Testing the Effects of the Presence of Uranium in Drinking Water from Individual Wells in the Village of Dubravica in the Braničev District on Public Health

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Abstract: The village of Dubravica is partially located over the western lignite deposits of the Kostolac basin. The examination of the dry residue obtained from drinking water from two individual wells by X-ray diffraction analysis, based on a typical peak, showed the presence of uranium in drinking water. The indirect method by Rajković and associates showed that, in sample I, the concentration of uranium in drinking water was 85.5 percent higher (3.71 μg/L) and that the concentration of uranium in sample II was only 22 percent lower (1.56 μg/L) than the Maximum Allowable Concentration (MAC) values required by the Regulations (2 μg/L). Analysis of the result of the introduction of uranium in the human body has shown that this way brings 0.84 to 2 mg of uranium in the human organism per annum or 0.09 to 0.22 mg of uranium is deposited annually in the kidney. Assessment of the potential health risk due to the presence of uranium in drinking water indicated that the population using drinking water from wells will be threatened by uranium in a short time interval. Regarding the long-term risk, the calculation has indicated that in the first sample of drinking water, about 25 inhabitants, and in the case of the second sample of drinking water, 10 inhabitants out of 1000 inhabitants are endangered. As the kidney is the organ in which uranium is deposited (accumulated) to the greatest extent, its presence causes weakening and failure of kidney function, which can destroy 75 percent of kidney function until the manifestation of the first clinical symptoms.

This phenomenon is observed among the population along the rivers the Kolubara, the Drina, the Sava and the Morava and is called endemic nephropathy.

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The elements found in trace amounts (Pb, Cd, Si), live agents (bacteria and viruses), fungal plant toxins, genetic factors and immune mechanism can be listed as possible causes. However, uranium in drinking water has not been listed so far.

The tests performed in this study clearly show the role of uranium in the epidemic, endemic nephropathy, which is growing and which is not at the acute phase of the disease but has already progressed to renal failure and end-stage kidney disease. Official data on the rise of endemic nephropathy and diabetes and increasing their share in diseases, as well as overall mortality rates, which amount to 18.19%, clearly indicate that the role of uranium in the Braničevo district environment should not be ignored. Since there are settlements on the terrain to be investigated, uranium and its migration through the environment must be monitored as all conditions for its migration are unfortunately favourable.

**Key words:** drinking water, heavy metals, uranium, endemic nephropathy.

**Introduction**

Population irradiation by natural radionuclides from drinking water is generally small and occurs due to the dissolution of radionuclides of the uranium and thorium series. One year of consuming drinking water must not contribute more than 5% of the average total irradiation of the population. To evaluate the similarity of drinking water from the radiological point of view, it is necessary to determine the concentration of certain radionuclides in drinking water for the recommended reference dose level of 0.1 mSv (Official Gazette of RS, 2018). Below this reference dose level, water can be used for drinking (for human purposes).

The first edition of recommendations on drinking water quality radiological aspects was printed in 1984 (WHO, 1984). Tolerant levels of radioactive substances in drinking water, recommended in the first edition of the WHO recommendations, were based on, at that moment, the available information on the degree of risk of exposure to ionizing radiation, concentrated mainly in the International Commission on Radiological Protection (ICRP) Publication 26 (ICRP, 1977). According to the ICRP issue, the WHO recommends radiological aspects of drinking water quality (ICRP, 1990). Also, in a separate chapter of a later release of recommendations (WHO, 1993), the WHO discusses the radiological aspects of drinking water quality and establishes methods for quality checking and tolerant levels of some radionuclide content or the total content of α- and β-non stable radionuclides, from natural and anthropogenic origins.

The recommended reference values for α-unstable radionuclides in drinking water are 0.1 Bq/L and for unstable β-unstable isotopes – 1 Bq/L. Higher values of the specific activity of α- and/or β-unstable radionuclides do not automatically mean that water should not be used for drinking (Official Gazette of FRY, 1998).
The dose of irradiation of the organism from radionuclides in water depends on the amount of the radionuclides and their metabolism and kinetics in the body. Therefore, the calculation of the tolerant (allowed) concentration of radionuclides in drinking water is based on the total amount of radionuclides taken into the body in one year, with consumption of 2L of water per day, taking into account the parameters of metabolism in an adult – reference man (ICRP Publication 23, 1975; Papić et al., 2012).

Maximum levels of radioactive contamination of drinking water were determined according to the Annual limits of intake of radionuclides (ALI) in the human body by ingestion and derived concentrations (IC) (Zamora et al., 1998; Official Gazette of RS, 2018). The method of calculating the value of uranium in drinking water is given in Rajković et al. (2008a).

In drinking water, the concentration of individual radionuclides and an unknown (or partially known) mixture of radionuclides must not be higher than the calculated concentration of radionuclides in the environment for 12 consecutive months.

Uranium migration through the environment

From a physical-chemical aspect, the term heavy metal includes metals whose density is higher than 5 g/mL or the regular (atomic) number greater than 20. In addition, the term is more often used for toxic metals, i.e. elements that exert their toxicity at low concentrations. One such metal is uranium (the regular number of 92), the last element found in nature, a heavy metal and a toxic and radioactive element.

Uranium occurs in nature as a mixture of three isotopes: uranium-238 (\(^{238}\text{U}\)), uranium-235 (\(^{235}\text{U}\)) and uranium-234 (\(^{234}\text{U}\)). The prevalence of these isotopes in nature is as follows: \(^{238}\text{U}\) (99.282%), \(^{235}\text{U}\) (0.712%) and \(^{234}\text{U}\) (0.006%). All uranium isotopes are radioactive with a half-life: \(^{238}\text{U}\) – 4.5\(\times\)10\(^9\) years, \(^{235}\text{U}\) – 7.07\(\times\)10\(^8\) years and \(^{234}\text{U}\) – 2.5\(\times\)10\(^5\) years.

