EVALUATION OF THE QUALITY OF DRINKING WATER IN RASINA DISTRICT, SERBIA: PHYSICOCHEMICAL AND BACTERIOLOGICAL VIEWPOINT

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Abstract. The water samples from Rasina District (Serbia) were evaluated for principal physical and chemical parameters, as well as for microbiological contaminants. Results were compared to National and *World Health Organization* (WHO) water quality standards. Several samples contained total organic matter, ammonia, residual chlorine, nitrite, nitrate, iron and manganese above proposed legislation limits. For samples contaminated with faecal bacteria, *Streptococcus faecalis*, aerobic mesophilic bacteria, coliform bacteria and *sulfite-reducing clostridia* special attention should be payed to drinking water disinfecting methods. The potential health risks of waterborne diseases due to consumption of water from contaminated sources could be implied.

Key words: Rasina area; drinking water; quality; physicochemical properties; microbiology.

1. INTRODUCTION

Water is a resource that has many uses, including recreation, transportation, and hydroelectric power, domestic, industrial and commercial uses [1, 2]. The quality of drinking water has a direct link with the human health and providing clean water to the consumers is one of the most important public health priorities [3]. The development of the science of water in recent decades is influenced by the rising awareness of the limited amount of unpolluted water which is at the disposal of mankind. Industrial development, urbanization and population growth have a negative impact on water quality [4].

Pollution of drinking water sources results from indiscriminate waste disposal; use of fertilizers and pesticides; industrial effluent discharges, surface run-off, lack of regulatory and remediation frameworks [5]. Although poor sanitation and food are the main sources for contamination with pathogen of gastrointestinal track, drinking water is the major source of microbial pathogens in developing regions [6].

The chemical characteristics of natural waters are a reflection of the soils and rocks with which the water has been in contact. Contaminants may include inorganic and organic substances. Some inorganic minerals dissolve quickly and change the composition of water rapidly, while other minerals, such as silicates, dissolve slower and have less conspicuous effects on the water composition [7, 8]. Organic compounds are derived from living organism as well as industrial sources. A wide variety of assortments of organic compounds are produced in the chemical and petrochemical industries. Benzene and *polycyclic aromatic hydrocarbons* (PAH's) as contaminants are known carcinogenic agents, while chloroform is a cancer-suspect agent [9].

Microbiological examination of the drinking water mainly covers detection of coliforms and total bacterial count. Coliforms are common bacteria that exist in the intestines of humans and mammals, and excreted out in the dejection. If large quantities of coliforms are present in the water, it is a prominent indicator of possible faecal contamination [10]. In developed countries, it is often regulated that coliforms must be undetectable in drinking water [11]. Too high total bacterial count means that the water is not perfectly disinfected and the water has already been polluted by microbes [12]. According to *World Health Organization* (WHO) estimation, about 1.1 billion people globally drink unsafe water and the vast majority (88%) of diarrheal disease reported across the globe is attributable to unsafe water, sanitation and hygiene [13]. There are numerous other diseases that are transmitted through polluted water [14, 15]. Additionally, the knowledge of the physical, chemical and biological parameters of water are very important for determining the type and quality of water [16–18]. According to WHO, drinking water should be clear, colorless, odorless, tasteless, and free of pathogens or other toxic chemicals [19].

The objectives of the present study were to determine selected physical and chemical parameters (color, turbidity, temperature, pH value, conductivity, the total organic matter content (TOM), NH_3, Cl_2, Cl^-, NO_2^-, NO_3^-, Fe, Mn) as well as microbiological parameters in water samples from the Rasina District (Serbia).

Results were compared to National and World Health Organization (WHO) water quality standards.

2. EXPERIMENTAL

2.1. STUDY AREA

Rasina District is in southern part of Central Serbia which occupies the surface of 2668 km^2. It has a population of 241.999. The Rasina District includes the municipalities of Kruševac (43°34′/N, 21°19′/E), Aleksandrovac (43°27′/N, 21°2′/E), Trstenik (43°27′/N, 21°0′/E), Brus (43°24′/N, 21°1′/E), Varvarin (43°43′/N, 21°21/E) and Ćićevac (43°43′/N, 21°26′/E). The administrative centre of the Rasina district is Kruševac. The district is bounded by several mountains and includes the parts of the basins of Zapadna Morava, Južna Morava and immediate basin of Velika Morava [20]. The geographical position of Rasina District and the sample locations are shown in Figure 1.

