# Microbiological composition of untreated water during different weather conditions

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**Abstract**

**Introduction**: Water can support the growth of different microorganisms which may result in contamination. Therefore, the microbiological examination is required for testing the hygienic probity of water. In the study of microbial composition of untreated, natural spring and mineral water differences in the presence and number of bacteria during the two periods, winter and summer, are detectable.

**Methods**: In our study, we analyzed and compared the following parameters, specified in the Rulebook: total bacteria and total aerobic bacteria (ml/22 and 37°C), total Coliform bacteria and Coliforms of fecal origin (MPN/100ml), fecal streptococci as *Streptococcus faecalis* (MPN/100ml), *Proteus spp* (MPN/100ml), and *Pseudomonas aeruginosa* (MPN/100 ml) Sulphoreducing *Clostridia* (cfu / ml). The paper is a retrospective study in which we processed data related to the period of 2005-2009 year. While working, we used the descriptive-analytical comparative statistical treatment.

**Results**: The obtained results show statistically significant differences in the microbial composition of untreated water in the two observed periods,

**Conclusions**: Findings were consequence of different weather conditions in these periods, which imply a number of other variable factors.


*Keywords: microbiological composition, untreated water, the time periods.*

## Introduction

Concerns for securing of sufficient quantities of hygienically proper drinking water have accompanied the mankind through all the stages of its development. The need for hygienically proper water is on the rise while existing reserves are reducing due to a continuous intense pollution. Therefore the water is becoming a restrictive factor for further economic development of the country, and a factor responsible for outbreak of contagious diseases (1). Bacteriological contamination of water is very frequent, and therefore the bacteriological examination is very important when determining the eventual risk of outbreak of contagious diseases. Bacterial contamination of water by pathogenic microorganisms is most often a consequence of inadequate disposition of waste fecal waters (2). The use of contaminated water may lead to the outbreak of epidemic spread of various contagious diseases. Bacteria such as *Escherichia coli, Salmonella, Shigella* and *Vibrio* from intestinal tract of humans and other warm-blooded animals frequently get into water in nature through feces and urine, leading to contamination (3). Underground waters are also frequently contaminated, and can hold even up to several million microorganisms in 1 ml of water. Microorganisms get into such waters from various sources: from the soil, air, atmosphere and surface waters, from the rocks themselves, but also as a consequence of the living organisms' activity. The quantity of microorganisms in underground waters depends on the temperature, presence of organic matter, dissolved oxygen, certain pH value of environment and so on. Richer and more versatile population of microorganisms develops in fresh waters than in salty ones. Besides the global significance for the circulation of matter, microorganisms in water are important factors for the process of bio-purification of waters. By origin, waters may be classified in three main groups: atmospheric, surface and underground. For human use, i.e. for drinking, all types of waters are being used, but often prior

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to their use their quality must be improved. Underground waters from greater depths are by composition and quality usually pure and most desirable as drinking water. Shallow underground waters are most frequently not good quality and are more rarely used for drinking purposes (4). Underground waters represent raw material of particular social interest, since in the majority of countries, and so in ours as well, due to their advantages, mainly they are the ones used for water supply for the population and the part of industry requiring quality water. The term "untreated water" denotes the water which has not been chemically treated, or filtered, or boiled in order to eliminate microorganisms. The underground water reserves are significantly greater than the needs for water, but the continuous increase in waste waters that are, in ours and other underdeveloped countries and developing countries, without prior purification, dumped into recipient or into the soil, represents a threat in terms of their contamination. The main sources of natural water contamination include: waste waters from urban environments, mineral fertilizers, inorganic matters, acid mine and drainage waters, waste waters from treatment and utilization of mineral raw materials, sedimentary and radioactive matters and waste heat. Composition and quality of water in nature are very much variable and depend on the series of factors. Also significant are their origin, history, chemical composition, but also the presence and composition of the living forms therein. The quality of water is influenced by: the temperature, the pH and the oxygen content in water, and other ecological factors which vary depending on the time of the day and night, as well as over the year. Underground water is destitute of organic matters and their composition depends on the soil they run through, and if they emerge from shallow depths their composition is similar to the composition of surface waters. When surface waters permeate through the soil to the underground waters they carry organic matters as well, and a part of these matters remains in the soil they run through. Underground waters from greater depths are either thermal or mineral, and frequently healing (5). The quality of water depends also on the geographical features of the area, particularly important of which are: the climate, altitude and fouling with vegetation because they have a direct influence on the type and quantity of precipitation. In the areas with mountain climate and at greater heights above sea level in the "colder" months it snows, and in the summer months it often rains (6). The objective of our work was to examine the microbiological characteristics of untreated spring, well and borehole waters, and to compare the results in various time periods in order to determine the eventual impact of external factors on the microbiological contamination of these categories of waters.

