Assessment of spa mineral water quality from Vrnjačka Banja, Serbia: geochemical, bacteriological, and health risk aspects

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[ДР РГФ]

Assessment of spa mineral water quality from Vrnjačka Banja, Serbia: geochemical, bacteriological, and health risk aspects

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Abstract The goal of this study is to evaluate the quality of seven natural mineral water sources in the Vrnjačka Banja area used in the treatment of various diseases. The 24 macro- and microelements were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The results show that element content was within allowed limits of concentration, except for B, As, and Se. The evaluated content of these elements can be associated with geological and anthropogenic factors. The sample S6 contains more than twice the concentration of As, meaning that the use of water from this source should be limited due to the negative impact of this element on human health. The calculated values of weekly (EWI) and oral (OI) intakes as well as the acute hazard quotient (HQ_A) of selected toxic and potentially toxic elements indicate no health risks to the consumers. The highest hazard quotient (HQ_L) observed for As in sample S6 ($2.0 \cdot 10^{-1}$) demonstrated a significant risk of carcinogenic diseases in long-term consumption from this source. The estimated non-carcinogenic (DI_A) and carcinogenic (DI_L) dermal intakes with acute (HQ_A) and long-term (HQ_L) hazard quotient values for sample S3 show that there is no health risk to those who use the water from this thermal source for bathing. The presence of aerobic mesophilic bacteria at 22 °C and 37 °C in samples S3, S6, and S7 as well as sulphite-reducing *Clostridia* species in sample S5 were detected, potentially indicating some non-faecal or faecal contamination.

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Introduction

Water quality is of a vital importance for mankind given the direct connection between water and human survival (Rajiv et al. 2012). Water is known to play essential roles in human health (Boe-Hansen 2001), such as regulation of body temperature, removal of harmful substances from the body, and transport of vital nutrients. Many water resources in developing countries are unhealthy due to the presence of harmful physical, chemical, and biological agents (WHO 2008). Unfortunately, industrial development, urbanisation, and population growth have a negative impact on water quality (Su et al. 2011). Generally, water can be chemically, physically, or microbiologically contaminated due to

excessive use of mineral and natural fertilizers, waste disposal, wastewater discharge, excessive use of pesticides, and air pollution (Ćurčić and Čomić 2002). Water is not an unlimited natural resource, and therefore, water quality should be a basic priority.

The chemical characteristics of natural waters depend on the composition of the rocks and the soil through which the water passes. On its path, water is filtered, dissolves various inorganic and/or organic chemical compounds, absorbs gases (SO_2, H_2S, and CO_2), and forms thermo-mineral, carbonic-acid, or sulphide water. Micro- and trace amounts of certain elements (such as Co, Cr, Cu, Fe, Se, and Zn) are essential for human life; however, lowered or elevated levels of these elements can have a negative impact on human health, either due to toxicity, persistence, and bioaccumulation capabilities (Bencko 1977; Huang 2003; Fail et al. 1998). Many metals (Fe, Cu, Zn, Cd, Mn, etc.) have an affinity to bind with organic substances to form organometallic compounds of a high liposolubility coefficient, thus potentially becoming toxic and, in some cases, carcinogenic (Van der Kooij et al. 1991; Chale 2002; Huang 2003).

Detection of bacteria in drinking water signifies the presence of pathogenic organisms, the source of water-borne diseases (Al-Khatib and Arafat 2009). Microbiological contamination of water may lead to serious human health issues such as gastrointestinal and urogenital tract infections, sepsis, meningitis (in the case of newborns), and lung inflammation (Craun et al. 2006; John and Rose 2005).

Mineral and thermal waters are a groundwater characterised by particular chemical and physical features such as greater mineralisation, dissolved gases (SO_2, H_2S, and CO_2), radioactivity, or increased/decreased temperature. Such waters are generally associated with a specific and unique geological and tectonic structure (Laboutka and Vylita 1983). Mineral waters from deeper aquifers often have an increased temperature and therefore qualify as thermo-mineral. According to the content of macro- and microelements and other physical and chemical parameters of mineral water quality, the territory of Serbia belongs to one of the most attractive areas of the European continent (Petrović et al. 2010). Mineral and thermal waters in Serbia are mostly used for health-related bathing and other balneological and recreational purposes. In addition, the bottling of these waters as mineral, spring, or table has become increasingly popular in the last few decades. These waters have a long use history in Serbia, but have only

relatively recently become the subject of scientific research (Todorović et al. 2016). One of the most famous and visited Serbian spa centres (resort) with thermal and mineral waters is Vrnjačka Banja. Thermo-mineral waters from the territory of Vrnjačka Banja are used in the treatment of digestion, rheumatic, cardiovascular, and various skin diseases. Therefore, a study of the quality of these waters from the perspective of physical, chemical, and microbiological parameters is of the utmost importance for both the local population and visitors (more than 200,000 in 2018).

The aim of this research is to investigate the quality of mineral waters from the Vrnjačka Banja area through analysis of physico-chemical parameters (temperature, pH value, turbidity, conductivity, total hardness (TH), total organic carbon (TOC), Cl^-, NH_3, NO_3^-). Furthermore, the study looks at the macro-, micro-, and trace element content, as well as bacteriological parameters (aerobic mesophilic and sulphite-reducing bacteria). Special emphasis is placed on oral and dermal intakes of increased concentrations of toxic elements and assessment of potential risk (acute and long-term) to human health.

Material and methods

Study area

The geographical position of the locations is shown in Fig. 1. The Vrnjačka Banja area is characterised by a very complex geological structure: from the oldest, Paleozoic formations (schists, with marble and serpentinite), through the Mesozoic flysch, to the youngest, Quaternary sediments (Neogene clay, marlstone, conglomerate, and tuff). The examination of mineral water sources in the wider area has shown that they drain from different types of fissured aquifers, formed in intensely cracked Paleozoic schists (Slatina and Beli izvor), marble (Jezero), or serpentinites (Topli izvor, Snežnik, and Borjak), as well as sandstones and breccias, from the deeper parts of the Neogene complex (Vrnjačko vrelo). Water samples from these mineral water sources were collected in the Vrnjačka Banja area: mineral source Snežnik (S1), thermo-mineral source Jezero (S2), thermo-mineral source Topli izvor, which patients used for bathing (S3), and mineral source Borjak (S4), as well as thermo-mineral source Beli izvor
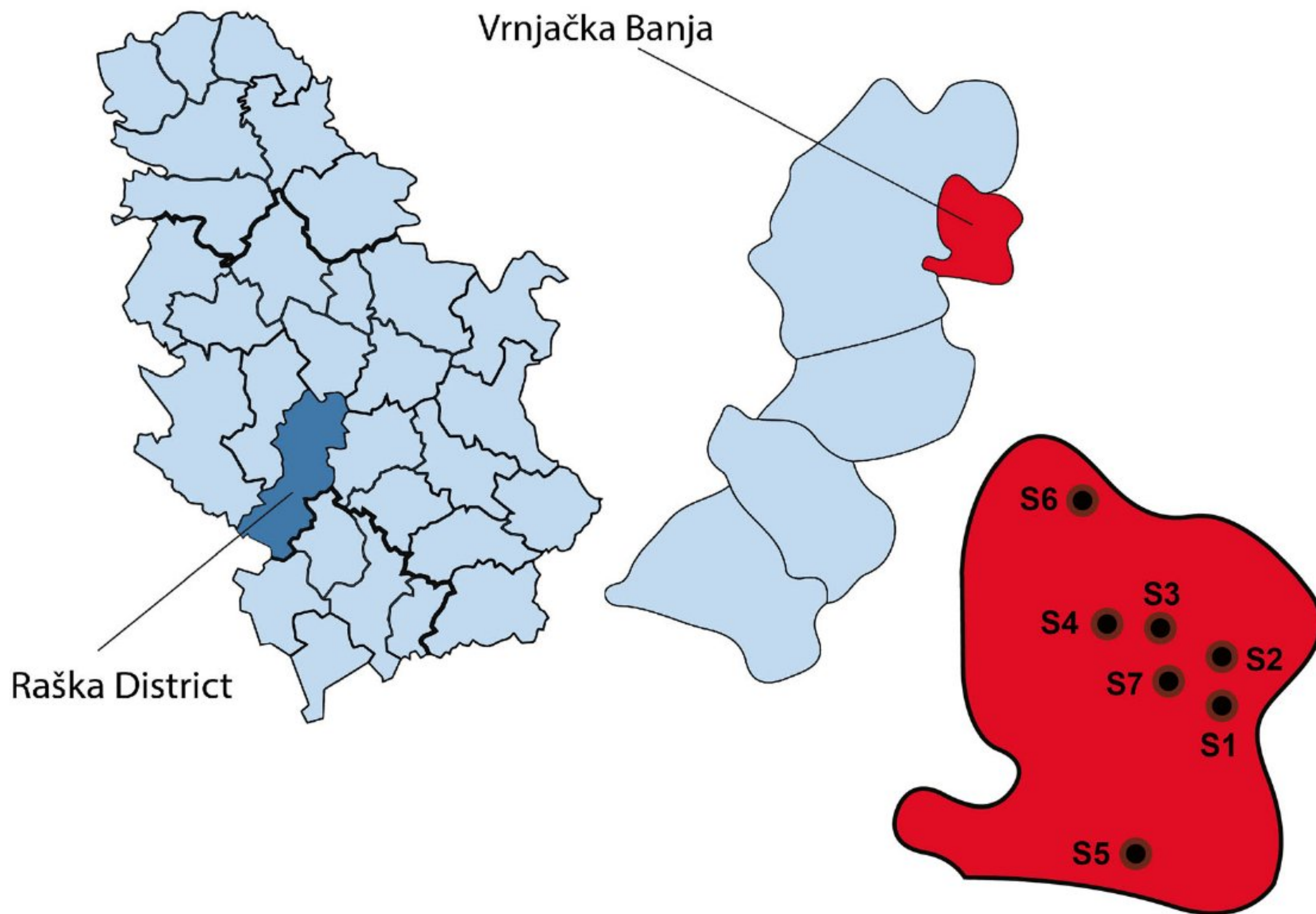
Fig. 1 Geographical position of Vrnjačka Banja area

(S5), mineral source Vrnjačko vrelo (S6), and mineral source Slatina (S7).

