

UDK 611(082)

ISSN 1512-8245



AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE  
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ  
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

# RADOVI

KNJIGA XCII

Odjeljenje medicinskih nauka

Knjiga 31

Centar za medicinska istraživanja

Knjiga 2

*Redakcioni odbor*

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redovni član Akademije nauka i umjetnosti  
Bosne i Hercegovine

SARAJEVO 2003

## WATER QUALITY AND MICROBIOLOGICAL STATUS OF THE DISTRIBUTION SYSTEM EVALUATED USING ESTABLISHED AND EMERGENT PARAMETERS

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### Abstract

The problem of the lack of good quality and quantity of water for all purposes has been increasing due to the war damage to water supply plants, the effects of the unique phenomena of subsidence of the area as well as flooding caused by recent heavy rain in the area of Tuzla Canton. The flood has resulted in pollution of the drinking water and, in the light of this emergency we carried out a study to determine drinking water quality by two methods: traditional tests required by law and specific laboratory tests.

The aims of the microbiological analysis of water were: to detect evidence of excretal biological pollution as a result of the flooding in the area of Tuzla Canton in 2002; to evaluate the required laboratorial procedures in Bosnia and Herzegovina for the detection of potent pathogens in the drinking water.

The study included the examination of 99 samples of water: 48 samples from municipal water supplies; 13 from closed sources and 38 from open sources. Samples of water were tested by routine bacteriological, parasitological and biological methods. Reverse transcription –polymerase chain reaction (RT-PCR) was applied for the detection of viruses.

Micro organisms were absent in four (4.04%) of the 99 samples of water. Out of 95 samples of water, 240 micro-organisms were isolated as follows: 114 strains of bacteria, 56 viruses, 52 bacteriophages (19 coliphages and 33 *Salmonella enteritidis* phage), 2 nematodes, 16 algae.

According to traditional tests required by law, water from 35.35% (35/99) sources was found suitable for drinking but using specific laboratory tests, only 10.10% (10/99) of samples were in compliance with the law. There was a significant difference in water quality ( $p<0.01$ ).



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These results call for a revision of water quality guidelines based only on indicator organisms without also making reference to the absence of viruses. We have pointed out the importance of all the parameters, which should be applied during emergencies such as the recent flooding. We also suggest that, along with routine examination of drinking water there should be periodically (per month or per year) incorporated into the current protocol extra measures for detection of enteroviruses and bacteriophages.

## Introduction

The biggest problem with the environment in many parts of the world is the lack of water and/or polluted water. As a potential carrier of pathogenic micro-organisms, water can endanger health and life. The World Health Organization (W.H.O.) has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water or unavailability of water for all purposes (W.H.O. 1981).

Water-borne pathogens are disease-causing bacteria, viruses, and protozoa that are transmitted to people when they consume untreated or inadequately treated water. The pathogens most frequently transmitted through water are those which cause infections of the intestinal tract; namely, hepatitis A, poliomyelitis, typhoid, paratyphoid, cholera, bacterial dysentery, giardiasis, amoebic dysentery, etc. The causative organisms of these diseases are present in the faeces or urine of an infected person and when discharged may gain entrance into a body of water that ultimately serves as a source of drinking water.

Water is also an important vehicle for extra intestinal infection. A number of pathogens can be acquired through occupational, recreational, and even therapeutic contact with water. Water-borne pathogens can enter the human body via intact or damaged skin, mucous membranes and by inhalation. Extra intestinal infections are primarily superficial infections involving the skin but severe systematic diseases may occur in those who are immunological deficient. The spectrum of microbial agents that can cause such water related diseases includes bacteria, fungi, viruses, and protozoa (Mandell GL, 1995).

Some parts of Bosnia and Herzegovina, especially the area of Tuzla Canton, lack good quality and quantity of water for all purposes. Since 1992 within Tuzla Canton, the problem of the lack of water has been increasing because of the war damage to water supply plants as well as the effects of the unique phenomena of subsidence of the area. Heavy rain has caused flooding in the area recently which, in some places, has resulted in the mixing of different kinds of water with

drinking water. Bacteria and other micro-organisms have polluted drinking-water supplies; sewage spills have occurred, forcing people to boil their drinking water. There was a high risk of an epidemic from water-borne pathogens and therefore it was necessary to carry out intensive controls by quick and simple tests for the detection of intestinal organisms.

