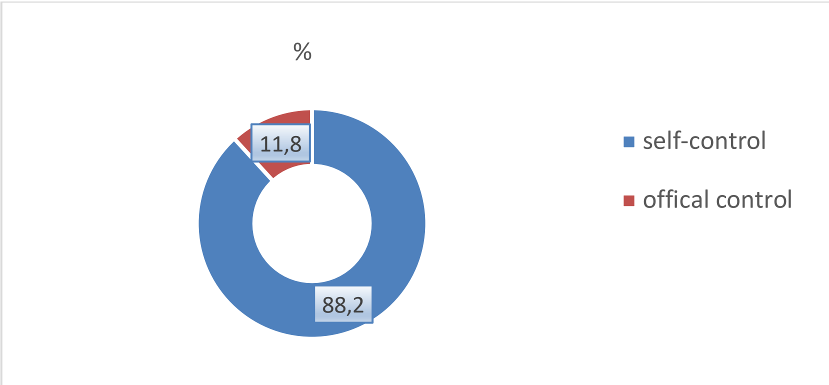

Figure 4. Water samples in relation to the control method for period from 2015 to 2017

For the period from 2015 to 2017, most of analyzed samples (88.20%) came from self-control, while 11.80% samples were submitted from official control.
Figure 5. and 6. show the analysed water samples by origin.

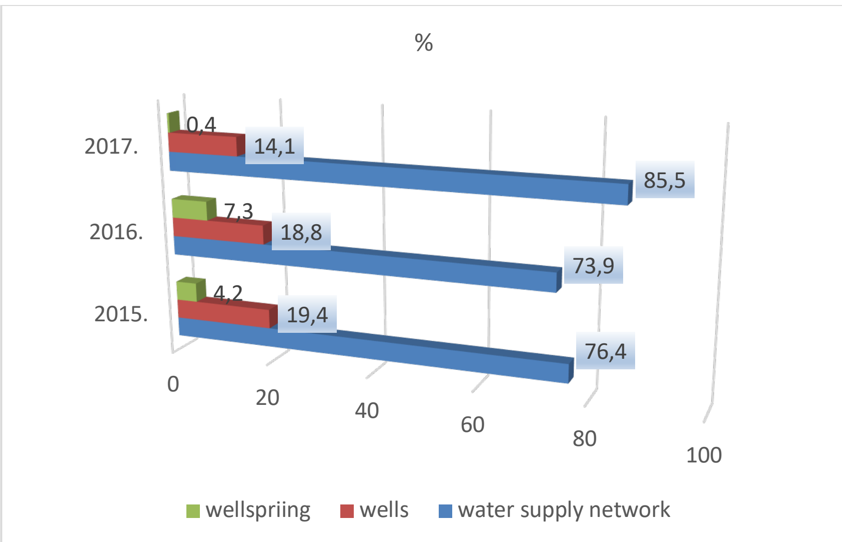

Figure 5. Water samples in relation to origin

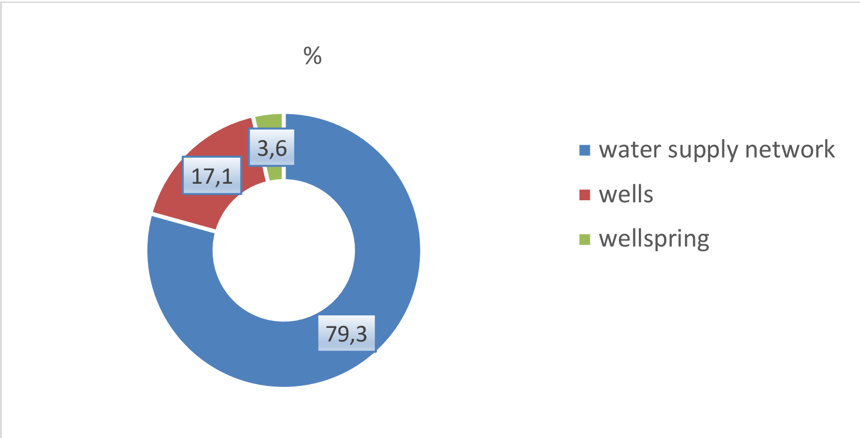

Figure 6. Water samples in relation to origin for perod from year 2015 to 2017

In relation to the origin, the analyzed samples originated from water supply networks, wells and wellsprings. For the observed period, most of the analyzed samples came from water supply networks (79.30%), followed by wells (17.10%), while the smallest number of samples came from wellspring water (3.60%). A noticeable decrease in the number of samples originating from wells and wellsprings, and increase in the number of samples originating from water supply networks, was observed in this period.
Figure 7. shows the distribution of analyzed samples by months.