## Methods

*Samples*

As a working material, we used the results of untreated water groups analyses by the contractors and companies from the Department for Microbiology of Food and Water of the Institute for Public Health of the Federation of Bosnia and Herzegovina, from 2005 to 2010. These include the unprocessed, untreated spring and well waters, as well as the waters from boreholes, which were analyzed monthly at this Department in accordance with the Rulebook. Every month, 10 samples were taken (120 on an annual basis, i.e. 600 in total). Water samples for bacteriological analysis were taken in accordance with the Directives of the Rulebook on the Manner of Taking Samples and Methods of Bacteriological Analysis of Drinking Water ("Rulebook on the Manner of Taking Samples and Methods of Laboratory Analysis of Drinking Water", Official Gazette of Socialist Federal Republic of Yugoslavia, No. 33/87).

*Procedures*

The bacteriological examination in MPN technique was conducted by standard laboratory methods (7). Bacteria were enumerated based on the turbidity in individual specimens by referenced to the MPN tables (8). Acceptable norms for individual groups of bacteria were construed upon the "Rulebook on Hygienic Properness of Drinking Water".

*Statistical analysis*

The following methods were used for the analysis of the research results: Descriptive statistics (minimum and maximum value, average

TABLE 1. The results of K-S tests for measurement of the "normality"

| 2005 – 2009 | Total microorg. | Aerobic bacteria | *E. coli* | *S. faecalis* | *Proteus spp.* | *P. aeruginosa* | Sulpho-reducing Clostridia | Sterile |
|---|---|---|---|---|---|---|---|---|
| K-S results – z value | 2,033 | 1,423 | 2,692 | 2,670 | / | / | 3,480 | 1,235 |
| K-S results – p value | 0,001 | 0,035 | 0,000 | 0,000 | / | / | 0,000 | 0,095 |

TABLE 2. Overview of positive samples by years 2005 – 2009

| Year | Samples with increase in total of bacteria % | Samples positive on aerobic bacteria % | Samples positive on *E.coli* % | Samples positive on *S. faecalis* % | Samples positive on Sulpho-reducing Clostridia % | TOTAL |
|---|---|---|---|---|---|---|
| 2005 | 9 (7,5) | 10 (8,3) | 6 (5,0) | 5 (4,2) | 2 (1,7) | 32 (26,7) |
| 2006 | 7 (5,8) | 7 (5,8) | 6 (5,0) | 6 (5,0) | 5 (4,2) | 31 (25,8) |
| 2007 | 7 (5,8) | 9 (7,5) | 4 (3,3) | 3 (2,5) | 4 (3,3) | 27 (22,5) |
| 2008 | 7 (5,8) | 9 (7,5) | 4 (3,3) | 6 (5,0) | 0 (0,0) | 26 (21,7) |
| 2009 | 7 (5,8) | 9 (7,5) | 6 (5,0) | 6 (5,0) | 1 (0,8) | 29 (24,1) |
| Total | 37 (6,2) | 44 (7,3) | 26 (4,3) | 26 (4,3) | 12 (2,0) | 145 (24,2) |

TABLE 3. Comperative overview of results of untreated water analyzes during the period (October-March and April-September) from 2005 to 2009.

| 2005-2009 | Total microorg. | | aerobic bacteria | | *E. coli* | | *S.faecalis* | | *Proteus spp.* | | *P. aeruginosa* | | Sulphoreducing Clostridia | | Sterile | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Period of year | 4/9 | 10/3 | 4/9 | 10/3 | 4/9 | 10/3 | 4/9 | 10/3 | 4/9 | 10/3 | 4/9 | 10/3 | 4/9 | 10/3 | 4/9 | 10/3 |
| Number of months | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Xmax | 179 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 7 |
| Xmin | 7402 | 410 | 6 | 3 | 3 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 6 | 2 | 9 | 10 |
| Avarage value | 2105,8 | 79,43 | 3,13 | 1,20 | 1,43 | 0,07 | 1,23 | 0,13 | 0,00 | 0,00 | 0,00 | 0,00 | 0,63 | 0,10 | 6,13 | 8,57 |
| Weighing error | 359,18 | 19,57 | 0,24 | 0,16 | 0,17 | 0,05 | 0,17 | 0,06 | 0,00 | 0,00 | 0,00 | 0,00 | 0,23 | 0,07 | 0,21 | 0,16 |
| St.deviation | 1967,31 | 107,19 | 1,33 | 0,89 | 0,94 | 0,25 | 0,94 | 0,35 | 0,00 | 0,00 | 0,00 | 0,00 | 1,25 | 0,40 | 1,14 | 0,86 |
| Coefficient of variation | 93,42 | 0,00 | 42,52 | 73,89 | 65,25 | 380,56 | 75,83 | 259,31 | | | | | 196,61 | 402,58 | 18,53 | 10,02 |
| z or t | -6,581 | | -5,226 | | -5,707 | | -4,887 | | 0,000 | | 0,000 | | -2,554 | | -9,35728 | |
| *p* | 0,000 | | 0,000 | | 0,000 | | 0,000 | | 1,000 | | 1,000 | | 0,011 | | 0,000 | |