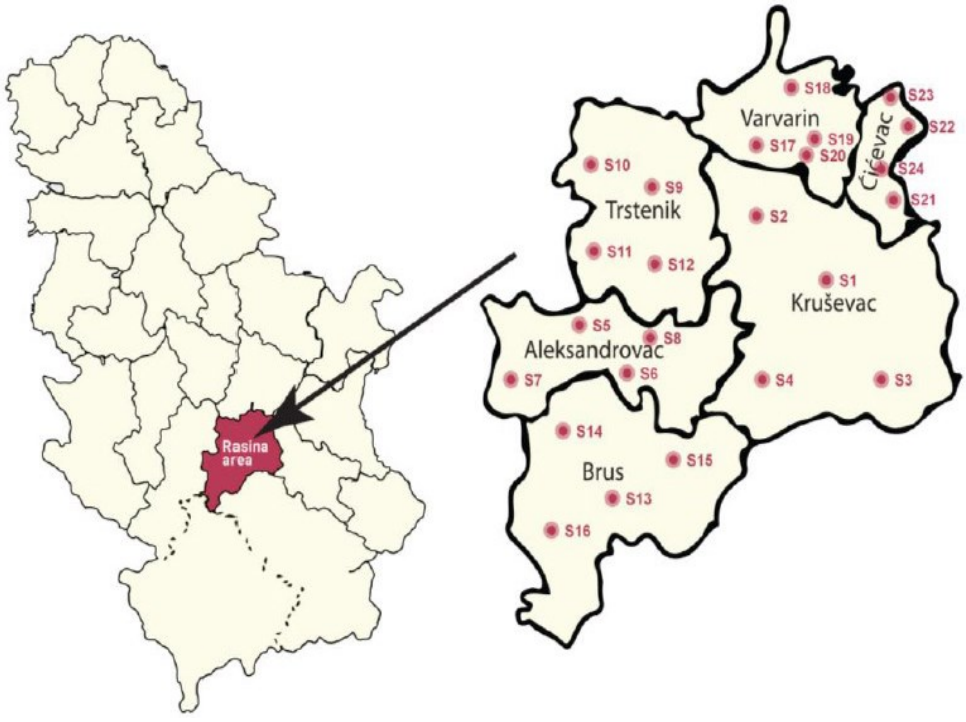

Fig. 1 – Geographical position of Rasina District and locations of water samples.

The samples were collected from municipalities of Kruševac: city's tap water (S1), water from village wells in Parunovac (S2), Jasika (S3) and Dedina (S4); wells water from territory of Aleksandrovac: Bratići (S5), Novaci (S6), Subotica (S7) and Gornja Velika Vrbnica (S8); Trstenik: Donji Dubič (S9), Milutovac (S10), Štulac (S11), Velika Drenova (S12); Brus: Brzeće (S13), Grad (S14), Dupci (S15), Vlajkovci (S16); Varvarin: Obrež (S17), Bačina (S18), Donji Katun (S19), Bošnjane (S20) and well water from territory of Ćićevac: Pločnik (S21), Stalać (S22), Pojate (S23) and Lučina (S24). All samples of water were collected in glass bottles which were previously rinsed with distilled water and sterilized with 70% alcohol. At the collection point, the containers were rinsed thrice with the sample water before being used to collect the samples, which were transported to the laboratory on the same day. Samples were collected and analysed in the period of June–August 2016.

2.2. DETERMINATION OF PHYSICOCHEMICAL PROPERTIES

Color was determined by standard platinum-cobalt method [21]. Turbidity was measured by nephelometry using Wagtech International Turbidity Meter (Wag-WT3020, Halma PLC Company) [21]. The temperature of the samples was measured by a thermometer and expressed in °C [22]. Thermometer was kept in the water until a constant reading was attained for two minutes. pH value was measured using a pH meter (MM multimeter 41, pH electrode 50 21 T) [21]. pH of each water sample was measured by inserting the probe into the water immediately after collection. It was rinsed and left standing in distilled water before being used for further pH measurement. Conductivity was measured using a conductometer (Crison, Multimeter MM 41, EC cell 50 70) [21]. The total content of organic matter (TOM) was determined using Kubel-Tiemann method (titration with a potassium permanganate in acid solution) [23]. The chloride content in water was determined by volumetric titration using standard solution of silver nitrate (0.1 $molL^{-1}$) with potassium chromate (K_2CrO_4) as an indicator (More's method) [24]. A Shimadzu (Model No: UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements. The colorimetric method was used for determination of ammonia (Nessler's reagent, 425 nm), residual chloride (*o*-tolidine, 470 nm), nitrate (220 and 275 nm), nitrite (sulfanilic acid, 525 nm), iron (1,10-phenanthroline, 510 nm) and manganese (sodium bismuthate, 510 nm) [21, 25, 26]. All chemicals used were of analytical reagent grade, and doubly distilled water was used in the preparation of all solutions in the experiments.