Determination of physico-chemical properties

The temperature of the samples was measured with a thermometer and expressed in °C (Yu et al. 2009). The pH value and conductivity were measured using a pH meter and conductometer (sensION+ MM 374 GLP 2 channel Benchtop Meter) (APHA 2012). Turbidity was measured by nephelometry using the Handheld Turbidimeter (Turb 430 IR) (APHA 2012). The chloride content was determined by volumetric titration using a standard solution of silver nitrate (0.1 mol/L) with potassium chromate (K_2CrO_4) as an indicator (Mohr's method) (Waters-Doughty 1924). Total water hardness (TH) was calculated as described in Milojković et al. (2018): TH in °dH = 0.1339 × [Ca in mg/L] + 0.2307 × [Mg in mg/L]. The colorimetric method was used to determine ammonia (Nessler's reagent, 425 nm) and nitrate levels (220 and 275 nm) (APHA 2012). A Shimadzu (model no: UV-2550) UV-Visible

spectrophotometer with 1 cm matching quartz cells was used for the absorbance measurements. All chemicals used were of analytical reagent grade, and deionised water was used in the preparation of all solutions in the experiments.

The determination of TOC was performed using Teledyne Tekmar Instruments—Apollo 9000 TOC analyser. The inorganic carbon component from samples was eliminated by acidification with HCl, pH range = 2–3 (Florescu et al. 2013). The remaining carbon was measured to determine total organic carbon, presented as TOC and expressed as mg/L.

ICP analysis

The content of elements in solution samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES), according to previously described procedures (Kostić et al. 2016).

Oral and dermal intake with health risk assessment

For the estimated oral intake of selected toxic elements, acute and long-term health risks (expressed as hazard

quotient values, HQ_A and HQ_L) were determined according to calculations presented in our previous work (Kostić et al. 2016), with one change—the average mineral water consumption in Serbia used for calculation was 0.19 L/day. Further, the possible dermal intake of selected elements in sample S3 and accompanying health risk assessment was added (Wang 2018; Nnorom et al. 2019), since the water at this location is used for thermal bathing. The RfD_dermal values for examined elements were calculated as RfD_oral × ABS_GI, where RfD_oral is the reference dose for oral intake (US EPA 2018), while ABS_GI is element absorption fraction in the gastrointestinal tract (US EPA 2004).

Bacteriological analysis

Bacterial analyses (aerobic mesophilic and sulphite-reducing bacteria) were performed according to National Standard Methods for the Hygienic Examination Drinking Water Standard Methods for Examination of Hygienic Correctness (1990). To determine aerobic mesophilic bacteria, a 2-mL water sample was added to a Petri dish and after the addition of 15 mL of dissolved substrate (tryptone, agar, dehydrated yeast extract, water), the mixture was gently shaken. The plates are incubated at 36 °C for 44 h and at 22 °C for 68 h. Thereafter, the bacterial colonies grown on each plate were counted and the results were expressed as the number of colonies per mililitre (CFU/mL) of sample for each temperature. For determining sulphite-reducing *Clostridia*, a 50-mL sample was heated to 80 °C for 15 min. Afterward, 50 mL of anaerobic sulphite

agar was added and the mixture was stirred and cooled to room temperature. The inoculated medium was incubated for 48 h at 37 °C. The appearance of black colonies is confirmation of the presence of sulphite-reducing *Clostridia*. The results were expressed as the number of colonies per 50 mL (CFU/50 mL) of sample at 37 °C.

Statistical analysis

The results given in this investigation are averages of triplicate measurements and presented as mean value ± SD. The means were compared through a *t* test with significant differences at $p < 0.05$.

Results and discussion

Physico-chemical parameters

The results of the basic physico-chemical parameters of the analysed water samples are shown in Table 1. The temperature of the investigated water samples was in the range 14–36 °C. The highest value of temperature was measured in sample S3, and this water belongs to moderately warm waters. It is important to note that mineral water from that source is used to help digestion, stomach problems, and gallbladder problems. This mineral water is applied orally (through drinking), through inhalation, as an enema, and vaginal spray in the treatment of these difficulties. Furthermore, it is used in the treatment of inflammatory and degenerative illnesses of the skeletal system as well as a mineral bath. All measured

Table 1 Physico-chemical parameters of investigated water samples

Sample	S1	S2	S3	S4	S5	S6	S7	MAC*
Temperature (°C)	17	27	36	19	22	17	14	–
pH	6.42	6.49	6.48	6.88	6.55	6.90	6.68	6.5–9.5
Turbidity (NTU)	2.77	1.44	0.89	5.24	4.24	1.35	2.27	5
Conductivity (μS/cm)	1397	2980	2150	1215	3460	657	2200	2500
TH (°dH)	20.68	30.94	21.66	18.23	27.98	10.50	31.66	–
TOC (mg/L)	7.65	6.20	8.98	6.45	8.48	7.05	6.95	–
Cl⁻ (mg/L)	21.62	35.09	34.74	20.56	46.79	33.59	28.91	250
NH_3 (mg/L)	0.03	0.186	0.014	0.134	0.106	0.100	0.108	0.5
NO_3⁻ (mg/L)	2.35	1.58	1.32	1.31	4.21	7.63	4.74	50

TH total hardness, *TOC* total of organic carbon

*Maximum allowed concentration (MAC) in natural spring water for human use (Official Gazette 2013)

temperatures were higher compared to values (7–13.5 °C) obtained for samples collected in Guber Spa (Bosnia and Herzegovina) (Nesimović et al. 2017).

The pH value range of tested water samples was between 6.42 and 6.90, which is within range recommended by UNESCO/WHO/UNEP (Chapman and Kimstach 1996). The highest pH value was measured in sample S6 (Vrnjačko vrelo), while the lowest was recorded in sample S1 (Snežnik). As expected, the obtained results have shown that the investigated water samples are moderately acidic but significantly higher than the more acidic waters from Guber Spa (Bosnia and Herzegovina) where recorded pH values were between 1.67 and 4.91 (Nesimović et al. 2017).

The turbidity of the water is the result of the optical activity of dissolved substances in the water. The level of turbidity can be increased by the presence of different organic matter and the content of suspended inorganic substances. All investigated water samples showed turbidity below 5.00 NTU, except sample S4 (5.24 NTU) where the measured value was slightly higher than allowed. Groundwater turbidity is a result of the presence of fine suspended colloidal substances, such as clay, silt, and other suspended organic and inorganic substances (Dragišić and Živanović 2014). It is connected to the geology of the surrounding environment. Additionally, it can be the consequence of the rapid influx of surface water in rainy season (Pantelić et al. 2017). If suspended organic matter is causing elevated turbidity, higher concentration of total organic carbon (TOC) is expected (Gorham et al. 1986).

Conductivity is the measure of the ability of water to conduct electrical current, which can be a basic indicator of its mineral content. The degree of conductivity depends on the concentration of ions present in the water, the mobility and the amount of electrical charge of each ion, and the temperature at which the conductivity was determined. According to the legislation of the Republic of Serbia (Official Gazette 2013), all samples, except for S6, are classified as natural mineral waters. Several of them can even be characterised as natural mineral waters rich in mineral salts, a category comprising mineral waters of mineralisation higher than 1500 mg/L. Sample S6 is in the category of natural mineral waters with low content of dissolved mineral solids, since its conductivity is only 657 μS/cm. This value was similar to result for water conductivity from the "Mali Guber" well— 700 μS/cm (Nesimović et al. 2017).

