Chapter 2

# Disinfection of Water Used for Human and Animal Consumption

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76430

**Abstract**

This chapter deals with disinfection of water used for human and animal consumption. Water is the most abundant chemical component of the Earth and is very extensively used by mankind. Anthropogenic pressure on the environment leads to decrease in water quality. The quality of water is determined using the most important range of parameters (physical, chemical, and microbiological). This chapter discusses major pollutants of water, protection of water sources, micro-organisms causing the main waterborne diseases and methods of treatment, and disinfection of water. Different methods are used to disinfect drinking water. One of the most frequently used methods is disinfection with active chlorine, which is the only method providing continuous protection against microbial regrowth. However, this method has also some disadvantages (e.g., formation of trihalomethane and haloacetic acid precursors) linked to increased risk of cancer. It is important to remember that none of the products used to disinfect water is capable of ensuring complete safety of treated water if the water comes from unsuitable sources.

**Keywords:** disinfection, chlorination, drinking water safety, farm animal watering, microbiological examination, physico-chemical examination

## 1. Introduction

Water is essential for the existence of life. It should be available to all at adequate quantity and quality. Access to safe drinking water is the basic requirement for ensuring good health of animals and humans, so every effort should be made to achieve this goal [1]. The safety of drinking water

is assessed on the basis of national standards or international guidelines. The WHO Guidelines for Drinking Water Quality form an authoritative basis for the setting of national regulations and standards for water safety in support of public health. In Slovakia, regulation of the government of the SR Act No. 368/2007 Coll. [2], which amends and supplements the Act No. 322/2003 Coll. [3], on protection of farm animals, specifies that all sources of water used for watering of animals must comply with the requirements on water intended for human consumption. The requirements on the quality of water used for human consumption are determined by the regulation of the government of the SR No. 496/2010 Coll. [4], which complies with the criteria set by European Communities regulations and WHO guidelines. This regulation specifies also methods for the control of quality of water used for human consumption.

Water sources can be contaminated by numerous man-made pollutants, classified into two categories of sources, point and diffuse. Industrial premises, towns, agricultural installations including animal farms and landfills—point sources—can be more easily identified and controlled. Diffuse sources, such as run-off from agricultural land and hard surfaces (roads and acid rains), are less obvious and more difficult to control. Such sources are responsible for considerable variations in the contaminant load over time [5].

Source protection zones (SPZs) form a key part of the approach to controlling the risk to groundwater supplies from potentially polluting activities and accidental releases of pollutants. The procedure for land-surface zoning related to the protection of groundwater against both point and diffuse pollution is hydrogeologically based but not so complex as to be unworkable in practice. The SPZ approach is primarily a policy tool used to control activities close to water supplies intended for human consumption. For source protection, three zones have typically been defined:

1. Inner protection zone is defined as the 50-day travel time from any point below the water table to the source. The minimum radius of this zone is 50 m.
2. Outer protection zone is defined by a 400-day travel time from a point below the water table.
3. Source catchment protection zone is defined as the area around a source within which all groundwater recharge is presumed to be discharged at the source.

In the case of diffuse pollution, it will also be necessary to consider the nature of the soil cover in the area where the polluting activity occurs [6, 7].

Many agents of infectious diseases of animals and humans are waterborne. The greatest risk of their transfer is associated with ingestion of water that is contaminated with human or animal faeces that may become a source of pathogenic bacteria, viruses, and parasites (protozoa, eggs of parasites). They may survive in water for different periods and cause diseases in many people throughout the world. Monitoring of safety of water sources involves physical, chemical, microbiological, biological, and radiological parameters. The most frequently determined parameters indicate pollution caused by sewage, animal excrements, storage of waste, animal manure, and artificial fertilisers [8, 9]. With regard to protection of water, one should also mention the Directive 2010/75/EU [10] on integrated prevention of pollution and control that applies to industrial and agricultural installations with large pollution potential and helps to eliminate pollution of water sources. However, there are many smaller sources, particularly the non-point ones that do not fall under this directive.

The safety of drinking water with regard to harmful micro-organisms has traditionally been determined by monitoring the counts of bacteria, which indicate faecal contamination. This monitoring is done at entry to the supply system and at certain fixed and randomly located points within the distribution system. Much effort has been made to find ideal indicator micro-organisms, but, at present, no single micro-organism meets satisfactorily all the desired criteria. When using disinfection technologies based on active chlorine, the only reliable indicator of chlorination performance for real-time control of bacteria and viruses is the existence of a target chlorine residual concentration after a specified contact time [7].

The heterotrophic plate count that includes all micro-organisms in water capable of growing on or in a nutrient-rich solid agar is determined to indicate the overall quality of water sources. At incubation for 24 hours at a temperature of 37°C (bacteria cultivated at 37°C, BC37), the counts of bacteria of animal origin are obtained, while at 22°C and cultivation for 72 hours (bacteria cultivated at 22°C, BC22), one can enumerate bacteria that are derived principally from environmental sources. Substantial increase of BC22 and particularly of BC37 above, normal values may be cause for concern. Faecal enterococci as an evidence of faecal contamination are capable of persisting longer in the environment in comparison with thermotolerant or total coliforms. They exhibit high resistance to drying. Faecal enterococci are cultivated in or on sodium azide containing medium, at incubation temperature ranging from 37 to 44°C [11].

According to WHO [12], *Escherichia coli* are the only true indicator of faecal contamination. These bacteria are exclusively of intestinal origin and are found in human and animal faeces. They are indicators of mostly fresh faecal contamination, and their presence suggests inadequate protection of the specific water source, deficient treatment of water, and need for improving its safety.

Leclerc et al. [13] clarified the diversified roles that coliforms have in the environment and the real meanings of the tests on total coliforms and faecal coliforms. He concluded that: (1) in the enterobacteria, *E. coli* are the only true and reliable indicator of faecal pollution in environmental waters; (2) the traditional total coliform test should be abandoned because it can detect bacteria that have no connection with faecal pollution; (3) the detection of faecal coliforms must be carried out at 44.5°C, and positive results confirmed by identification to species levels in order to exclude false positives such as *Klebsiella pneumoniae*.

The intestinal enterococci group has been used as an index of faecal pollution. In human faeces, the numbers of intestinal enterococci are generally about an order of magnitude lower than those of *E. coli.* However, caution should be taken with interpreting the results obtained by the enterococci procedure in water analysis. Enterococci and other group D-streptococci are present in many foods, especially those of animal origin [14].

Managing microbial risks in water supply relies primarily on identifying catchment risk and, as far as possible, applying control measures to mitigate it—treatment and disinfection systems designed to deal effectively with expected microbial loads, raw water quality, preventing microbial contamination in distribution system and at consumers. This is consistent with the Drinking Water Safety Plan (DWSP) approach for water supply risk, which is a risk-based approach to managing water quality that is designed to ensure delivery of safe drinking water in terms of both quality and quantity. Then, effectiveness of controls and barriers has to be validated and action plan to reduce risks to an acceptable level identified [7].