2.3. BACTERIOLOGICAL ANALYSIS

Bacterial analyses were performed according to National Standard Methods for the Hygienic Examination of Drinking Water [23].

2.4. STATISTICAL ANALYSIS

The results are expressed as the mean ± SD. Statistical differences were determined by Duncan's test ($p < 0.05$) using Statistica 8.0. software system.

Principal component analysis (PCA) and *Hierarchical cluster analysis* (HCA) were carried out by PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a). All data were autoscaled prior to any multivariate analysis. PCA was carried out by using a singular value decomposition algorithm and a 0.95 confidence level for Q and T2 Hotelling limits for outliers. Results of HCA are presented as a dendrogram where steps in the hierarchical clustering solution and values of the distances between clusters (Euclidean distance) are represented.

3. RESULTS AND DISCUSSION

3.1. PHYSICOCHEMICAL ANALYSIS

The results of physicochemical parameters of water samples are shown in Tables 1 and 2. The most of the investigated samples are colorless with *total color units* (TCU) value bellow 1. Three samples (S4, S7 and S11) possessed color with TCU between 2 and 4 which is still less than maximum allowed value (MAC) according to Serbian legislation. Additionally, this is significantly lower than 15 TCU when the color would be noticeable in a glass of water by most people. It is important to know origin of color of water since if it is not true but apparent color could be eliminated by filtration process [27]. Turbidity of water are caused by suspended inorganic and dispersed organic substances, and is the result of the optical activity of substances dissolved in it. In most tested water samples, measured turbidity was < 0.2 NTU, while samples S21 and S23 were on the edge of maximum allowed value (MAC = 5). Increased turbidity values of the samples S4, S7 and S8 may be linked to several factors such as the geology of the surrounding terrain, the presence of organic and inorganic materials, and sudden inflow of surface water in rainy season. According to EPA [28], turbidity should be below 1 NTU, because in this case there is no possibility of occlusion of microorganisms present in the colloidal particles. Additionally, all samples of water were odor- and tasteless.

Table 1

Physicochemical parameters of drinking water of Rasina area

Sample	Color °Pt-Co	Turbidity [NTU]	Temperature [°C]	pH	Conductivity [µS/cm]
S1	< 1	< 0.2	18.0	7.05	625
S2	< 1	< 0.2	17.6	7.37	360
S3	< 1	0.3	14.2	7.28	570
S4	2.3	**8.0**	20.1	6.87	658
S5	< 1	0.9	15.5	7.15	807
S6	< 1	< 0.2	14.0	7.30	707
S7	2.9	**13.5**	20.0	7.29	498
S8	< 1	< 0.2	15.5	6.97	248
S9	< 1	< 0.2	16.2	7.00	537
S10	< 1	< 0.2	13.9	7.01	863
S11	3.7	**7.7**	19.5	7.42	547
S12	< 1	< 0.2	18.0	7.33	963
S13	< 1	< 0.2	17.5	7.35	243
S14	< 1	< 0.2	17.0	7.40	178
S15	< 1	< 0.2	20.2	7.26	478
S16	< 1	< 0.2	15.5	8.00	171
S17	< 1	< 0.2	18.2	7.12	661
S18	< 1	< 0.2	20.5	7.22	624
S19	< 1	< 0.2	19.0	7.42	702
S20	< 1	< 0.2	14.8	7.24	**1005**
S21	< 1	5.0	17.5	7.38	565
S22	< 1	< 0.2	18.5	6.88	498
S23	< 1	3.3	20.5	7.81	460
S24	< 1	< 0.2	14.6	6.85	703
MAC*	**5**	**5**	/	**6.8-8.5**	**1000**

*maximum allowed concentration (MAC) in water for human use (Official Gazzet, 1999)