Uranium and its isotopes at the same time are radioactive and highly chemically toxic elements. Toxicity of uranium consists of two toxicokinetic mechanisms: the first is non-radiation, chemical toxicity, characterised by heavy metals such as Pb, Hg, Cd and Bi, and the second is ionisation due to \(\alpha\)-emissions (radiation), and other natural and artificial radionuclides (ATSDR, 1997). Uranium radioactivity can cause the problems to the human body, such as cancer which can be manifested several years after exposure to uranium. However, the higher risk is of its chemical toxicity, which manifests itself in a very short period of time (weeks or months) after contact with it (for example, kidney, leukaemia, etc.) (Domingo, 1995).
Soluble uranium shows the same chemical toxicity as soluble lead (Rajković, 2001). In this sense, the impacts of uranium and its chemical effect as a toxic element are limited through the following concentrations (Domingo et al., 1987; Domingo, 1995; Maynard et al., 1953; NIOSH, 1994):

- in the air – 0.01 mg/L (0.01 ppm);
- water – 2·10⁻³ mg/L (0.002 ppm or 2 μg/L);
- lethal dose is 100 mg/kg body weight (BW).

Uranium in drinking water originates from natural sources: the lithosphere (3–4 ppm), volcanic rocks (0.1–5 ppm), sedimentary rocks (0.5–4 ppm), phosphate rocks (30–300 ppm) and soil (1–4 ppm) (Harmsten and DeHaan, 1980); or it is of anthropogenic origin: from different industries (mining, metal smelting, metallurgy, chemical industry, etc.), uncontrolled use of organic and mineral fertilisers and pesticides from sewage sludge.

After 1999, the drinking water used in our country could contain the uranium originating from the ammunition with depleted uranium (DU) used in the NATO bombing (Rajković, 2001). According to official data, about 112 strikes were carried out, of which 98 were in Kosovo and Metohija, 12 in southern Serbia, and two in Montenegro. Of that, 60 percent were civilian targets. In Kosovo, the area of Podujevo with the surrounding villages, the area around Kosovska Mitrovica, the part around Dečani, Đakovica, Prizren and other places were most affected. According to the data of our military experts, a total of 8,112 bullets with depleted uranium were fired (Zaric et al., 2001), while the exact number of fired bullets was never determined with certainty.

Although it is different toxicity of these two forms of uranium, the effect of the presence of depleted uranium in drinking water seems the same as natural uranium – it is a risk to human health (Rajković and Đorđević, 2006).

The migration potential of uranium depends on the physical and chemical properties of soil and soil solution and the oxidation product of uranium. The mobility of the dissolved uranium products predominantly affects pH value, Eh and the presence of complex organic and inorganic agents in a local groundwater area.

Hexavalent uranium U(VI) exists in solution as uranyl ion (UO₂²⁺) and it is more mobile than four-valent uranium U(IV), as it easily builds soluble complexes with ligands present in the soil solutions. The presence of carbonate and phosphate is also influenced by these processes. The transport of the soluble forms of uranium can be affected by dilution, as this reduces its concentration in groundwater and surface water. These reactions include ion exchange and specific adsorption of uranium organic substances, mineral clays, Fe(III) and hydroxide present in the soil.

The lifecycle of uranium in the environment and the potential risk to human health are shown in Figure 1.
The solubility of natural uranium occurs in two phases (Dong et al., 2006; Laue et al., 2004):

The first phase, the oxidation of metallic uranium to uranium dioxide or uranium(IV) oxide, which builds up in the natural mineral, uraninite (UO$_2$) according to the equations (1) and (2):

\[
\begin{align*}
    U(s) + 2H_2O &\rightarrow UO_2(s) + 2H_2 & (1) \\
    U(s) + O_2 &\rightarrow UO_2(s) & (2)
\end{align*}
\]
The second phase, the oxidation of uranium dioxide to uranium trioxide, according to equations (3) and (4):

\[ \text{UO}_2(\text{s}) + 2\text{H}^+ + 1/2\text{O}_2 \rightarrow \text{UO}_{2}^{2+}(\text{aq}) + \text{H}_2\text{O} \] (3)

\[ \text{UO}_2(\text{s}) + 1/2\text{O}_2 + 2\text{H}_2\text{O} \rightarrow \text{UO}_3\cdot2\text{H}_2\text{O} \] (4)

The first phase is favourable from the aspect of environmental protection because it results in insoluble products, which, under certain soil conditions (pH value and redox potential), pass to the second phase, which leads to the formation of soluble kinds of products, such as uranyl ions (equation 3) and mineral schoepite (\(\text{UO}_3\cdot2\text{H}_2\text{O}\)) (equation 4). \(\text{UO}_2\cdot2\text{H}_2\text{O}\), under certain conditions, can dismiss \(\text{UO}_2^{2+}\) ions, which are easily transported and the territorial solutions are included in the food chain (equation 5):

\[ \text{UO}_3\cdot2\text{H}_2\text{O}(\text{s}) + 2\text{H}^+ \rightarrow \text{UO}_{2}^{2+}(\text{aq}) + 3\text{H}_2\text{O} \] (5)

Uranium oxidised in nature due to the inflow of oxygen, or a rise in its fugacity. The oxygen affinity is such that the first hydrogen sulfide oxidises to sulfate ion, uranium(IV) to uranium(VI), and only at a higher redox potential, Fe(II) to Fe(III), according to equations 6–8:

\[ \text{H}_2\text{S} + 4\text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 10\text{H}^+ + 8\text{e}^-; \quad E^0 = 0.303\text{V} \] (6)

\[ \text{U}^{4+} + 2\text{H}_2\text{O} \rightarrow \text{UO}_{2}^{2+} + 4\text{H}^+ + 2\text{e}^-; \quad E^0 = 0.327\text{V} \] (7)

\[ \text{Fe}^{2+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3 + 3\text{H}^+ + \text{e}^-; \quad E^0 = 1.06\text{V} \] (8)