The physico-chemical properties of water, particularly pH, temperature, the presence of organic matter (chemical oxygen demand, COD), low level of dissolved oxygen (DO), electric conductivity (EC), turbidity, content of ammonium ion, presence of heavy metals, and others, affect the quality of drinking water, and some of them have direct effect on the health of consumers [15]. In addition, these parameters can affect the survival of potential disease agents, the effectiveness of the performed disinfection [16].

Although the groundwater is filtered when passing through the soil, it is often susceptible to microbial contamination and must be checked periodically and disinfected if necessary. A major groundwater pathogen occurrence study supported by the American Water Works Association (AWWA) Research Foundation and the U.S. Environmental Protection Agency (EPA), involving testing for total coliform bacteria, *E. coli*, coliphage, and human viruses, indicated positivity for one or more indicators of faecal contamination that in 60% of vulnerable wells and about 50% of wells initially considered not vulnerable.

### 1.1. Disinfection of drinking water

The current drinking water regulations specify parametric values for various chemicals in drinking water, and compliance with the limits for microbiological parameters is of primary concern in the protection of human health. Different disinfectant technologies can be used to eliminate the risks consequent to the presence of organic and inorganic impurities in source waters and to meet the pathogen inactivation demands, as a part of a treatment process and/or subsequent disinfection processes.

The control of residual organic or inorganic compounds in water before disinfection limits disinfection by-products in water supplied to consumers. The maintenance of a disinfectant residual within the distribution system that is not ensured by all disinfection technologies is an important factor that prevents the regrowth of microorganisms in water.

The following key factors influence the selection of a disinfection system: the effectiveness of the disinfectant in destroying pathogens of concern; the quality of the water to be disinfected; the formation of undesirable by-products as a result of disinfection; the ability to easily verify the operation of the chosen disinfection system; the ease of handling and health and safety implications of a disinfectant; the preceding treatment processes; and the overall cost [7].

Chlorination is a chemical disinfection based on the application of various substances with different concentration of active chlorine ranging from gaseous chlorine, through sodium or calcium hypochlorite and chloramines, up to chlorine dioxide. Chlorine-based compounds are the only major disinfectants ensuring residual levels of the disinfectant agent capable of providing continuous protection against microbial regrowth [17].

When chlorination is performed with gas chlorine, the active forms of chlorine in water are a hydrolysis product, hypochlorous acid. At pH values below 6, the chlorine exists almost exclusively as hypochlorous acid, and at pH values above 9, it exists as hypochlorite. Since hypochlorous acid is a more potent disinfectant, chlorination under slightly acidic conditions is recommended [18].

The dose of chlorine is affected by the quality of the treated water and the form of the chlorine preparation used for disinfection. The above factors affect the residual active chlorine levels

present in water supplied to the consumer. Active chlorine preparations have been considered the most suitable way of disinfection of on-farm groundwater (wells) for numerous reasons. Such disinfection is cost effective, reliable, relatively simple, measurable and provides a protective residual level of active chlorine [17, 19].

Different techniques of chlorination have been developed. Breakpoint chlorination uses active chlorine dose sufficient to rapidly oxidise all the ammonia nitrogen present in the water and to leave free residual chlorine capable of protecting the water against reinfection from the point of disinfection always up to the consumer. Superchlorination/dechlorination is based on the addition of a large dose of chlorine ensuring rapid disinfection by-products of relevant chemical reaction, followed by reduction in the excess of free chlorine residual, which must be removed to prevent taste problems and reduce corrosion of pipelines. The latter method is used mainly in case of variable bacterial load or inadequate detention time in the tank. Marginal chlorination is used for disinfection of high quality water supplies. It involves simple dosing of chlorine to produce a desired level of free residual chlorine. The chlorine demand of water from these sources is very low, and the breakpoint might not even occur [1].

The original WHO recommendations for the use of chlorine as a disinfectant stipulated a minimum free chlorine concentration of 0.5 mg/L after 30 min contact time at a pH of less than 8 provided that the turbidity is less than 1 nephelometric turbidity unit (NTU). A site specific approach may need to take into account: the levels of contamination with pathogens expected, and any specific pathogens of concern for the site (catchment risk); the extent and performance of treatment prior to final disinfection; the design of the contact tank, in relation to short-circuiting; and expected variations in temperature and pH [7].

The by-products (BPs) of chlorine disinfectants can affect the health of consumers of the disinfected water or induce in them various responses. Their extent depends on numerous factors such as the period of action, concentration, and frequency of exposure [20].

Chlorine dioxide is a more powerful disinfectant than chlorine and does not form trihalomethanes (THMs) by reaction with humic substances. However, its generation is also associated with some BPs, such as chlorites and chlorates [21]. One of the most undesirable BPs in generators is the toxic chlorate ion [18]. It cannot be stored in compressed form in tanks because it is explosive under pressure and must be generated on site and thus is likely to be substantially more expensive than chlorine.

Chloramination of water is based on the formation of monochloramine, which is formed when ammonia and chlorine are dosed, and react, under well-controlled conditions. It is essential to control the process to prevent the formation of strong tastes and by-products. The disinfection capability of monochloramine is poor when compared with chlorine. The key advantage of monochloramine is that it does not form THMs but still provides a disinfectant residual [7].

Chloramine-T is an organic N-chloramine. Chloramine-T is a slow-release chlorinating agent, and it is an exception to the organic chloramines because of its considerable value as a disinfectant and a sanitiser. The hydrolysis mechanism involves the production of aqueous free chlorine (HClO, $ClO^-$). Organic chloramines in general are thought to be considerably less toxic to aquatic life than the inorganic chloramines, such as mono-, di-, and trichloramine. Inorganic chloramines usually exist as monochloramine in aqueous solutions [22]. The detailed hydrolysis mechanism of chloramine-T varies with pH and is quite complex. In aqueous solutions of

chloramine-T, caused by dissociation, hydrolysis, and disproportionation processes, seven different kinds of molecules emerge (HClO, $ClO^-$, R─NCl─, R─NHCl, R─$NCl_2$, R─$NH_2$, and R─NH─ [R═$CH_3$─$C_6H_4$─$SO_2$]) [23]. The use of chloramine-T solutions for disinfection of water includes its use in aquaculture. Tests performed on brook trout by Cipriano et al. [24] substantiated the therapeutic value of single treatment with chloramine-T (15 mg/L) against *Aeromonas salmonicida*, which was more successful than that treatment with formalin or salt.