The temperature of analysed water samples was in the range 13.9–20.5°C; pH-value of the analysed water samples was in the range 6.85–8.00, which is within the values defined by recommendations of UNESCO/WHO/UNEP [29]. The lowest temperature (13.9°C) was recorded in a well water sample from Milutovac (Trstenik, S10) while the highest value of temperature was in sample

S18 (Bačina, Varvarin), Table 1. pH value may be affected by humic substances that alter the balance of the carbonate, the biological activity of flora and fauna, as well as hydrolysing salts. Due to the influence of pH on the chemical and biological properties of water, determining pH value is very important [30]. The results showed that the studied water samples were neutral to moderately alkaline. The lowest pH value (6.85) was measured in samples from Lučina (Ćićevac, S24) while the highest pH value (8.00) was recorded in Vlajkovci (Brus, S16). Conductivity is the electrical property of water, and depends on the ions present in the water – their concentration, mobility and the charge, as well as of the temperature at which the conductivity is measured. The maximum allowed value of conductivity of drinking water is up to 1000 μS cm^{-1} [32]. The minimum conductivity was measured in S16, 171 μS cm^{-1}, and the maximum in S20 – 1005 μS cm^{-1} (Table 1).

In the water containing organic substances of human, animal, plant or industrial origin, a certain amount of potassium permanganate is spent for their oxidation depending on the amount of organic matter in water [29]. Twenty three samples contained 0.09 to 5.90 mg L^{-1} of organic substances which is within the permitted limits, while the water sample from Vlajkovci (Brus, S16) has a 2.5 times higher than allowed value (Table 2). The organic substances present in the water do not have to be of polluting type, but may naturally be present in a sample because of the field geology.

Ammonia is a biologically active compound found in most waters as a normal biological degradation product of nitrogenous organic matter (protein). In water ammonia reacts to form ammonium (NH_4^+) and hydroxyl (OH^-) ions. When pH is above 7.2, some free NH_3 remains and this increases with increasing pH. It has been known that ammonia (NH_3) is toxic to fish and that its toxicity increases with increasing pH and temperature of the water [31]. According to the legislation of the Republic of Serbia the maximum allowed presence of ammonia in drinking water is 0.1 mg L^{-1} [32].

Only water samples from Milutovac (Trstenik, S10) contained higher level of ammonia (0.5 mg L^{-1}) while the values of the other samples were far below the maximum permissible value (Table 2).

The presence of ammonia at higher than geogenic levels is an important indicator of faecal pollution [33].

In all tested samples the presence of residual chlorine and chloride were in the range of allowed values (Table 2). A slight deviation of residual chorine was

observed in a water sample from Brzeće, S13 (0.51 mg L^{-1}). The values obtained for the chloride content ranged from 5.2 to 84.4 mg L^{-1}.

It was observed that two samples contained nitrite above MAC value. The presence of nitrite indicates possible bacterial contamination. Nitrite is the end product of aerobic decomposition of organic nitrogenous matter. According to the legislation of the Republic of Serbia the maximum allowed presence of nitrite in drinking water is 0.03 mg L^{-1} [32]. A slight increase of the concentration was detected in a water sample from Dedina, S13 (0.034 mg L^{-1}), while in sample S11 was 2 times higher concentration (0.069 mg L^{-1}) (Table 2). This strongly indicates a need for bacterial test [34]. On the other side, in the present investigation, mean nitrate values were in range between 1.2 mg L^{-1} and 156.0 mg L^{-1}. The nitrate content of water varies according to season [35]. World Health Organization and legislation of the Republic of Serbia has fixed the maximum value of nitrate in drinking water at 50 mg L^{-1}. The lowest nitrate value of the sample water was found in Dupci, Brus (S15) but in samples S4, S12 and S20 were obtained 2 to 3 times higher concentration than maximum allowed (Table 2). It has been demonstrated that if a pregnant women consumes regular water rich in nitrates, it could increase the risk of methemoglobinemy in newborn baby [36]. Therefore, it is important to monitor the level of nitrate in the area where was measured higher concentration of nitrate than allowed.