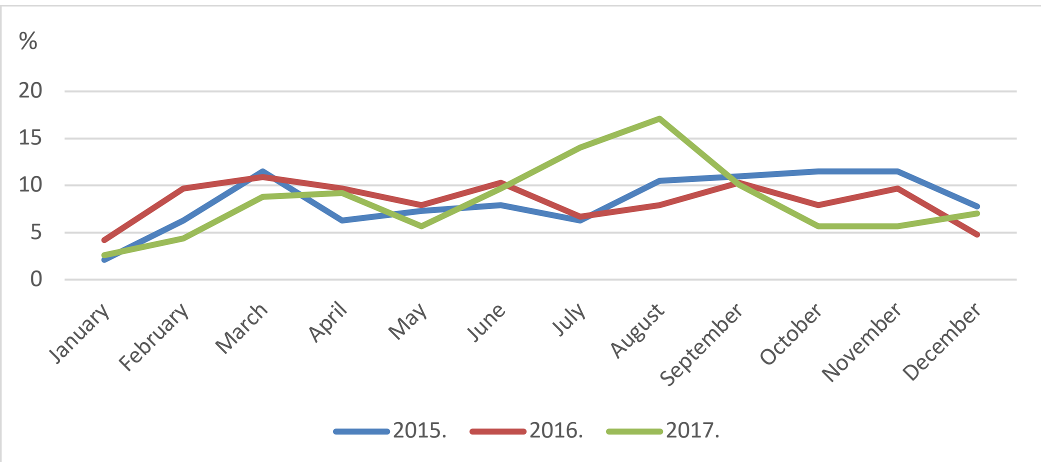

Figure 7. Distribution of analyzed water samples by months

Observed by months, it can be concluded that the sampling was performed unevenly, without an established sampling plan. However, no seasonal effect on sampling was

determined, with a slightly smaller number of analyzed samples taken at the beginning of all three calendar years.
Table 3. and figure 8. show the results of water samples testing in relation to microbiological criteria.

Table 3. Results of water samples testing in relation to microbiological criteria

Year	%	
	meet the criteria	does not meet the criteria
2015.	70.70	29.30
2016.	77.60	22.40
2017.	73.70	26.30

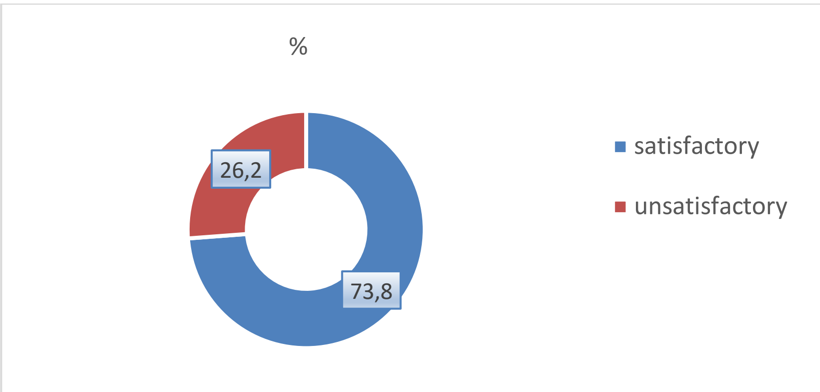

Figure 8. Results of water samples testing for the period from year 2015 to year 2017

Of the total number of tested water samples in the period from year 2015 to 2017, 26.20% of samples did not meet the requirements of the Regulation (2010; 2015), with the number of unsatisfactory samples ranged from 22.40 to 29.30%. The greatest percent of unsatisfactory samples was observed in 2015 (29.30%), and the lowest in 2016 (22.40%).
All the samples submitted within the official control were satisfactory, while the share of unsatisfactory samples submitted within self-control is shown in figure 9.