Schmidt et al. [22] presented detailed environmental assessment of the effects of chloramine-T use in and discharge by freshwater aquaculture. Intensive aquaculture facilities discharge into streams, rivers, and lakes. Both before and after discharge, chloramine-T can remain unchanged, release its chlorine as aqueous free chlorine, or donate its chlorine directly to produce ammonia chloramines or other chlorinated organic-N or non-N compounds. Since chloramine-T is used as an antiseptic and a surface sanitising agent, toxicity to bacteria is to be expected at some concentration level. Chloramine-T was an effective microbicide against *Pseudomonas aeruginosa* at 300 mg/L (reduced colony forming units by $10^5$) and at 5000 mg/L against *Vibrio cholerae* [25].

Ozone is more powerful disinfectant when compared with either chlorine or chlorine dioxide. It is the only chemical that can ensure effective inactivation of either Giardia or Cryptosporidium. It also destructs organic micropollutants (pesticides, odour compounds). However, its residual is insufficiently low lasting for distribution.

The non-chemical disinfection system involves ultraviolet (UV) radiation. It is necessary to ensure suitable intensity and duration of UV radiation to give a UV "dose," which will depend on the application. Dose of 40 mJ/$cm^2$ is commonly used for UV disinfection systems as it is capable of inactivating a broad spectrum of waterborne pathogens. It is effective for protozoa, bacteria, and most viruses but less effective for viruses than chlorine [7].

The main drawback of disinfection with gaseous chlorine and active chlorine releasing preparations is that chlorine can react with natural organic matter (NOM) present in water to generate various types of disinfection BPs, such as trihalomethane and haloacetic acid. The BPs are associated with increased incidence of the risk of cancer in areas served with chlorinated water [26, 27]. Zhao et al. [28] mentioned chloro- and bromobenzochinones as additional by-products of chlorination.

The presence of NOM in water and their chemical and physical characteristics can be investigated by excitation emission matrix (EEM) fluorescence spectroscopy that serves as a powerful tool [27].

As the effectiveness of chlorination can be affected by NOM, it is important to obtain adequate information about this parameter. As the content of NOM in water from natural sources may vary considerably, the optimum dose of chlorine disinfectants necessary for complying with the respective legislative requirements on active chlorine residuals should be determined, for example, by experimental chlorination [29].

With regard to the negative effects of gaseous chlorine and stricter legislation, new methods and technological procedures were searched for to find a way of ensuring hygiene safety of drinking water. Of the physical methods, Jirotkova et al. [30] proposed the use of electrolytic methods, and Hussain et al. [31] presented the combination of adsorption and electrochemical disinfection. Recently, UV technologies with online fluorescence detection were employed for disinfection of

secondary water sources [32], for example, the combination of mechanical filtration and disinfection by solar radiation [33], or combined action of UV radiation and chlorine [34]. With these new approaches, one could achieve reduction in the level of undesirable BPs and elimination of negative effects on physical properties of water, resembling that after disinfection with ozone [35]. However, the majority of them do not ensure the residual disinfection power.

The aim of this study was to monitor the quality and safety of three groundwater sources located in the eastern Slovakia and to determine experimentally the optimum dose of chloramine-T (commercial preparation) needed for their adequate disinfection that could ensure hygiene safety of water in terms of devitalisation of potential pathogens and observation of the relevant limit for residual active chlorine (0.3 mg/L) in drinking water [36].

## 2. Materials and methods

The study involved monitoring of three groundwater sources supplying water to three farms, two cattle farms, and one farm keeping both cattle and sheep, located in a hilly area in the Prešov region (eastern Slovakia), about 4 km apart. The samples of groundwater from these wells were collected from January to May, in intervals specified below. The quality of water in the investigated sources and its potential to form disinfectant BPs was assessed on the basis of microbiological, physico-chemical, and fluorescence analyses. After obtaining unfavourable bacteriological results during preliminary sampling in January and February, experimental chlorination of water was carried out for each source. Subsequently, the effectiveness of such dose was then checked under field conditions.

The experimental chlorination was conducted using a chloramine-T (sodium tosylchloramid; sodium salt of N-chloro-4-methylbenzene-1-sulfonamide) as disinfectant. It involves the determination of optimum dose of chloramine T and intervals between application of this disinfectant necessary to prevent transmission of waterborne diseases and ensure such level of residual chlorine, so that the water can be used for watering of animals (complying with the national limit for residual active chlorine 0.3 mg/L) and for other related processes [37, 38].

### 2.1. Description of the monitored water sources

**Source 1:** It was located on a farm in eastern Slovakia at a distance of approximately 13 km from the town Prešov. The farm focused on fattening and rearing of cattle and included milk-producing dairy cows and a calf rearing section. The farm was well-known abroad because of fattening of bulls [39]. Five groundwater sources with a capacity of about 8000 L/d and depth in the range of 6–11 m were situated in close proximity of this farm. Water from these wells was brought to a storage tank of capacity about 40,000 L/d, from which the water was supplied to animals and used for other related operations. Water samples were collected from the common storage tank.

**Source 2:** It was located on a farm situated 15 km northeast of Prešov. Sheep of Tsigai breed and Slovak-spotted cattle were kept on this farm. The source was a 23-m deep well. Water from this well was collected in a storage reservoir located up on a hill above the farm, of capacity 150,000 L/d. Water samples were collected from the storage reservoir.

**Source 3:** A well of depth of 20 m and a reservoir of capacity of about 90,000 L/d were located on a farm 12.5 km away from Prešov. On this farm, young cattle and dairy cows were kept. The water samples for examination were collected from a tap in a cow house.

### 2.2. Microbiological examination

Determination of counts of relevant bacteria complied with the regulation of the government of the SR 496/2010 Coll. We determined colony forming units (CFUs) of bacteria cultivated at 22°C (BC22) and 37°C (BC37) (heterotrophic count) according to STN EN ISO 6222 [7], coliform bacteria (CB) and *E. coli* according to STN EN ISO 9308–1 [40], and faecal enterococci (FE) according to STN EN ISO 7899–2 [41]. A pour-plate method was used to determine counts of BC22 and BC37 in nutrient agar medium after aerobic incubation. The number of colony forming units (CFUs) per mL of sample was determined. According to the regulation of the government of the SR 496/2010 Coll. [4], the limit value is 200 CFU/mL for BC22 and 20 CFU/mL for BC37.

Coliform bacteria (CB) and *E. coli* were cultivated on Endo agar (HiMedia, India) for 24 hours at 37 and 43°C, respectively, and the characteristic colonies were counted. In the absence of colonies, the incubation was prolonged for additional 24 hours. According to respective regulation, lactose fermentation test was performed for the confirmation of coliform bacteria. According to WHO (2008) [1], neither *E. coli* nor thermotolerant coliform bacteria can be detected in any 100-mL sample. The same applies to total coliform bacteria that must not be detected in any 100-mL sample (WHO, 1996, STN EN ISO 9308-1:90) [11, 40].