The process of precipitation of uranium by reduction is of significant importance since it excludes the uranium from aqueous streams and therefore suspends its expansion process through the environment. The reduction of mobile ions of uranium (\(\text{U}^{6+}\)) to insoluble forms of uranium (\(\text{U}^{4+}\)) is carried out when the fugacity of oxygen is declined in the solution so that the above reaction takes place at the expense of the oxidation of iron and sulfur. If there is more \(\text{Fe}^{2+}\) than oxygen, the oxygen will run out, and uranyl ion will be used as an oxidising agent, which transforms (oxidises) \(\text{Fe}^{2+}\) to \(\text{Fe}^{3+}\), or sulfide to sulfate. Also, uranyl ion reduces to \(\text{UO}_2\), as shown in equations 9–11 (Milačić et al., 2004):

\[ \text{UO}_2^{2+} + 2\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{UO}_2^{0} + \text{Fe}_2\text{O}_3 + 6\text{H}^+ \] (9)

\[ 2\text{Fe}^{3+} + 1/2\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{FeO(OH)} + \text{H}^+ \] (10)

\[ 2\text{UO}_2^{2+} + \text{S}^{2-} + 4\text{H}_2\text{O} \rightarrow 2\text{UO}_2^{0} + \text{SO}_4^{2-} + 8\text{H}^+ \] (11)

Experiment

In the municipality area of Požarevac, there are three ways of the water supply of the population (Official Gazette of Požarevac, 2012):

1. Central waterpipe supplies in the cities of Požarevac and Kostolac, and rural areas such as Čirikovac, Klenovnik, Stari Kostolac, Drmno and Bradarac;
2. The supply of local water facilities – public fountains;
3. Individual supplies from wells in the rest of settlements.
For verification the safety of drinking water, in our earlier works are presented and sampled models of drinking water from individual wells from the village of Dubravica in the territory of the city of Požarevac in the Braničevo district (Rajković et al., 2014; Milojković, 2014). Basic and target tests comprised examining drinking water samples taken from rural areas of the Braničevo district, where it is determined whether the water is physico-chemically and microbiologically correct and to determine the presence of heavy metals in drinking water (Rajković et al., 2017).

With a control method of testing the quality of drinking water, additional water tests from the village of Dubravica were carried out. Two samples from different wells are examined by the indirect method for determining toxic elements (heavy and light metal) in drinking water, proposed by Rajković and associates (Rajković et al., 2008b; Rajković et al., 2009). Testing has proved their presence in drinking water, and heavy metals, such as Fe, Ti, Pb, Cd and Cr are found at a concentration that exceeds the Regulations (Official Gazette of FRY, 1998; Rajković et al., 2015a).

According to the Regulation (Official Gazette of the FRY, 1998), drinking water may not contain radionuclides, so that their presence in normal conditions without accidents or incidents is challenging to prove (Official Gazette of FRY, 1999). In drinking water from individual wells from the village of Dubravica revealed the presence of uranium (Rajković et al., 2015b), which is in one sample approaching the allowable concentration envisaged by Regulation on hygienic quality of drinking water (EPA, 2009; Official Gazette of FRY, 1998).

One of the work tasks was to fortify the concentration of uranium in drinking water from individual wells from the village of Dubravica. Since it is a radioactive element, and highly toxic substance, in this paper, we analyse the consequences of the presence of uranium in drinking water on human health caused by long-term consumption of these waters.

Location

The village of Dubravica is located north-west of Požarevac at 75 m above sea level at 44°41'13.8" north black latitude (N) and 21°42'11.6" east longitude (E) beyond the Danube coastal zone in the municipality of Požarevac (Braničevo district).

According to morphological characteristics, the Dubravica village is a lowland type, belonging to the Danube villages. Water supply in the village is provided through the local water supply and channelling wastewater, mainly through septic tanks (Official Gazette of Požarevac, 2012).

Sampling methods, tests and the interpretation of the results are in compliance with Regulations on hygienic safety of drinking water (Civil Engineering Faculty,
Sample I was taken from the location in the centre of a densely populated village at a distance of about 2.5 km from the Danube, while sample II was taken from the entrance to the village from the direction of Požarevac with a distance of about 1 km in relation to sample code I, and about 3.5 km from the Danube. Both wells are at the same depth of 12 m.

Materials and Methods

A sample of scale formed by precipitation on a water-heater surface during a long period of time has been used in this research. The content of all solids, which actually represents scale, has been determined by boiling 1.0 dm³ of drinking water to obtain the corresponding dry residue. The composition of scale was determined using an atomic absorption spectrophotometer the Perkin-Elmer AAnalystModel 300, according to SRPS B.B8.070 (by methods DM 10 – 0/4, 0/6, 0/7, 0/8, 0/9, 0/10, 0/11, 0/12, 0/13, and 0/17) (the standard method of ITNMS).

The share of the elements, in the form of compounds or the elemental form, in %, is compared with the Maximum Allowable Concentration (MAC) of inorganic substances in water, which are prescribed by the Regulative (Official Gazette of the FRY, 1998) and the Act provided for bottled water for drinking and natural disasters (Official Gazette of the FRY, 1999).

With a control method of testing the quality of drinking water, additional water tests from the village of Dubravica were carried out. Two samples from different wells were examined by the indirect method of determining toxic elements (heavy and light metal) in drinking water, proposed by Rajković and associates (Rajković et al., 2008b; Rajković et al., 2009).

To determine the presence of uranium in drinking water, an X-ray diffraction analysis of scale was carried out. The scale originating from drinking water was previously milled into a fine powder fraction which was further examined. The diffractogram was recorded with the Energy Dispersive X-Ray Fluorescence (EDXRF) by the MiniPal 4 X-Ray Fluorescence Spectrometer.