In the light of such an emergency we carried out a study to determine drinking water quality. In addition to traditional tests recommended by the W.H.O. and required by "The rule book of hygienic quality of drinking water" (37°C and 20°C viable count, total and faecal coliforms and faecal streptococci) Yugoslav Official Register 13/91 we carried out tests for *Pseudomonas aeruginosa*, other Gram negative bacilli, Gram positive aerobic and anaerobic sporogenic bacilli, *Mycobacterium* species, enteroviruses, bacteriophages, algae and nematodes.

## Goals

The aims of the microbiological analysis of water were:

- to detect evidence of excretal biological pollution as a result of the flooding in the area of Tuzla Canton in 2002.
- To evaluate the required laboratorial procedures in Bosnia and Herzegovina for the detection of potent pathogens in the drinking water.

## Materials and methods

The study included the examination of 99 samples of water, which were collected in the area of Tuzla Canton. Three kinds of water sources were examined: 48 samples of municipal drinking water (potable water); 13 from closed sources and 38 from open sources.

Samples of water for bacteriological testing were collected in sterile bottles of 250 ml capacity and for virological, biological and parasitological testing in sterile containers of 10-litre capacity. The samples included the following information:

- Code number of the sample.
- Where the water had been collected (open source, closed source and municipal water supplies)
- Whether the water has been chlorinated.
- Temperature of the source of the sample.
- Date and time when the sample was taken and dispatched.

## **Residual chlorine detection**

Residual chlorine in the water was measured by the DPD (N, N-diethyl-1, 4-phenylenediamine) colorimetric technique (Cheesbrough M, 1992).

## **Bacteriological examination**

The routine bacteriological procedures were applied to detect the presence of: coliform bacteria, *Escherichia coli*, *Enterobacter*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus*, *Serratia*, *Pseudomonas*, other genera of Gram negative, nonsporulating bacillus, Gram positive coccus, *Enterococcus faecalis*, sulphate-reducing bacteria, Gram positive sporulating bacillus, *Mycobacterium* species (Poček B, 1990.).

## **Viral examination**

Bacterial viruses (bacteriophages) were isolated and cultivated in young cultures of *Salmonella enteritidis* and *Escherichia coli*. Agar culture medium showed a mass of bacterial growth on which bacteriophages produced clear zones or plaques, becoming visible after 8 to 24 hours (Armon R, Kott Y, 1993).

The enteroviruses, which included poliovirus, A coxackievirus, echoviruses, and human enteroviruses types 68 to 71, were detected after concentrating 10 litres of water by a filter absorption method, which resulted in a concentrate containing viruses. All samples were analysed by performing reverse transcription–polymerase chain reaction (RT-PCR) analyses for enteroviruses (De Leon et al 1990; Abbaszadegan et al 1993).

## **Biological and parasitological examination**

Algae and parasites from water samples were concentrated from 10 litres of water by filtration through cellulose acetate filters. They were fixated with 4% formaldehyde and analysed under the microscope. The present micro-organisms were determined according to size, shape and internal morphology by the key for determination (Hindak et al 1978).

## **Statistical processing**

The obtained indicators were processed by counting the percentages of occurrence and numerical values, which then represented their co-relation. The statistical significance of results was analysed by  $\chi^2$  test

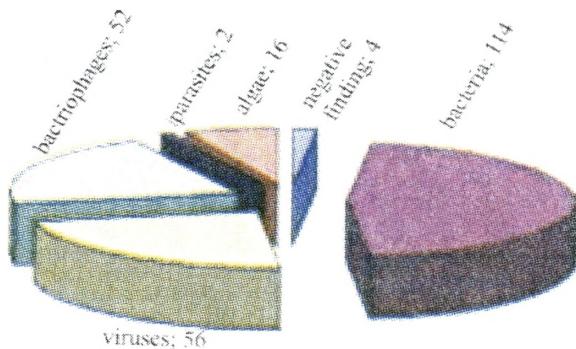
and Student test (Computers programme SPSS for Windows release). The statistical significant difference between compared results was considered  $p<0.05$ .