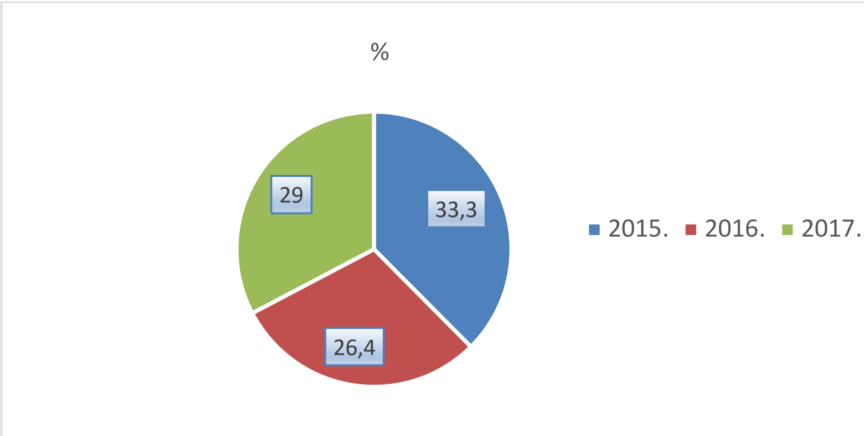

Figure 9. Unsatisfactory samples originating from self-control

The number of unsatisfactory samples originating from self-control, observed by years, was equal and ranged from 26.40% to 33.30%. Figure 10. and 11. show unsatisfactory water samples by origin.

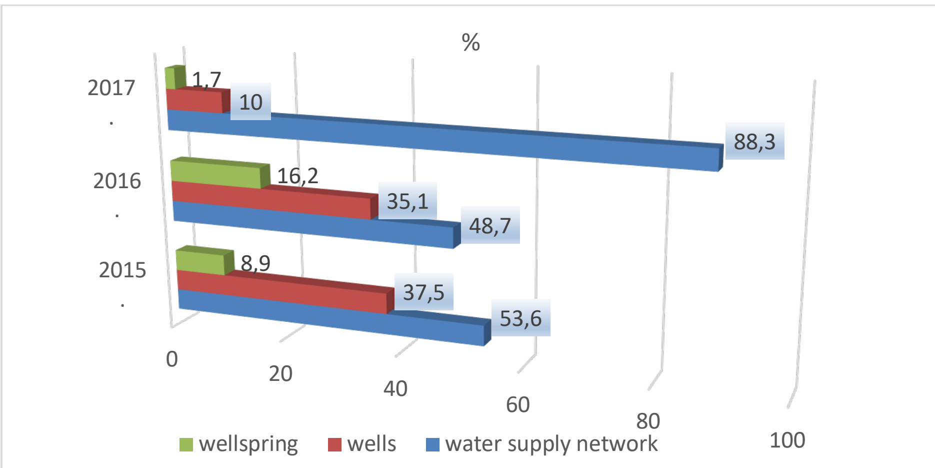

Figure 10. Unsatisfactory water samples related to origin

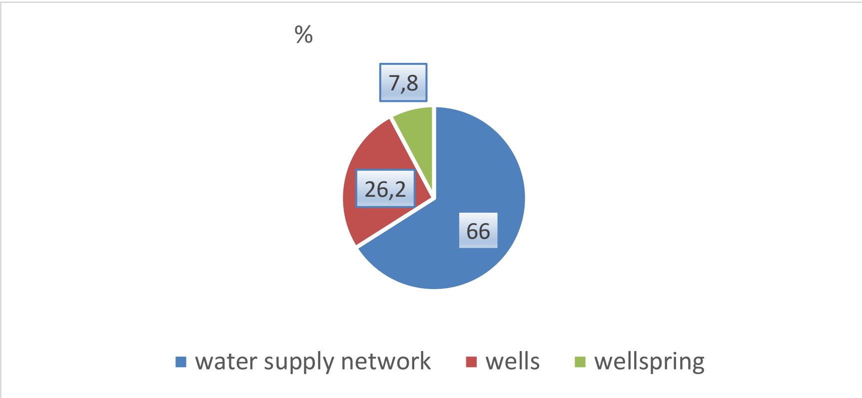

Figure 11. Unsatisfactory water samples related to origin for period from year 2015 to year 2017