The quantitative content of uranium was determined by the fluorimetric method based on the linear dependence with fluorescence intensity of uranium compounds from their concentration (Anonym, 2004). There was a linear dependence for a vast range of low concentrations (about four orders of magnitude). The decrease in fluorescence intensity was reduced to a minimum using “standard addition” after the extraction of uranium with the synergistic alloy TOPO (tri-n-octyl phosphine oxide) – ethyl acetate.

The fluorescence intensity was measured by the fluorometer 26-000 Ash Jarrah Division (Fisher Scientific Company, Waltham, 1978). It should be noted that the fluorimetric method only gives information about the mass of the uranium
isotope U-238 in the sample and the extrapolation to the total activity of natural isotopes U-234 and U-235 is unreliable (Stojanović and Martinović, 1993).

**Results and Discussion**

The diffractograms of tested scales (Figures 2 and 3) proved the presence of uranium, which was detected in our previous work (Rajković et al., 2015b).

![Figure 2](image1)

**Figure 2.** The diffractogram of the scales formed in sample I of drinking water.

On the diffractogram of scales, the pick dots are at 13, 13.5, 16, 17 and 20 keV, which clearly indicates the presence of uranium in the drinking water.

![Figure 3](image2)

**Figure 3.** The diffractogram of the scale formed in sample II of drinking water.
Results of the tests of urine composition by this process obtained in our previous paper (Rajković et al., 2015a; Rajković et al., 2015b) have indicated the presence of uranium (probably isotopes $^{234}\text{U}$, $^{235}\text{U}$, $^{238}\text{U}$) at concentrations shown in Table 1. They are compared with the maximum permissible concentration of uranium in drinking water based on literature data of 0.002 ppm or 2 µg/L (Domingo et al., 1987; Domingo, 1995; Maynard et al., 1953; NIOSH, 1994).

Table 1. The presence of uranium in the drinking water from individual wells from the village of Dubravica, calculated on the basis of the scale composition.

| Scale sample | Uranium (ppm) | The calculated concentration by weight in drinking water (µg/L) (Rajković et al., 2015b) | The MAC value in drinking water (ppm or µg/L) (Domingo et al., 1987; Domingo, 1995; Maynard et al., 1953; NIOSH, 1994) |
|--------------|---------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Sample I     | 8.84          | 3.71                                                                                       | $2 \cdot 10^{-3}$ mg/L (0.002 ppm or 2 µg/L)                                                |
| Sample II    | 3.29          | 1.56                                                                                       | (0.002 ppm or 2 µg/L)                                                                         |

where: MAC – Maximum Allowable Concentration.

Based on the data from Table 1, it can be seen that in the case of the first water sample, this value was exceeded 1.855 times, and in the case of the second sample of water, this value was only 22% smaller. This means that one must be careful about the possible consequences of the long-term use of this water for drinking.

The human organs primarily affected by uranium are kidneys (Harley et al., 1999; Rostker, 1998). They retain the uranium input ranging from 0.05% to 12% with a period of elimination from 6 to 1500 days. Uranium is highly toxic to the kidney, while its radiation effect at a single intake is fragile. Therefore, long-term ingestion of uranium present in drinking water by the people does not mean that it will lead to losing kidney function or chronic disease. Still, it is undoubtedly the first step that leads to progressive and irreversible kidney damage (ATSDR, 1997).

People exposed to uranium have a higher risk of renal disorder than those who are not exposed. Also, before any changes in kidney function are observed, 25% of kidney function can be lost, and more than 75% can be lost before showing severe clinical symptoms (Rajković, 2002).

The earliest recorded data on the maximum permitted concentration of uranium in the kidney, which does not cause a significant deviation from the normal functioning of kidneys, is 3 mg/kg (i.e. 3 ppm) (Rajković et al., 2014). An extensive study of the state laboratory in Oak Ridge recommended maximum concentration of 300 µg/kg (0.3 ppm) of uranium to which the people can be exposed (Edwards, 1999). Because of the chemical toxicity of uranium in soluble form, the amount of uranium of any isotopic composition should not exceed any daily limit of 150 mg of uranium inputted into the body of water and food.
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(ingestion) (Official Gazette of SFRJ, 1987). For a one-time introduction and timely treatment, changes are reversible (Rajković et al., 2003).

Uranium primarily affects the proximal tubule in the kidney. The dissolved complex of uranyl carbonate decomposes in this acidic environment on the uranyl and hydrogen carbonate ions. As Hg, Cd, and other heavy metals, uranyl ions reduces glomerular function, secretion of organic anion tubes, and reabsorption of filtered glucose and amino acids in the proximal wrapped tube (Harley et al., 1999; DOE, 1999). The dissolved uranyl ion, similar to the ions of heavy metals (e.g., Hg and Cd), reacts with outstretched chelate compounds in the form of a relatively stable and inert complex. These inert complexes were then filtered and extracted in the urine (Figure 7).

Uranium can be detected in the urine for several years (even after 7 years) after ingestion (intake), but when ingesting small doses, it is best to perform a urine test for the first six months, a maximum of one year.

Complete necrosis and renal tubules of the nephron occur in the chronic exposition. Kidney insufficiency occurs slowly. Damages occur on the first tubular cells and then the glomerulus. As the tubular cells regenerate very quickly, but at the same time, they often change (become atypical), the sensitivity of these structures changes (Miler et al., 1998; Hendee and Edwards, 1996; McDiarmid et al., 2000).

Soluble uranium absorbed into the blood circulates through the body and is quickly eliminated through the kidneys into the urine. About 67% of uranium is excreted on the first day without its deposit in the body. About 11% of the initial amounts are deposited in the kidney and excreted with a half-life of 15 days. Out of the remaining 22%, most of it is deposited in the bones (more than 20%), which are the main storage of uranium in the body, and the rest is distributed to other organs and tissues of a man (Rajković et al., 2003) (Figure 4).

