Science of the Total Environment 408 (2010) 3507–3512

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Fluoride in drinking water and dental fluorosis

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ARTICLE INFO

Article history:
Received 23 October 2009
Received in revised form 8 April 2010
Accepted 13 April 2010
Available online 23 May 2010

Keywords:
Fluoride
Drinking water
Hair
Schoolchildren
Dental fluorosis

ABSTRACT

In this study we determined the fluoride content in drinking water and hair of 12-year-old schoolchildren from different Serbian municipalities, *i.e.* Valjevo, Veliko Gradiste, Kacarevo and Vranjska Banja. The analyses were performed using composite fluoride ion-selective electrode. Average fluoride levels were 0.10, 0.15, 0.79 and 11 ppm in well water, 0.07, 0.10, 0.17 and 0.15 ppm in tap water, 19.3, 21.5, 25.4, and 32.5 ppm in hair samples, in Valjevo, Veliko Gradiste, Kacarevo and Vranjska Banja, respectively. Correlation analysis indicated statistically significant positive relationship between fluoride in wells water and fluoride in hair, for all municipalities: correlation coefficients were 0.54 ($p<0.05$), 0.89, 0.97 and 0.99 ($p<0.001$), in Vranjska Banja, Valjevo, Veliko Gradiste, and Kacarevo, respectively. Positive correlation was obtained also between fluoride in tap water and hair samples in all regions under the study, with statistical significance only in Valjevo municipality, $p<0.05$. Dental examination of schoolchildren confirmed dental fluorosis only in the region of Vranjska Banja. Moreover, in endemic fluorotic region of Vranjska Banja, positive and statistically significant correlations were confirmed between fluoride in well water and dental fluorosis level ($r=0.61$; $p<0.01$) and additionally between fluoride in hair and dental fluorosis level (0.62; $p<0.01$). The primary findings from this study have shown that fluoride content in hair is highly correlated with fluoride content in drinking water and dental fluorosis level, indicating that hair may be regarded as biomaterial of high informative potential in evaluating prolonged exposure to fluorides and to individuate children at risk of fluorosis regardless of the phase of teeth eruption.

1. Introduction

Fluorides occur naturally in soil, water, plants, animals and humans in trace quantities. The natural concentration of fluoride in water depends on several contributing factors such as pH, total dissolved solids, alkalinity, the porosity and acidity of the soil and rocks, the temperature, the depth of wells, etc. (Kahama et al., 1997; Grobler et al., 2001; Harrison, 2005; Msonda et al., 2007; Viswanathan et al., 2009). For the general population the intake of fluoride derives mainly from drinking water and to a lesser extent from foodstafs. The exposure from other sources may originate from industrial production of phosphatic fertilizers and volcanic activities that might result into wide distribution of fluoride in the atmosphere (Czarnowski and Krechniak, 1990a,b; Kaseva, 2006; Stewart et al., 2006; Yoshimura et al., 2006; Bellomo et al., 2007). It is well known that fluorides exert both, beneficial and detrimental effects on dental health (Erdal and Buchanan, 2005). The "optimum" or recommended level of fluoride in drinking-water, associated with the maximum level of dental caries protection and minimum level of dental fluorosis, is considered to be approximately 1 ppm (WHO, 1994, 2002, 2006; Pendrys, 2000; Grobler et al., 2001; Kaseva, 2006; Zemek et al., 2006; Tamer et al., 2007). Exposure to fluoride that is above optimal levels during enamel formation may induce appearance of dental fluorosis (Pendrys, 2000; Grobler et al., 2001; Whelton et al., 2004). It has been confirmed that there is significant positive relationship between fluoride intake by water and the prevalence of dental fluorosis (Binbin et al., 2004; WHO, 2006; Viswanathan et al., 2009; Mandinic et al., 2009). Impact of environmental contamination on body burden is usually based on biomonitoring data on fluoride content in urine and/or blood. Due to its specific informative potential, hair analysis has been also used for the purpose of exposure assessment (Kono et al., 1993; Hać et al., 1997).

According to our state of knowledge, statistical/mathematical interpretation of fluoride cross relation in different media and its influence on dental health has not been completely evaluated, so far. In this study attempt was made to establish correlation between fluoride concentration in hair and in drinking water, and between fluoride concentration in hair and the occurrence of dental fluorosis among 12-year-old schoolchildren from non fluorotic (Valjevo, Veliko Gradiste and Kacarevo) and fluorotic (Vranjska Banja) municipalities in Serbia.

