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INFLUENCE OF CARCINOGEN COMPOUNDS ON HYDROGEN BONDS IN WATER

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Abstract: Citizens of the town of Zrenjanin Serbia have been exposed, to high levels of arsenic in drinking water for a long time. By looking at the structure of categories of leading causes of death, and registered diseases in adults and children, it is evident that among them there are those associated with the exposure to arsenic.
In our previous studies we reported the effects of Zrenjanin tap water on tissues and organism on the whole, using the method of opto-magnetic spectroscopy. Here, we report our first results of Zrenjanin tap water analysis in comparison with other drinking waters that satisfy WHO recommendations, by using NIR spectroscopy and a new conceptual approach to studying aqueous systems - aquaphotomics.
Keywords: carcinogen, arsenic, water, near infrared spectroscopy, aquaphotomics.

1. INTRODUCTION

Arsenic is class one human carcinogen [1]. The primary route of exposure to arsenic comes from the drinking water, where it is mainly present in inorganic species such as arsenate [As(V)] and/or arsenite [As(III)].

The WHO recommended value for arsenic concentration in drinking water is 10μg/l and the maximum concentration limit is 50μg/l [2].

Multiple studies have confirmed that a long-term exposure to arsenic leads to a number of various cancerous (skin cancer, lung cancer, bladder cancer etc.) and non-cancerous diseases (skin diseases, vascular diseases, reproductive toxicity, diabetes mellitus…), some of which are very puzzling such as diabetes mellitus [3]. Biological mechanisms by which arsenic exerts its toxic and carcinogenic activities are not well understood.

The citizens of town Zrenjanin in Serbia have been for a long time exposed to high levels of arsenic in drinking water. By looking at structures of categories of leading causes of death (Fig.1a), or registered diseases in adults (Fig.1b) and children (data not shown, for further information see ref. [4]) it is evident that among them there are those associated with the exposure to arsenic. In our previous studies we reported the effects of Zrenjanin tap water on tissues and the organism as a whole, using opto-magnetic spectroscopy [5]. Here, we report our first results of Zrenjanin tap water analysis in comparison with other drinking waters that satisfy WHO recommendations, by using Near infra red spectroscopy and a new conceptual approach to studying water systems – Aquaphotomics [6].

2.MATERIAL

The samples investigated were Millipore pure water (Milli Systems Co., USA), one kind of commercial natural mineral water from Serbia (Aqua Viva), two kinds of tap water from Serbian cities of Belgrade (Belgrade tap water) and Zrenjanin (Zrenjanin tap water). Commercial mineral water was bought at a supermarket and stored in original plastic bottle with a plastic screw cap. Samples of tap waters were collected using polyethylene bottles thoroughly rinsed with pure water prior to collecting.
Basic information about particular water sample was obtained from the manufacturer label on the bottle as well as the data from the manufacturers' website [7]. Physico-chemical properties of tap waters were obtained from the last published data from Health Institutions in Zrenjanin and Belgrade. These data are summarized in Table 1.

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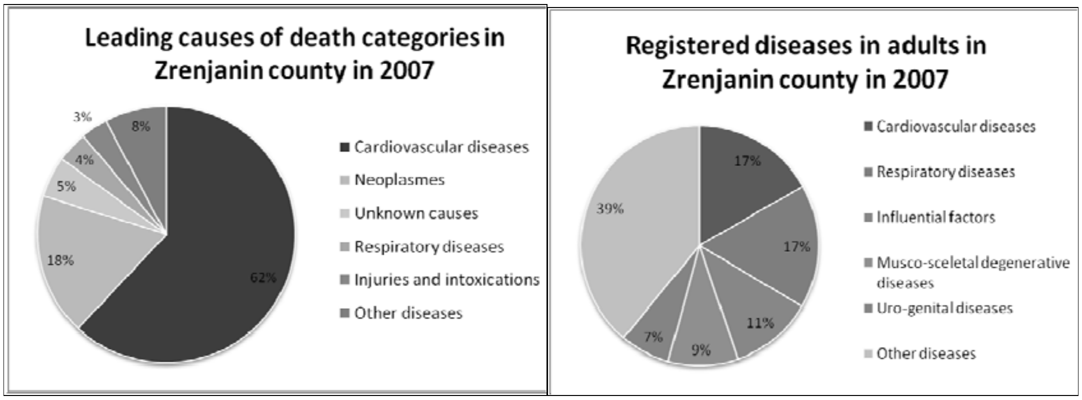