value, standard deviation and variance coefficient), Kolmongorov-Smirnov test (K-S test) for the "normality of distribution" in the period observed, nonparametric U test and T test. We started our research with the results of the Kolmongorov-Smirnov test (K-S test) for measurement of the "normality of distribution" of treated samples in the period of 2005 – 2009. (Table 1). For *Proteus spp.* and *Pseudomonas aeruginosa* types on all measurements the values were equal 0, so we do not have the test results, since this is a series of constants, and not the frequency distribution. Only for variable "sterile", the *p* value was greater than 0.05 and therefore satisfies the assumption of normality. All other variables failed to satisfy the assumption of normality considering that the associated p value of the K-S test was less than 0.05. Hence have we used for comparisons of the variable "sterile" the parametric t - test, and for others the non-parametric U - test.

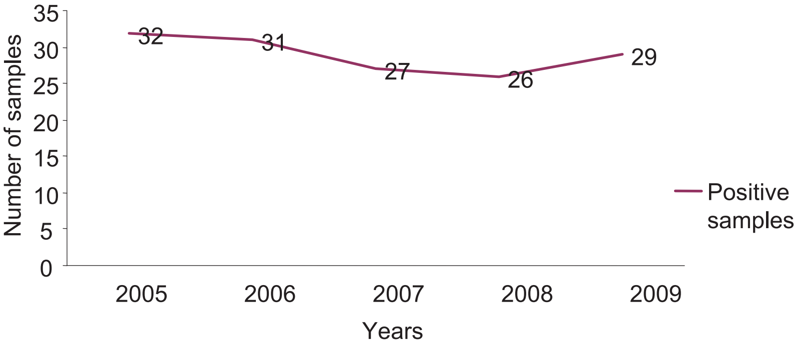


FIGURE 1. Positive samples by years of observation

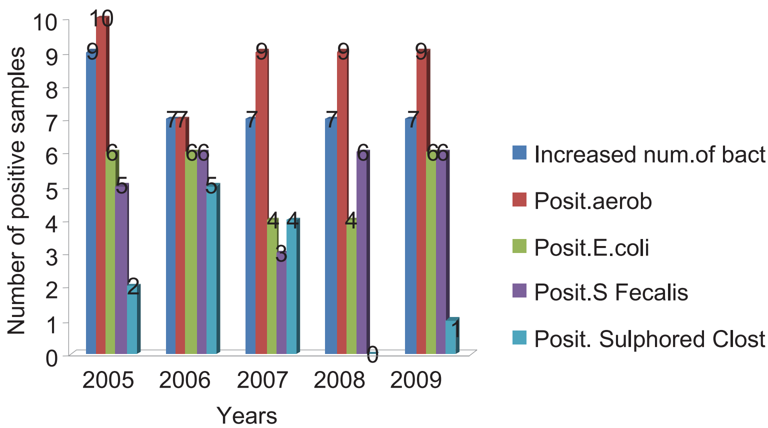


FIGURE 2. Presence of some contaminants in water

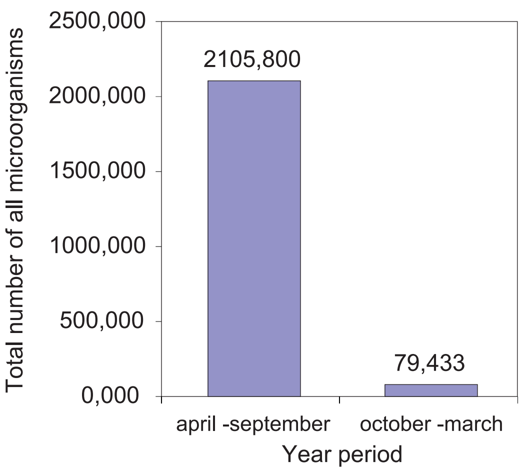


FIGURE 3. Average value for total bacteria by periods

## Results

Our research is based on the samples which were taken in the five years (from 2005. to 2009.), by individual parameters. (Table 2, Figure 1). Each year 120 samples were taken, out of which various were positive, and varied from 26 (2008.) to 32 (2005.). The greatest number of positive samples, 32 was in found in year 2005, which is 26.7% of samples taken in that year. The most frequent parameter that was not in accordance with the Rulebook was the presence of aerobic bacteria which were found in 44 samples in the five-year period observed, which is 7.3% of total taken samples (Figure 2). Our research was directed