



Relationship between fluoride intake in Serbian children living in two areas with different natural levels of fluorides and occurrence of dental fluorosis

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ABSTRACT

The amount of fluoride present naturally in drinking water is highly variable, being dependent upon the individual geological environment from which the water is obtained. Chronic exposure to exceeding fluoride doses induces set of toxic effects, i.e. fluorosis. The aim of this study was to examine fluoride content in water and in the most frequently used vegetables, potato and bean, grown in two different Serbian regions, i.e. control region (Valjevo) and high naturally occurring fluoride region (Vranjska Banja), and moreover, to correlate estimated daily intake with dental fluorosis occurrence as an adverse effect of fluoride exposure of schoolchildren in Serbia. Study confirmed significant difference in fluoride content in water, potato and bean, consumed by 12-year-old children in two investigated municipalities. Results of the study indicated positive and statistically significant correlation between daily intake of fluoride and dental fluorosis level in the fluorotic municipality of Vranjska Banja ($r = 0.61$; $p = 0.000017$). Obtained relationship could be evaluated by means of binary logistic regression analysis, whereas probability for fluorosis occurrence could be predicted using the following equation: fluorosis occurrence (%) = $(34.852 \times C_{\text{water}} - 12.644 \times C_{\text{potato}} - 9.362 \times C_{\text{bean}} - 7.673) \times 100$ (Chi-Square (3) = 33.033; $p < 0.001$).

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1. Introduction

From the first reports by Dean and colleagues published in the 1930s, fluorides are still considered as the most effective mean in reducing dental caries. However, assessment on the quantitative relationship between dental fluorosis and fluoride intake conducted by WHO (1994), initiated a large number of further studies on this topic. Studies in the 1950s and 1960s estimated that 7–16% of children reared in an optimally fluoridated area showed signs of mild or very mild fluorosis in the permanent dentition, which is confirmed by, recently performed meta-analysis (McDonagh et al., 2000; Whelton et al., 2004). Public health programmes, seeking to maximize the beneficial effects of fluoride on dental health through the introduction of fluoridated drinking water have, at the same time, strived to minimize its adverse effects on teeth (WHO, 1987; Petersen, 2003; Jones et al., 2005). Dental fluorosis is hypomineralisation of the enamel caused by intake of an amount

of fluoride that is above optimal levels during enamel formation (Pendry, 2000). From various publications, it became clear that dental fluorosis varies within the population. Factors responsible for these variations could be fluoride intake by drinking water, dietary intake, especially intake of food grown in soil or irrigated with water rich in fluoride. However, systemic and topical fluoride administration, could also contribute to fluorosis occurrence (WHO, 2002).

The aim of this study was to examine fluoride content in water and in the most frequently used vegetables, potato and bean, grown in two different Serbian regions, i.e. regions with low and high naturally occurring fluoride, and moreover, to correlate estimated daily intake with dental fluorosis as an adverse effect of fluoride exposure of schoolchildren in Serbia.

2. Materials and methods

2.1. Subjects and sample collection

This study was conducted in two areas in Serbia with significantly different levels of fluoride in drinking water, municipalities of Valjevo and Vranjska Banja. Region of Vranjska Banja is well documented particularly in national literature as fluorotic endemic area, whereas region of Valjevo as non-fluorotic area (Ivanovic et al., 1991). It should be added that in these areas there are no other relevant sources of exposure, i.e. industries that cause the pollution of the environment by fluoride emission.

Abbreviations: CFI, community fluorosis index; DI, daily intake; EPA, environmental protection agency; FAO, Food and Agricultural Organisation; FE, fluoride exposure; NOAEL, non-observed adverse effect level; TISAB, total ionic strength adjustment buffer; WHO, World Health Organisation.

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Seventy six healthy, 12-year-old schoolchildren, both gender (33 boys and 43 girls) were participated in our study. Prior to the study, the objectives of the study and entire procedure were thoroughly presented to their parents and after that written consent was obtained. Ethical Committee of School of Dentistry, Belgrade University, approved this study (No. 727/1; 2006).

Samples of water were collected from the community water supplies and wells in spring season in 2006. Portions of soil were taken near well springs from the depth of 20–30 cm. Vegetable items used in this study (potato and bean) shown to participate predominantly in schoolchildren diet in both regions, were purchased from the local markets (FAO, 2006). Until analysed, samples of water were stored in polyethylen bottles, whereas samples of soil and vegetables were stored in plastic bags in dry and dark place at the temperature of 4 °C.