The function of the iron in the body is limited almost exclusively to the oxygen transport in the blood, through the hemoglobin [37, 38]. In the human body, the richest organs in iron are the liver and the spleen. However, iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage, as a result of formation of free radicals [39]. In this study, the range of iron concentration in samples within the allowed values was from < 0.05 to 0.21 mg L^{-1}, while in samples S4 and S7 were observed almost 2 times higher values than the maximum permissible (Table 2). The increased concentration of iron in the analysed samples can be correlated with geological or anthropogenic factors [40]. Increased concentrations of Fe may lead to its accumulation in the body, and long-term accumulation may have a carcinogenic effect on humans [41]. The trace amounts of Mn were observed in almost all tested samples (Table 2), except in samples S6 and S10 where it was found in 2 to 6 times higher concentrations, respectively than allowed. Olias *et al.* determined that Mn had a strong correlation with sulfates [42]. Manganese is an important nutrient that is involved in forming connective tissue and bones, clotting the blood, producing sex hormones, metabolizing carbohydrates, absorbing calcium and regulating blood sugar. It also plays a role in brain and nerve function [43].

Table 2

Chemical parameters of drinking water of Rasina area

Sample	Total organic matter [mg L^{-1}]	NH_3 [mg L^{-1}]	Cl_2 [mg L^{-1}]	Cl^- [mg L^{-1}]	NO_2^- [mg L^{-1}]	NO_3^- [mg L^{-1}]	Fe [mg L^{-1}]	Mn [mg L^{-1}]
S1	2.5±0.1[a*]	0.02±0.001[a]	0.05±0.002[a]	44.8±0.7	n.d[**]	15.5±0.6	0.05±0.002[a]	0.02±0.001[a]
S2	1.6±0.05[b]	0.02±0.001[a]	0.05±0.002[a]	18.0±0.5[a]	n.d.	6.9±0.4[a]	0.05±0.002[a]	0.02±0.001[a]
S3	1.9±0.1	0.02±0.001[a]	0.05±0.002[a]	32.8±0.7[b]	n.d.	24.7±0.9[b]	0.07±0.004	0.02±0.001[a]
S4	4.3±0.2[c]	0.02±0.001[a]	0.05±0.002[a]	47.2±0.9[c]	**0.034±0.003**	**116.5±1.5**	**0.55±0.03[b]**	0.02±0.001[a]
S5	3.7±0.1[d]	0.02±0.001[a]	0.05±0.002[a]	87.2±1.0	0.014±0.001[a]	**62.5±0.9**	0.05±0.002[a]	0.034±0.002[b]
S6	4.0±0.2[c,d]	0.02±0.001[a]	0.02±0.001[b]	56.8±0.9	0.003±0.0001[b]	17.5±0.8[c]	0.05±0.002[a]	**0.295±0.005**
S7	2.5±0.1[a,e]	0.02±0.001[a]	0.02±0.001[b]	23.6±0.7[d]	n.d.	22.7±0.9[b,d]	**0.59±0.02[b]**	0.02±0.001[a]
S8	0.9±0.05	0.02±0.001[a]	0.05±0.002[a]	10.0±0.5	n.d.	7.1±0.3[a]	0.05±0.002[a]	0.02±0.001[a]
S9	3.1±0.1[f]	0.02±0.001[a]	0.05±0.002[a]	22.0±0.3[e]	n.d.	29.5±0.9	0.05±0.002[a]	0.02±0.001[a]
S10	4.0±0.2[c,d]	**0.5±0.03**	0.05±0.002[a]	48.8±0.9[c]	0.012±0.001[a]	12.9±0.7	0.05±0.002[a]	**0.098±0.002**
S11	5.9±0.3[g]	0.02±0.001[a]	0.05±0.002[a]	22.0±0.9[d,e]	**0.069±0.002**	**57.3±0.9**	0.21±0.01	0.02± 0.001[a]
S12	1.2±0.05[h]	0.02±0.001[a]	0.05±0.002[a]	78.0±1.0	n.d.	**130.8±1.8**	0.05±0.002[a]	0.02±0.001[a]
S13	4.4±0.2[c]	0.02±0.001[a]	**0.51±0.03**	13.2±0.7[f]	n.d.	2.7±0.1	0.05±0.002[a]	0.02±0.001[a]
S14	2.7±0.1[a,e]	0.02±0.001[a]	0.05±0.002[a]	5.2±0.2	n.d.	3.0±0.1	0.05±0.002[a]	0.02±0.001[a]
S15	1.5±0.1[b]	0.02±0.001[a]	0.05±0.002[a]	6.0±0.2	n.d.	1.2±0.05	0.05±0.002[a]	0.02±0.001[a]
S16	**19.6±0.5**	0.02±0.001[a]	0.05±0.002[a]	8.0±0.2	n.d.	1.6±0.05	0.05±0.002[a]	0.02±0.001[a]
S17	5.5±0.2[g]	0.02±0.001[a]	0.05±0.002[a]	28.4±0.5	n.d.	19.1±1.0[c,e]	0.05±0.002[a]	0.02±0.001[a]
S18	3.4±0.1[d,f]	0.02±0.001[a]	0.02±0.001[b]	14.4±0.4[f]	n.d.	22.6±0.6[d]	0.05±0.002[a]	0.02±0.001[a]
S19	1.2±0.05[h]	0.02±0.001[a]	0.05±0.002[a]	22.0±1.0[d,e]	n.d.	**53.8±1.8**	0.05±0.002[a]	0.02±0.001[a]
S20	2.2±0.1	0.02±0.001[a]	0.05±0.002[a]	84.4±1.0	n.d.	**156.0±2.0**	0.05±0.002[a]	0.02±0.001[a]
S21	3.4±0.1[d,f]	0.02±0.001[a]	0.02±0.001[b]	30.8±0.9	0.003±0.0001[b]	**75.3±0.9**	0.21±0.01	0.034±0.002[b]
S22	1.2±0.05[h]	0.02±0.001[a]	0.02±0.001[b]	18.0±0.9[a]	n.d.	8.9±0.4	0.05±0.002[a]	0.02±0.001[a]
S23	3.7±0.2[d]	0.02±0.001[a]	0.05±0.002[a]	18.0±0.8[a]	n.d.	40.4±1.1	0.06±0.004	0.02±0.001[a]
S24	3.7±0.2[d]	0.02±0.001[a]	0.05±0.002[a]	34.0 ±1.0[b]	n.d.	19.9±0.9[e]	0.05±0.002[a]	0.02±0.001[a]
MAC[***]	**8.0**	**0.1**	**0.5**	**200.0**	**0.03**	**50.0**	**0.3**	**0.05**