Figure 1. a) Leading causes of death categories in Zrenjanin county in 2007, b) Registered diseases in adults in Zrenjanin county in 2007 (based on data from ref. [4])

Table 1. Physico-chemical properties of investigated waters

	pH	EC [µS/cm]	As	B	Ca^{2+}	Fe^{2+}	K^{+}	Mg^{2+}	Na^{+}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
			[mg/L]									
Zrenjanin	7.56	928	71.9	895	27.4	87.5	1.2	13.6	275	846	17.4	0.97
Belgrade	7.56	318	0.29	22.2	61.1	2.34	1.1	11.1	6.5	194	11.3	37.5
Aqua Viva	7.62	504	0.0006	0.1	90.1	0.05	2.1	13.3	13.7	341	20.6	28.1

3. METHOD

3.1 Near-infrared spectral analysis

NIR transmission spectra of water samples in the 400-2500nm region were recorded using NIR Systems 6500 spectrophotometer (FOSS-NIRSystems). A quartz liquid sample cell was used as a container.

The experiment was conducted 5 times, and each time the ambient conditions were recorded. These recorded data are presented in Table 2.

3.2 Multivariate analysis

All multivariate analysis was carried out by Pirouette ver.4.0 (Infometrics, USA) software program. Multivariate data analysis in the form of Principal Component Analysis (PCA), Partial Least Squares Regression (PLS) and Soft Modeling of Class Analogies (SIMCA) was applied to obtain quantitative and qualitative information from the spectra. All multivariate spectral analysis was carried out by Pirouette ver.4.0 (Infometrics, USA) software program.

Principal component analysis is a method for compressing the data by using orthogonal matrix decomposition. Soft modeling of class analogies employs principal components analysis of spectra for the construction of mathematical models for each class to be analyzed. The goal of partial least squares regression is to predict or analyze a set of dependent variables (for example concentration of elements in water) from a set of independent predictors (e.g. IR spectra).

Before all analysis, the data were mean-centered and a smoothing transformation (11 points) was applied. The models were validated using cross-validation (leave-three-out).

Only 1st overtone region of the water spectra was used in the analysis (Fig. 2).

Table 2. Experimental conditions

Exp. No	**Date**	**p [hPa]**	**t [C°]**	**Humidity [%]**
1	03/07/2011	998.9	24	19
2	03/08/2011	1000.3	23	15
3	03/09/2011	1001.9	24.4	9
4	03/10/2011	1003.7	23.8	13
5	03/16/2011	992.8	24.4	14

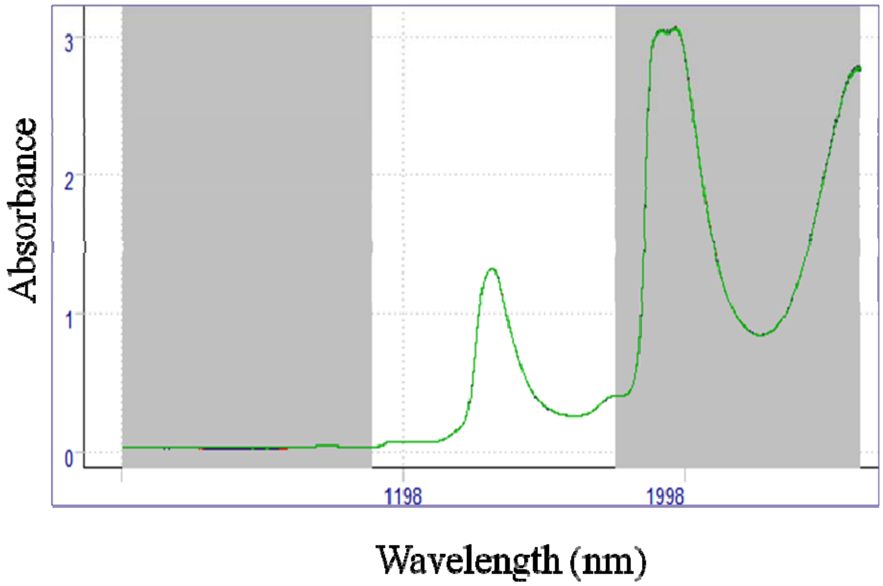

Figure 2. First overtone water absorbance region (1300-1600nm). Only this region of the water spectra was used in the analysis.