TOC is defined as the total concentration of carbon, found in organic matter form existent in the water, and accounts for water taste, colour, and odour and therefore may be a useful indicator of water quality. TOC consists of dissolved and particle material and is consequently influenced by fluctuations in suspended solids. The maximum allowable value of the total organic carbon in natural mineral waters is not legally defined by the Republic of Serbia (Official Gazette 2013), but it is considered that it should not exceed 5 mg/L. In all analysed water samples, the calculated values of TOC were in the range of 6.20 to 8.98 mg/L, mostly consistent with the elevated turbidity, except for the samples S3 and S4 (Fig. 2), where the presence of organic substances is not the cause of water turbidity. Sample S3 has the highest TOC value (8.98 mg/L), which does not cause increased turbidity (only 0.89 NTU). On the other hand, sample S4 has one of the lowest TOC

Fig. 2 Relationship between TOC (mg/L) and turbidity (NTU) in analysed water samples
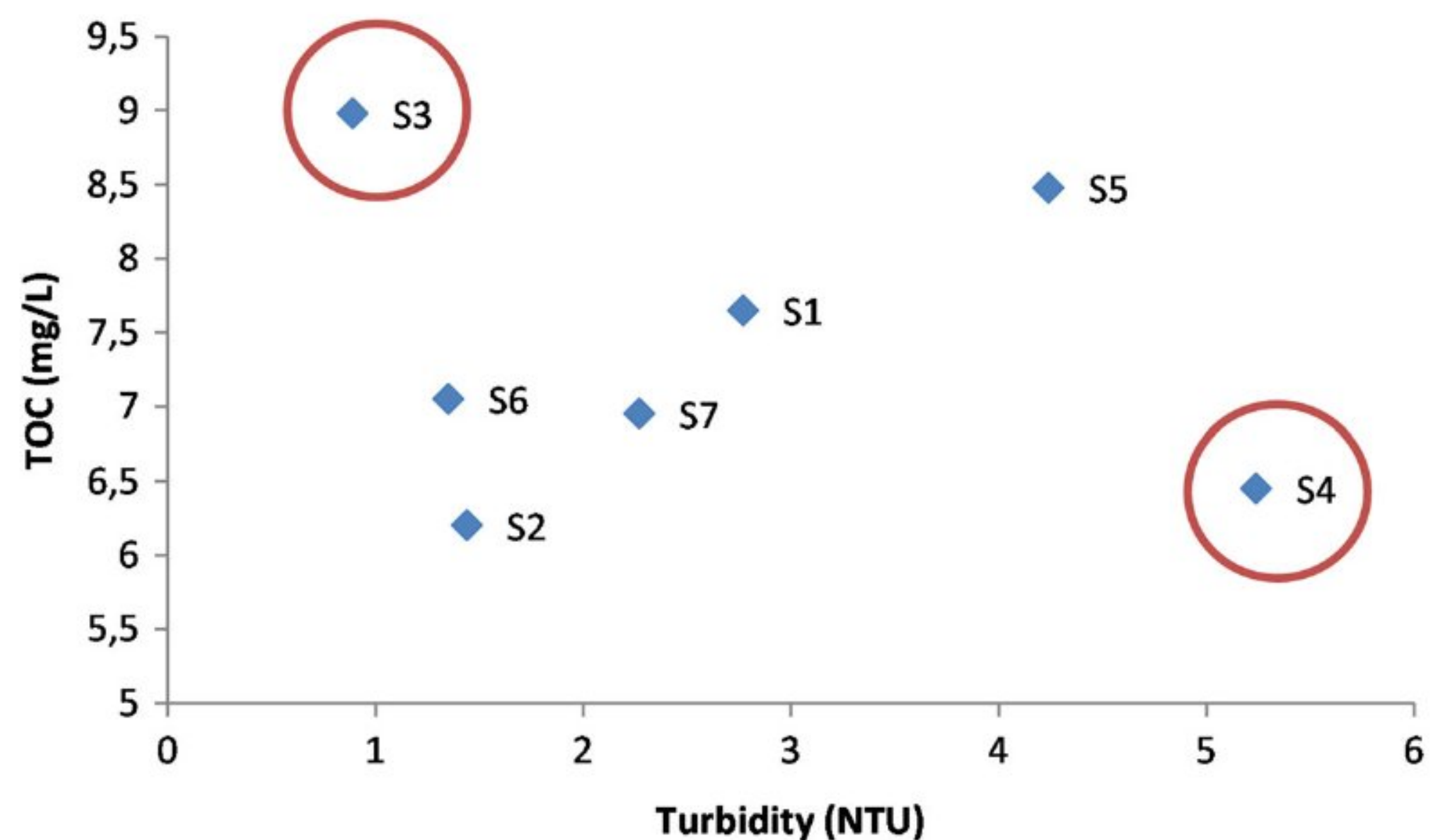
values on site (6.45 mg/L), but the turbidity is elevated (5.24 NTU), probably due to the presence of dispersed inorganic substances (such as clay, sludge, etc.).

In groundwater, chlorine mostly occurs in the form of chloride ions. There is no proof that increased concentration of chloride influences human health; nevertheless, chloride ions can have an effect on water flavour and colour (Pantelić et al. 2017). In winter periods, due to road salt sprinkling, the concentration of chloride is significantly increased in groundwaters (Chapman and Kimstach 1996). In the analysed samples, the chloride content was relatively low, ranging from 20.6 to 46.8 mg/L (Table 1), leading to the conclusion that there is no negative effect on organoleptic characteristics of analysed waters. The highest concentration of chloride ions was recorded in sample S5, while S4 showed the lowest value of this parameter.

In an aqueous solution, ammonia occurs in equilibrium with the ammonium ion. Total ammonia presents the sum of the concentration of both forms and depends on temperature as well as the water pH value. In natural mineral waters, the presence of ammonia is normal due to degradation of nitrogenous organic compounds (such as proteins). Higher concentrations, however, may indicate organic pollution of water, and it is, therefore, very important to monitor its level in water for human use. By legislation of the Republic of Serbia, the maximum allowed ammonia concentration in natural spring waters is 0.5 mg/L (Official Gazette 2013). All investigated samples of mineral waters had a significantly lower value than the standard specified, as well as a significantly lower concentration of nitrate ion than the maximum permitted value.

The content of macro- and microelements in water samples

The median concentrations of the investigated elements in mineral water samples from the Vrnjačka Banja area are given in Tables 2 and 3. Sodium is the dominant macrocomponent in all these mineral waters (Fig. 3), generally due to the lithology of the aquifers, comprising schists, among other rock (Dragišić et al. 2012). For the same reason, these waters are rich in potassium. As shown in Table 2 and Fig. 3, the water samples S2, S3, S5, and S7 contain significantly higher amounts of sodium compared to other macrocomponents, with 748.6 mg/L from sample S5 the highest amount. There

Table 2 The content of macroelements in water samples

Sample	Na*	K*	Li**	Ca*	Mg*	Ba**	Sr**	Si*	S**
S1	189.9 ± 8.7	32.7 ± 1.8a	364.2 ± 18.4	70.9 ± 3.1a,c	48.5 ± 2.5a,b	264.9 ± 4.2	864.5 ± 42	23.7 ± 0.8a	14,660 ± 640
S2	569.9 ± 20.9	78.8 ± 2.7	872.5 ± 40.6	155.1 ± 5.0b	44.1 ± 1.4a,c,d	651.3 ± 5.0	2031.0 ± 91.0	11.2 ± 0.6b	1273 ± 58a
S3	394.6 ± 16.6	56.9 ± 1.1	618.6 ± 28.3	72.7 ± 3.7a	51.7 ± 1.5b	523.4 ± 4.7	1454.0 ± 54.0	20.9 ± 0.9c,d	4757 ± 156
S4	158.3 ± 5.3	31.0 ± 1.4a	292.5 ± 12.4	64.3 ± 3.1c	41.7 ± 1.4c	194.9 ± 5.2	730.0 ± 18.0	22.4 ± 1.1a,c,d	18,010 ± 810
S5	748.6 ± 26.6a	91.2 ± 2.3b	989.5 ± 42.9a	122.5 ± 5.9	50.2 ± 1.2b	820.2 ± 3.8^A	2424.0 ± 104.0a	19.3 ± 0.9c	834 ± 34^A
S6	27.9 ± 1.4	7.2 ± 0.2	29.4 ± 1.2	6.7 ± 0.2	41.7 ± 1.8c,d	21.1 ± 5.6	141.4 ± 6.0	16.4 ± 0.8	3024 ± 124
S7	715.4 ± 27.4a	92.7 ± 4.7b	985.2 ± 40.1a	158.9 ± 7.7b	45.0 ± 1.9a,c	770.1 ± 4.7	2327.0 ± 87.0a	11.3 ± 0.5b	1188 ± 48a
MAC†	/***	/***	/***	150	50	1000	/***	/***	/***

Results with the same lowercase letter (a–d) in the same column are not significantly different ($p < 0.05$). Results with the same superscript uppercase letter (A) for the same row/sample are not significantly different ($p < 0.05$)

*Concentration of elements in mg/L

**Concentration of elements in μg/L

***Not regulated by Official Gazette (2013)

† Maximum allowed concentration (MAC) in water for human use (Official Gazette 2008)

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Table 3 The content of microelements in water samples [μg/L]