[*]a,b,c – means with same letter within the same column are not significantly different ($p < 0.05$); [**]n.d. – not detected; [***] – maximum allowable concentration.

3.2. MICROBIOLOGICAL ANALYSIS

According to results obtained for microbiological analysis, a half of studied samples were contaminated with some type of coliform bacteria, meanwhile presence of faecal coliform were detected in samples S4, S9, S16, S18 and S23, Table 3.

Table 3

Bacteriological analysis of drinking water of Rasina area

Sample	*Total coliform* [CFU/100mL]	*Faecal coliform*	Aerobic mesophilic [CFU/1mL]	*Enterococcus faecalis*	*Proteus*	*Sulfite-reducing clostridia* [CFU/100mL]	*Pseudomonas aeruginosa*
S1	0	–	70	–	–	0	–
S2	0	–	80	–	–	0	–
S3	0	–	10	–	–	0	–
S4	21	+	300	+	–	10	–
S5	3	–	50	–	–	0	–
S6	0	–	20	–	–	0	–
S7	3	–	160	–	–	0	–
S8	0	–	150	–	–	0	–
S9	**161**	+	150	–	–	0	–
S10	0	–	0	–	–	0	–
S11	2	–	200	+	–	0	–
S12	14	–	100	–	–	0	–
S13	0	–	0	–	–	0	–
S14	0	–	6	–	–	0	–
S15	0	–	80	–	–	0	–
S16	24	+	300	–	–	0	–
S17	1	–	80	–	–	0	–
S18	3	+	20	+	–	0	–
S19	0	–	80	–	–	0	–
S20	8	–	20	–	–	0	–
S21	0	–	300	–	–	0	–
S22	0	–	100	–	–	0	–
S23	**161**	+	50	–	–	0	–
S24	1	–	30	–	–	0	–
MN*	**100**	/	**300**	/	/	**10**	/

**Maximum number* (MN) in water for human use (Official Gazzet, 1999).

Official Gazette of the Republic of Serbia (1999) established *maximum number* (MN) of coliform's at 100 *colony forming units* (CFU) per 100 mL. Comparing present results with MN it can be concluded that only samples from Donji Dubič (S9) and Pojate (S23) having number of total coliform bacteria more than proposed MN value. Presence of faecal bacteria is proved in both of samples. Although in most of the contaminated samples non-faecal coliforms are present regardless of their origin presence of this type of bacteria indicates that some correction and improvements on water collection and/or treatment should be done [44].