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doi:10.1016/j.scitotenv.2010.04.029

2. Materials and methods

2.1. Area under study

This study was conducted in four municipalities in Serbia with different levels of naturally occurring fluoride in drinking water: Valjevo, Veliko Gradiste, Kacarevo and Vranjska Banja. Region of Vranjska Banja has been well documented as fluorotic endemic area *i.e.*, results from national studies indicated fluorosis prevalence of about 30% (Gajic, 1989; Ivanovic et al., 1991; Carevic et al., 1993). Kacarevo represents a region with moderate fluoride levels, whereas Valjevo and Veliko Gradiste are non-fluorotic locations. In these municipalities there are no additional sources of exposure, *i.e.* industries that cause the pollution of the environment by fluoride emission.

2.2. Population under study

Totally, 164 healthy, 12-year-old schoolchildren, both genders (66 boys and 98 girls), who were born and grew up in the same municipality participated in our study. Age of 12 has been selected as it is expected that by that age children will have almost exclusively permanent dentition. Prior to the study, the objectives of the study and the entire procedure were thoroughly presented to their parents and after that written consent was obtained. This study was approved by the Ethical Committee of School of Dentistry, Belgrade University (No.727/1; 2006).

2.3. Samples and sampling

Samples of drinking water were collected from the community water supplies and wells, during spring season in 2006. Following reports of respective regional public health institutes there were no significant seasonal variations in fluoride content in drinking water.

In all four regions, 209 samples of drinking water were selected randomly. Of all the samples 65 were tap water samples and 144 water samples from wells. Totally, 140 samples of hair were collected from the occipital zone of the scalp. Due to a small hair length, samples of hair from 24 children were not collected.

Until analysed, samples of water were stored in polyethylene bottles, whereas samples of hair were stored in plastic bags at temperature of 4 °C.

2.4. Analytical procedure

All chemicals were purchased from commercial sources, and were of analytical grade purity. In water samples, fluoride was determined directly using ion-selective electrode (Orion 9609, Cambridge, Mass, USA), while hair samples were previously homogenised, weighted and mineralised before fluoride determination according to procedure given by Nedeljkovic et al. (1991). Portion of homogenised samples of 2–3 g was transferred into the diffusion cell and after that dried in a laboratory oven at 55 °C. On the diffusion cell cover, thin layer was formed by the evaporation of 0.5 mL 1 mol/L NaOH in ethanol in laboratory oven at 55 °C. Mineralisation was done using 1.5 mL 40% $AgClO_4$ and 1.5 mL 70% $HClO_4$. The diffusion cell was immediately covered. During the micro diffusion process (for 24 h at 55 °C), the fluoride, released under the influence of 70% $HClO_4$, reacted with the NaOH to form NaF. The constituents of the thin layer coating the diffusion cell cover was dissolved in 5 mL of deionised water, than quantitatively transferred into a polyethylene dish and mixed with the TISAB buffer solution in ratio 1:1. TISAB was made of 57 mL of glacial acetic acid, 58 g NaCl, 300 mg of sodium citrate and water up to 500 mL. Thereafter, the solution was neutralised to pH 5–5.5 with 5 mol/L NaOH, while immersed in a cooling water-bath. The buffer was than diluted to 1 L with water (Nedeljkovic et al, 1991). Analytical method for fluoride determination in water was linear in the range of 0.05 to 20 ppm ($r = 0.9991$). Repeatability was confirmed by corresponding coefficient of variation of 3.03%. Obtained limits of determination (LOD) and quantification (LOQ) were 0.02 ppm and 0.04 ppm, respectively. For fluoride determination in hair samples linearity was confirmed in the range of 10 to 50 ppm ($r = 0.9995$), while repeatability was 5.67%. Calculated LOD and LOQ values for hair samples were 3 and 8 ppm, respectively. *Recovery* values of 96% (water) and 93% (hair) indicated adequate accuracy of the method.