Sample	B	Al	As	Se	Pb	Fe	Co	
S1	417.6 ± 1.4	6.06 ± 0.18a	5.66 ± 0.16	3.83 ± 0.12	0.922 ± 0.020a,A	754.5 ± 34.2	1.47 ± 0.07	
S2	1570.0 ± 79.0	6.49 ± 0.26a	n.d.	7.12 ± 0.28	n.d.	87.6 ± 1.6	n.d.	
S3	848.1 ± 39.9	3.43 ± 0.12	1.37 ± 0.06a	14.10 ± 0.70a	0.123 ± 0.005	0.8 ± 0.1^A	0.61 ± 0.01	
S4	233.1 ± 12.1	4.83 ± 0.13b,c,A	5.01 ± 0.10^A	13.73 ± 0.68a	0.915 ± 0.045a	403.6 ± 22.4	1.01 ± 0.02^B	
S5	1860.0 ± 70.0a	5.20 ± 0.23b	2.40 ± 0.12	17.36 ± 0.81	0.060 ± 0.003	34.5 ± 1.4	0.94 ± 0.03^B	
S6	0.90 ± 0.05^A	5.15 ± 0.26b	23.91 ± 0.81	n.d.	1.005 ± 0.025	38.9 ± 1.9a	0.69 ± 0.01	
S7	1890.0 ± 89.8a	4.62 ± 0.12c	1.46 ± 0.05a	6.03 ± 0.25	0.676 ± 0.016	39.9 ± 2.0a	1.65 ± 0.05	
MAC*	1500	/**	10	10	10	/**	/**	
Sample	Ni	Cu	Zn	Cd	P	Mn	Mo	Cr
S1	16.26 ± 0.71	n.d.	4.83 ± 0.21	1.02 ± 0.02a	0.90 ± 0.05a,A	96.9 ± 3.1a	n.d.	0.382 ± 0.012a
S2	1.56 ± 0.06	n.d.	8.37 ± 0.33	0.92 ± 0.02a,A	0.90 ± 0.05a,A	179.8 ± 7.3	0.192 ± 0.007	0.369 ± 0.019a
S3	n.d.	n.d.	5.43 ± 0.21	1.14 ± 0.05b	0.90 ± 0.05a,A	101.1 ± 4.2a	n.d.	0.107 ± 0.004
S4	2.74 ± 0.08	n.d.	5.82 ± 0.24a	1.03 ± 0.03a,B	0.90 ± 0.05a	115.3 ± 5.7	n.d.	0.387 ± 0.017a
S5	9.58 ± 0.46a	n.d.	14.34 ± 0.46a	1.23 ± 0.04b	0.90 ± 0.05a,B	49.6 ± 2.5	n.d.	0.393 ± 0.012a
S6	n.d.	5.84 ± 0.22^B	15.03 ± 0.61a	0.87 ± 0.02a,A	32.27 ± 1.45^C	5.8 ± 0.2^B	2.348 ± 0.061	0.074 ± 0.001
S7	10.44 ± 0.46a	n.d.	7.73 ± 0.29	0.95 ± 0.04a,A	0.90 ± 0.05a,A	235.1 ± 7.4	n.d.	0.537 ± 0.026
MAC*	20	3000	/**	3	/**	500	/**	50

Results with the same lowercase letter (a–c) in the same column are not significantly different ($p < 0.05$). Results with the same uppercase letter (A–C) for the same row/sample are not significantly different ($p < 0.05$)

n.d. not detected

*Maximum allowed concentration (MAC) in water for human use (Commission Directive 2003/40/EC)

**Not regulated by Commission Directive 2003/40/EC

is no MAC for sodium in mineral waters. However, the maximum allowable sodium in drinking water for everyday use is 200 mg/L (Official Gazette 2008), and therefore, the consumption of these waters should be limited. The concentration of this macroelement in samples S5 and S7 is comparable to those in mineral water springs in Kanjiža, Serbia (Bogdanović and Besermenji 2000), and Vitinički Kiseljak, Bosnia and Herzegovina (Lazić et al. 2011). On the other hand, the same samples contain twofold higher concentration of Na compared to mineral water sources in Lukovska and Vranjska Banja, Serbia (Šaraba et al. 2017), and Permet, Albania (Eftimi and Frashëri 2016). Furthermore, all tested samples from Vrnjačka Banja contain approximately 3 to 69

Fig. 3 Concentration of macroelements (in mg/L) in analysed water samples
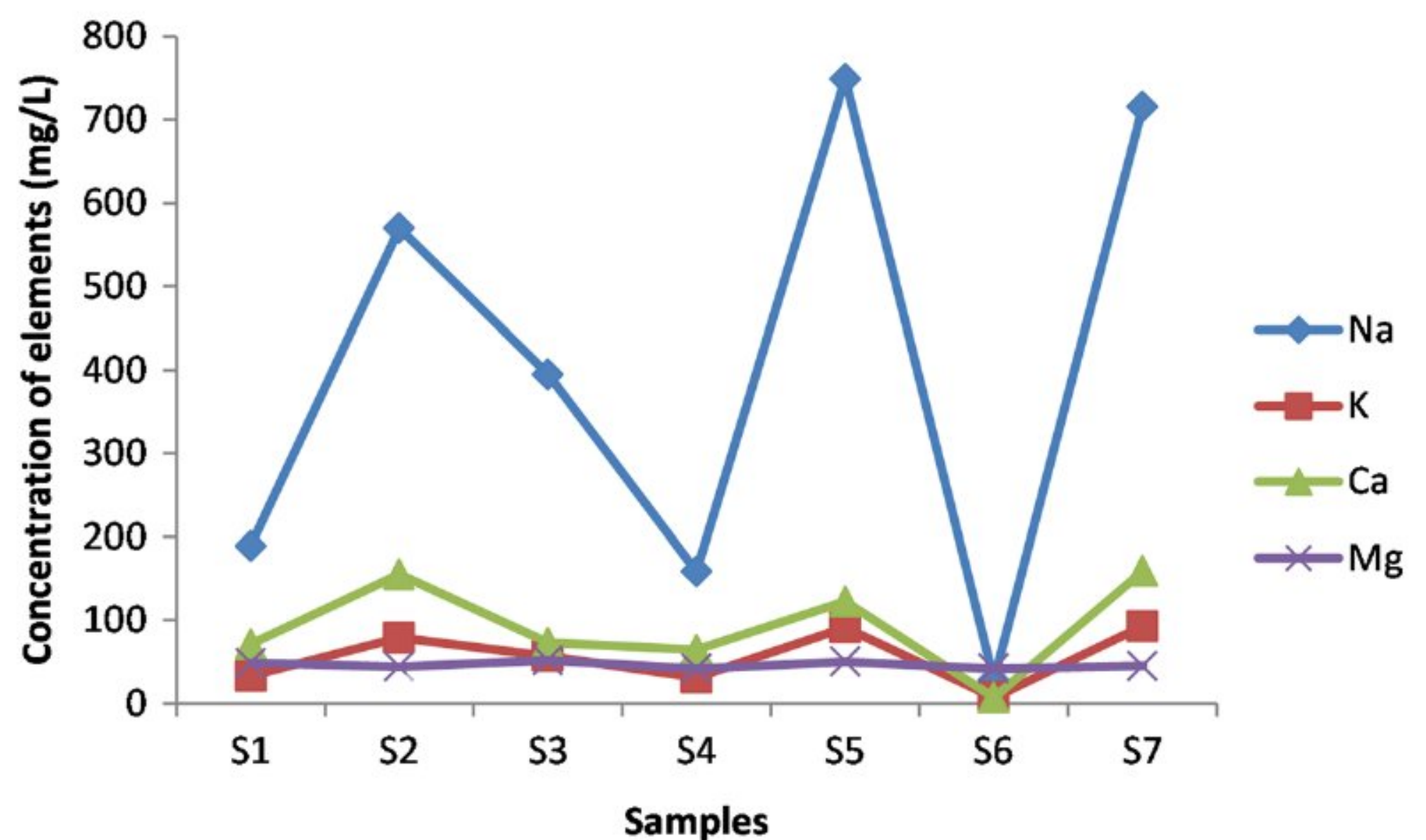
times higher amount of Na than the mineral water in Krapinske Toplice, Croatia (Borović et al. 2016) and even 7 to 187 times more than the Iliča, Montenegro water spring (Burić et al. 2016). The concentrations of potassium in all analysed samples (Table 2) were significantly higher than the median potassium concentration for European bottled waters (2.1 mg/L) (Reimann and Birke 2010) and for Serbian mineral waters (10 mg/L) (Stojković 2013). The maximum allowable concentration of lithium is not defined in either the legislation of the Republic of Serbia or the WHO, and obtained results of tested samples indicate that the highest concentration was recorded in samples S5 and S7 (989.50 and 985.20 μg/L, respectively), while a rather low value was measured in sample S6, only 29.36 μg/L.

The source of calcium and magnesium in these mineral waters is mostly marble (for calcium and magnesium) and serpentinite (for magnesium), both present in the aquifers (Dragišić et al. 2012). Most of the analysed water samples had calcium and magnesium contents within the limits for everyday drinking water (150 and 50 mg/L, respectively) (Official Gazette 2008). All analysed samples had a higher concentration of calcium and magnesium compared to mineral water springs in Radanjska, Lukovska, and Vranjska Banja, Serbia (Šaraba et al. 2017), Vitnički Kiseljak, Bosnia and Hercegovina (Lazić et al. 2011), Krapinske and Tuheljske Toplice, Croatia (Borović et al. 2016), and Iliča, Montenegro (Burić et al. 2016). The concentration of calcium in sample S5 is comparable to that in spring Permet, Albania (Eftimi and Frashëri 2016). The conclusion is that most investigated samples from Vrnjačka Banja contain a higher concentration of these essential macroelements compared to other spa mineral waters in Serbia and surrounding countries. It should be noted that sample S6 had a rather low concentration of calcium (6.7 mg/L), consistent with the fact that this is low mineralised water from a complex fractured aquifer in Neogene sandstones and breccias of serpentinite character. The described lithology favours magnesium over calcium in this sample, distinguishing it from the rest (Dragišić et al. 2012; Protić 1995). Sample S6 had the lowest concentrations of other analysed macrocomponents as well (Fig. 3). In addition, the hardness is imparted to the water mainly by calcium and magnesium ions. Hard water is generally undesirable, due to soap residue and a high boiling point, making it unsuitable for cooking. In the present study, the mean total hardness ranged from 10.50 to 31.66 °dH

