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CONSUMPTION OF RAW WATER – THE HEALTH RISKS RELATED TO THE PRESENCE OF HEAVY METALS AND *ESCHERICHIA COLI*

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Abstract:

This research aimed to investigate the quality of drinking water from the rural area of village Pajsijević (Šumadija, central Serbia). The water is consumed as raw since it is not purified or chlorinated before consumption. The water was collected at three sampling points – in the spring of Kotlenik Mountain stream (W1 sample), in the local reservoir (W2 sample), and from the tap (W3 sample). Also, the sediment samples (soil and sand) were analyzed, too. The health risks related to the presence and concentration of some major and trace elements (Ca, Cr, Cu, Fe, Mg, Mn, Ni, Zn) and N, NO, NN_3, NH_4, P, P_2O_5, and PO_4 were evaluated. Additionally, the presence and the number of total coliform bacteria and *Escherichia coli* (as an indicator of fecal contamination) were evaluated. The concentrations of analyzed major and trace elements in all water samples were below those at which toxic effects may occur. The exception was the concentrations of Fe (2.02 – 2012 mg/L), which were higher than is allowed. The origin of Fe in water is from sediment (soil and sand), which also showed high content of Fe (3006.0 mg/g and 2229.9 mg/g, respectively). The results of the Colorimetric test indicated the presence of coliform bacteria as well as the presence of *E. coli* in all water samples. Further research needs to include characterization of isolated coliform bacteria and serological investigation of *E. coli* strains in order to evaluate the risks of consumption related to waterborne illness.

Key words: water quality, coliform bacteria, *Escherichia coli*, health risks, heavy metals

1. Introduction

Water is necessary for a normal life, but many people do not have access to clean, quality, and safe drinking water. Research by the World Health Organization [1] has shown that there are major challenges for people's water supply systems, which include constant population growth in big cities, growing shortages of clean water sources, excessive urbanization, and climate changes. The supply of households with clean and purified water is implied in large cities, but in rural areas access to clean water is often denied, and water-borne infections are common.

The presence of heavy metals is an important parameter in drinking water because they pose a threat to human health. Humans are exposed to heavy metals primarily through the consumption of water that contains them, but a small number of heavy metals can bioaccumulate in the human body and cause certain diseases. Therefore, determining the levels of heavy metals in different water sources is important for the proper assessment of human health risks [1, 2]

The aim of this investigation was to evaluate the potential health risks related to the consumption of raw water in rural area. These risks are associated with the presence of major and trace elements in water samples as well as with the presence of total coliform bacteria, with particular emphasis to the presence of *Escherichia coli*.

2. Material and methods

2.1. Sampling of water and sediment

Investigated raw water samples were collected in the three target points in the village Pajsijevic (Sumadija area, central Serbia) during the autumn of 2020, in the dry period. The first target point is the mountain spring of stream (Kotlenik Mountain) (W1 sample), where the water passes through layers of small and large rocks (natural filtration) and goes into the pipes which transmit water into the reservoir in the village (W2 sample). There is no water purification and chlorination in the reservoir. At 50 cm from the bottom of the reservoir, there is a pipe from, by natural fall transmit water into the households (W3 sample - the tap in the household).

Water samples were collected in sterile glass bottles by directly dipping the bottles into the surface of the water. The water samples from the tap were collected directly into the sterile bottles, after letting the tap run for a minute. The samples were labeled properly and transported on ice to the laboratory for analysis.

The sediment samples (soil and sand) were also investigated to determine the origin of major and trace elements in the water. Soil samples (3 replicates) were taken from the forest which is around of mountain spring coast. Sand samples (3 replicates) were taken directly from the mountain spring. A composite mixture of sand samples was air-dried at room temperature for 48 h before analysis.