## Results

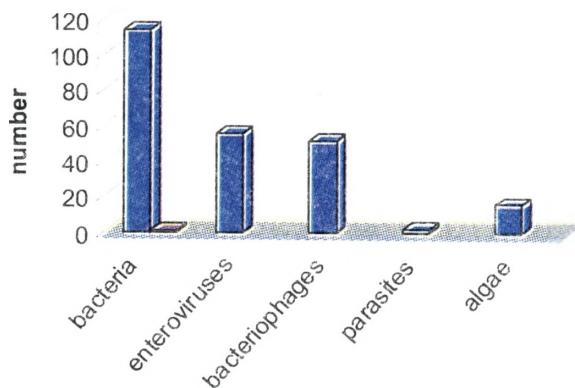
Residual chlorine was detected in 20.83% (10/48) of water samples originating from municipal water supplies (potable water). Residual chlorine was not found in the water from closed or open sources

Micro-organisms were absent in four (4.04%) of the 99 samples of water. Out of 95 samples of water, 240 micro-organisms were isolated as follows: 114 strains of bacteria, 56 viruses, 52 bacteriophages (19 coliphages and 33 *Salmonella enteritidis* phages), 2 nematodes, 16 algae (Figure 1.).

**Figure 1:** *The presence of members of different groups of micro-organisms in the drinking water.*



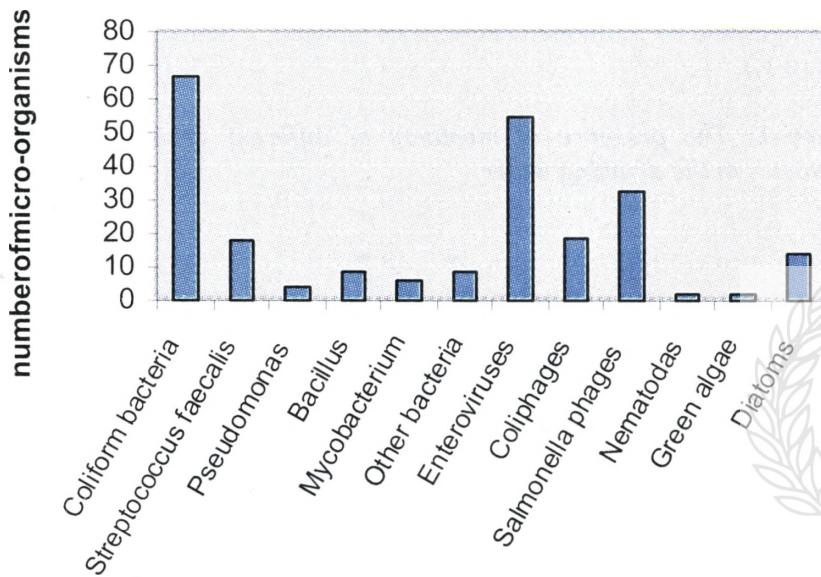
**Figure 2:** *Number of members from different groups of micro-organisms in the drinking water*



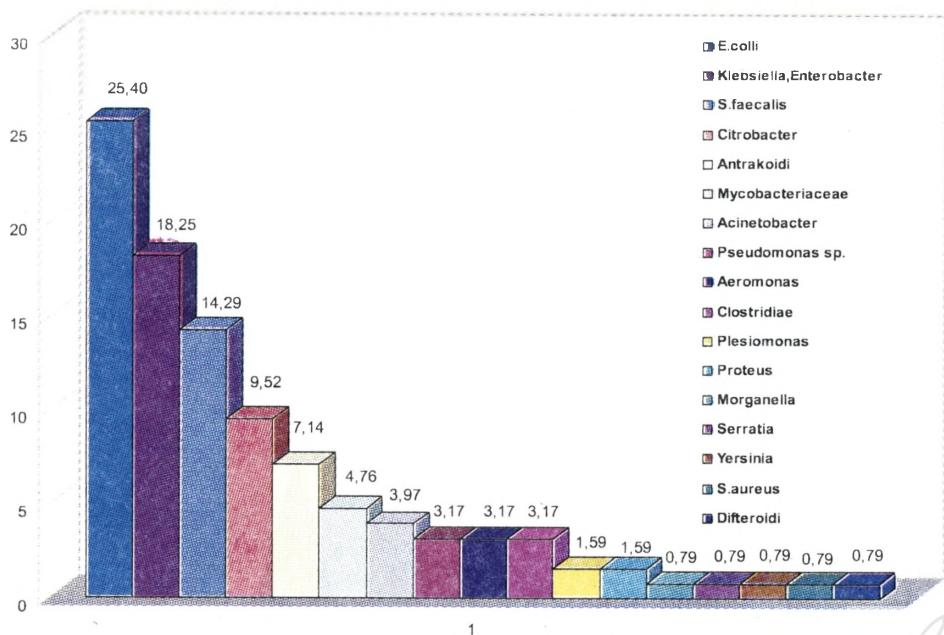
The most frequently detected micro-organisms in the water were bacteria, enteroviruses and bacteriophages. Nematodes were found in 2 samples (Figure 2).