(Table 1). The results of water hardness analysis (°dH) show that samples S1, S3, and S4 belong to the category of hard waters (15–25 °dH). Furthermore, water samples S2, S5, and S7 belong to the category of very hard waters (> 25 °dH), while water from spring S6 belongs to the category of medium soft waters (10–15 °dH). Nevertheless, the presence of another alkaline earth metal − barium – was below the nationally allowed limit for mineral waters, which is 1 mg/L (Official Gazette 2008). Concentrations of strontium were in the interval 141.4–2424.0 mg/L and mostly higher than the median strontium concentration for European bottled waters, which is 326 μg/L, and for mineral waters from Serbia—660 μg/L (Reimann and Birke 2010; Stojković 2013). The concentration limit of silicon is not given in the regulations on the quality and safety of natural mineral waters of the Republic of Serbia. Nevertheless, the appearance of this macroelement in mineral waters is entirely expected, due to the widespread distribution of silicate minerals, whose weathering leads to silicon enrichment of groundwater. Although silicon is present practically in all groundwater, concentrations are usually low, due to poor solubility of silicate minerals. In the tested samples, the concentrations of this element were in the range of 11.2 to 23.7 mg/L (Table 2), which are considered average groundwater silicon concentrations, typical for the sandstones and other rocks with the predominant presence of practically insoluble quartz (Stojković 2013). Sulphur also occurs naturally in soil and rocks in the form of various minerals. In groundwater, depending on the pH of the medium, sulphur can appear in the form of sulphates (SO_4^{2-}), hydrogen sulphide (HS^-), and hydrogen sulphide (H_2S). Although the Official Gazette of the Republic of Serbia does not specify the upper limit of the concentration of total sulphur, it is well known that a higher amount of sulphur in water can influence its taste and colour, and can in some cases cause a laxative effect. Sources of natural mineral water in Vrnjačka Banja are characterised by relatively low-sulphur content, most likely originating from oxidation of sulphide minerals, scattered across the investigated area. Based on the sulphur content, the analysed samples can be classified as waters with low (S5; 0.8 mg/L) and moderate content of this biogenic element (S4; 18.0 mg/L).

In the van Wirdum diagram (van Wirdum 1991; van der Aa 2003), based on conductivity and ion ratio (IR), the position of the analysed groundwater samples is given relative to standard reference samples of

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atmospheric water (*atmocline*), calcium-rich groundwater (*lithocline*), and seawater (*thalassocline*) (Fig. 4). The ion ratio is calculated as follows: IR (%) = ½ [Ca^{2+}] / {½ [Ca^{2+}] + [Cl$^-$]} × 100, where Ca and Cl concentrations are given in meq/L. The majority of analysed samples were taken from the vicinity of lithocline water, slightly inclined towards the transitional area to the thalassocline water, since these are samples of groundwater in which sodium is the dominant cation. Sample S6 stands out, by its proximity to the atmocline water, indicating lower mineralisation and overall different chemical composition, consistent with the different lithology of the aquifer of the Vrnjačko vrelo source.

The contents of examined microelements in this study are presented in Table 3. Boron is a natural component of water that arises from the leaching of soil, rocks, and other natural processes. In rocks, boron is mainly present in the form of its borate and borosilicate minerals and some studies have mentioned that B^{3+} ion can substitute silicon in silicates naturally present in the rocks (Kostić et al. 2016). High concentrations of this element are typical for the deepest horizons of artesian basins, for aquifers composed of fine granulation sediments (e.g. sands with clay). Boron enrichment is also expected in thermal waters in areas of recent volcanic activity and for groundwater from serpentinite and shale rocks (Stojković 2013). The maximum permitted values of boron in mineral water vary by country. In the countries of the European Union, this value is 1500 μg/L (Commission Directive 2003/40/EC). The presented results in Table 3 indicate that samples S2, S5, and S7 contain an elevated concentration of boron (1570.0; 1860.0; 1809.0 μg/L, respectively), most likely related to presence of serpentinite and shale in the area

(Dragišić et al. 2012), while in S6, that value was below the limit of detection of the apparatus (< 0.1 μg/L). The recommendation for an excess of boron in water (above 5 mg/L) is not to be used as drinking water, but it can be used for special health-related bathing.

Arsenic is one of the most toxic and carcinogenic microelements, appearing in both inorganic and organic forms (Lin et al. 2015). Due to its high noxiousness to the heart, stomach, lungs, kidneys, and liver, the arsenic limit in drinking and mineral waters is only 10 μg/L. Sample S6 showed a significantly increased concentration of arsenic (29.9 μg/L), while the other analysed samples had concentrations within permitted limits. Generally, arsenic migrates well in absence of dissolved oxygen and sulphate ions (< 5 mg/L SO$_4$), i.e. reducing conditions (Eh < 50 mV), in alkaline groundwater rich in iron, manganese, and ammonia, and with elevated concentrations of organic substances (Smedley and Kinniburgh 2002). The described conditions apply partially to water sample S6 (elevated Fe, Mn, and TOC and relatively low sulphate concentrations), so increased arsenic content is expected. Arsenic in soil can be found in the form of various arsenic sulphide minerals which, under certain conditions (temperature and pH), can be dissolved, leading to the accumulation of arsenic in groundwater. In the legislation of the Republic of Serbia and EC directive 2003/40, the maximum allowed value of selenium in mineral waters is 10 μg/L, and the tested samples S3, S4, and S5 had a higher concentration of this oligoelement (14.1; 13.7; 17.4 μg/L, respectively). Selenium can be found in sulphide minerals, so its elevated concentrations in groundwater can be the result of oxidation of these minerals (e.g. at Topli izvor) (Dragišić et al. 2012). Selenium can be found in soils

Fig. 4 van Wirdum diagram of analysed groundwater samples. Vertical axis represents ionic ratio (IR, %) and the horizontal axis represents conductivity (μS/cm). Circles stand for standard reference water samples of atmospheric water (*atmocline*), groundwater (*lithocline*), and seawater (*thalassocline*)
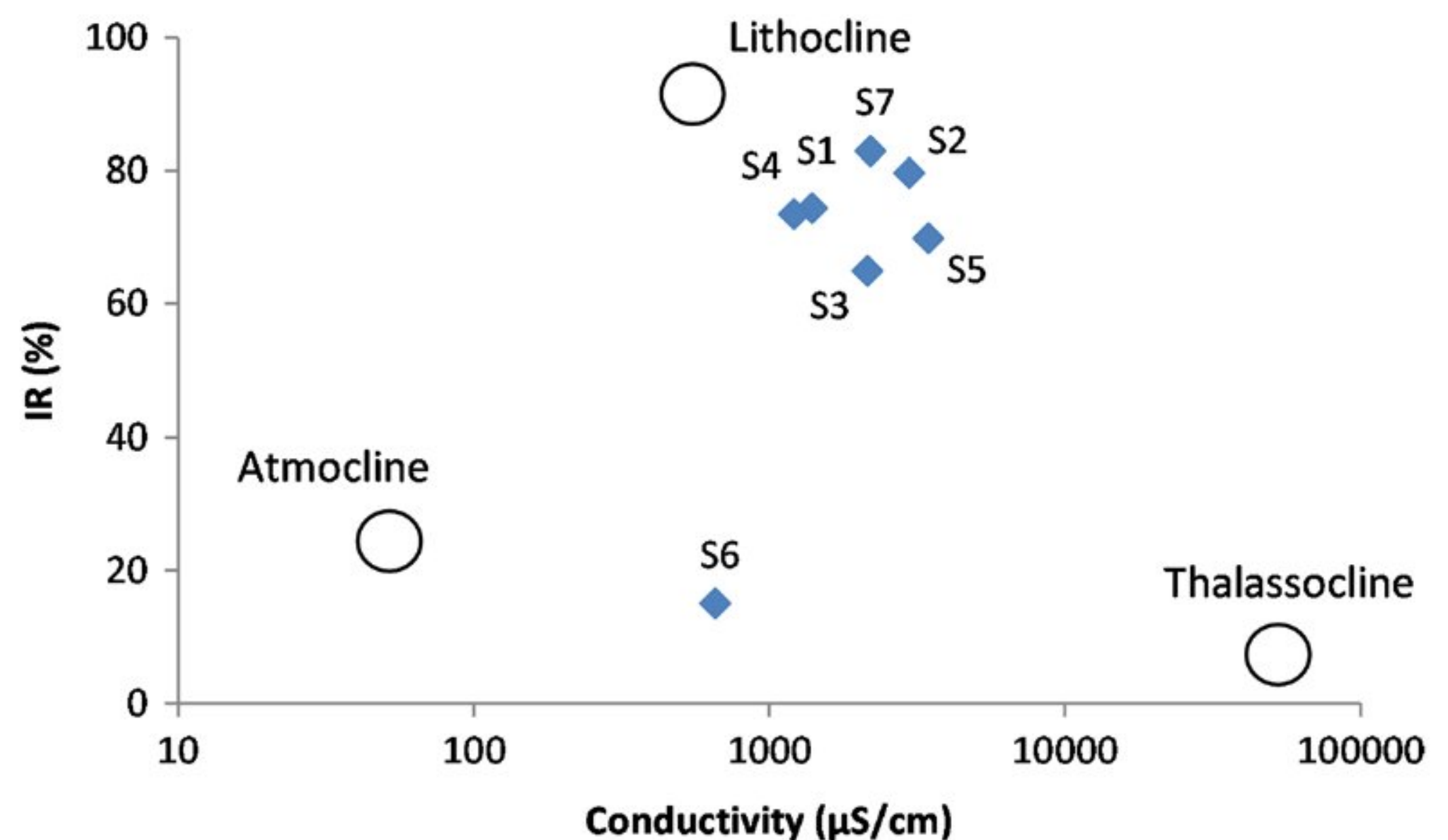
as selenite (SeO_3^{2-}) and selenate forms (SeO_4^{2-}), both of which are highly soluble and therefore easily reach groundwater (Mehdi et al. 2013). As can be seen in Table 3, the recorded concentration of Pb was within the permitted limits (Commission Directive 2003/40/EC). In all investigated samples, the concentration of phosphorus was < 1 μg/L, except sample S6 where it was 32.3 μg/L (Table 3). Phosphorus occurs in many of the more common rock-forming minerals, such as pyroxenes and amphiboles (Atanacković et al. 2013), which are most likely present in the heterogeneous mineralogical composition of sandstones, breccias, and other Neogene rocks of this locality.