Determination of faecal enterococci (FE) consisted of filtering 100 or 10 mL of water sample (for water intended for mass consumption or individual consumption, respectively) through a membrane filter (filter size 0.45 μm). The filter was then placed onto a solid selective medium containing sodium azide (to suppress growth of Gram-negative bacteria) and colourless 2,3,5-trifenyltetrazolium chloride, which is reduced by intestinal enterococci to red formazan. The regulation stipulates that faecal enterococci must not be detected in any 100 mL sample of water [42].

### 2.3. Experimental chlorination of water

The preliminary bacteriological examination of water from all three sources showed the need to carry out experimental chlorination of water. This allowed us to determine appropriate doses of chloramine-T necessary for disinfection of water in the investigated sources.

Procedure—Horakova et al. [29]: We used 0.1% solution of chloramine-T for experimental chlorination (active ingredient Tosylchloramide sodium, 81% active chlorine, manufactured by Bochemie—http://www.bochemie.cz/en-US/contact) [43]. The dosage recommended by the manufacturer is 10 g per 1000 L of water (this presumes maximum pollution of water). After measuring equal volumes of water into a series of bottles, we added to them increasing doses of 0.1% solution of chloramine-T, allowed it to act for the prescribed time (30 min) and determined content of residual free chlorine in each bottle. The optimum dose of chloramine-T (g/L) for each source was determined by recalculation on the basis of the volume of 0.1% chloramine-T added to the bottle with the residual free chlorine within the range stipulated by the legislation (0.05–0.3 mg/L).

The doses of chloramine-T determined by experimental chlorination and dissolved in a sufficient volume of water before added to each source were used to disinfect water in the investigated sources three times in regular intervals during the first half of 2015. On the 5th day after disinfection, we carried out bacteriological examination of water. On the basis of results, the chloramine-T dose originally determined by experimental chlorination (100 g) for Source 1—100 were doubled after heavy rain in April to 200 g. The dose for Source 2 was 360 g for reservoir with a capacity of about 150,000 L/d and 180 g (90,000 L/d) for Source 3.

### 2.4. Physico-chemical examination of water

The water was examined on site for sensorial properties (colour, odour, turbidity) and checked again after transported to a laboratory. No changes were detected, and the results met the requirements set by legislation for drinking water. The temperature of samples was measured at sampling and ranged between 7 and 10.5°C. Water was sampled and examined from January to May 2015.

The pH was determined according to STN ISO 10523 by means of a pH-meter HACH and a WATERPROF pH Tester 30. Conductivity was determined by a conductometer WTW InoLab Cond 720 (Germany).

Quantitative determination of nitrates was carried out with ion-selective nitrate electrode WTW (InoLab pH/ION 735P, Germany), and chlorides and active chlorine were determined by titration (STN ISO 9297 [44] and EN ISO 7393-3 [45], respectively) and $Ca^{2+}$ and $Mg^{2+}$ by titration method according to Horakova et al. [29]. Dissolved oxygen was determined electrochemically using an oxygen probe LDO HQ Series Portable Meters, supplied by HACH (STN EN ISO 5814:2013 [46], ion selective method), and for determination of chemical oxygen demand, the samples were oxidisied with $KMnO_4$ using the procedure specified in STN EN ISO 8467 [47].

In parallel with collection of samples for microbiological and physico-chemical analysis, samples of water from all three water sources were taken for FEEM spectroscopy and examined by a luminescence spectrophotometer Perkin Elmer LS 55 (USA) at the following settings: excitation wavelength in the range 250–450 nm with a gradual increment increase (10 nm), range $\lambda$ = 250–600 nm (excitation/emission slit: 5/10 nm, quartz cuvette of width 1 cm, scanning rate of emission monochromator: 20 nm/s). Excitation-emission matrices (EEMs) were obtained using a FIW Inlab programme [48].

## 3. Results and discussion

Water problems face virtually every nation in the world. Major water supply problems are related to shortages, overexploitation of supplies, flooding and insufficient protection of water sources, either surface or ground, against contamination with human and animal wastes, and other human activities. Good quality of water intended for human consumption and watering of animals is essential for its safety and prevention of disease transfer.

Surface water serves as a recipient not only for rain water from relevant catchment areas but also of wastewater (treated and untreated) and waters penetrated by infiltration from

landfills. Because removal of some pollutants is very difficult and expensive, pollution of surface water that is used for drinking after appropriate treatment must be prevented. This is achieved by zones of protection, the size of which depends on particular situation [18].

The primary pollution of groundwater can be caused by substances naturally occurring in groundwater and the mineral environment or by all types of wastewater, industry, agriculture, transportation, and exploitation of minerals. Therefore, groundwater sources also require protection, regular monitoring, and some treatment—the process of converting raw water from subsurface source into a potable form, suitable for drinking and other domestic uses. The method used for the treatment of groundwater will depend on the contaminants involved [49]. Although scientists look for new methods of disinfection or combine several technologies in order to reduce some harmful by-products associated with some ways of disinfection [50], processes based on active chlorine releasing substances are still most frequently used owing to their effectiveness, relatively low cost and residual disinfection power.

### 3.1. Results of microbiological examination of disinfected groundwater

Because we monitored water that should meet the limits for drinking water, we compared our results with those set by the relevant legislation Act 496/2010 Coll. [1, 4, 11, 40].

#### *3.1.1. Source 1*

In the period from January to May 2015, this source was disinfected five times, and on 5th day post each chlorination, the bacteriological quality of water was checked. The results are presented in **Table 1**.

The first chlorination was performed using 20 g of chloramine-T dose for one well, based on previous experimental chlorination. Because bacteriological results obtained 5 days after

| Parameter | CB (CFU) | *E. coli* (CFU) | BC 37 (CFU) | BC 22 (CFU) | FE (CFU) | $Cl_2$ (mg/L) |
|---|---|---|---|---|---|---|
| Before disinfection | | | | | | |
| Mean value | 160 | 1 | 18 | 23 | 1 | 0 |
| 5 days after disinfection by chloramine-T | | | | | | |
| 1 sample (20 g) | 130 | 0 | 42 | 2 | 5 | 0 |
| 2 sample (40 g) | 15 | 0 | 12 | 15 | 0 | 0 |
| 3 sample (40 g) | 0 | 0 | 0 | 2 | 2 | 0 |
| 4 sample (40 g) | 21 | 0 | 6 | 8 | 0 | 0 |
| 5 sample (40 g) | 0 | 0 | 0 | 3 | 1 | 0.05 |
| Limit (CFU) | 0[a] | 0[a] | 20[b] | 200[b] | 0[a] | 0.05–0.3 |

[a]CFU in 100 ml.

[b]CFU in 1 ml.