The accumulation of uranium in the bones and other organs is consequently returned to the bloodstream with at least two different half-lives, both longer than the half-life for kidney extraction (ICRP, 1978; Arlt et al., 2002). Since uranium retains in the bones for a long time, it causes significant radiation effects. U(IV) is accumulating in kidney and bone tissue. In other tissues (liver, pancreas, and spleen), 0.03 to 12% of ingested uranium, as U (VI), is bound before redistribution in the kidney and skeletal system. Erythrocytes are the most vulnerable in the blood. Due to membrane damage, their lifespan is significantly shortened (Duraković, 1999).

Due to the toxic effects of radiation, pancytopenia (decreased number of blood cells) occurs, causing infection, fever, spontaneous bleeding from the mucous tissues and later from internal organs, anaemia, weakness, fatty degeneration of the centrilobular liver necrosis (Hendee and Edwards, 1996; Mc Diarmid et al., 2000). In the chronic exposition, neurologic disorders (vertigo, loss of balance) appear.
After a very long period (20–25 years), late effects – malignant changes, osteosarcomas, leukosis, tumours of the liver – occur. Potential mutagenic effects of uranium are still unknown, but it is experimentally demonstrated that the content of uranium in urine is positively correlated with urinary mutagenic effects (Miller et al., 1998).

Figure 4. The flowsheet diagram of the distribution of uranium in the human body (Rajković et al., 2008a).

Based on the data in Table 1, the input of uranium in the human organism based on the consumption of both samples of drinking water on daily, monthly and annual bases is calculated (Table 2).

Table 2. The input of uranium in the human organism based on the consumption of drinking water.

| Scale sample | One glass of water (μg U) | Daily intake (μg U) | Monthly intake (μg U) | Annual intake (mg U) |
|--------------|---------------------------|---------------------|-----------------------|----------------------|
| Sample I     | 0.74                      | 5.56                | 166.95                | 2.00                 |
| Sample II    | 0.31                      | 2.34                | 70.2                  | 0.84                 |

The data in Table 2 were calculated based on the data that the volume of one glass is 0.2L, and assuming that the person normally drinks 10 glasses of water daily – 1.5L of water (Official Gazette RS, 2018). The calculation is based on the content of the corresponding element in the dry residue. Therefore, the monthly intake is based on 30 days, and the annual intake is based on 12 months (365 days).

Based on the intake of uranium through drinking water (Tables 1 and 2), the distribution of the uranium in the human body can be calculated (Table 3).
Table 3. The distribution of uranium in the human body based on the consumption of drinking water.

| Sample   | The precipitated uranium in the bones | Uranium deposited in the kidneys |
|----------|--------------------------------------|----------------------------------|
|          | day (μg U) | month (μg U) | year (mg U) | day (μg U) | month (μg U) | year (mg U) |
| Sample I |            |              |              |            |              |              |
|          | 1.22       | 36.70        | 0.44         | 0.61       | 18.35        | 0.22         |
| Sample II| 0.51       | 15.44        | 0.185        | 0.26       | 7.72         | 0.09         |

The rapid absorption of uranium for 5 days results in a concentration of uranium (c) in the blood:

$$c = \frac{T}{(\ln2) \cdot (k \cdot U) / m \cdot (1 - 2^{\tau / T})}$$  \hspace{1cm} (12)

where: \(T = 15\) days is the time for extracting the accumulated uranium, \(\tau = 5\) days from the moment of entry, \(U\) – an average daily value of the uranium which enters the blood, for example, 2.1 mg/day, for \(k = 11\%\), the amount of uranium which is deposited in the blood, \(\ln 2 = 0.693 = 0.29\) kg of the total weight of the two kidneys (Harley et al., 1999).

The increasing of uranium concentrations in the kidney, according to this calculation, is shown in Figure 5.

Figure 5. The uranium concentration in the kidney calculated by the model of exposure of Gulf War veterans to depleted uranium (Eckerman et al., 1998).
However, the rapid absorption of "caught" and ingested uranium in the blood is not a realistic assumption. When also including the period of 5 and 6 days in the calculation (equation 12) (ICRP, 1995), there will be a small absorption of uranium from the lungs and gastrointestinal tract (GI tract). The maximum concentration in the kidneys will be noticed on the 20th day as 90% of the maximum value. This maximum value will be extracted for 15 days (Figure 5 – a tinted area).

Figure 6 shows the organs in which uranium is stored after ingestion through drinking water at a concentration of 1 µg/L.

As can be seen from Figure 6, a constant amount is retained in the GI tract, while the excretion of uranium takes place via the faeces and of a small extent via urine. Uranium slowly accumulates over time, partly in the lungs but in kidneys and other soft tissues as well, so that its concentration is continuously growing (WISE, 2005).

Figure 7 shows concentrations of uranium in the urine of adults with continuous ingestion of drinking water containing 1 µg/L of uranium and daily intake of 1.4L (at the speed of introduction of 1.4 L/day) (according to ICRP’s biokinetics model for uranium (ICRP, 1995)).

Figure 7 indicates that the continuous introduction of uranium through drinking water concentration of only 1 µg/L makes the concentration of uranium in urine grow rapidly, but it still shows a tendency to rise to about 20 ng/L for adults (WISE, 2005). With increasing concentrations of uranium in drinking water over time, the concentration of uranium increases in the urine.
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United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) takes as a reference value for uranium in drinking water of 1 mBq U-238/kg (UNSCEAR, 2000), which corresponds to 81 ng U nat/L and results in a level of uranium in the urine of about 1.6 ng/L, according to biokinetics model for adults (WISE, 2005).

Roth et al. (2001) sum the years as a conditional value for uranium in the urine of the German population: median values were 11 ng/L for the age of 20 years and 21 ng/L for the age of 50 years. Maximum values are: 21 ng/L for the age of 20 years and 50 ng/L for the age of 50 years (value converted with an intake of 1.4 L/day).