2.5. Procedure of dental examination

Dental examinations were made using dental mirrors and explorers, under daylight by a single dentist previously well calibrated. Except for the third molars, all partly or fully erupted permanent teeth were examined. The teeth of the schoolchildren were assessed for fluorosis, using Dean's criteria and according to the WHO guidelines (WHO, 1987). Criteria for Dean's fluorosis index are described as follows (Dean, 1942). Normal (0): the enamel represents the usual translucent semivitriform type of structure, and the surface is smooth, glossy, and usually of a palecreamy white color. Questionable (1): the enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilized in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and a classification of "normal" is not justified. Very mild (2): small opaque, paper white areas scattered irregularly over the tooth but not involving as much as 25% of the tooth surface. Frequently included in this classification are teeth showing no more than about 1–2 mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars. Mild (3): The white opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50% of the tooth. Moderate (4): all enamel surfaces of the teeth are affected, and the surfaces subject to attrition show wear. Brown stain is frequently a disfiguring feature. Severe (5): includes teeth formerly classified as "moderately severe and severe." All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance (WHO, 1987). Intra-examiner reproducibility was calculated using Cohen's kappa index (Landis and Koch, 1977). The kappa index for repeated scores was 0.82, when considering all levels of fluorosis (0–5).

2.6. Statistical analysis

Statistical analysis of schoolchildren gender profile was performed using a Chi-squared test. One way analysis of variance, ANOVA, was used to obtain statistical analysis regarding the fluoride level in drinking water or hair samples. Pearson's correlation was applied to establish the relationship between fluoride in water, fluoride content in hair and fluorosis level. Values of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered significant. All statistical calculations were performed using STATISTICA version 5.0.

3. Results

According to our previously published data, fluoride contents in soil in these four regions were 29.63, 9.83, 15.71 and 22.9 ppm in Vranjska Banja, Valjevo, Veliko Gradiste and Kacarevo, respectively, indicating diversity in ground mineral resources that are natural fluoride donors (Fig. 1) (Mandinic et al., 2009).

Results of measurements in drinking water indicated significant differences among municipalities included in the study: in Vranjska Banja, fluoride levels in wells water were 50 to 100 times higher than in other three municipalities, which are considered as moderate fluorotic or non-fluorotic areas. Concentrations of fluoride measured in wells

Fig. 1. Location map of municipalities under the study.

water collected from regions of Kacarevo, Valjevo and Veliko Gradiste as well as in all tap water samples were below 1 ppm (Table 1).

A total of 78 schoolchildren participated in low-fluoride groups, 44 in moderate-fluoride group, and 42 schoolchildren participated in the high-fluoride group. Frequency distribution regarding gender did not show statistically significant differences among study participants in area of residence (Pearson Chi-square 6.12; $p = 0.12$). According to annual reports of regional public health institutes almost all participants consumed water from public water supply (71–91%).

Fluoride levels, measured in hair samples of children from Vranjska Banja were the highest, while the lowest values were obtained in hair samples of children from Valjevo municipality (Table 2). Expectedly, significant difference of fluorides in hair was obtained between samples collected in Vranjska Banja locality and other three regions, and moreover between Kacarevo and the rest of two regions of Valjevo and Veliko Gradiste.

Dental examination provided information on fluorosis level indicating the prevalence of dental fluorosis (scores 1, 3 and 5) of 16.7% in Vranjska Banja, while in Valjevo, Veliko Gradiste and Kacarevo, dental fluorosis was not observed, *i.e.* all of children examined exhibited normal dental score (Table 3).

Relationship between fluoride levels in hair of schoolchildren and wells or tap water was described using Pearson's parametric correlation analysis. Positive and statistically significant correlation was obtained between fluorides in wells water and hair samples in all three non-fluorotic regions (correlation coefficients were 0.89, 0.97 and 0.99, in Kacarevo, Valjevo, and Veliko Gradiste, respectively; $p < 0.001$). Positive correlation was obtained also between fluoride in tap water and hair samples in all regions under the study, with statistical significance only in Valjevo municipality, $p < 0.05$ (Table 4 and Fig. 2).

For the fluorotic region of Vranjska Banja results of statistical analysis showed also positive and significant correlation between fluoride levels in hair and wells water samples (Table 4 and Fig. 3), and furthermore between fluorides in hair and fluorosis level, and fluoride in wells water and fluorosis level ($r = 0.54$, 0.62 and 0.61, respectively; $p < 0.05$ and $p < 0.01$).

Table 1
Fluoride content in water samples.

Region	Wells					Tap water				
	Fluoride (ppm)					Fluoride (ppm)				
	Mean	SD	Min.	Max.	*n*	Mean	SD	Min.	Max.	*n*
Kacarevo	0.79	0.22	0.54	1.13	44	0.17	0.03	0.11	0.22	16
Valjevo	0.10	0.02	0.08	0.11	32	0.07	0.01	0.06	0.09	14
Veliko Gradiste	0.15	0.02	0.13	0.17	37	0.10	0.03	0.07	0.14	18
Vranjska Banja	11.00	2.47	8.01	14.07	31	0.15	0.02	0.12	0.19	17

Table 2
Fluoride content in hair samples.