Observing unsatisfactory samples in relation to origin, the greatest number refers to water that originated from water supply network. This could be expected, because most of the analyzed samples originated from water supply networks. For the observed three-year period, it refers to 66% unsatisfactory samples originating from water supply network, 26.20% from wells, and 7.80% from wellsprings. In this period, there is noticeable decrease in the number of unsatisfactory samples originating from wells and wellsprings, and an increase in the number of unsatisfactory samples originating from water supply networks. Figure 12. shows the distribution of unsatisfactory water samples by months
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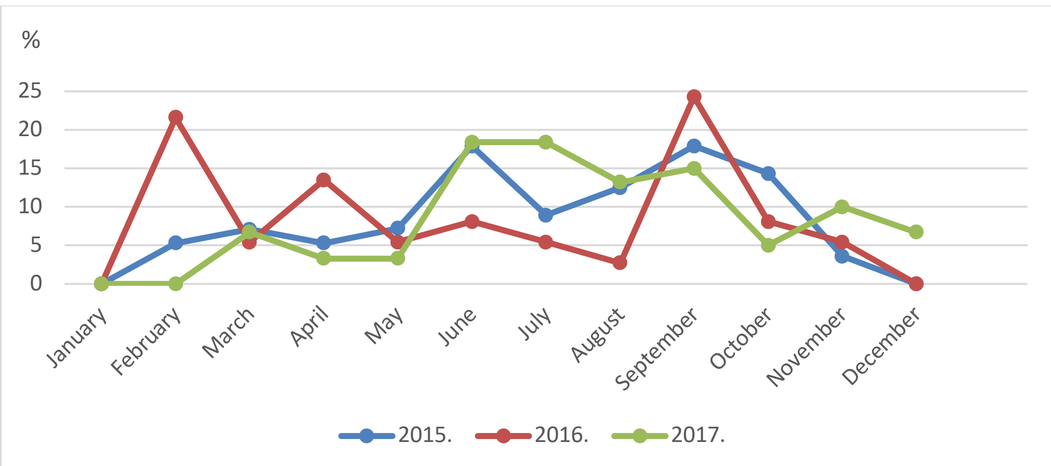

Figure 12. Distribution of unsatisfactory water samples by months

Observed by months, it can be concluded that there was no seasonal effect on the number of unsatisfactory samples. Nevertheless, there was a decreases in the number of

unsatisfactory samples at the beginning and end of three calendar years.
Figure 13. and 14. show unsatisfactory samples according to examined parameters.