3.3. Aquaphotomics

Aquaphotomics is a new term introduced to describe a concept of approaching water as a multi-element system that could be well described by its multidimensional spectra [6]. In a series of experiments, vis-NIR spectra were collected and regression models of respective perturbations were analyzed in order to see if there were common bands in various biological systems and it was found that a group of WAMACS (water matrix coordinates – characteristic water absorbance bands) repeatedly occurred in different combinations in the spectral models predicting the investigated perturbations [6].

It was found that in the area of the first overtone of water there were 12 such characteristic wavelength ranges [6]. Therefore, characteristic absorbance pattern for water or any aqueous system can be described using this 12 WAMACS while the graphical representation of this pattern is presented in the form of aquagrams.

4. RESULTS AND DISCUSSION

In this study we applied NIR spectral analysis to investigate the differences between 4 types of water: Zrenjanin tap water known to have large amounts of arsenic compounds proven to be a human carcinogen; Belgrade tap water that satisfies all WHO recommendations for drinking water, Aqua Viva commercial mineral water with optimally balanced mineral complex according to the producers' marketing and Milli-Q pure water.

Raw spectra for all investigated waters recorded in all experiments are presented in Fig. 3. It is evident that spectra of different waters, or different experiments are hard to recognize by visual inspection.

4.1 Aquaphotomics approach

Waters were investigated from the Aquaphotomics point of view: a concept of approaching the water as a multielement system. Thus, special attention was paid to the area of 1st overtone of water, where 12 characteristic wavelength ranges were identified as especially important water bands where the highest spectral variations were likely to occur, with respect to biological systems functioning.

Waters in different experiments showed slightly different spectral signatures in the region of 1st overtone of water, which is reflected in water aquagrams (Fig. 4 – 9).

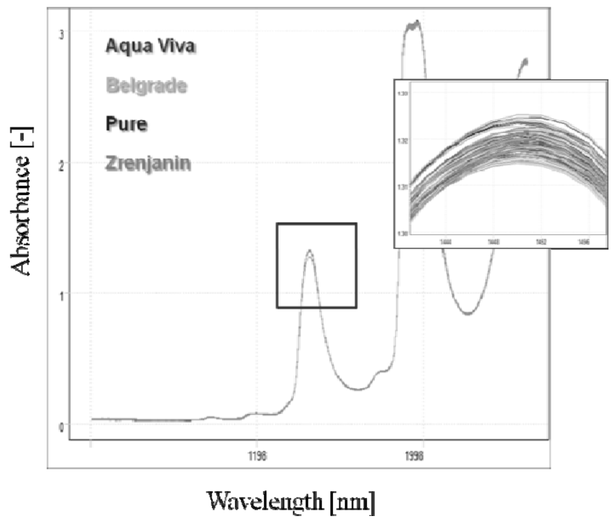

Figure 3. IR spectra of all analyzed water samples recorded in all experiments.