2.2. The total concentration/content of major and trace elements in water and sediment samples

2.2.1. Instruments and apparatus: The flame atomic absorption spectrophotometer (FAAS) model Perkin Elmer 3300 with D_2 lamp as a corrector was used for the determination of metals: Mn (λ = 279.8 nm), Ca (λ = 422.7 nm), Mg (285.2 nm), Fe (248.3 nm), Zn (213.9 nm), Cu (324.8 nm), Ni (λ = 232.0 nm), Cr (357.9nm). Standard solutions of the appropriate concentrations were used to prepare the calibration. The range of standard solutions was 0.5-2.0 mg/L for Cu, Zn, Mg, and 1.0-5.0 mg/L, for Mn, Fe, Ca. All samples were analyzed by FAAS using acetylene flame (2.0:10.0) for Cu, Zn, Mg, Mn, Fe, and (3.8:10.0) for Ca.

2.2.2. Preparation of samples: In order to determine the total metal content in soil and sand, the samples were prepared by digestion with nitric acid and hydrogen peroxide according to EPA 3050B [3] in relation to HNO_3:H_2O_2 = 5:1; The ratio of soil sample: digestive mixture was 1:12. A sample of water with a volume of 500 mL was evaporated to dryness, 3 mL of a mixture of HNO_3 and HCl in a ratio of 1 to 3 was added, evaporated to dryness, and then the dry residue was dissolved in 30 mL of distilled water. The solution was filtered in a normal vessel of 50 mL and the vessel was filled to the line. The metal concentration was determined from the obtained solution. The results are shown as a mean value of five repeated measurements.

In order to check the accuracy of the applied method, blank tests and standard reference materials were used: MEES-3 (Trace elements in sediments). The values obtained ranged in the range of ± 5% of the certified values.

The concentration of total phosphorus (P), phosphate (PO_4), phosphorus pentoxide (P_2O_5), total nitrogen (N), nitric oxide (NO), ammonium (NH_4), and ammonia (NH_3) were determined by the Photometer system AL400 Aqualytic, immediately after raw water samples were collected.

2.3. Microbiological analysis

2.3.1. Enumeration of total aerobic mesophilic bacteria and total coliform bacteria: The total aerobic mesophilic bacteria were evaluated as follows: 1 ml of water sample was aseptically transported

in nutrient agar plates in two repetitions and plates were incubated at 37°C/24 h. After incubation, a total number of aerobic mesophilic bacteria were enumerated as follows:

Number of bacteria (in ml) = number of colonies/V x 10^n

where V is the volume of inoculated sample and 10^n is the dilution coefficient.

The total coliform bacteria were enumerated by colorimetric test, which includes three types of tests. The first stage is called the Previous Colimetry Test and it is done as described below. The lactose Andrade peptone water (LAP) with Durham tubes is prepared in two concentrations:

1. In one test tube with 50 ml of 2 x LAP is added as per 50 ml of water that we test
2. In five test tubes with 10 ml of 2 x LAP is added as per 10 ml of water that we test

These test tubes are incubated at 37°C/24 h. If there is a change in indicator color from light pink to dark pink and the Durham tubes have more than 1/10 of volume with gas, the test is positive.

The second stage is called the Confirmatory Colimetry Test. In this stage, the sample from every positive test tube is aseptically transported onto endo-agar and the samples are incubated at 37°C/24 h. If there is an occurrence of purple colonies with metallic-green shine, the coliform bacteria are present in the water. To confirm the presence of *E. coli*, the specific colonies from endo-agar are inoculated into MacConkey broth with Durham tubes. One test tube is incubated at 37°C/24 h and the other one is incubated at 44°C/24 h. The gas production at 37°C confirms the presence of coliform bacteria, while the production of gas at 44°C indicated the presence of *E. coli*.

The third stage is called the Final Colimetry Test. In this stage, the identification of *E. coli*, by gram straining, catalase and oxidase test, and other biochemical tests is done. The identification of Gram-negative, catalase positive, and oxidase negative rods with specific biochemical characteristics confirm the presence of *E. coli* in water.

The most probably number of coliform bacteria in 1 ml of a water sample is determined by results from the Previous Colimetry Test, by using tables by Swaroop.