CB: coliform bacteria; BC 37 or BC 22: bacteria cultivated at 37 or 22°C; FE: faecal enterococci; $Cl_2$: free chlorine; CFU: colony forming unit.

**Table 1.** Results of microbiological examination and the level of free chlorine for Source 1 before and after disinfection with chloramine-T.

the first chlorination indicated that the dose cannot ensure effective disinfection, this dose was doubled in subsequent months to 40 g of chloramine-T per well. After the 4th chlorination, we observed that at the beginning of May no residual chlorine was present in water, and total coliform bacteria were detected in the relevant sample. Their presence suggested that a source of pollution may exist in the vicinity of one or more wells and that this source should be identified and eliminated in order to ensure safety of water. After the fifth chlorination at the end of May, before which there was a period without precipitations, the 40 g dose of chloramine-T appeared sufficient again. The wells that supplied water to the reservoir were not very deep (6–11 m), so in the period of intensive precipitations, they were more susceptible to contamination with various groups of bacteria including those of faecal origin, which could reach through run-off the relevant aquifer, as the wells were situated in an agricultural area. In such periods, we recommend more frequent disinfection of water with the 40 g dose.

Bonton et al. [51] observed that bacteriological pollution of groundwater in an agricultural area varied in space and time, and its contamination was higher during summer. Contamination exceeding the drinking water standard for treated water was determined in only 2% of the raw water samples. Total coliforms appeared to be a good precursor of *E. coli* or enterococci contamination.

Cho et al. [52] observed that heavy rainfall supports the transport of pathogenic bacteria. If these bacteria are introduced into groundwater, they can survive in a viable state but may or may not be culturable.

The studies of groundwater pollution focus usually on two to three indicator bacteria (e.g., total coliforms, faecal coliforms, and faecal enterococci) that were used to evaluate water quality. Because the combination of different kinds of pollution indicator bacteria provides better picture about faecal contamination in a given environment, we also used such approach in our study and determined heterotrophic counts besides indicator bacteria.

#### *3.1.2. Source 2*

After the experimental chlorination, Source 2 was disinfected with a dose of 180 g chloramine-T, which, however, appeared insufficient at checking on day 5 post disinfection as total coliforms and faecal coliforms were detected in the sample. This again required to increase the dose of chloramine-T to 360 g (**Table 2**). This increased dose was used in all four subsequent chlorinations and appeared effective up to May. After using 360 g dose, increased coliform counts were detected in this source at the beginning of May after intensive precipitations. Although the groundwater source has a depth of 23 m, it is located again in agricultural area where it can also run-off from the farm supplied from this source. Similar to the previous farm, change in intervals between disinfection is recommended in dependence on weather in order to ensure bacteriological safety of water.

#### *3.1.3. Source 3*

On the basis of experimental chlorination of water from Source 3, 180 g of chloramine-T was proposed as the optimum single dose. This amount was sufficient, and neither *E. coli* nor enterococci were detected after disinfection. The increased counts of coliform bacteria in the samples

| Parameter | CB (CFU) | *E. coli* (CFU) | BC 37 (CFU) | BC 22 (CFU) | FE (CFU) | $Cl_2$ (mg/L) |
|---|---|---|---|---|---|---|
| Before disinfection | | | | | | |
| Mean value | 150 | 0 | 35 | 88 | 20 | 0 |
| 5 days after disinfection by chloramine-T | | | | | | |
| 1 sample (180 g) | 55 | 0 | 195 | 192 | 2 | 0 |
| 2 sample (360 g) | 1 | 0 | 32 | 125 | 0 | 0 |
| 3 sample (360 g) | 1 | 0 | 15 | 30 | 0 | 0.05 |
| 4 sample (360 g) | 8 | 0 | 85 | 136 | 0 | 0 |
| 5 sample (360 g) | 0 | 0 | 0 | 2 | 0 | 0.15 |
| Limit (CFU) | 0[a] | 0[a] | 20[b] | 200[b] | 0[a] | 0.05–0.3 |

[a]CFU in 100 ml.

[b]CFU in 1 ml.

CB: coliform bacteria; BC 37 or BC 22: bacteria cultivated at 37 or 22°C′; FE: faecal enterococci; $Cl_2$: free chlorine; CFU: colony forming unit.

**Table 2.** Results of microbiological examination and the level of free chlorine for Source 2 before and after disinfection with chloramine-T.

after fourth chlorination together with the detection of 1 CFU of *E. coli* and the absence of residual free chlorine could be ascribed to heavy rain, so more frequent disinfection is recommended in such period (**Table 3**).

| Parameter | CB (CFU) | *E. coli* (CFU) | BC 37 (CFU) | BC 22 (CFU) | FE (CFU) | $Cl_2$ (mg/l) |
|---|---|---|---|---|---|---|
| Before disinfection | | | | | | |
| Mean value | 10 | 0 | 2 | 11 | 0 | 0 |
| 5 days after disinfection by chloramine-T | | | | | | |
| 1 sample (180 g) | 1 | 0 | 3 | 8 | 0 | 0 |
| 2 sample (180 g) | 3 | 1 | 0 | 19 | 1 | 0.05 |
| 3 sample (180 g) | 2 | 0 | 0 | 12 | 0 | 0.05 |
| 4 sample (180 g) | 9 | 1 | 13 | 38 | 0 | 0 |
| 5 sample (180 g) | 0 | 0 | 1 | 2 | 0 | 0.05 |
| Limit (CFU) | 0[a] | 0[a] | 20[b] | 200[b] | 0[a] | 0.05–0.3 |

[a]CFU in 100 ml.

[b]CFU in 1 ml.

CB: coliform bacteria; BC 37 or BC 22: bacteria cultivated at 37 or 22°C; FE: faecal enterococci; $Cl_2$: free chlorine; CFU: colony forming unit.

**Table 3.** Results of microbiological examination and the level of free chlorine for Source 3 before and after disinfection with chloramine-T.

Our results indicated better quality of water in this source in comparison with Sources 1 and 2. The depth of this source was considerable, and soil should ensure sufficient filtration of water. However, potential infiltration of pollutants is affected by many factors, such as the aquifer itself, immediate environment of well, geological conditions, existence of potential sources of pollution, and others.

### 3.2. Results of physico-chemical examination

Physico-chemical examination of water is important for assessment of its acceptability and potential health risks. Some chemical parameters indicate the risk of faecal or environmental contamination of water sources and may help to identify the sources of such contamination and take preventive measures.

Active chlorine added to water reacts to form hypochlorous acid and hypochlorite ion that are referred to as "free" or "available" chlorine. Their relative amounts vary with pH, when the pH rises above 8, the free chlorine loses most of its disinfectant power [53].