The following analysed elements belong to *d*-elements—heavy metals (Fe, Co, Ni, Cu, Zn, Cd, Mn, Mo, and Cr). In groundwater, iron is mainly present in the form of bivalent salts of carbonates, bicarbonates, and sulphates, while trivalent iron is usually not in dissolved form, since at pH values greater than 4.5, it precipitates in the form of insoluble colloidal hydroxide. Redox conditions are also very important for the migration of iron in groundwater: ferrous iron is mobile under reducing conditions in a wide pH range, while the oxidised, ferric iron is typically only soluble under relatively acid conditions (for example, in acid mine drainage). Upon atmospheric exposure, dissolved ferrous iron is usually oxidised and relatively rapidly precipitated (especially at high pH), in the form of ferric oxy-hydroxides. Consequently, scales of characteristic ochre colour appear at points of emergence of iron-rich groundwaters. Microbial activity is often important in these reactions (Reimann and Birke 2010; Hitchon 1999). Among the analysed mineral water samples, S1 and

S4 stand out with high iron concentrations: 754.5 μg/L and 403.6 μg/L, respectively (Table 3, Fig. 5). Although these values were 2 to 4 times lower compared to Vrelo, Svinčine, and Talpora springs that belong to natural mineral resources in Bukovačka Banja, Serbia (Rajković et al. 2012), they were still in the range of values (148–2069 μg/L) recorded in samples from Guber Spa (Nesimović et al. 2017). This concentration of iron in the tested water samples can be associated with geological and anthropogenic factors. Among geological factors, the most probable are as follows: oxidation of sulphide minerals (e.g. pyrite, present in serpentinite veins), hydrolysis of serpentine minerals (hydrous magnesium iron phyllosilicate minerals), present at several locations in the Vrnjačka Banja are, or hydrolysis of micas, chlorite, hornblende, etc., from schists also present at several aquifers. Sometimes, occurrence of iron in groundwater can result from corrosion of iron or steel well casings or water pipes (anthropogenic factor). The monitoring of iron content is very important because increased levels can lead to its accumulation in humans (Kostić et al. 2016). On the other hand, the highest cobalt concentrations were observed in samples S1 and S7 (1.5 and 1.6 μg/L, respectively), while in sample S2, cobalt was not detected. Further, the content of nickel in all tested samples was below the maximum allowed value. As for other *d*-elements, increased concentrations of manganese (Table 3, Fig. 5) were measured in all tested samples (except for S5 and S6), of more homogeneous content than iron. All obtained values for manganese were significantly higher compared to results Nesimović et al. (2017) obtained for water samples from Guber Spa

Fig. 5 Concentration of microelements (in μg/L) in analysed water samples
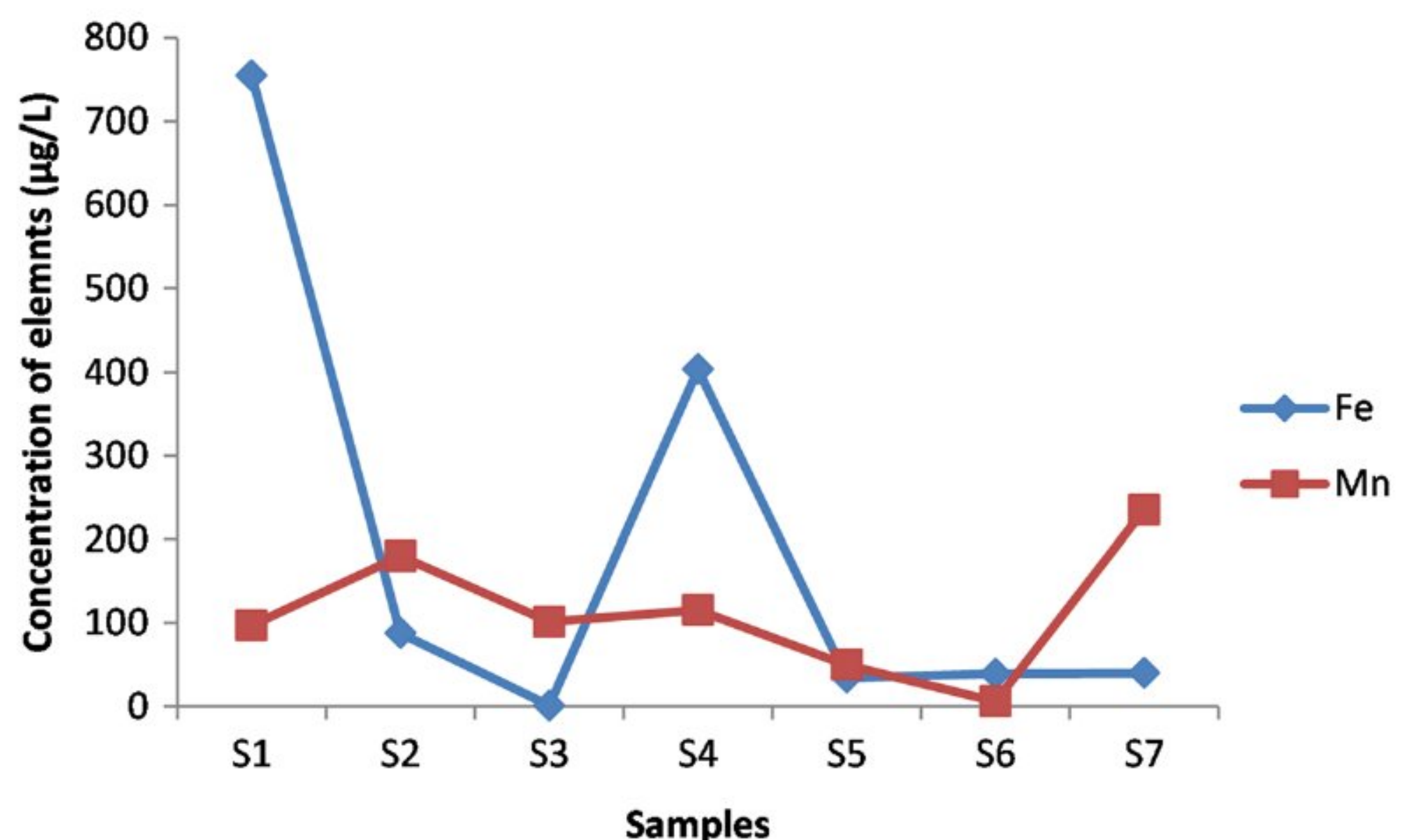
(1.42–14.06 μg/L) except in the case of S6. The possible reason for these manganese concentrations lies in the geological structure of the area. Manganese is scattered in silicate minerals, in rock such as serpentinite and schists, across the Vrnjačka Banja area, and hydrolysis of these minerals, along with manganese oxides and oxy-hydroxides, releases manganese into mineral water. Similarly to iron, the mobility and form of manganese in groundwater strongly depend on Eh and pH conditions. The most common form of manganese migration in groundwater is Mn^{2+} and this form is more stable than Fe^{2+} under oxidising conditions, although it tends to slowly oxidise to manganese(IV) oxide on exposure to the atmosphere (Reimann and Birke 2010; Hitchon 1999). Mn^{2+} ion can replace Fe^{2+} ions perfectly in all oxide minerals and in some cases Mg^{2+} in silicates (Kostić et al. 2016). Increased concentrations of manganese can lead to changes in the intellectual development of children, damage to the respiratory system, and modification of the skin. Zinc content in all analysed samples was in range with the results for Guber Spa: 2.29–13.20 μg/L (Nesimović et al. 2017). All other tested *d*-elements (Cu, Cd, Cr) were within the allowed values according to the regulations on mineral water quality of the EC Directive 2003/40 (Table 3).

Health risk assessment

Results for estimated weekly intake of selected toxic and potentially toxic elements as well as for acute health risk assessment (expressed through hazard quotient value, HQ_A) are given in Table 4.

According to the obtained results, the weekly intake of all examined elements is low. Consequently, all HQ_A values were significantly lower than 1. Based on this, there is no acute health risk to the consumer of mineral waters from these spots. The calculation of long-term (carcinogenic) health risk assessment (HQ_L) of oral intake (OI) was also low (Table 4). However, since a significant time period is applied in this case for some samples and elements, the obtained HQ_L values were not completely negligible. The HQ_L value higher than 10^{-3} indicates some measurable risk to human health (Momot and Synzynys 2005; Kostić et al. 2016). For instance, in the case of sample S1, the calculated value for carcinogenic risk incurred by the presence of arsenic in mineral water sample was $4.8 \cdot 10^{-2}$. It means that around 50/1000 citizens/visitors who have used water from this location are potentially at carcinogenic risk. A

similar situation is with samples S2 (presence of boron), S3 (presence of arsenic and boron), S4 (presence of arsenic), S5 (presence of arsenic, boron, barium, and strontium), and S7 (presence of arsenic and boron). The highest HQ_L value was calculated for arsenic in sample S6 ($2.0 \cdot 10^{-1}$). The concentration of arsenic in this sample was sufficient to cause potential risk of carcinogenic diseases in up to 200 inhabitants using the water from this specified source over a long period of time.