Region	Fluoride (ppm)		
	Mean	SD	*n*
Kacarevo	25.4^{b}	3.7	41
Valjevo	19.3^{a}	1.0	30
Veliko Gradiste	21.5^{a}	2.5	38
Vranjska Banja	32.5^{c}	0.3	31

Values sharing different letters are significantly different, $p<0.05$ (one-way ANOVA and post-hoc LSD test).

4. Discussion

Fluorides are ubiquitous in the environment and the amount of fluoride occurring naturally is dependent upon the individual geological environment (Kahama et al., 1997; Harrison, 2005). Especially high fluoride levels in water have been found in China, India and Africa (Yoder et al., 1998; Cao et al., 2000; Gikunju et al., 2002; Fantaye et al., 2004; Tekle-Haimanot et al., 2006; WHO, 2006). In Serbia, fluoride concentration in soil and water varies among the regions (Gajic, 1989; Ivanovic et al., 1991; Carevic et al., 1993). Fluoride content in soil — in all four municipalities, in present study, is highly related to assignation of region as fluorotic or non-fluorotic area (Fig. 1). Vranjska Banja has been chosen as a location with elevated naturally occurring fluoride (Ivanovic et al., 1991; Carevic et al., 1993; Mandinic et al., 2009). In other three regions fluoride levels in both water sources were below value of 1 ppm, recommended by WHO (1994). As a matter of concern, average level of fluoride in wells obtained for endemic fluorotic municipality of Vranjska Banja was 11 times higher than recommended value. However, prevalence of dental fluorosis in this region was significantly lower compared to the similar studies (Binbin et al., 2004; Meenakshi Garg and Kavita Renuka Malik, 2004; WHO, 2006; Mascharenhas and Mashabi, 2008; Indermitte et al., 2009) and the studies conducted over 20 years ago in the same region (Gajic, 1989; Ivanovic et al., 1991; Carevic et al., 1993). This finding can be explained by the fact that public water supply with extremely low concentration of fluoride (<0.14 ppm) was introduced for the first time in this region approximately 10 years ago. Our results showed that most inhabitants today are drinking water from public water supply as indicated by public health institutes.

Table 3
Dental fluorosis scores.

Dental fluorosis score	Kacarevo		Valjevo		Veliko Gradiste		Vranjska Banja	
	n	%	*n*	%	*n*	%	*n*	%
0 = normal	44	100	34	100	44	100	35	83.3
1 = questionable	–	–	–	–	–	–	4	9.5
2 = very mild	–	–	–	–	–	–	–	–
3 = mild	–	–	–	–	–	–	2	4.8
4 = moderate	–	–	–	–	–	–	–	–
5 = severe	–	–	–	–	–	–	1	2.4
Total	34	100	44	100	44	100	42	100
Pearson Chi-Square	36.12, $p = 0.001^{*}$							

* Statistically significant, $p<0.05$ (Pearson Chi-Square statistical test).

Table 4
Correlation coefficients between fluoride content in water and hair.

	Non-fluorotic regions			Fluorotic region
	Valjevo	Veliko Gradiste	Kacarevo	Vranjska Banja
	F^-_{hair}			
$F^-_{well\ water}$	0.89*	0.97*	0.99*	0.54#
$F^-_{tap\ water}$	0.91#	0.53	0.51	0.39

* Statistically significant, $p<0.001$.
# Statistically significant, $p<0.05$ (Pearson's correlation analysis).