on presence of contaminants in water during two periods: from April until September and from October until March. Overview of results is presented in Table 3. Preciseous analyzes were conducted by years, for every five years. Observed parameters were monitored in the periods from April until September and from October until March. During this two observed periods there were large differences in „total bacteria“ (Figure 3). Our results showed that a smaller total number of microorganisms was identified, as well as the presence of aerobic bacteria, *Escherichia coli, Streptococcus faecalis* in the period from October to March, compared to the period April-September, when it was sterile and a number of measurements. We observed a statistically significant difference in the total number of microorganisms, the number of samples with aerobic bacteria, *Escherichia coli* and the presence of sterile measurements, because the *p* values correspond-

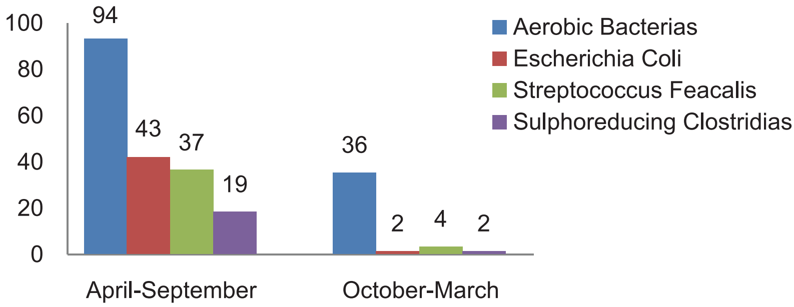


FIGURE 4. Number of positive samples per period, according to the contaminants

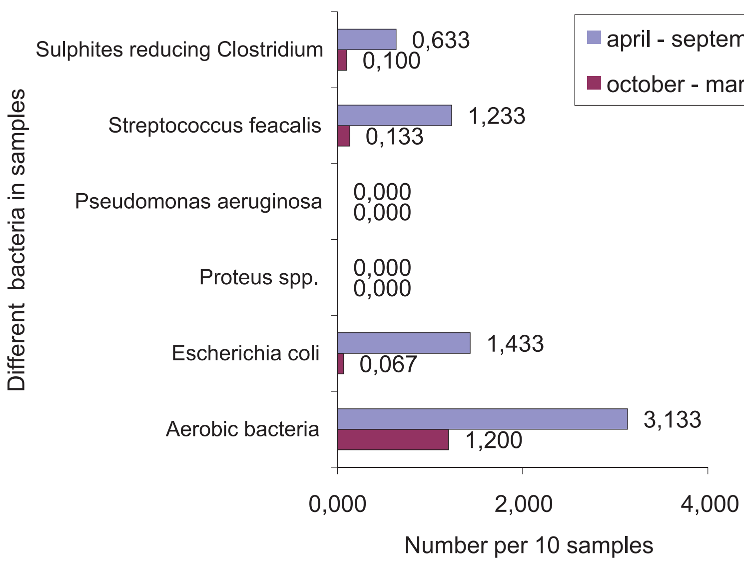


FIGURE 5. Average value for different bacteria by periods

ing U or t - test, were lower than 0.05 (Figure 4). Sulphoreducing Clostridias were significantly higher in the period April-September for all observed years except 2009, when they were increased, but not statistically significant. The most frequently isolated, observed in both periods, were the aerobic bacteria, but with much higher frequency in the period April-September. During this period there were followed by the presence of *Escherichia coli, Streptococcus faecalis* and *Sulphoreducing Clostridias* (Figure 5). In the period April-September, after the aerobic bacteria the most frequent were *Streptococcus faecalis* and *Sulphoreducing Clostridias*, while *Escherichia coli* was rarely isolated.