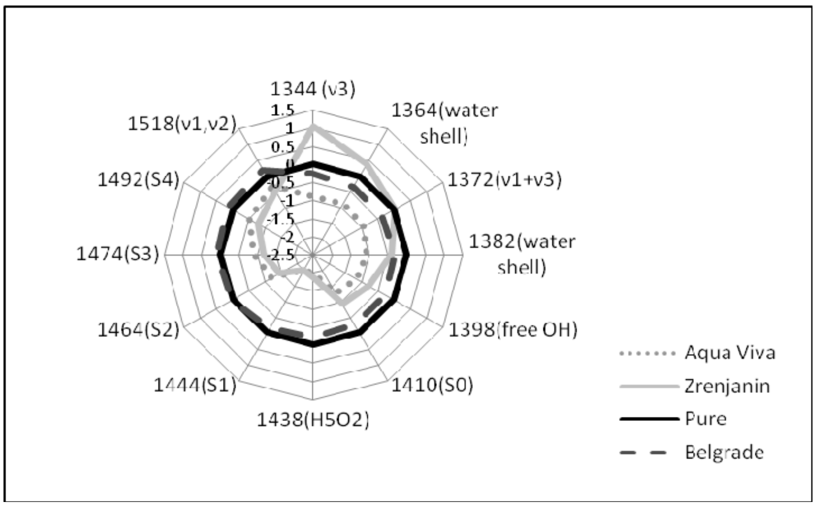

Figure 4. Aquagram of analyzed waters in Experiment 1

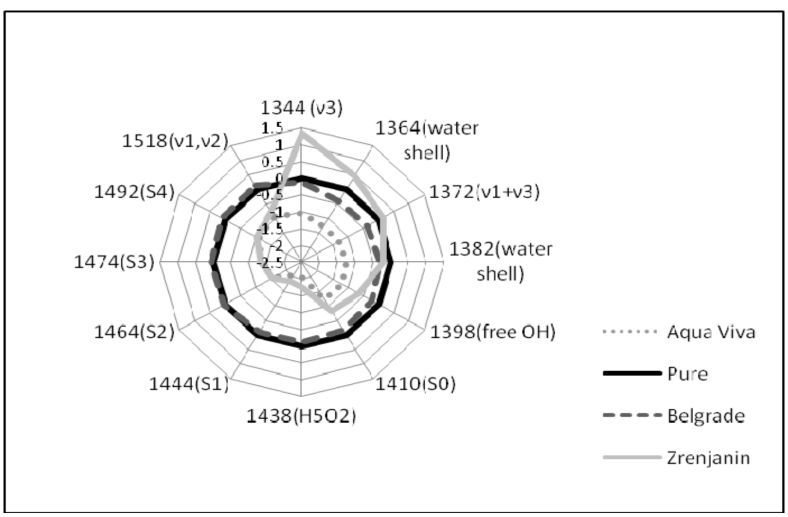

Figure 5. Aquagram of analyzed waters in Experiment 2

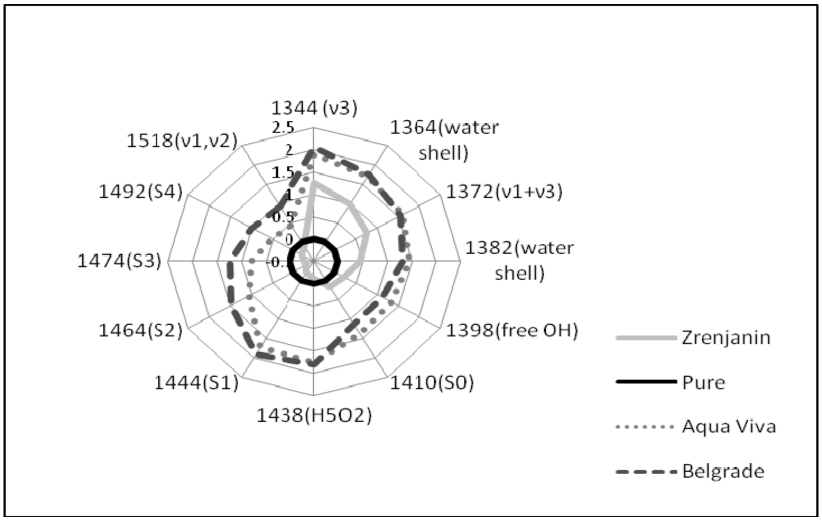

Figure 6. Aquagram of analyzed waters in Experiment 3

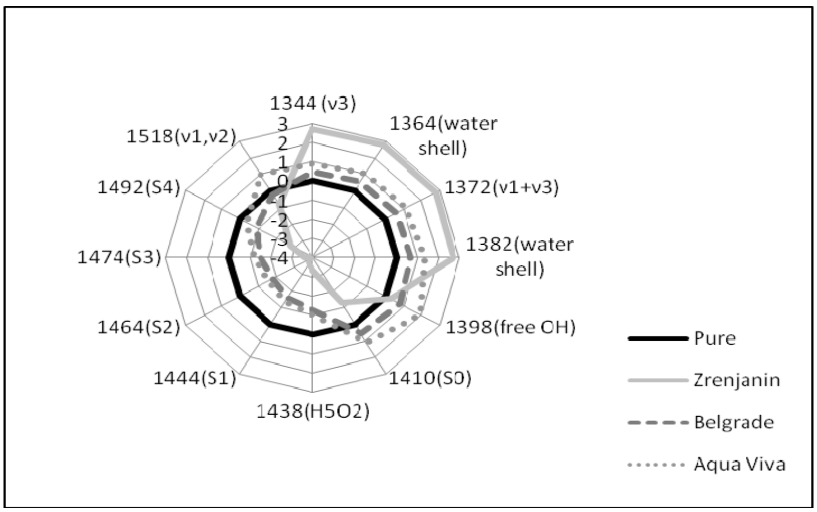

Figure 7. Aquagram of analyzed waters in Experiment 4

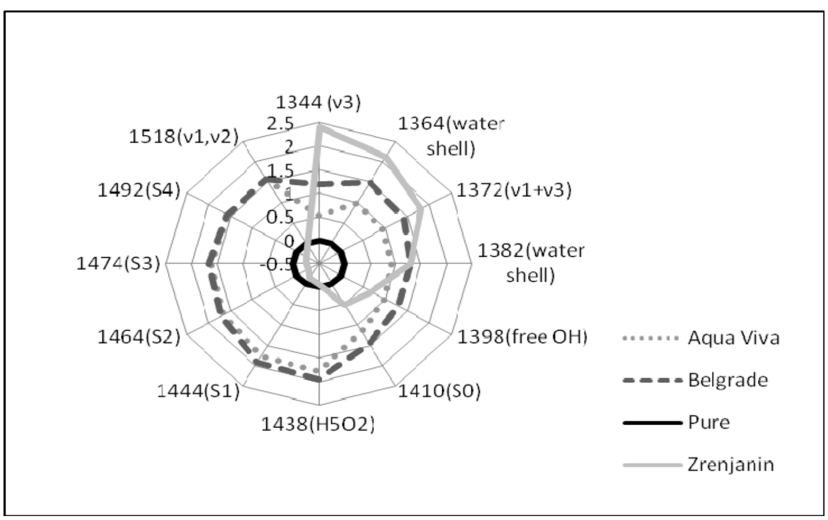

Figure 8. Aquagram of analyzed waters in Experiment 5

When comparing these aquagrams, it is clear that despite different experiment conditions, Zrenjanin tap water shows similar behavior. It has lower absorbance at S0 (free molecule species), S1, S2, S3, S4, H_5O_2 (water molecules with *i* hydrogen bonds) bands when compared to other waters, while consistently higher absorbance at 1344 (ν3), 1364 (water shell), 1372 (ν1+ ν3). The exception to this happens in Exp. 3, but we believe that this exception is rather a consequence of experiment conditions.

4.2 Soft modeling of class analogies