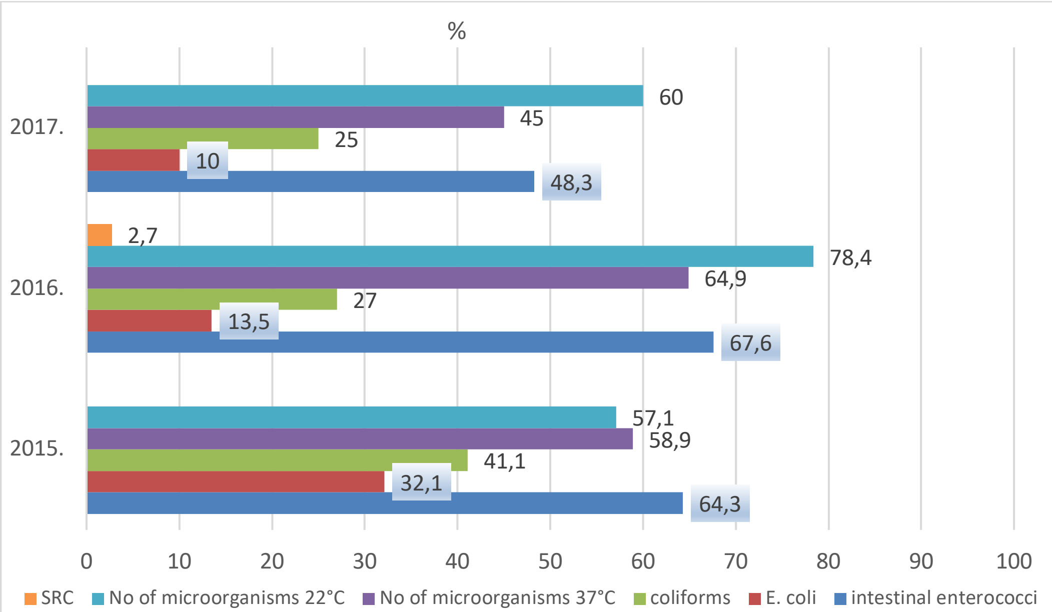

Figure 13. Unsatisfactory water samples accodring to examined parameters

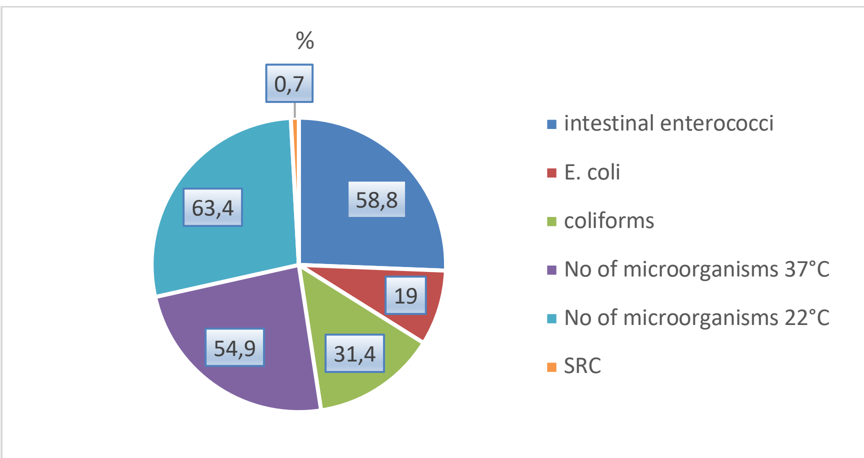

Figure 14. Unsatisfactory water samples related to examined parameters for period from year 2015 to 2017

Analyzing unsatisfactory samples in relation to examined parameter, for the period from year 2015 to 2017, it can be observed that the largest number of unsatisfactory samples was due to increased number of microorganisms at 22°C (63.40%) and 37°C (54.90%), and when it comes to pathogenic microorganisms, the presence of intestinal enterococci was on the first place (58.80%). Sulfite-reducing clostridia were found in water samples in year 2016 (2.70%) while in years 2015 and 2017, they were not found, leading to total of 0.70% of unsatisfactory samples for observed three-years period. It is important to emphasis that from one year to another, starting from 2015 to 2017, the number of coliforms and *E.coli* decreases significantly, from 41.10% to 25% for coliforms and from 32.10% to 10% for *E. coli*. In contrast, the number of intestinal enterococci is constantly maintained at a high level, with minor variations from one year to another. Figure 15. shows unsatisfactory water samples by examined parameters and origin for the period from year 2015 to 2017.

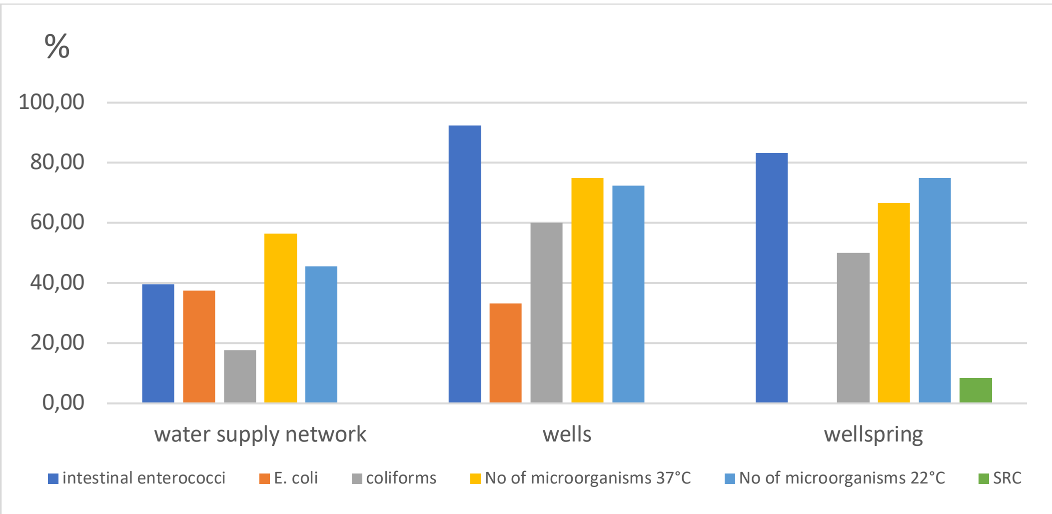

Figure 15. Unsatisfactory water samples related to examined parameters and origin from year 2015 to 2017.