The presence of N-$NH_4^+$ in groundwaters is one of the most important indicators of fresh faecal pollution of water sources as a product of microbiological decomposition of organic matter and unused nutrients in the animal excrements. Although ammonium ions are retained by the cation exchange complex in the soil, Fridrich et al. [8] and Bartel-Hunt et al. [54] detected increased levels of ammonium nitrogen in shallow groundwater of the wells downstream from the pig housings and slurry lagoons. Natural levels of N—$NH_4^+$ in groundwater and surface water are usually below 0.2 mg/L, and anaerobic groundwaters may contain up to 3 mg/L [1].

Nitrates found in water as a final product of oxidation of N—$NH_4^+$ may also serve as indicators of older pollution. Due to various activities, such as excess application of inorganic nitrogenous fertilisers and animal manures, wastewater disposal, or leaking septic tanks, nitrates can reach both surface water and groundwater. While the concentration of nitrates in surface water can change rapidly as a result of run-off from the surface, application of fertilisers, uptake by phytoplankton, and action of denitrification bacteria, their concentrations in groundwater generally exhibit relatively slow changes. Although the most important sources of human exposure to nitrates and nitrites are vegetables and meat in the diet, under some circumstances, drinking water can significantly contribute to nitrate and, occasionally, nitrite intake [55]. Exposure of bottle-fed infants to nitrates and nitrites through drinking water can result in serious consequences.

In the majority of countries, the contribution of surface waters to nitrate levels in drinking water does not exceed 10 mg/L. However, nitrate levels in groundwater are often higher, exceeding the acceptable limit for adults (50 mg/L), particularly in agricultural areas. Nitrite levels are usually lower, rarely exceeding a few milligrammes per litre. Bonton et al. [51] monitored quality of groundwater and its variations in an agricultural area and reported considerable spatial and temporal variations in nitrate concentration from 6 to 125 mg/L.

Drinking water contains chlorides that originate from natural sources, sewage and industrial effluents, urban run-off containing de-icing salt, and saline intrusion. Urine of animals and humans contains relatively high levels of chlorides; therefore, values above 250 mg/L indicate risk of pollution of water with faeces [42].

The mineral content of natural and treated waters varies in considerable range. It could be important for individuals who are marginal for calcium and magnesium intake that drinking water may contribute to calcium and magnesium in the diet. Although epidemiological studies provided some information about a protective effect of magnesium or hardness on cardiovascular mortality, the evidence is being debated and does not prove causality. Further studies are being conducted in this respect. Because we lack sufficient data to suggest either minimum or maximum concentrations of minerals at this time, no guideline values for calcium and magnesium (hardness) are proposed [12].

**Source 1:** In water from Source 1, the pH was in the range of 6.9–7.4, which corresponded with the requirements on drinking water. Saturation with oxygen ranged from 55.4 to 80.9%. Saturation below the recommended level was determined in May (45.4% vs. recommended min. 50%), which could be related to intensive precipitations in the first half of this month. The contamination caused by increased run-off could result in processes with increased demand on oxygen.

Conductivity was in the range of 94.9–100.3 mS/m and was lower than the limit for this parameter (125 mS/m). Chemical oxygen demand ranged from 0.9 to 1.3 mg/L (limit 3.0 mg/L). Negative results were obtained for ammonium ions and nitrites. Nitrate levels ranged from 5.0 to 24 mg/L (limit 50 mg/L) and chlorides from 18.0 to 24.8 mg/L (limit 250 mg/L). Determination of calcium and magnesium showed that the recommended maximum concentration of these two elements (5 mmol/L) was exceeded at all samplings (5.18–5.78 mmol/L).

Contrary to the positive results for bacterial indicators, the physico-chemical examination of groundwater from Source 1 failed to indicate increased faecal contamination, even in the period of heavy precipitation.

**Source 2:** Determination of pH showed that all samples complied with the recommendations for drinking water. Level of dissolved oxygen (DO) in drinking water serves as an indication of its pollution and potability. Depletion of DO in water supplies can result in microbial reduction in nitrate to nitrite and sulphate to sulphide [1]. Saturation of water in this source was in the range of 81.9–95.6%, and thus well above the minimum limit, indicating good quality of water.

Electrical conductivity (EC) is a measure to the capacity of water to conduct electrical current, and it is directly related to the concentration of salts dissolved in water and therefore to the total dissolved solids (TDSs) principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulphates and some small amounts of organic matter that are dissolved in water. The EC of the groundwater is a general indicator of manure pit leakage [56]. Conductivity of water in Source 2 ranged from 76.0 to 83.1 mS/m and complied with the standard (125 mS/m). Oxidisability (chemical oxygen demand—$COD_{Mn}$) ranged from 1.2 to 1.24 mg/L, i.e., well below the maximum limit (3.0 mg/L). The level of nitrates was in the range of 6–18 mg/L, i.e., well below the 50 mg/L limit. With regard to the level of calcium and magnesium, water from this reservoir was within the recommended range (1.1–5.0 mmol/L) as it ranged from 3.8 to 3.9 mmol/L.

Overall, similar to Source 1, the results of physico-chemical examination of water from Source 2 did not indicate significant pollution with faeces.

**Source 3:** pH values determined in all samples were within the recommended range as they varied between 6.6 and 7.7. Compliance with the standard was also observed for saturation

with oxygen (64.5–98.3%). Conductivity of water (40.3–77.2 mS/m) is directly related to the concentration of salts dissolved in water. The level of this parameter was lower than in Sources 1 and 2, so were also the values of oxidisability $COD_{Mn,}$ which ranged from 0.16 to 0.8 mg/L. These values indicated very low level of chemically oxidisable pollutants, and therefore low possibility of development of disinfection BPs at chlorination.

When disinfecting water with active chlorine, the level of chemical oxygen demand (COD) or oxidisability is very important. COD is a measure of the capacity of **water** to consume oxygen during the **decomposition** of organic **matter** and the oxidation of inorganic chemicals, such as ammonia and nitrite. Thus, it indicates potential risk of development of BPs, such as THMs and haloacetic acid, which are linked to increased risk of cancer [26, 27]. When assessing the vulnerability of groundwater, there is an assumption that the water closer to the soil surface is of greater risk of contamination by pollutants, including N compounds. The proportion of N forms in groundwater is also affected by the depth [57].

Nitrites and ammonium ions were not detected in Source 3 and nitrates ranged between 25 and 32 mg/L and only at one sampling exceeded the limit by 8 mg/L. Chloride levels persisted well below the limit of 250 mg/L (6.8–22.3 mg/L). The sum of calcium and magnesium in water from Source 3 ranged between 2.0 and 2.4 mmol/L, which was in the recommended range.