The assessment of the potential health risks due to the presence of uranium in drinking water

Short-term risk

Intake of toxic elements (heavy and light metals) and a risk to human health caused by the consumption of drinking water from individual wells from the village of Dubravica are determined on a weekly basis (short-term risk) over the estimated weekly intake through water (PNU) and the coefficient of risks to health (KR).

These coefficients are determined based on the following equation (Lin et al., 2015):

\[
PNU = \frac{\text{PPV} \cdot c \cdot 7}{\text{PTM}} \tag{13}
\]

\[
KR = \frac{PNU}{TNU} \tag{14}
\]
where: \textbf{PPV} – the average water consumption per citizen (1.5L per day) (Papić et al., 2012; Official Gazette of RS, 2018), \(c\) – the concentration of elements in the analysed samples of water expressed in \(\mu\)g/L, \textbf{PTM} – the average body mass of the population, which amounts to 75.65 kg (Pavlica et al., 2010). \textbf{TNU} is a tolerant weekly intake of toxic metals expressed as \(\mu\)g/kg body weight. As for the oral intake of uranium, it amounts to 0.6 \(\mu\)g/day for each kilogram of body weight (WHO, 1998; WHO, 2011).

When assessing the short-term risk to human health, it is considered to be high risk if there is a risk coefficient (KR) for an element greater than 1 (Kostić et al., 2016).

Potential long-term carcinogenic risks to human health

In addition to short-term risks caused by consuming water with a high content of toxic elements, it is possible to determine the potential long-term, carcinogenic risks to human health (Wu and Sun, 2015).

To assess this type of risks, the oral intake of toxic elements (by consuming drinking water) \(U_{\text{oral}}\) and the risk coefficient caused by the oral intake of toxic elements, \(KR_{\text{oral}}\), are defined based on the following equations:

\[
U_{\text{oral}} = \frac{\text{PPV} \cdot c \cdot 365 \cdot 30}{\text{PTM} \cdot 10950}
\]

\[
KR_{\text{oral}} = \frac{U_{\text{oral}}}{RfD_{\text{oral}}}
\]

where: \textbf{PPV} – the average water consumption per citizen (1.5L per day) (Papić et al., 2012, Official Gazette of RS, 2018), \(c\) – the concentration of elements in the analysed samples of water expressed in \(\mu\)g/L, \textbf{PTM} – the average weight of the population of Serbia, which is 75.65 kg (Pavlica et al., 2010) and \textbf{RfD\text{oral}}\ are reference values for the intake and potentially carcinogenic contaminants prescribed by American agency for environmental protection – EPA (Momot and Synzynys, 2005; CHMP, 2007). The minimum level of risk for long-term intake of toxic metals in the population prescribed by the Agency for Toxic Substances and Disease Registry (ATSDR) is 1.1 mg/day/kg (ATDSR, 1997).

Based on the data on the concentration of uranium in drinking water (samples I and II), short-term and long-term potential health risks for people who use this water are assessed (Table 4).

Based on the results obtained for the tested samples of drinking water from the village of Dubravica, the value of the coefficient of risk \(KR < 1\) for potentially harmful uranium, analysed in drinking water can be seen. It should be noted that the KR for the first sample of drinking water amounted to only 14.18% less than the value of the coefficient of risk (Kostić et al., 2016), suggesting the possible short-term (acute) danger of uranium present in drinking water in this water sample.
Testing the effects of the presence of uranium in drinking water

Table 4. The assessment of short- and long-term risks to the health of people who use this water for drinking expressed through risk coefficients (KR) and risk coefficients of oral intake (KR_{oral}) for uranium.

| Sample     | The concentration of uranium in drinking water | Short-term risk | Long-term risk |
|------------|-----------------------------------------------|-----------------|----------------|
|            |                                               | PNU             | KR             | U_{oral} | KR_{oral} |
| Sample I   | 3.71 µg/L                                     | 0.515           | 0.858          | 7.4·10^{-5} | 0.0245    |
| Sample II  | 1.56 µg/L                                     | 0.216           | 0.361          | 3.0·10^{-5} | 0.0100    |

where: TNU (tolerant weekly entry) – 0.6 µg/kg (WHO, 1998; WHO, 2011), RFD_{oral} (reference value of the oral intake of uranium) – 0.003 mg/day/kg (ATDSR, 1997; US EPA, 1989).

However, based on the calculated estimates of long-term health risk, it can be seen that there is a health risk in the case of consumption of both water samples. Of 1000 inhabitants, 24.5 inhabitants (from water sample I) and 10.3 inhabitants (from water sample II) show a potential risk of cancer, based on the present concentration of uranium in drinking water in the village of Dubravica.

In this analysis, the risk of uranium concentration in drinking water during the long-term consumption of drinking water from individual wells in the village of Dubravica is compared with the standard values prescribed by the EPA (Environmental Protection Agency) and the WHO (World Health Organization).

The standard for the concentration of uranium in drinking water has not been regulated for a long time. Only recently, there has been an increased activity to elaborate and establish reference values (standards).

Table 5 shows some examples of current legal standards on the concentration of uranium in drinking water.

Table 5. The standards for uranium concentrations in drinking water.

| Standard                        | Concentration of U in drinking water | Concentration of U in urine* | Concentration of U in the kidneys* | Annual radiation doses** |
|--------------------------------|-------------------------------------|-----------------------------|------------------------------------|--------------------------|
| EPA Standard (US EPA, 2000)     | 30 µg/L                             | 600 ng/L                    | 8.9·10^{-3} µg/g                  | 0.018 mSv/year           |
| WHO recommendations (WHO, 2004) | 15 µg/L                             | 300 ng/L                    | 4.5·10^{-3} µg/g                  | 9·10^{-3} mSv/year       |
| Sample I                        | 3.71 µg/L                           | 74.2 ng/L                   | 1.1·10^{-3} µg/g                  | 2.2·10^{-3} mSv/year     |
| Sample II                       | 1.56 µg/L                           | 31.2 ng/L                   | 4.6·10^{-4} µg/g                  | 9.4·10^{-4} mSv/year     |

where: * by application of ICRP biokinetics model for the uranium, based on the introduction of 500L of drinking water per year; ** Annual radiation dose data for natural uranium (U_{nat}) – Natural uranium of isotopic composition (0.71% of isotope 235U).
The standards for uranium content in the drinking water are based upon chemical toxicity and are intended for protecting the kidney. Maximum concentrations obtained for the kidney after 30 years of continuous ingestion of 30 µg/L of drinking water (US EPA standard 2000) are still 30 times lower than the recommended value of 0.3 g/g for a kidney, and the annual radiation is 50 times lower than the value recommended by the ICRP biokinetics model (ICRP, 1995).