If unsatisfactory water samples are examined related to origin and in relation to observed parameter, it may be noticed that the lowest risk of the presence of coliforms and *E. coli* is in water supply network water, while it is significantly higher in well and wellspring water. When it comes to intestinal enterococci, their presence is significant in all three types of drinking water, with the fact that they are present in well and wellspring water in over 80% of unsatisfactory samples. The risk of sulfite-reducing clostridia, i.e. their presence, was determined only in wellspring water (8.30%).
The supply of water for drinking, food production or watering of livestock depends on the origin and quality of water, i.e. the quality of springs and the number of consumers. Local water supply meets the needs of a smaller number of consumers. These facilities rarely provide quality and safe drinking water. Central water supply meets the needs of a larger

number of consumers. This water is taken from rivers or lakes and treated to comply with drinking water requirements. Every water supply network system must have arranged and protected water source, catchment, reservoir and water supply network, and the quality of the water supply network must be constantly controlled (Marjanović, 2010).

The most important group of microorganisms in the overall bacteriological examination of drinking water are bacteria indicators of fecal pollution. Many types of enterococci are of fecal origin and may be indicators of fecal contamination. Enterococci belong to the normal intestinal flora and their pathogenic potential is small. Since enterococci disappear from water much faster than coliform bacteria, their presence in the water indicates that contamination has recently occurred or that there is a large number of enterococci. For these reasons, enterococci may be better indicators of pollution than *E. coli.* They are significantly more resistant to chlorination than enterobacteria. Monitoring the number of enterococci can be used to assess the success of water treatments (Kalaba et al., 2015).

E. coli belongs to the group of enetrobacteria. The feces of humans and animals are rich in the bacteria, which lives in the lower part of digestive tract of mammals and participates in the process of digestion of food, i.e. in the intestinal flora activities. *E. coli*, in water, occurs as a result of human, animal, or certain agriculture activities. Compared to many pathogenic bacteria, viruses and protozoa, *E. coli* is much more sensitive to the action of disinfectants. The total number of coliform bacteria give us information about water quality. The presence of coliform bacteria in the water indicates the possibility of contamination of the source and potential distribution across the water which can cause an epidemic (Agrawal and Rajwar, 2010; Kalaba et al., 2015; Đukić et al., 2000).

Although they are not indicators of fecal pollution, the presence of a significant number of aerobic mesophilic bacteria in drinking water indicates the possibility of contamination with human or animal organic matters, the unhygienic water treatment and the insufficient purification efficiency (Singh et al., 2010; Delević et al., 2012; Kalaba et al., 2015)

Anaerobic bacteria in water are given less attention than aerobic ones. It is thought that anaerobic bacteria indicate organic decomposition and fecal contamination. Clostridia are much more resistant to external conditions than other bacteria and therefore their findings in water, without the presence of *E. coli* or enterococci, indicates that it is an old contamination of water with feces, although *Clostridium perfringens* may also originate from the ground (Đukić and Ristović, 2005).

Their findings in water proves the hygienic malfunction of water and the use of such water is risky especially if it is used for the preparation of foodstuff in which they can reproduce and cause food poisoning (Kalaba, 2014).

CONCLUSION

Based on the results obtained in the study, the following conclusions are drawn:

In the period from year 2015 to 2017, 26.20% of water samples did not meet the requirements defined by regulation.

In relation to the origin, the largest number of unsatisfactory water samples refer to tap water (66.00%), followed by wells (26.20%) and wellsprings (7.80%).

In relation to the tested parameters, the largest number of unsatisfactory samples was due to the increased number of microorganisms at 22°C (63.40%) and 37°C (54.90%), and when it comes to pathogenic microorganisms due to the presence of intestinal enterococci (58.80%).

For the period from year 2015 to 2017, the number of coliform bacteria decreased significantly from 41.10% to 25.00% and from 32.10% to 10.00% for *E. coli.* In contrast, the number of intestinal enterococci is constantly maintained at a high level.

In relation to pathogenic microorganisms, it is noticed that the lowest risk of the presence of coliforms and *E. coli* is in tap water, while it is significantly higher in well and wellspring water. The presence of intestinal enterococci is significant in all three types of drinking water, with the fact that they are present in well and wellspring water in over 80% of unsatisfactory samples. The risk of sulfite-reducing clostridia, i.e. their presence, was determined only in wellspring water (8.30%).

In relation to sampling period and water analysis, no seasonal impact on the number of unsatisfactory samples was determined.

A relatively high percentage of microbiologically defective water samples indicates environmental pollution by waste materials, poor sanitary condition of water supply facilities, and insufficient purification of drinking water before use and before disinfection. The supply of drinking water from local water supply facilities (wells and wellsprings) is unsafe and poses a risk of water-borne diseases and generates an epidemiologically unsafe situation.

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