### 3.3. EEM fluorescence spectra of water from Sources 1 to 3

Contamination of treated drinking water may occur while passing through the distribution system consumers. Elevated levels of dissolved organic matter (DOM) by the consumer compared to the water leaving the treatment plant indicates potential contamination that can be measured sensitively, inexpensively, and potentially online via fluorescence and absorbance spectroscopy. However, we lack the knowledge how much natural variation can be expected in a stable distribution system [58].

DOM plays an essential role in biogeochemical cycles and in transport of organic matter throughout the hydrological continuum. Fox et al. [59] used excitation-emission matrix (EEM) fluorescence spectroscopy to characterise microbially derived organic matter from common environmental microorganisms (*E. coli, Bacillus subtilis*, and *P. aeruginosa*). Their study showed that bacterial organisms can produce fluorescent organic matter (FOM) in situ and, furthermore, that the production of FOM differs at a bacterial species level. Fluorescence spectroscopy is a reliable and highly sensitive optical technique that allows one to carry out rapid monitoring of DOM in both natural and engineered systems. Fluorescence excitation emission matrices (EEMs) provide plenty of information about DOM [60].

EEM indicates the presence of pollutants by means of fluorescence characteristics, namely position of fluorophore in EEM, or excitation and emission maximum. Recent studies showed that different ways of disinfection of water affect its fluorescence properties due to development of various disinfection BPs [61]. The basis for correct evaluation of EEM of respective samples is the determination of a standard that can be used for comparison of quality at the absence of previous chemical analysis. Sample of drinking water taken from public drinking water supply (**Figure 1**) was used as a graphic standard in our study.

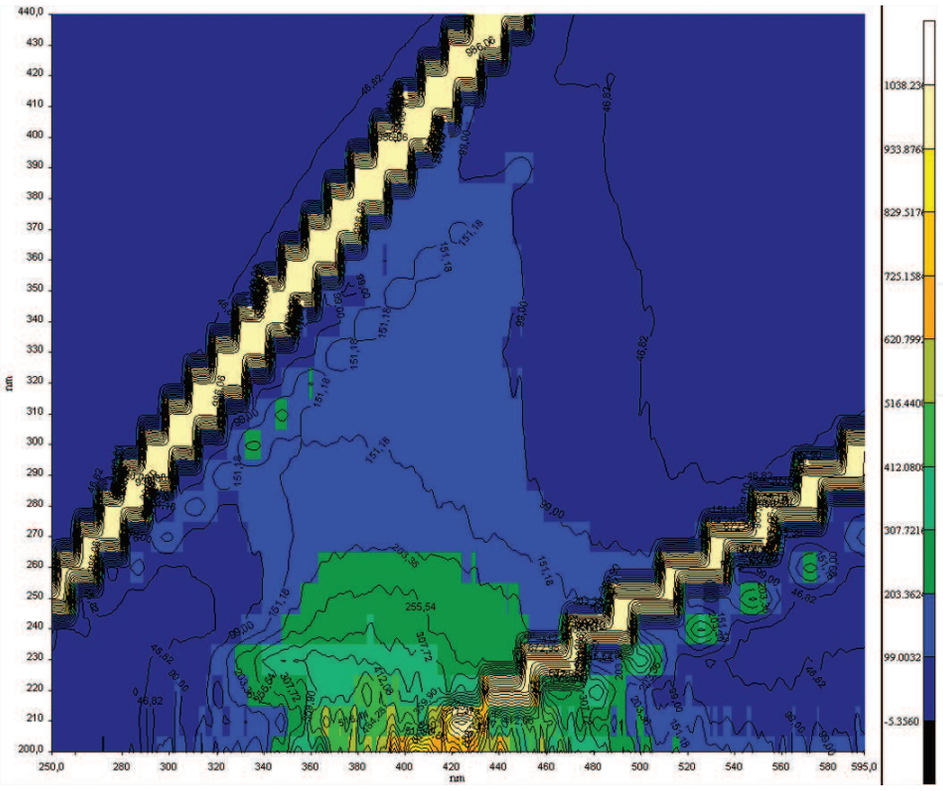


**Figure 1.** EEM of potable water sample from public water main.

## 4. Conclusion

Physico-chemical, microbiological examination, and EEM fluorescence spectroscopy used to investigate water from three monitored sources showed that the Source 3 provided water of better quality than Sources 1 and 2 (**Figures 2–4**). The results obtained did not indicate pollution of water with animal or human wastes. Some discrepancies between results of EEM spectroscopy and other analyses could be explained by limited number of EEM examinations and inability to identify the sources of NOM detected by this method.

Our results also suggested that weather (precipitations) was most likely the reason why quality of water was adversely affected at some samplings. The presence of total coliform bacteria indicated potential risk to animals consuming this water. However, according to some sources, total coliform testing can detect bacteria that have no connection with faecal contamination. Also, results of physico-chemical examination did not indicate faecal pollution. This is a complex issue requiring additional more detailed investigations.

The dose of chloramine-T determined by experimental chlorination and used for disinfection of investigated sources appeared effective only for Source 3, while they have to be doubled for Sources 1 and 2, and even these increased doses were much lower than the dose recommended by the manufacturer of this preparation. This is important from the point of view of decreasing production of potential BPs of water disinfection with active chlorine preparation. It may be more appropriate to adjust the intervals between individual treatments (disinfection) to weather conditions (heavy rain) instead of significantly increasing the active chlorine doses.