The data presented in Table 5, compared with the standards recommended by the EPA and WHO, indicate that with the prolonged ingestion of the drinking water (water samples I and II), the concentration of uranium in the kidneys may not be negligible, even though the concentration of uranium in drinking water is lower than recommended.

These data confirm the first result obtained by analysing the uranium found in the scale of the individual wells. These wells are located in different soil types, which directly affects the quality of water.

Where does uranium found in drinking water in the village of Dubravica come from?

The village of Dubravica is partially located over the western lignite deposit of the Kostolac basin (Official Gazette of Požarevac, 2012). Although coal is mainly composed of organic matter, some trace elements in coal are naturally radioactive and constitute naturally occurring radioactive material (Normally Occurring Radioactive Material – NORM). These radioactive elements include U, Th, and their numerous products of disintegration, Ra and Rn (Kisić et al., 2013). As a result, with contents of potentially hazardous trace elements (As, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Th, and U), the role model of coal from the coal liner fields of Drmno was examined (Životić et al., 2008).

By analysing the geometric middle values, the Kostolac coal basin contains relatively high concentrations of Mn, Ni, Cr, As, Cu, and Co. These concentrations are much higher than average world values. However, the content of other elements is lower compared to the average world values.

Content of other elements close to the world average values. Based on the results in our previous works (Rajković et al., 2015a; Rajković et al., 2015b), it was found that the concentration of Cr in sample I amounted to 54.5 µg/L, which is higher than the allowed value by Regulation, 50.5 µg/L, while in sample II, this value was much lower than this value (4.04 µg/L), which is in agreement with these studies.

The contents of U and Th in the coal of the Kostolac basin were lower than the world average, although there were some parts of the coal with a high content of uranium. Trials of the content of natural radionuclides $^{238}$U, $^{226}$Ra, $^{232}$Th and $^{40}$K in samples of the lignite coal basin in Kostolac (WHO, 2004) revealed the content of
the isotope $^{238}$U of 0.60 to 70.10 mg/kg and of the isotope $^{232}$Th from 0.20 to 2.60 mg/kg. These data perhaps indicate the origin of uranium in drinking water since the drinking water was obtained from the individual wells. This is especially pronounced for sample I (3.71 $\mu$g/L) although the uranium value in sample II (1.56 $\mu$g/L) was only slightly lower than the value permitted by regulations (2 $\mu$g/L).

In this region, a systemic examination of radionuclides in the environment is provided twice a year in residential areas - in the working environment, as well as in the soil (Official Gazette of Požarevac, 2012). Also, the General Urban Plan of Požarevac envisages that in the period of 2016–2020 preparations for relocating (parts of) Dubravica settlements that are located above the western lignite deposits of the Kostolac basin may be made (Official Gazette of Požarevac, 2012). The data obtained in this study clearly indicate that the location and the water from which sample I was taken are in the zone of lignite deposits, while the location from which sample II was taken is not in the zone.

What is especially warning is the occurrence of endemic nephropathy (EN) along the rivers of the Kolubara, the Drina, the Sava and the Morava (Institute of Public Health, Požarevac, 2011). Since the aetiology of endemic nephropathy is unknown, certain elements that are found in trace amounts (Pb, Cd, Si), live agents (bacteria and viruses), fungal and plant toxins, genetic factors and immune mechanisms have been listed as possible causes.

Some experts believe that this insidious disease is caused by aristolochic acid (AA) and ochratoxin A (OTA) found in certain plants.

The disease is undetectable, relatively asymptomatic and quite well-tolerated, sometimes for decades. The general weakness occurs only in the stage of kidney insufficiency when the anaemia expresses because of the retention of nitrogenous products in the blood. Patients complain of vague gastric ailments. Elevated blood pressure is encountered in about 30–40% of elderly patients with advanced stages of the disease. There is no oedema of renal origin. The disease progresses to kidney failure and end-stage kidney disease.

The previous analysis clearly indicates that long-term ingestion of uranium by humans can lead to disturbances in the functioning of kidneys and that the introduction of uranium in elevated concentration is the first step that leads to progressive and irreversible kidney damage. Thus, in addition to all the causes that lead to the occurrence of endemic nephropathy, uranium in the environment should be also taken into account.

**Conclusion**

Based on tests of drinking water, from the aspect of uranium content from individual wells in the village of Dubravica in Braničevo district, we can draw the following conclusions:
Naturally occurring radioactive materials (NORM) may move out of the ground into the drinking water, so the analysis of drinking water may establish their presence in the environment. Thus, it can indirectly determine the degree of danger to human health, and also explain the origin of many diseases that are now linked to other causes.

The village of Dubravica, according to official data, lies over the western part of lignite deposits of the Kostolac basin, as tests of drinking water unambiguously confirmed based on the different contents of uranium from different locations.

Uranium is a natural element of the soil, and the degree of its migration depends on the physical and chemical properties of soil, soil solution and the oxidation product of uranium. The biggest danger for migration represents U(VI) in the form of uranyl ions because they easily build soluble complexes with ligands present in the soil solution. In this way, uranium enters groundwater from individual wells from which water is used for drinking.