## Discussion

Research of the microbiological composition of the spring untreated water during different months of the year was conducted within our research. According to available information, so far in our area, similar researches were not done. We analyzed 10 samples each month, or 120 samples per year, and this is a total of 600 samples for the 5 years that we observed. In statistical analyzes, the results are divided into two periods, „winter" (October - March) and „summer" (April - September). We monitored 7 different parameters including: total number of present microorganisms - quantitatively, and for positive samples: aerobic bacteria at 22° and 37° C, *Escherichia coli, Streptococcus faecalis, Proteus spp, Pseudomonas aeruginosa* and *Sulphoreducing Clostridias* - qualitatively. The total number of microorganisms showed significantly higher values during the "summer" period, then "winter", and applied statistical tests showed statistically significant difference. Findings are confirming that different weather conditions, especially those who have influence on the melting snow and runoff water from higher to lower areas have a significant impact on the contamination of underground waters, including waters used in our research. Cabral et al. (4) also did research on effects fecal contaminations on environment. He points out importance of testing on coliform bacteria as important indicator of contaminated waters. Samples that were analyzed come from traps located in mountain area of Bosnia and Herzegovina at the altitude of 820, 1000 and 1200 m. Only one trap was on 450 m above the sea, though its waters come from nearby mountain with height of more than 1000 m. In these areas the mountain climate is predominant. Characteristics of it are increased amount precipitations during the entire year – snow during the winter and rain at springs and summers. Snowfalls has no important direct impact on contamination of underground waters, though at the season of malting snow brings to significant increase of the mount of the waters on the surface and its pouring into the ground. That often results into contamination of underground waters. It shows indirectly that snowfalls significantly influence on contamination of the underground waters. During the summer time in this areas are frequent rainfalls what results into erosion. It makes easier and increases contamination of untreated wells and drills. Similar researches are conducted in Guinea Bissau on 1998. During the rainy season when epidemics of cholera broke out as well as other diseases related to water. Research was targeting the termination of microbiological characteristic of drinking water during the rainy season (9). Microbiological analyzes were including the termination of total number of coliform bacteria, *Escherichia coli* and *Enterococcus faecalis*, what coincide with microbiological parameters followed in our research. What was obvious was increase of fecal contamination during the rainy season showed in our researches as well and significant statistically differences were detected. Researches and quantitative estimation of pathogen Pearl River Delta in China, as well as springs of the drinking water were conducted on samples of water from six reservoirs in two seasons during 2006. Results showed that outside environment factors – falls, location as well as certain internal environmental factors, physical and chemical characteristics of water have direct impact on presence of coliforms (10). Analyzes done by the seasons showed the level of coliform bacteria during the fall season as well as melting the snow showed increase of one to two lines size compare to a dry season, what is exactly the same as shown in our researches. Basic elements of analysis showed the level coliform is tightly connected to physical and chemical characteristics while fecal coliform are more connected to the external components brought in by the influence of the seasons. Contamination of the ground water is connected to human activities and pollution of the waters on the surface (11,12). Aiming to test environmental effects of bacterial content of sediment of Dongping lakes in China, researches were done in six positions for sampling in two periods – July and October to determine bacterial diversities, what in many aspects is similar to our research. Little differences are founded amount samples. However, bacterial indexes of difference where significantly different samples. Abundance, prevalence and evenness found samples during the dry season (October) where generally higher compare the same found during wet season (July), while domination of the bacterial species was higher during the wet season, what again is similar with findings in our researches. As a conclusion of this experiment it has been established that environmental factors are affecting prevalence of bacteria in tested lake's sediment (13). The other researches showed the level of contamination of the waters directly connected to a season (14). Some researches were conducted aiming to assess risks of appearance of diseases caused by drinking water in the US. Contamination of the drinking water was not spread correctly, though under the influence of the certain factors: number of pathogen in the source of the spring, agents of the water system, quality of it, as well as changes in climate (15,16). Microbiological contamination of waters often brings to emergence of different human diseases, what emphasizes important of the clearness of water as one of the basic preventive measures for combating diseases of that kind (17,18). This research is preliminary in this area and it presents a screening of possible influence of certain climate and other outside factors on microbiological content of spring untreated waters, heavily used in process of further consuming and bottling. Results could signalize coordination of dynamics of control of this waters and taking of appropriate measures in controlling of these waters as well as taking measures in the contamination of springs in certain period of times during a year, aiming prevention of emergence or reduction of risks of spreading infectious diseases spread by the water, what is pointed out by Jha M. and Gu R. (19). Due to potential increasing problem of supplying enough quantity of drinking water much more attention has to be dedicated to the laboratory control of microbiological quality of bottled waters (20).

## Conclusions

In this paper we present a research of microbiological contamination of spring water taken from the trap in mountainous parts of the country. Our research has shown that the contamination is much higher during the period April-September compared to the period October-March, which can be associated with melting snow and groundwater contamination. It would be useful to continue this research with detailed monitoring of single climate and other factors of external environment in order to determine which factor is most related to water contamination.

## Competing interests

Authors declare that they do not have any conflict of interest.

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