There were found: coliform bacteria (68.69%), viruses (56.57%), bacteriophages (52.52%), parasites (2.02%) and algae (16.16%). Frequency of different genera and/or species of micro-organisms is shown in Figure 3.

**Figure 3: Overview of the micro-organisms present in all the sources of water**



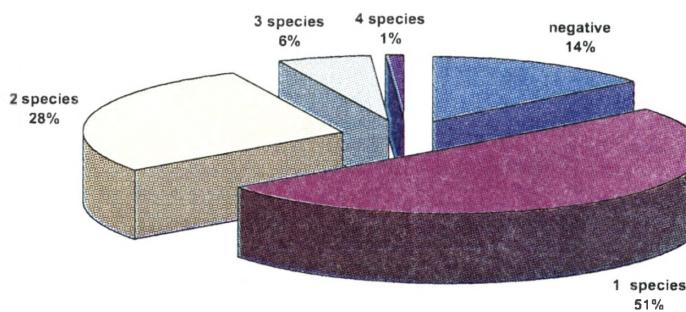
Different genera and species of bacteria were found in all the types of water. The most frequently found bacteria were: Escherichia coli (25.40%), Enterobacter / Klebsiella (18.25%) and Streptococcus faecalis (14.20%), while Pseudomonas aeruginosa and sulphate-reducing bacteria were present in 3.17%. Frequency of genera and/or species of bacteria is shown in the Figure 4.



**Figure 4:** Frequency of different genera / species of bacteria in the drinking water.

Between 1 and 4 different kinds of bacteria were present in some samples of water (Figure 5).

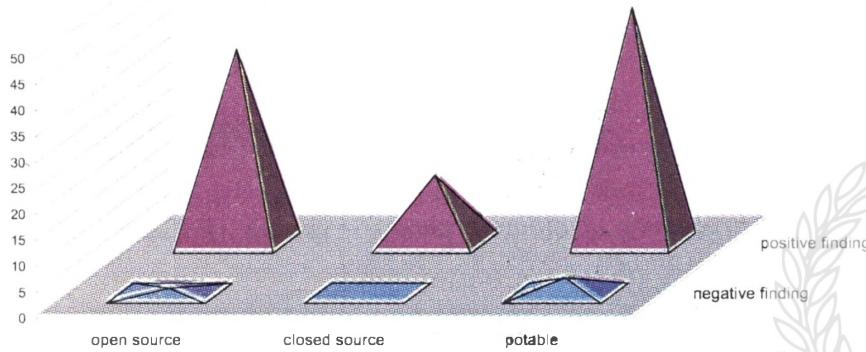
**Figure 5:** Overview of isolated bacteria according to number of positive / negative finding per sample.



In addition, a combination of different kinds of bacteria with other groups of micro-organisms were found: bacteria – bacteriophages 7; bacteria - virus 17; bacteria - virus – bacteriophages 24; bacteria - virus - algae 4; bacteria - virus - bacteriophages – algae 2; bacteria - bacteriophages – algae 3; bacteria - algae 3; bacteria - nematode - algae 1; bacteria + nematode 1.

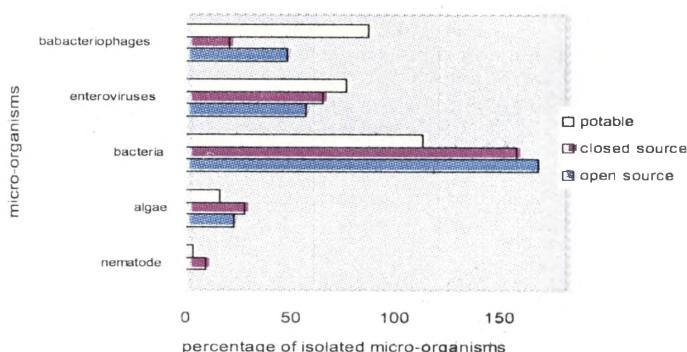
The finding of micro-organisms in the samples of water correlated with the type of water sources (Figure 6). All samples of water from closed sources were positive.