Determined high concentrations of fluoride in drinking water refer only to the drinking water from wells, which was used before as the only available drinking water, since in the past there was no public water supply. Today the water from wells are mostly used for garden watering and livestock feeding, since it is free of charge and the region of Vranjska Banja is well known as low living standard region. Therefore, the main sources of natural fluorides today in the region of Vranjska Banja comes from agricultural products (potatoes, beans, tomato, cucumbers, watermelons and etc.), which were still traditionally grown in domestic gardens and probably much less from wells drinking water which are still high in fluoride content. In fluorotic areas all over the world, where endemic fluorosis has been well documented, fluoride concentrations in drinking water ranges from 3 to more than 20 mg/L (Cao et al., 2000; Yoder et al., 1998; Gikunju et al., 2002; Fantaye et al., 2004; Tekle-Haimanot et al., 2006; WHO, 2006). Taking into consideration that water is the major source of fluoride intake in humans, consuming appropriate and relatively small amounts of fluoride by water is generally conceived to have a beneficial effect on the rate of occurrence of dental caries, particularly among children, while excessive intake of water reach in fluoride, results in pathological changes in teeth, such as dental fluorosis (Czarnowski and Krechniak, 1990a,b; Czarnowski et al., 1996; WHO, 2006). The later includes statistical verification of relationship between fluoride content in drinking water, in biological media and the occurrence of dental fluorosis.

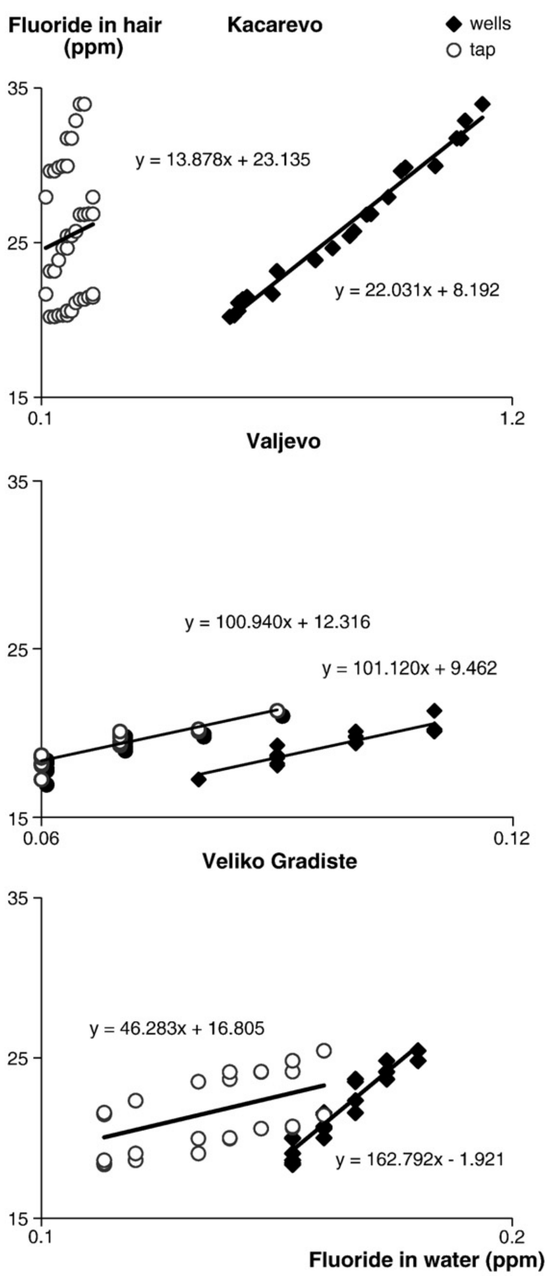

Fig. 2. Correlation analysis between fluoride content in water and hair in non-fluorotic regions.

Daily level of fluoride in biomaterials such as urine and blood has been proposed as the most reliable indicator of exposure to fluoride (Kokot and Drzewiecki, 2000). However, results from this study indicated that hair could be also useful biomaterial for fluoride exposure monitoring. Fluoride levels presented in Table 2 reached the value of up to 33.0 ppm in hair samples of children from Vranjska Banja, unlike lower mean values of 25.4, 19.3 and 21.5 ppm, measured in hair samples of children from Kacarevo, Valjevo and Veliko Gradiste, respectively. Variation in fluoride content among hair samples was the highest in the moderate fluorotic region of Kacarevo with the standard deviation of 3.7 ppm (14.6%). In other municipalities variations were 1.0, 2.5 and 0.3 ppm (5.2, 11.6 and 1%) in Valjevo, Veliko Gradiste and Vranjska Banja, respectively. Fewer data are available on fluoride in hair (Czarnowski and Krechniak, 1990a,b, Czarnowski et al., 1996; Kono et al., 1993; Hać et al., 1997; Kokot and Drzewiecki, 2000). In the study of Hać et al. (1997) it was shown that average level of fluoride in hair of 1.4 ppm in a population aged between 17 and 87 years, corresponded to the mean concentration of 0.27 ppm in drinking water. Findings of Czarnowski and Krechniak (1990b) on fluoride in hair range from 2.1 to 2.9 ppm in population of fertiliser workers. Kokot and Drzewiecki (2000) reported on concentration range of <0.5 to 26 ppm for fluoride in hair of non-exposed children and adults, who live in the region of Poland where fluoride concentration in water was mainly less than 1 ppm. In the same study, fluoride levels in hair samples of exposed workers range from 113 to 5459 ppm. Wide range obtained in workers, professionally exposed, authors explained by dependence on the type of work exposure to fluoride. In comparison with the results published previously, higher levels of fluoride in hair samples of non exposed children in present study, could be ascribed to seasonal variation in water intake, various methods used and/or certain contribution of fluoride intake by food.