Spectra of the waters from different experiments were analyzed separately. In each case classes were well separated (Mahalanobis distance >>3) and distinctive. The discrimination power in cases of all experiments analysis was plotted in order to observe which wavelengths are most influential in terms of separating waters into different classes. The illustration of this process is given in Fig. 9 for the waters spectral data recorded in the first experiment. Wavelengths with the biggest influence on discrimination between different waters are summarized in Table 3.

4.3 Partial least squares regression

Spectra of waters from different experiments were analyzed separately. Regression vectors for each experiment model had a similar shape to the one presented in the Fig. 10 which is a regression vector from the results of partial least squares regression applied on spectral data from the first experiment.

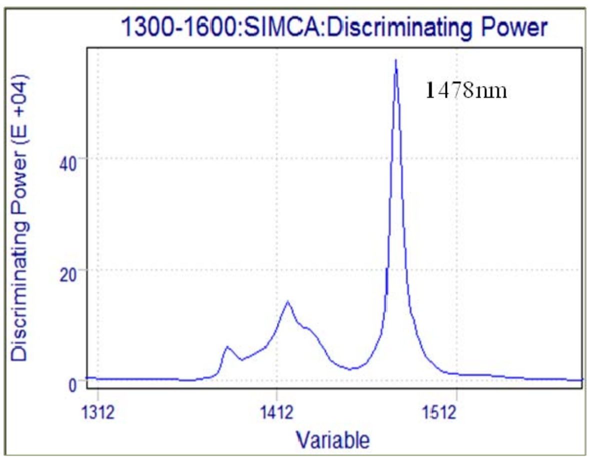

Figure 9.Discriminating power plot from the SIMCA analysis of the spectra of waters from the first experiment

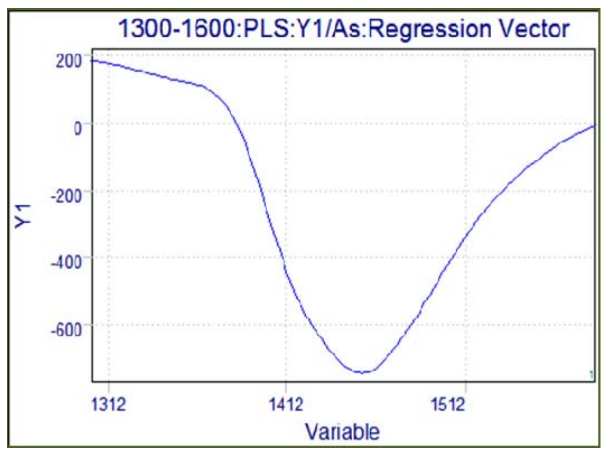

Figure 10. Regression vector from the partial least squares regression applied on the spectra of waters from the first experiment. Regression vectors for all experiments had a similar shape.