**Figure 6: Frequency of micro-organisms in the drinking water correlating with the type of sources**



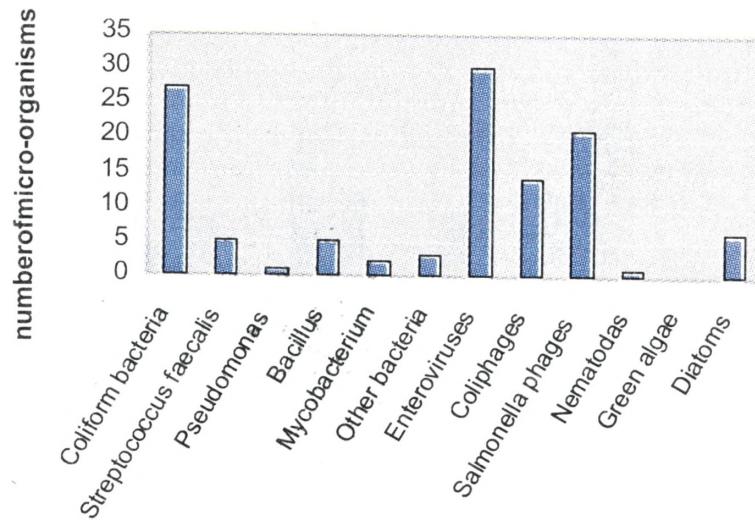
Frequency of different groups of micro-organisms was different in samples originating from the different sources: enteroviruses and bacteriophages were dominant in potable water; bacteria in water from open sources; algae and nematodes in water from closed sources (Figure 7).

**Figure 7: Groups of micro-organisms detected in the different kinds of drinking water.**



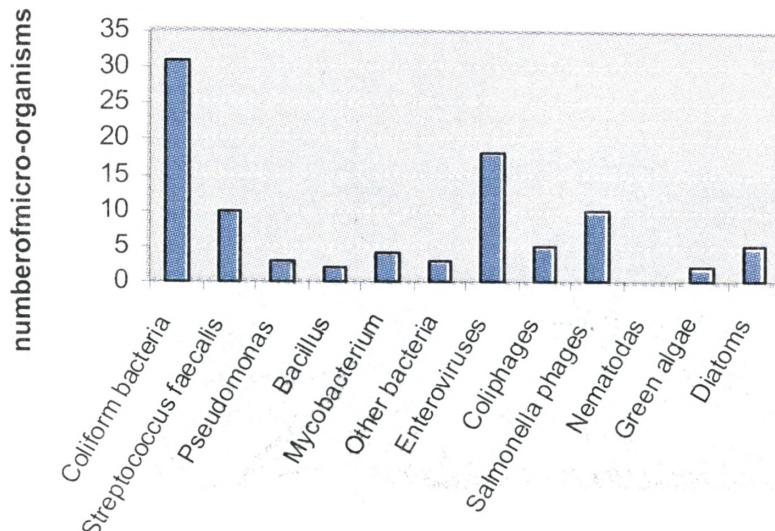
Order of frequency of genera / species of micro-organisms in water from different sources is presented in Figures 8, 9 and 10.

**Figure 8: Overview of the micro-organisms present in potable water.**



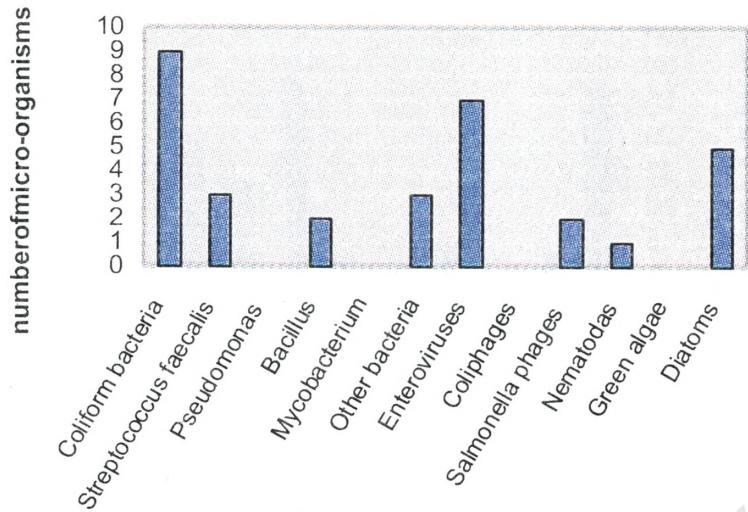
30 (62.5%) enteroviruses, 35 (72.9%) bacteriophages and coliform bacteria 27 (58.70%) were isolated from 48 samples of potable water.