Since water from the thermo-mineral source Topli izvor (S3) is used for bathing, an estimation of health risk through dermal intake has been calculated (Table 5). Based on results given in Table 5, there is no significant health risk for anyone using water from this thermal source for bathing.

Bacteriological analysis

Results of the bacteriological analysis of water samples from Vrnjačka Banja are shown in Table 6.

The samples S1, S2, S4, and S5 can be characterised as bacteriologically safe for consumption since all values were equal or lower than 1. Comparing the obtained values from Table 6 with national standards (Official Gazette 2005), three water samples (S3, S6, and S7) can be said to have an elevated presence of aerobic mesophilic bacteria at 22 °C and 37 °C. In particular, mineral source Slatina (S7) was threatened by these microbes, since significantly elevated levels were recorded. The occurrence of mesophilic bacteria is a good indicator of non-faecal water contamination and can be used for long-term health risk assessment. On the other hand, sulphite-reducing *Clostridia* strains could originate from sources of faecal wastewater. In that sense, the presence of these bacteria in sample S5 could indicate faecal contamination. It is advisable to eliminate this issue through certain prevention measures. In addition, with respect to time of year when the samples were collected (summer), the possibility of bacterial contamination during the sampling process cannot be excluded.

Conclusion

Seven sources of natural mineral waters from the Vrnjačka Banja area have been investigated through the basic physico-chemical parameters obtained by standard analytic methods, as well as 24 macro-

Table 4 Estimated weekly (EWI) and oral (OI) intakes, acute (HQ$_A$), and long-term (HQ$_L$) hazard quotient values for examined samples

Table 4 (continued)

Sample	Element	EWI	OI	HQ$_A$*	HQ$_L$**
S1	As	$1 \cdot 10^{-4}$	$1.5 \cdot 10^{-5}$	$1.01 \cdot 10^{-2}$	$4.8 \cdot 10^{-2}$
	B	$7.4 \cdot 10^{-3}$	$1.1 \cdot 10^{-3}$	$6.3 \cdot 10^{-3}$	$5.3 \cdot 10^{-3}$
	Ba	$4.7 \cdot 10^{-3}$	$6.7 \cdot 10^{-4}$	$3.2 \cdot 10^{-3}$	$3.4 \cdot 10^{-3}$
	Cd	$1.8 \cdot 10^{-5}$	$2.5 \cdot 10^{-6}$	$3.1 \cdot 10^{-3}$	$5.1 \cdot 10^{-3}$
	Mn	$1.7 \cdot 10^{-3}$	$2.5 \cdot 10^{-4}$	$6.2 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$
	Ni	$2.9 \cdot 10^{-4}$	$4.1 \cdot 10^{-5}$	$3.4 \cdot 10^{-3}$	$2.0 \cdot 10^{-3}$
	Se	$6.8 \cdot 10^{-5}$	$9.7 \cdot 10^{-6}$	$2.8 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$
	Sr	$1.5 \cdot 10^{-2}$	$2.2 \cdot 10^{-3}$	$1.7 \cdot 10^{-2}$	$3.7 \cdot 10^{-3}$
	Zn	$8.5 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$	$4.1 \cdot 10^{-5}$
S2	As	0	0	0	0
	B	$2.8 \cdot 10^{-2}$	$4.0 \cdot 10^{-3}$	$2.3 \cdot 10^{-2}$	$2.0 \cdot 10^{-2}$
	Ba	$1.2 \cdot 10^{-2}$	$1.7 \cdot 10^{-3}$	$7.9 \cdot 10^{-3}$	$8.3 \cdot 10^{-3}$
	Cd	$1.6 \cdot 10^{-5}$	$2.3 \cdot 10^{-6}$	$2.8 \cdot 10^{-3}$	$4.6 \cdot 10^{-3}$
	Mn	$3.2 \cdot 10^{-3}$	$4.6 \cdot 10^{-4}$	$1.1 \cdot 10^{-3}$	$3.3 \cdot 10^{-3}$
	Ni	$2.9 \cdot 10^{-5}$	$4.1 \cdot 10^{-6}$	$3.4 \cdot 10^{-4}$	$2.0 \cdot 10^{-4}$
	Se	$1.3 \cdot 10^{-4}$	$1.8 \cdot 10^{-5}$	$5.3 \cdot 10^{-4}$	$3.6 \cdot 10^{-3}$
	Sr	$3.6 \cdot 10^{-2}$	$5.2 \cdot 10^{-3}$	$4.0 \cdot 10^{-2}$	$8.6 \cdot 10^{-3}$
	Zn	$1.5 \cdot 10^{-4}$	$2.1 \cdot 10^{-5}$	$2.1 \cdot 10^{-5}$	$7.1 \cdot 10^{-5}$
S3	As	$3.1 \cdot 10^{-5}$	$4.3 \cdot 10^{-6}$	$3.0 \cdot 10^{-3}$	$1.4 \cdot 10^{-2}$
	B	$1.5 \cdot 10^{-2}$	$2.2 \cdot 10^{-3}$	$1.3 \cdot 10^{-2}$	$1.1 \cdot 10^{-2}$
	Ba	$9.3 \cdot 10^{-3}$	$1.3 \cdot 10^{-3}$	$6.3 \cdot 10^{-3}$	$6.7 \cdot 10^{-3}$
	Cd	$2.0 \cdot 10^{-5}$	$2.8 \cdot 10^{-6}$	$3.4 \cdot 10^{-3}$	$5.6 \cdot 10^{-3}$
	Mn	$1.8 \cdot 10^{-3}$	$2.5 \cdot 10^{-4}$	$6.4 \cdot 10^{-4}$	$1.8 \cdot 10^{-3}$
	Ni	0	0	0	0
	Se	$2.5 \cdot 10^{-4}$	$3.6 \cdot 10^{-5}$	$1.0 \cdot 10^{-3}$	$7.2 \cdot 10^{-3}$
	Sr	$2.6 \cdot 10^{-2}$	$3.7 \cdot 10^{-3}$	$2.8 \cdot 10^{-2}$	$6.1 \cdot 10^{-3}$
	Zn	$9.6 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$	$4.6 \cdot 10^{-5}$
S4	As	$8.9 \cdot 10^{-5}$	$1.3 \cdot 10^{-5}$	$8.9 \cdot 10^{-3}$	$4.2 \cdot 10^{-2}$
	B	$4.1 \cdot 10^{-3}$	$5.9 \cdot 10^{-4}$	$3.5 \cdot 10^{-3}$	$3.0 \cdot 10^{-3}$
	Ba	$3.5 \cdot 10^{-3}$	$5.0 \cdot 10^{-4}$	$2.4 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$
	Cd	$1.8 \cdot 10^{-5}$	$2.5 \cdot 10^{-6}$	$3.1 \cdot 10^{-3}$	$5.1 \cdot 10^{-3}$
	Mn	$2.1 \cdot 10^{-3}$	$2.9 \cdot 10^{-4}$	$7.6 \cdot 10^{-4}$	$2.1 \cdot 10^{-3}$
	Ni	$4.8 \cdot 10^{-5}$	$6.9 \cdot 10^{-6}$	$5.7 \cdot 10^{-4}$	$3.4 \cdot 10^{-4}$
	Se	$2.4 \cdot 10^{-4}$	$3.5 \cdot 10^{-5}$	$1.0 \cdot 10^{-3}$	$7.0 \cdot 10^{-3}$
	Sr	$1.3 \cdot 10^{-2}$	$1.9 \cdot 10^{-3}$	$1.4 \cdot 10^{-2}$	$3.1 \cdot 10^{-3}$
	Zn	$1.0 \cdot 10^{-4}$	$1.5 \cdot 10^{-5}$	$1.5 \cdot 10^{-5}$	$4.9 \cdot 10^{-5}$
S5	As	$4.3 \cdot 10^{-5}$	$6.1 \cdot 10^{-6}$	$4.3 \cdot 10^{-3}$	$2.0 \cdot 10^{-2}$
	B	$3.3 \cdot 10^{-2}$	$4.7 \cdot 10^{-3}$	$2.8 \cdot 10^{-2}$	$2.4 \cdot 10^{-2}$
	Ba	$1.5 \cdot 10^{-2}$	$2.1 \cdot 10^{-3}$	$9.9 \cdot 10^{-3}$	$1.0 \cdot 10^{-2}$
	Cd	$2.1 \cdot 10^{-5}$	$3.1 \cdot 10^{-6}$	$3.7 \cdot 10^{-3}$	$6.1 \cdot 10^{-3}$
	Mn	$8.9 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$	$3.2 \cdot 10^{-4}$	$9.1 \cdot 10^{-4}$
	Ni	$1.7 \cdot 10^{-4}$	$2.4 \cdot 10^{-5}$	$2.0 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$
	Se	$3.1 \cdot 10^{-4}$	$4.4 \cdot 10^{-5}$	$1.3 \cdot 10^{-3}$	$8.8 \cdot 10^{-3}$
	Sr	$4.3 \cdot 10^{-2}$	$6.1 \cdot 10^{-3}$	$4.7 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$
	Zn	$2.5 \cdot 10^{-4}$	$3.6 \cdot 10^{-5}$	$3.6 \cdot 10^{-5}$	$1.2 \cdot 10^{-4}$
S6	As	$4.3 \cdot 10^{-4}$	$6.1 \cdot 10^{-5}$	$4.3 \cdot 10^{-2}$	$2.0 \cdot 10^{-1}$
	B	0	$2.5 \cdot 10^{-6}$	0	$1.3 \cdot 10^{-5}$
	Ba	$3.7 \cdot 10^{-4}$	$5.3 \cdot 10^{-5}$	$2.5 \cdot 10^{-4}$	$2.7 \cdot 10^{-4}$
	Cd	$1.6 \cdot 10^{-4}$	$2.2 \cdot 10^{-6}$	$2.7 \cdot 10^{-2}$	$4.4 \cdot 10^{-3}$
	Mn	$1.1 \cdot 10^{-4}$	$1.5 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	$1.1 \cdot 10^{-4}$
	Ni	0	0	0	0
	Se	$1.8 \cdot 10^{-6}$	$2.5 \cdot 10^{-7}$	$7.4 \cdot 10^{-6}$	$5.1 \cdot 10^{-5}$
	Sr	$2.5 \cdot 10^{-3}$	$3.6 \cdot 10^{-4}$	$2.7 \cdot 10^{-3}$	$5.9 \cdot 10^{-4}$
	Zn	$2.7 \cdot 10^{-4}$	$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$
S7	As	$2.7 \cdot 10^{-5}$	$3.8 \cdot 10^{-6}$	$2.7 \cdot 10^{-3}$	$1.3 \cdot 10^{-2}$
	B	$3.4 \cdot 10^{-2}$	$4.8 \cdot 10^{-3}$	$2.8 \cdot 10^{-2}$	$2.4 \cdot 10^{-2}$
	Ba	$1.4 \cdot 10^{-2}$	$2.0 \cdot 10^{-3}$	$9.3 \cdot 10^{-3}$	$9.8 \cdot 10^{-3}$
	Cd	$1.7 \cdot 10^{-4}$	$2.4 \cdot 10^{-6}$	$2.9 \cdot 10^{-2}$	$4.8 \cdot 10^{-3}$
	Mn	$4.3 \cdot 10^{-3}$	$6.0 \cdot 10^{-4}$	$1.5 \cdot 10^{-3}$	$4.3 \cdot 10^{-3}$
	Ni	$1.8 \cdot 10^{-4}$	$2.5 \cdot 10^{-5}$	$2.1 \cdot 10^{-3}$	$1.3 \cdot 10^{-3}$
	Se	$1.1 \cdot 10^{-4}$	$1.5 \cdot 10^{-5}$	$4.5 \cdot 10^{-4}$	$3.1 \cdot 10^{-3}$
	Sr	$4.1 \cdot 10^{-2}$	$5.9 \cdot 10^{-3}$	$4.6 \cdot 10^{-2}$	$9.9 \cdot 10^{-3}$
	Zn	$1.4 \cdot 10^{-4}$	$2.0 \cdot 10^{-5}$	$2.0 \cdot 10^{-5}$	$6.5 \cdot 10^{-5}$