Counting of aerobic mesophilic bacteria (also known as heterotrophic plate counts, HPC) can be useful as information for numbering of non-faecal pollution bacteria. According to Serbian legislation maximum allowed number of this type of bacteria in drinking water is 300 CFU/mL. All of the investigated samples were within proposed limits with only three samples that were on upper limits – S4, S16 and S21. Since S21 did not contain coliform bacteria it can be stated that total contamination was with non-faecal origin. Although HPC does not have significant sanitary importance it can be used for long term assessment of drinking water quality [44].

Sulfite-reducing clostridia is a type of bacteria that can or can not be of faecal origin (except if *Clostridium perfigens* as specific specie is determined) [44]. Only one sample (S4) was positive with 10 CFU/100mL, which represent upper limits for sulfite-reducing bacteria, and since presence of faecal coliform was detected in it there is a great possibility of faecal origin of clostridia.

Studied samples were uncontaminated with *Proteus* and *Pseudomonas aeruginosa*. Meanwhile, presence of *Enterococcus faecalis* was detected in three samples – S4, S11 and S18. This type of contamination could point out on the human faecal pollution of well water [44].

3.3. PC AND HC ANALYSIS

In order to obtain a more detailed insight into the structure of data and identify similarities and specificities of groupings of objects, *Principal component analysis* (PCA) and *Hierarchical cluster analysis* (HCA) were conducted based on the parameters used in assessing the quality of water in Rasina County. The contents of ammonia, chlorine, nitrites, iron and manganese were not taken into consideration since their concentrations were below the detection limit, Table 2. A PCA resulted in a three-component model which explains 87.96% of total variance. Statistical parameters (the number of principal components and the percentage of variance they explain) are shown in Table 4.

Table 4

Statistical parameter

	PC1	PC2	PC3
Eigenvalue %	46.33	22.89	18.74
Cumulative %	46.33	69.22	87.96

Results obtained in PCA based on the content of nitrates, chlorides, total organic matter, pH value, conductivity, and temperature in the Rasina county water samples are shown in score plots and loading plots (Fig. 2). Results obtained in a hierarchical cluster analysis are shown in the dendrogram (Fig. 3). HCA results in the division of samples into four clusters. The first cluster includes samples S3–S6, S10, S12, 20 and S24. Within this cluster, two subclusters can be noted: Ia with samples S3, S6, S10 and S24, and Ib with samples S4, S5, S12 and S20. The second cluster consists of samples S7, S11, S15, S18, S19, S21 and S23; the third cluster of samples S1, S2, S8, S9, S13, S14, S17 and S22; and the fourth cluster of sample S16.

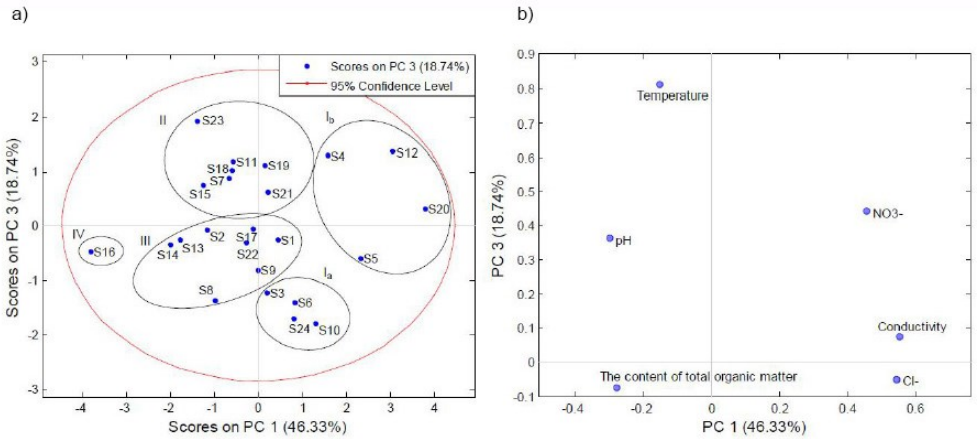

Fig. 2 – Score plot (a) and loadings plot (b).