**Figure 9: Overview of the micro-organisms present in open sources of water.**



There were 53 bacteria and 33 viruses (enteroviruses 18 and bacteriophages 5) in open sources (38).

**Figure 10:** Overview of the micro-organisms present in closed sources of water.



Numbers of isolated micro-organisms in closed sources were: bacteria 18, viruses 9, algae 3 and nematode 1.

Rapidly growing mycobacterium (Figure 11), *M. fortuitum* and *M. gordone*, were detected in six out of a total of 98 (6.06%) samples; potable water 4.35% and open sources 10.53%.

**Figure 11:** *Mycobacterium gordoneae* isolated from drinking water.



Coliphages were found in 19 samples of water while salmonella-phages were found in 33 samples.

**Figure12:** *Agar culture medium of E. coli shows a mass of bacteria growth on which bacteriophages have produced visible plaques.*

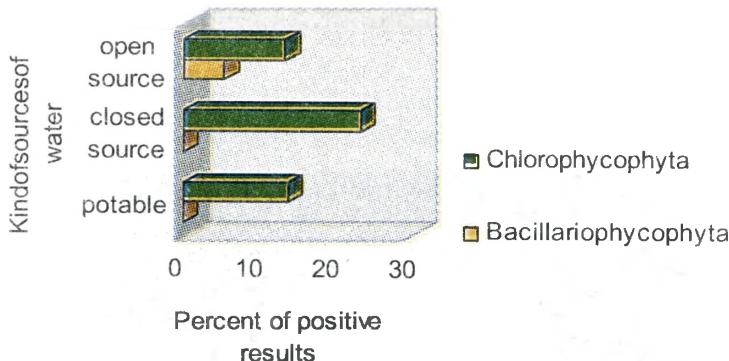


There were found different combinations of viruses, bacteriophages and bacteria: viruses and bacteriophages 5; viruses and / or bacteriophages and bacteria 57; whilst viruses only were isolated in 4 samples and bacteriophages only in 1 sample.

Microscopic nematodes were found in two samples from potable and closed sources.

Members of algae, group Bacillariophycophyta (the Diatoms) and Chlorophycophyta (the Green algae), were found in 16 samples of water. Members of Chlorophycophyta were present in all kinds of water while Bacillariophycophyta was found only in the open sources (Figure 13).

According to the routine method for examination of water recommended by law, water from 35.35% (35/99) of sources was found suitable for drinking. After using a broad spectrum of tests for detection of enteroviruses, bacteriophages, nematodes and algae, 10.10% (10/99) of samples were in compliance with the law. There was a significant difference between positive results, which were obtained using two different methods ( $p<0.01$ ).



**Figure 13: The finding of algae in different sources of water**

### Discussion and Conclusions

Urbanization and consequently the growing demand for water by communities has resulted in the establishing of different agencies in each country of the world who exercise jurisdiction over the many aspects of natural sources of water. The World Health Organisation has suggested physical and chemical water standards, which can be found in the W.H.O. Guidelines for drinking water quality. Protection from physical, chemical and biological contamination of water as well as monitoring of water quality is legally required in Bosnia and Herzegovina. Suggested standards of water quality and required tests can be found in "The rule book of hygienic quality of drinking water" Yugoslav Official Register 13/91 ("Pravilnik o higijenskoj ispravnosti vode za piće", Službeni list SFRJ 13/91).

Characteristics of good water quality as well as methods of examination are very similar all over the world. All guidelines for drinking water quality recommend the detection of coliform bacteria, *Escherichia coli*, *Streptococcus faecalis* as the most important sign of fresh faecal contamination of water and the detection of the presence of *Pseudomonas aeruginosa* and sulphates-reducing bacteria as the sign of old faecal contamination (WHO 1981, WHO 1982, Poček B 1990, Yugoslav Official Register 1992).

Following these guidelines for examination and estimation of water quality we found that 35.35% (35/99) of samples were in compliance with the law. From other samples (63/99) were isolated: coliform bacteria was 67 (E.coli 25,40%, Enterobacter/ Klebsiella 18,25%); *Streptococcus faecalis* (18); *Pseudomonas aeuginosa* (4); sulphate-reducing bacteria 4. Two, three or four kinds of bacteria were isolated from 35% of samples. The total number of coliform bacteria was quite high probably as a consequence of mixing different kinds of

water with drinking water. The positive total coliform bacteria test typically indicates surface intrusion in drinking water during the flood, but is not a specific indication of faecal contamination. It may serve as a screening test, because if total coliforms are absent, faecal bacteria are probably absent also.