Hair has certain advantages over other media; it can be collected easily and painlessly and it is easy to transport and store (Mikasa et al., 1988; Kono et al., 1993). Moreover, the analysis of hair strands may provide information on long-term exposure from environment, in contrast to transient information obtained from blood and urine samples (Grandjean, 1984; Mikasa et al., 1988; Hać et al., 1997; Nohara et al., 1998; Kokot and Drzewiecki, 2000; Stolarska and Czarnowski, 2000; Zakrzewska et al., 2000; Souad et al., 2006; Mandinic et al., 2007; Sukumar and Subramanian, 2007).

In conclusion, correlation analysis of the results of our study confirmed positive statistically significant relationship between fluoride levels in hair and fluoride in wells water, in both fluorotic and non-fluorotic areas (Figs. 2 and 3, Table 4). In endemic fluorotic region of Vranjska Banja, positive and statistically significant correlations between levels of fluoride in hair and dental fluorosis were also obtained. Dental fluorosis in children from this region has been directly

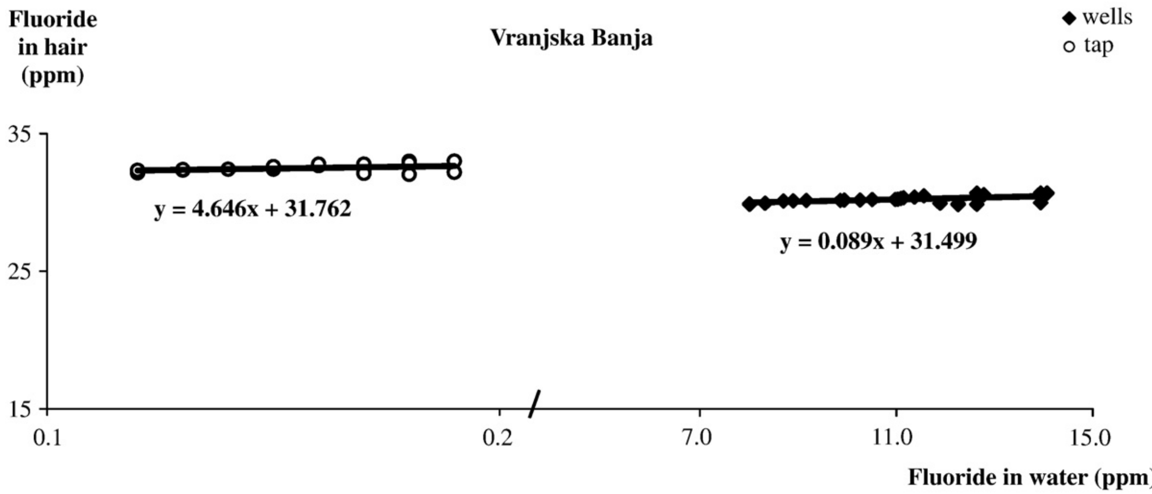

Fig. 3. Correlation analysis between fluoride content in water and hair in Vranjska Banja.

linked not only to the content of fluoride in drinking water, but also to the content of fluoride in self-grown agricultural products, traditionally present in the diet of local inhabitants.

The primary findings from this study have shown that fluoride content in hair is highly correlated with fluoride content in drinking water and dental fluorosis level, indicating that hair may be regarded as biomaterial of high informative potential in evaluating prolonged exposure to fluorides and to individuate children at risk of fluorosis regardless the phase of teeth eruption.

Acknowledgements

The authors would like to thank to Prof. Marko Vulovic and to Prof. Mirjana Nedeljkovic, for disinterestedly support in this study.

This study is part of the Project No. 20212 granted by Ministry of Science and Technological Development of the R. Serbia.

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