This classification of samples was confirmed by the principal component analysis (Fig. 2a). Mutual projections of factor scores and their loadings for the first and the third PCs have been presented in Fig. 2. Taking into account PC1 and PC3 score values (Fig. 2a) four distinctive groups of samples were obtained. The loading plot (Fig. 2b) revealed that the conductivity, nitrate and chlorides contents have the most positive influence on the separation of the first group of objects (Ia and Ib) along the PC1 axis (Fig. 2b). The concentration of chlorides influenced the separation of samples S3, S6, S10 and S24 (subcluster Ia), while the concentration of nitrates influenced the separation of samples S4, S5, S12 and S20

(subcluster Ib). Samples of the second and the third group of objects were separated along the PC2 axis. Temperature had the strongest influence on the separation of the second group of objects (samples S7, S1, S15, S18, S19, S21 and S23), while the separation of the third group of objects (samples S1, S2, S8, S9, S13, S14, S17 and S22) was most strongly influenced by the total contents of organic matter (Fig. 2b). Sample S16 was separated from the remaining samples as an independent group (cluster IV) (Figs. 2 and 3). The pH value as well as the total content of organic matter had the strongest influence on its separation, which is in accordance with the fact that this sample had the highest values of these parameters (Tables 1 and 2). Additionally, this sample had the lowest levels of chloride and nitrate contents and conductivity (Table 2), which was confirmed by the PCA, since these parameters had the least influence on the separation of this sample (Fig. 2).

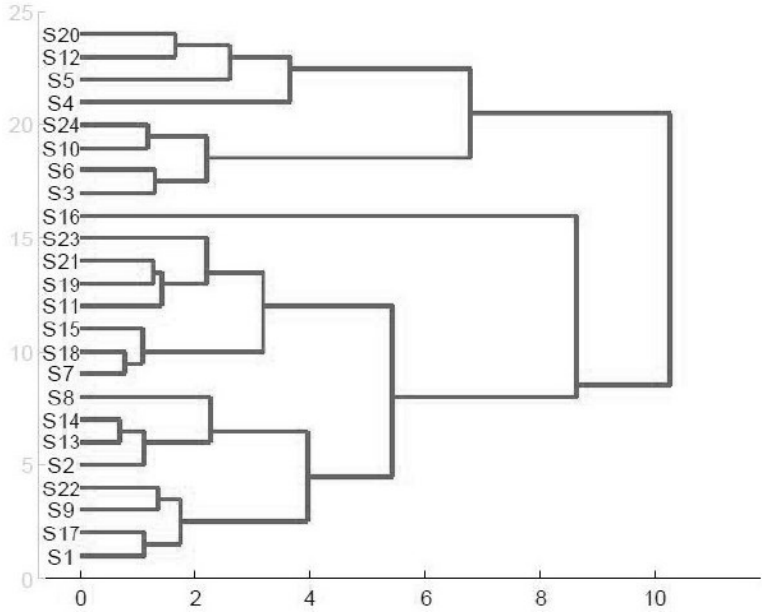

Fig. 3 – Dendrogram.

4. CONCLUSIONS

The results of the physico-chemical parameters were mostly agreed with relevant regulations except for turbidity (S4, S7 and S11) and conductivity in sample S20. The obtained values for Cl^- were within proposed legislation limits, as well as values for TOM, ammonia and residual Cl_2 except for samples S16, S10 and S13 respectively. Content of nitrite (S4, S11), iron (S4, S7) and manganese

(S6, S10) ions were increased in two samples, whereas contents of nitrate ion were above MAC in seven samples (S4, S5, S11, S12, S19, S20 and S21), indicating that these wells are nearby agriculture production areas. Microbiological analysis has shown that samples S4, S9, S16, S18 and S23 were contaminated with faecal bacteria and three samples with *Streptococcus faecalis*. Aerobic mesophilic bacteria in samples S4, S16 and S21, while in samples S9 and S23 coliform bacteria were found above MN values. One sample was contaminated with *sulfite-reducing clostridia*. Generally, the results of chemical parameters in studied samples, have shown that drinking water in Rasina area were of good quality and in accordance with national regulations or with WHO/EPA recommendations. It can be concluded that sample S4, had elevated levels of most chemical and microbiological parameters, so it should take care of maintenance of the that tap, as well as the proximity of potential sources of faecal pollution. The study pointed to the potential risks of waterborne diseases that may affect the heath of population due to consumption of contaminated water. According to results for microbiological analysis of water samples it would be important to pay attention to method which was applied for disinfecting of drinking water.

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