*Mycobacterium fortuitum* and *Mycobacterium gordone* are prevalent in aquatic environments and they are usually saprophytic organisms but can be associated with a variety of infections in patients with immunodeficiency (Fujita Y at all. 2000). In this study they were found in 6.6% samples of water. Their being so prevalent in the potable water along with the increasing number of patients undergoing ambulatory dialysis in the area of Tuzla, infections from these organisms may emerge as a significant problem.

Until now, water has not been examined for mycobacterium in our country, but the finding of *M. gordonae* is common in some parts of the world and it is known as "tip water bacillus" (Kozumi T et al 2000).

Bacteriophages, coliphages and salmonella phages are bacterial in origin and could be used as an indicator of water polluted with *Salmonella* and *Escherichia*. Although *Salmonella enteritidis* was not detected in the water, its bacteriophages were found in 33/99 samples. These findings can explain the recent outbreak of *Salmonella*-infections. It was surprising that coliphages were detected in a lower percentage (19.19%) than *Salmonella* phage (33.33%). The frequency of bacteriophages in potable water was much higher (71,71%) than the finding of coliform bacteria (58,70%). The significance of coliform bacteria in detecting water pollution is known, but we consider it very important, as organisms indicative of pollution, to test for bacteriophages and viruses. In a study, which took place in Israel and Spain in 1997, the frequency of isolation of bacteriophages (4.4% to 6.1%) was significantly higher than the frequency of isolation of coliform bacteria (1.9%) (Armon R at all 1997). Samples of drinking water from different sources in Greater Cairo, Egypt, were positive for both total coliform and coliphage in 2.72%; 53% of samples were positive for coliphage but negative for total coliforms and faecal coliforms (el-Abagy et al 1990)).

Despite other intestinal micro-organisms, such as intestinal viruses (polio, coxackie, echo, hepatitis A, and rotaviruses), being potent pathogens which can cause serious diseases, we are not obliged by law to examine water for their presence. This study showed that virus pollution of water was very high in drinking water: potable water 63%; water from closed sources 53%; water from open sources 47%. Viral pollution of water is a very real problem in many countries. For example, over a period of two years, viruses were detected in 23% of 413 drinking water samples and 73% of 224 raw water samples in South

Africa. Enteroviruses were detected in 17% of drinking water samples (Grabow WO et al 2001).

Bacteria were associated with bacteriophages and viruses in 57 (58.16%) of samples whilst only viruses and bacteriophages were detected in 10 (10.2%) of samples. Coliforms concentrations could be correlated with enteric virus concentrations as well as with concentrations of coliphages but an absent of coliform bacteria does not exclude the present of viruses and bacteriophages.

Still other micro-organisms are regarded mainly as saprophytic, nuisance organisms. They create problems of odour, colour, and taste, or cause obstruction to the free flow of water. Among the most important nuisance organisms are slime-forming bacteria, iron bacteria, sulphur bacteria, algae and nematodes.

Members of the group *Bacillariophycophyta* and *Chlorophycophyta* are principally freshwater species. They are also found in moist soil and seawater. In this study algae (*Bacillariophycophyta* and *Chlorophycophyta*) were present in 16 samples of water. Their presence in drinking water in such high numbers is a sign a water inadequate chlorination or damage to a water-purification plant and/or supply system. Our results indicating high percentages of bacterial water pollution along with low levels or absence of chlorine serve to conform this statement.

Nematodes were found in two samples. There are thousands of different types of the microscopic worms on earth, many of them parasites of insects, plants or animals. Free-living species are abundant, including nematodes that feed on bacteria, fungi, and other nematodes. Their presence in drinking water is rare and is a sign of insufficient water purification and bacterial pollution.

Microbiological quality of water was absolutely insufficient in the area of Tuzla Canton in the specific time after the flood. Extremely high levels of bacterial pollution of drinking water were consistent with defects or breaks in the disinfections process.

Residual chlorine was found in sufficient concentration in 21.74%(10/48) of samples of water originating from municipal water supplies (potable water), while in other kinds of water, residual chlorine was not found. Different level of chlorination in different sources of water influenced the relationship between bacteria and viruses /bacteriophages. Numbers of isolated bacteria decreased and numbers of viruses increased in the water treated with an inadequate concentration of chlorine. This finding deserves further examination as it can be the consequence of the different methods that we have applied (using methods provided for the detection of live and dead viruses, we detected only live bacteria) and/or of liberation of viruses and bacteriophages from cells and their different biology.