*Tolerable weekly element intake (TWI) values [mg/L] used for HQ$_A$ calculation (WHO 2017) are as follows: As 0.01; Ba 1.47; B 1.19; Cd 0.0058; Mn 2.8; Sr 0.91 (WHO 2010); Se 0.24; Ni 0.084; Zn 7

**Referent oral dose (RfD$_{oral}$) values [mg/kg/day] used for HQ$_L$ calculation (US EPA 2018) are as follows: As 0.0003; Ba 0.2; B 0.2; Cd 0.0005; Mn 0.14; Se 0.005; Sr 0.6; Ni 0.02; Zn 0.3

and microelements determined using inductively coupled plasma optical emission spectrometry (ICP-OES), with the aim to estimate the quality of these spa mineral waters. The conductivity results suggest that several samples can be characterised as natural mineral waters rich in mineral salts (> 1500 mg/L), while sample S6 belongs to the category of low dissolved mineral component concentration. Furthermore, the content of analysed essential and toxic elements was in the range of maximum allowed concentrations according to national and World Health Organization water quality standards, except for B, As, and Se. The results have shown that sodium is the predominant macroelement in all tested samples. Moreover, samples S2, S3, S5, and S7 contain a multiply greater quantity of sodium in comparison to other macroelements, with a

Table 5 Estimated non-carcinogenic (DI$_A$) and carcinogenic (DI$_L$) dermal intakes with acute (HQ$_A$) and long-term (HQ$_L$) hazard quotient values for sample Topli izvor (S3)

Element	DI$_A$*	DI$_L$*	HQ$_A$**	HQ$_L$**
As	$2.0 \cdot 10^{-4}$	$8.0 \cdot 10^{-5}$	$7.0 \cdot 10^{-4}$	$3.0 \cdot 10^{-4}$
Ba	$7.0 \cdot 10^{-2}$	$3.0 \cdot 10^{-2}$	$5.0 \cdot 10^{-3}$	$2.0 \cdot 10^{-3}$
Cd	$2.0 \cdot 10^{-4}$	$7.0 \cdot 10^{-5}$	$1.6 \cdot 10^{-2}$	$6.0 \cdot 10^{-3}$
Mn	$1.4 \cdot 10^{-2}$	$6.0 \cdot 10^{-3}$	$2.0 \cdot 10^{-3}$	$7.0 \cdot 10^{-4}$
Ni	$3.0 \cdot 10^{-6}$	$1.0 \cdot 10^{-6}$	$4.0 \cdot 10^{-6}$	$1.0 \cdot 10^{-6}$
Se	$2.0 \cdot 10^{-3}$	$8.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-4}$

*Permeability coefficient (K_p) values [cm/h] in water for selected elements used for calculations (US EPA 2004; Wang 2018; Nnorom et al. 2019) are as follows: As 0.001; Ba 0.001; Cd 0.001; Mn 0.001; Ni 0.0002; Se 0.001

**Referent dermal (RfD$_{dermal}$) doses [μg/kg/day] used for HQ$_L$ calculations (US EPA 2004; US EPA 2018) are as follows: As 0.285; Ba 14; Cd 0.0125; Mn 8.4; Ni 0.8; Se 1.5

maximum value of 748.6 mg/L (S5). Based on the content of magnesium and calcium, the analysed sources of water belong to moderate to very hard water. The elevated concentrations of boron were recorded in samples S2, S5, and S7, which can be related to the presence of serpentinite and shale in the area. It is important to emphasise that sample S6 contains more than twofold the concentration of arsenic, which, given its toxicity and potentially carcinogenic quality, can limit the use of water from this source. Additionally, slightly elevated

Table 6 Results of bacteriological analysis of water samples from Vrnjačka Banja area

Sample	Aerobic mesophilic count at 22 °C* [CFU/mL]	Aerobic mesophilic count at 37 °C* [CFU/mL]	Sulphite-reducing *Clostridia*** [CFU/50 mL]
S1	1	1	< 1
S2	< 1	< 1	< 1
S3	45	26	< 1
S4	< 1	< 1	< 1
S5	< 1	< 1	15
S6	25	6	< 1
S7	90	28	< 1

*Maximal allowed values: 20 CFU/mL at 22 °C; i.e. 5 CFU/mL at 37 °C

**Maximal allowed value: 0 CFU/50 mL

selenium contents of samples S3, S4, and S5 were measured, probably the result of oxidation of sulphide minerals, present in the aquifers. Although the maximum allowed concentration of iron is not given by national regulation for mineral waters, a high content of this element was observed in samples S1 and S4, which can be attributed to processes of oxidation and hydrolysis of iron-containing minerals in the study area.

Further, the calculated values of weekly (EWI) and oral (OI) intake as well as acute (HQ$_A$) hazard quotient of selected toxic and potentially toxic elements indicate no acute health risk for the consumer of mineral waters from the tested sources. However, the highest long-term hazard quotient (HQ$_L$) observed for As in sample S6 ($2.0 \cdot 10^{-1}$) indicates a significant risk of carcinogenic diseases over long-term consumption of water from that source. On the other hand, the estimated non-carcinogenic (DI$_A$) and carcinogenic (DI$_L$) dermal intakes for acute (HQ$_A$) and long-term (HQ$_L$) hazard quotient values for sample S3 show that there is no health risk to those who use the water from this thermal source for bathing.

Bacteriological assays have shown the presence of aerobic mesophilic bacteria at 22 °C and 37 °C in samples S3, S6, and S7, which could be an indicator of non-faecal water contamination, while sulphite-reducing *Clostridia* species, observed in sample S5, may be the result of some faecal wastewater.

To our best knowledge, this is the first study of quality assessment of natural mineral waters from the Vrnjačka Banja area that includes the content of macro- and microelements as well as bacteriological, geological, and health risk aspects. The findings of the research indicate that all investigated mineral sources, except Vrnjačko Vrelo (S6), contain a high concentration of essential elements and therefore can have a beneficial effect on human health if used in moderate and/or controlled quantities. Since this was the first, preliminary detailed investigation of Vrnjačka Banja mineral waters, a further monitoring of examined water samples is needed, as well as additional research. Due to the fact that Vrnjačka Banja is one of the most famous spa resorts with thermal and mineral waters in this part of the Balkans, these findings could be very useful for both the local population and visitors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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