Table 3. Most influential wavelengths for separating different waters' spectra into distinctive classes

Experiment No.	1	2	3	4	5
Wavelengths with highest discrimination power [nm]	1478	1462	1414	1456	1478

Table 4. Most prominent wavelengths in regression vectors from the regression models built on spectral data from different experiments

Experiment No.	1	2	3	4	5
Most prominent wavelengths [nm]	1454	1458	1442	1450	1466

5. CONCLUSION

The citizens of town Zrenjanin in Serbia have been for a long time exposed to arsenic in drinking water, and we previously investigated the effect of drinking of this water in animal studies using opto-magnetic spectroscopy [5]. Although arsenic is a proven carcinogen, it is not well understood how the exposure to arsenic from drinking water leads to developing cancer; its association with diabetes mellitus is even more puzzling [8].

In this study, we applied NIR spectral analysis to investigate the differences of Zrenjanin drinking water compared to other waters which satisfy standard regulation requirements in order to understand how the presence of arsenic influences the organization of water molecules.

All waters showed different behavior depending on their composition, and small variations as a result of differences in pressure and temperature, which was expected given that water is very sensitive to changes in pressure and temperature [9]. We believe that the different water dynamics observed through repeated experiments is a result of macro and micro heterogeneity of water samples.

However, some spectral features consistently appeared in all experiments. By studying the absorbance pattern through aquagrams it is evident that Zrenjanin water shows lower absorbance in bands of free water molecules (S0) or water clusters (Si) with i hydrogen bonds (i=1,2,3,4), and also at 1438nm H2O-R where both lone pairs of the oxygen electrons bound to water clusters. Also, this water consistently showed higher absorbances at 1344nm (ν3), 1364 nm (1st overtone OH-stretch [OH-$(H_2O)_2$]), and 1372nm (ν1+ ν3). This means that Zrenjanin tap water has actually very few free water molecules and water clusters available. It has been particularly suggested that water molecules making two hydrogen bonds might be of special importance for water dynamics. All these findings imply that Zrenjanin water may not have enough significant water species for proper hydration of biological structures in living systems.

The number of free water molecules and water clusters proved to be a basis for discrimination between water types (SIMCA analysis). Most prominent wavelengths in regression vector having correlation with arsenic are found to be in C8: 1450 nm [1st overtone DDA symmetric stretch [OH-$(H_2O)_{4,5}$]) water absorbance band and C9 : (S2) water band.

It is our intention to continue and expand this research on skin tissues and blood in living organisms in order to find out how specific molecular organization of water affects hydration of organisms. We believe that this can lead to further understanding of a specific mode of action that arsenic has in causing cancer.

6. ACKNOWLEDGMENTS

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УТИЦАЈ КАНЦЕРОГЕНИХ СУПСТАНЦИ НА ВОДОНИЧНЕ ВЕЗЕ У ВОДИ

Сажетак: Становништво града Зрењанина у Србији дугорочно је изложено високим концентрацијама арсеника у води из градског водовода. Прегледом највише заступљених категорија водећих узрока смрти и регистрованих обољења код одраслих и деце, евидентна је присутност обољења која се доводе у везу са дугорочном изложеношћу арсенику. У претходним студијама са овом водом, објављени су ефекти конзумирања зрењанинске воде на кожи и другим ткивима, добијени коришћењем методе опто-магнетне спектроскопије. У овом раду, дати су прелиминарни резултати анализе зрењанинске воде у поређењу са узорцима вода које задовољавају препоруке Светске здравствене организације, добијени коришћењем блиске – инфрацрвене спектроскопије и применом новог концептуалног приступа за испитивање воде и водених система - аквафотомике.

Кључне речи: канцероген, арсеник, вода, блиска инфрацрвена спектроскопија, аквафотомика.

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