In conclusion, after using a broad spectrum of tests for detection of bacteria, enteroviruses, bacteriophages, algae and nematodes, only 10% (10/98) of water samples were in compliance with the law. The routine method for examination of water recommended by law was not sufficient to detect serious contamination of water in 25.25% of cases. The difference is significant between positive results, which were obtained using two different methods ( $p<0.01$ ). These results call for a revision of water quality guidelines based only on indicator organisms without also making reference to the absence of viruses.

We have pointed out the importance of all the parameters, which should be applied during emergencies such as the recent flooding. We also suggest that, along with routine examination of drinking water there should be periodically incorporated into the current protocol extra measures for detection of enteroviruses and bacteriophages.

### **Acknowledgements**

We acknowledge Centro Regionale d' Intervento per la Cooperazione (C.R.I.C.) for funding the research and the authorities of Tuzla Canton for making available the resources for this work.

Special thanks due to:

All representatives from Health Centres from the area of Tuzla Canton who did the sampling of water in the area.

Mrs. Mubera Kutlovac for kindly giving so generously of her time to prepare some of the laboratory procedures.

Mrs. Patricia Prestidge for proof reading this document.

### **Apstrakt**

Jedan od krucijalnih problema na području Tuzlanskog kantona tokom prethodna dva desetljeća je, sasvim izvjesno, deficit potrebnih kolicina vode, odgovarajuće kvalitete za humanu upotrebu. Nastao kao posljedica dugogodišnjeg slijeganja zemljišta uslijed eksploatacije mineralnih sirovina, a potom oštećenja vodo-opskrbnih objekata uslijed ratnih dejstava, te poplava koje su nedavno zahvatile ovo područje, uz neočekivano brzi porast broja stanovnika u toku ratnih i poratnih migracija, nedostatak vode pokazuje upozoravajuće tendencije daljeg rasta. Istovremeno, obilate oborine tokom ove godine, rezultirale su poplavama koje su, s obzirom na staru i oštećenu vodoopskrbnu infrastrukturu, dovele do zagadenja vode za piće. Bio je to razlog da pristupimo istraživanju kvalitete vode za piće, koristeći dvije

relevantne metode; standardnu metodu za pregled voda, uz dodatne posebne laboratorijske testove čiju korisnost potvrđuju dobijeni rezultati.

Ciljevi mikrobiološke analize vode bili su:

a) ispitivanje vanjskog biološkog zagadenje, kao posljedice poplave u području Tuzlanskog Kantona u 2002. godini i

b) evaluacija propisanih laboratorijskih procedura u Bosni i Hercegovini za detekciju patogenih mikroorganizama u vodi za piće.

Ispitivanjem su obuhvaćena 99 uzorka vode: 48 uzoraka iz gradskih vodovoda, 13 iz zatvorenih izvorišta i 38 iz otvorenih izvorišta. Uzorci vode su testirani rutinskim bakteriološkim, parazitološkim i biološkim metodama, a reverzno transkripciona lančana reakcija sa polimerazom (reverse transcription –polymerase chain reaction / RT-PCR) je korištena za detekciju virusa.

Mikroorganizmi nisu nadeni u 4 (4.04%) od p99 uzorka vode. Iz preostalih 95 uzorka vode izolovano je: 240 microorganizama i to: 114 sojeva bakterija, 56 virusa, 52 bakterofaga (19 faga *Escherichia coli*, i 33 *Salmonella enteritidis*faga), 2 nematode, 16 algi.

U skladu sa osnovnim mikrobiološkim pregledom vode za piće propisanim zakonom, voda iz 35,35% (35/99) izvorišta bila je odgovarajućeg kvaliteta, dok je nakon pregleda dodatnim testovima samo 10.10% (10/99) uzorka bilo u skladu sa zakonskim propisima. Nadena razlika je visoko statistički značajna.

Ovi rezultati ukazuju na potrebu revizije osnovnog pregleda «Pravilnik o higijenskoj ispravnosti vode za piće», baziranog na detekciji indikatorskih mikroorganizama, bez pregleda na prisustvo virusa. Smatramo da je neophodno pratiti sve te parametre mikrobiološke ispravnosti vode u toku elementarnih nepogoda, kao periodično (mjesečno ili godišnje) u toku rutinskih pregleda.

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