Examining the uranium contents in drinking water from individual wells by the indirect method, based on the deposited lime, found significant differences in the content of uranium, since sample I recorded 2.4 times more uranium in comparison to the second pattern. In addition to uranium, in sample I, higher values of Fe (2.1 times higher), Mn (1.4 times), Ti (not found in sample II), Cr (13.5 times more), one of the causes of EN) were recorded, while, as for the heavy metals, higher contents of Pb (4.15 times), Zn (11 times), Cu (6.4 times) and Cd (1.4 times) were observed in sample II. The contents of elements found naturally in the soil, Si and Al, were higher in sample II: Si (2.7 times), Al (190 times). These data clearly show that the individual wells are found on different soils, and all the elements can affect the EN found in both samples of drinking water.

Uranium as a radioactive element and highly toxic substance is extremely dangerous to human health, so that its allowable concentration always decreases. Thus, the initially permitted concentration of 3 ppm in drinking water has decreased 10 times. Today, this value is 1000 times lower than the original (0.002 ppm) due to the risk of long-term ingestion of uranium, which is shown in this paper.

Although most of uranium is taken out through the urine (67%), the rest is deposited in the soft tissues so that its concentration is continuously growing, even with low concentrations of uranium in drinking water.

In a study examining the effects of chronic ingestion of uranium in the drinking water on people, it was found that kidney function is disturbed by the presence of uranium despite the conclusion that the examination is not done on animals in the laboratory. The fact is that endemic nephropathy (EN) occurs in hot spots along the rivers of the Kolubara, the Drina, the Sava, and the Morava. Also, in Serbia, an inhabitant has kidney problems. Because of this, it is of great importance to monitor the concentration of uranium in drinking water or to...
discover the cause of its presence. Evaluation of short-term health risk showed the pattern that drinking water indicates a risk to human health. In contrast, long-term risk assessment indicates a threat to human health for the population in consuming both samples of drinking water.

Official data show that, in the period between the two censuses, the population of the village of Dubrava fell by 15.92%. The reasons can be either migration flows or effects on the quality of the environment, primarily on drinking water.

Toxic effects of radiation lead to the reduction in the number of blood cells which arise due to infection, fever, spontaneous bleeding from mucous membranes and the internal organs, anaemia, loss of appetites with this, fatigue, and also lead to fatty degeneration of the liver with centrilobular necrosis. Neurological outbursts can appear in the chronic exposition. After a very long period (20–25 years), malignant changes, the most common cancers of the bronchi, osteosarcoma, leucosis, and liver tumours can occur. Potential mutagenic effects of uranium are still unknown, but it is experimentally demonstrated that uranium content in urine is positively correlated with urinary mutagenicity.

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ISPITIVANJE POSLEDICA PRISUSTVA URANA U VODI ZA PIĆE IZ INDIVIDUALNIH BUNARA U SELU DUBRAVICA U BRANIČEVSKOM OKRUGU PO ZDRAVLJE STANOVNIŠTVA

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Rezime

Selo Dubravica se delimično nalazi iznad zapadnog lignitskog ležišta Kostolačkog basena. Ispitivanjem suvog ostatka dobijenog iz vode za piće iz dva individualna bunara rendgenskom difrakcionom analizom, na osnovu karakterističnih pikova, utvrđeno je prisustvo urana u vodi za piće. Indirektnom metodom Rajkovića i saradnika pouzdano je dokazano da je u prvom uzorku koncentracija urana u vodi za piće za 85,8% viša (3,71 μg/L), dok je u drugom uzorku koncentracija urana svega 22% niža (1.56 μg/L) od vrednosti MDK predviđene Pravilnikom (2 μg/L).

Analiza posledica unošenja urana u čovekov organizam ukazala je na to da se na ovaj način unosи 0,84–2 mg urana u čovekov organizam na godišnjem nivou, odnosno 0,09–0,22 mg deponuje na godišnjem nivou u bubreg.

Procena potencijalnog zdravstvenog rizika usled prisustva urana u vodi za piće ukazala je na to da je stanovništvo koje koristi vodu za piće iz prvog bunara u kratkom vremenskom intervalu ugroženo od urana. Što se tiče dugoročnog rizika, proračun je ukazao da su u slučaju prvog uzorka vode za piće ugroženo oko 25, a u slučaju drugog uzorka vode za piće 10 stanovnika od 1000 stanovnika. Kako je bubreg organ u kome se uran deponuje u najvećoj meri u čovekovom organizmu, a dejstvo urana dugotrajno, slabljenje i otkazivanje funkcije bubrega može biti toliko da je čak 75% funkcije bubrega uništeno da bi se pokazali prvi klinički simptomi. Ova pojava se zapaža među stanovništvom duž reka Kolubare, Drine, Save i Morave i naziva se endemska nefropatija. Kao mogući uzročnici ističu se elementi koji se nalaze u tragovima (Pb, Cd, Si), živi agensi (bakterije i virusi), gljivični i biljni toksini, genetski faktori i imuni mehanizmi, ali ne i uran u vodi za piće.

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Ispitivanja izvršena u ovom radu jasno ukazuju i na ulogu urana u epidemiji endemske nefropatije koja je u porastu i koja nema akutnu fazu već bolest progredira ka bubrežnoj insuficijenciji i terminalnom stadijumu bolesti bubrega.

Zvanični podaci koji govore o porastu endemske nefropatije i šećerne bolesti i porastu njihovog udela u bolestima, kao i stope opšteg mortaliteta koja iznosi 18,19%, nedvosmisleno ukazuju na to da se uloga urana u životnoj sredini Braničevskog okruga ni na koji način ne sme zanemarivati. Zbog konfiguracije terena na kojima se nalaze naselja mora se pratiti uran i njegova migracija kroz životnu sredinu, za čije kretanje postoje, nažalost, svi uslovi.

Ključne reči: voda za piće, teški metali, uran, endemska nefropatija.