3. Results and Discussion

3.1. The total concentration/content of major and trace elements in water and sediment samples

The results of the comparative analysis of the quantity of major and trace elements Ca, Cr, Cu, Fe, Mg, Mn, Ni, and Zn in the water and sediments samples are presented in Table 1. The mean value of the concentration of the analyzed elements in water samples was ordered in the following way: Ca > Mg > Fe > Mn > Zn > Cu > Ni > Cr. The concentrations of Cd and Pb were under the detection limits in all water and sediment samples. The concentrations of analyzed major and trace elements in all water samples were below those at which toxic effects may occur. The exception was the concentration of Fe, which was higher than is allowed. However, the concentration of Fe is not of health concern, but it affects the acceptability of water for consumers. As it can be seen in Table 1, the origin of these elements in water is from sediment since they are present in it.

The concentrations of total P, PO_4, and P_2O_5 were in the optimum range for water, while concentrations of N, NO, NH_3 and NH_4 were under the range of detection in all water samples. In addition, the nitrogen and phosphorus contents are relatively low or under detection limits, which makes algae growth hardly. Therefore, there is a low risk of pollution to water quality, such as algae growth and reproduction [4].

Table 1. The concentration/content of major and trace elements in water and sediment samples

Origin of sample	Water samples			Sediment samples	
	W1	W2	W3	Soil	Sand
Element/quantity	mg/L	mg/L	mg/L	mg/g	mg/g
Ca	10.68 ± 0.40	9.99 ± 0.06	9.88 ± 0.07	1356.24 ± 14.51	1130.6 ± 15.13
Cr	0.005 ± 0.00	0.005 ± 0.00	0.005 ± 0.00	9.84 ± 0.08	5.29 ± 0.07
Cu	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.05	5.15 ± 0.05	3.35 ± 0.06
Fe	2.12 ± 0.03	2.02 ± 0.03	2.05 ± 0.05	3006.0. ± 4.59	2229.9 ± 11.81
Mg	4.07 ± 0.05	3.73 ± 0.09	3.78 ± 0.04	1314.92 ± 4.13	927.9 ± 5.37
Mn	0.06 ± 0.003	0.06 ± 0.003	0.05 ± 0.001	329.28 ± 1.49	302.7 ± 1.87
Ni	0.003 ± 0.00	0.02 ± 0.01	0.003 ± 0.00	5.10 ± 0.07	4.19 ± 0.04
Zn	0.01 ± 0.00	0.01 ± 0.001	0.03 ± 0.001	9.87 ± 0.05	5.09 ± 0.05
P	0.11 ± 0.00	0.04 ± 0.00	0.08 ± 0.00	/	/
PO_4	0.34 ± 0.00	0.12 ± 0.00	0.24 ± 0.00	/	/
P_2O_5	0.26 ± 0.00	0.90 ± 0.00	0.18 ± 0.00	/	/
N	u.d.	u.d.	u.d.	u.d.	u.d.
NO	u.d.	u.d.	u.d.	u.d.	u.d.
NH_3	u.d.	u.d.	u.d.	u.d.	u.d.
NH_4	u.d.	u.d.	u.d.	u.d.	u.d.

The values are presented as mean value ± standard deviation; u.d. – Under detection limit; / - not evaluated

3.2. Microbiological safety of examined water samples

The total count of aerobic mesophilic bacteria was enumerated on the nutrient agar and ranged between 36 (W1 sample) and 78 (W2 sample) colonies in 1 ml of the water sample, which is in accordance with the regulation in Serbia. According to the Rules on hygienic drinking water [6], 300 colonies of aerobic mesophilic bacteria in 1 ml of drinking water are allowed.

The results of the Previous colorimetric test indicated the presence of coliform bacteria in water samples (MPN index between 2 and 5). The results of the Confirmatory and Final test showed the presence of *Escherichia coli* in all water samples since the purple colonies with metallic-green shine were detected on endo-agar plates and the gas was produced into MacConkey broth with Durham tubes. At 44°C and all biochemical tests indicated the presence of *E. coli* in tested drinking water, which is not allowed according to the Rules on hygienic drinking water [5].

4. Conclusions

The results of this paper indicated the need for monitoring of raw water used for drinking in rural areas since there is an increased danger for water-borne illness in humans. If the presence of *E. coli* is confirmed in drinking water, that water is epidemiologically dangerous and should not be consumed for drinking. Also, the presence and content of major and trace elements need to be evaluated before consumption.

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