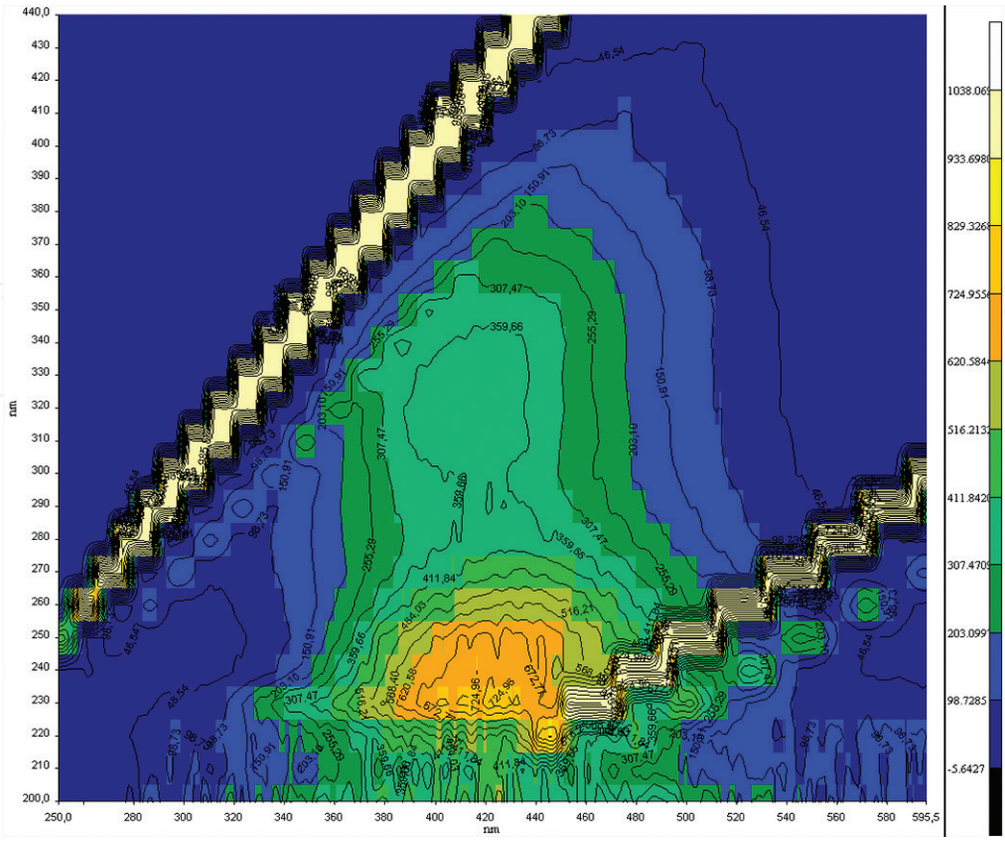


**Figure 2.** EEM of water sample of Source 1.

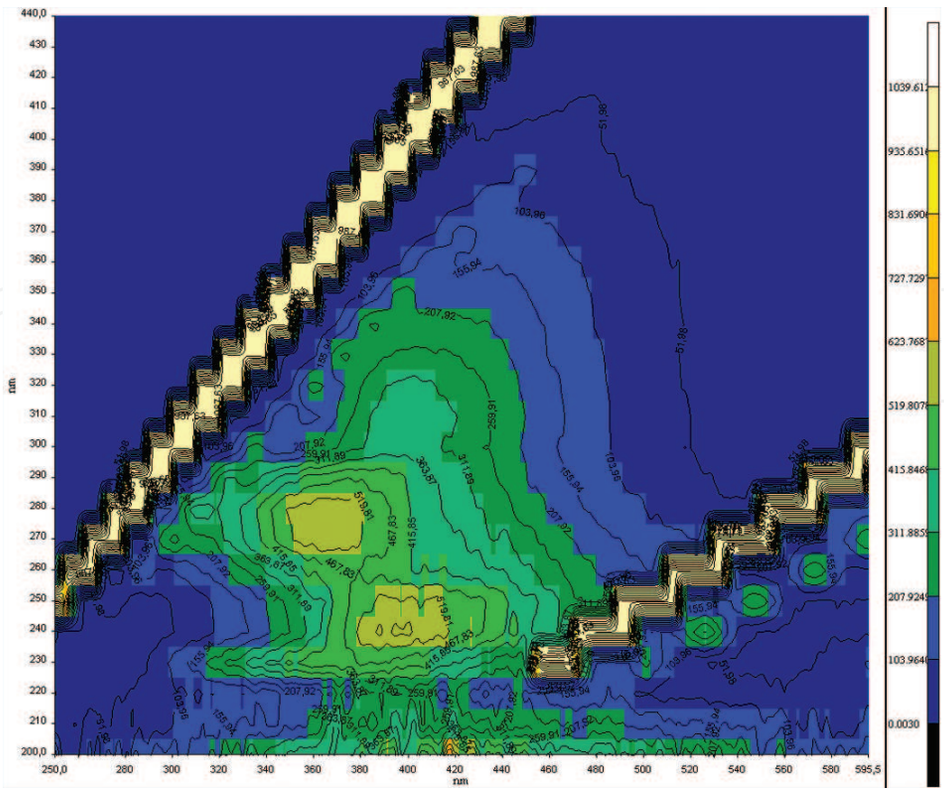


**Figure 3.** EEM of water sample of Source 2.

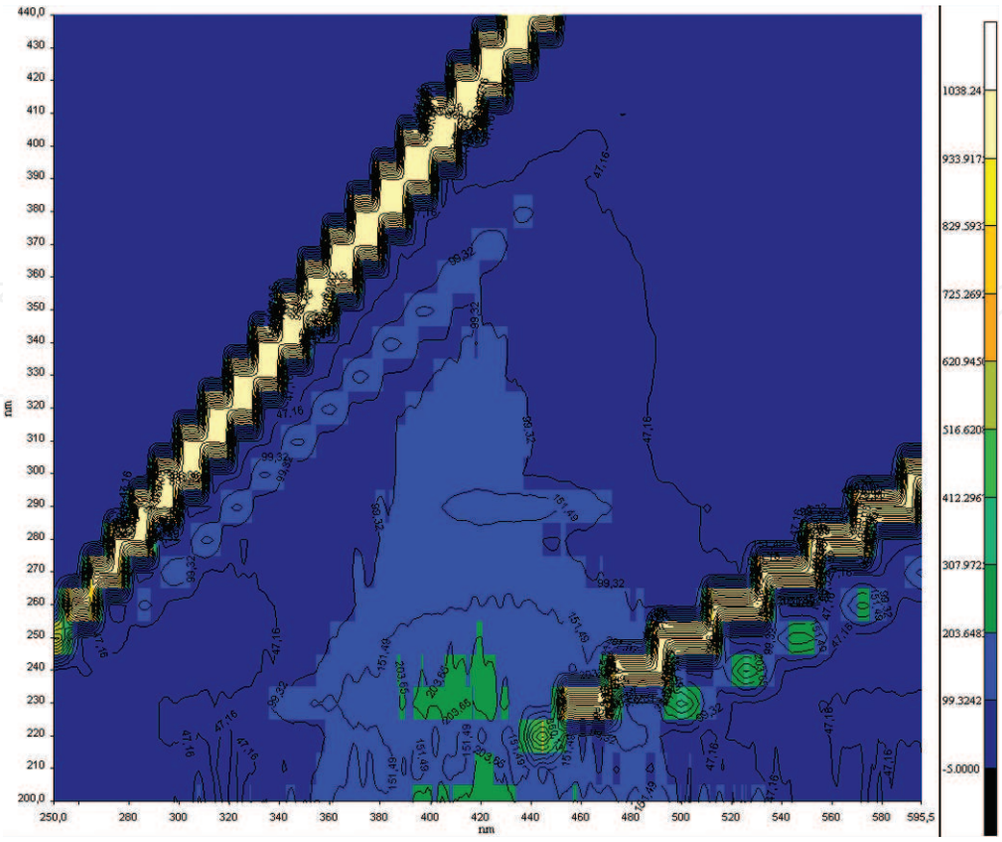


**Figure 4.** EEM of water sample of Source 3.

## Acknowledgements

The study was supported by the project VEGA 2/0125/17 and Slovak Ministry of Culture and Education Grant Agency No. 003UVLF-4/2016.

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