2.2. Sample preparation and fluoride determination

In water samples, fluoride was determined directly using ion-selective electrode (Orion 9609, Cambridge Mass, USA). Soil, potato and bean samples were previously homogenised, weighted and than mineralised. Complete analytical procedure for fluoride determination in either water, vegetable or soil samples were published by Nedeljkovic et al. (1991). A 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% AgClO₄ and 1.5 mL 70% HClO₄. 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% HClO₄, reacted with the NaOH to form NaF. The constituents of the thin layer coating the diffusion cell cover was dissolved in 5 mL of de-ionised 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. After dissolution, the solution was neutralized 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.

The separated fluorides were subsequently determined by composite fluoride ion-selective electrode (Orion 9609, Cambridge Mass, USA). All chemicals, obtained from commercial sources, were of analytical grade purity.

2.3. Examination of dental fluorosis

Randomised sampling was performed for examination of dental fluorosis occurrence expressed as CFI score.

$$CFI = \frac{\sum(\text{frequency} \times \text{statistical weight})}{\text{number of individuals}}$$

Dental examinations were made using dental mirrors and probes, under indirect sunlight by one calibrated and well trained dentist. Except for third molars, all partly or fully erupted teeth were examined. The teeth of the schoolchildren were assessed for fluorosis, using Dean's criteria according to the WHO guidelines (WHO, 1987). Criteria for Dean's fluorosis index are described as follows. Normal (0): the enamel represents the usual translucent semivitriform type of structure, and the surface is smooth, glossy, and usually of a pale creamy 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 bicuspid 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). The kappa index for repeated scores was 0.82, when considering all levels of fluorosis (0–5).

2.4. Exposure assessment

In order to evaluate apparent risk of dental fluorosis on the bases of estimated dietary exposure, two different sets of data were used in the present study: fluoride concentration database and consumption database. Fluoride content in water and vegetables was originally measured in this study. Estimated daily intake of fluoride was calculated using formula based on daily intake data for potato and bean taken from the GEMS/Food Consumption Cluster Diets built up by the FAO (FAO, 2006). Integration of food consumption database and contaminant concentration data has been done by the use of a simple, deterministic procedure. Average body weight of 12-year-old children was obtained from the scale (Fisher et al., 2001), which

recommends that body weights of 12-year-old children at 50th percentile are 37.7 kg and 40.5 kg, for boys and girls, respectively, and at 90th percentile are 49.0 kg and 54.7 kg, for boys and girls, respectively.

The following formula was used for calculation of FE

$$DI = \text{daily intake (kg)} \times \text{fluoride concentration (mg/kg)}$$

$$FE = DI/\text{body weight at 50th or 90th percentile (mg/kg bw)}$$

2.5. Statistical analysis

Statistical analysis was performed using a Chi-squared test. Pearson's correlation was performed for the relationship between fluorosis score and fluoride content in water and vegetable items. Fluoride content in all samples was correlated with prevalence of dental fluorosis and consequently binary logistic regression was used for probability of fluorosis occurrence. Values of $p < 0.05$ were considered significant. All statistical calculations were performed using STATISTICA version 5.0.

3. Results

Approximately 230 km far from Valjevo municipality, region of Vranjska Banja is well known as fluorotic endemic area (Fig. 1). Soil fluoride contents in Valjevo and Vranjska Banja regions reach the values of 9.83 ppm and 29.63 ppm, respectively, indicating significant difference between these two regions with respect to natural resources of certain minerals that are principal donors of fluoride.

Regarding to the region and gender, results of Pearson's Chi-Square statistical analysis, did not show statistically significant differences (Table 1).

Results of fluoride content in water indicated significant difference between two regions included in the study: in the region of Vranjska Banja, fluoride levels in water were more than 100 times higher than in Valjevo region, which was considered as control one. Consequently, fluoride intake by water was higher in Vranjska Banja area. Concerning consumption rate of potato and bean, there is significant difference between daily intakes of these two vegetables. High value for potato daily intake of 244 g could be ascribed to the certain socio-economic status and to the specific dietary habits due to intake of vegetable carbohydrates are assured predominantly by potato consumption. Therefore, potato intake contributes to total daily intake of fluorides largely (Table 2).

Total daily intake of fluoride was further recalculated taking into account body weight. In comparison with NOAEL value of 0.06 mg/kg bw/day (EPA, 2002), obtained values for fluoride exposure in the region of Vranjska Banja were higher, whereas values at 50th percentile representative for the population of schoolchildren in Valjevo, were slightly higher than NOAEL (Fig. 2). The NOAEL value of 0.06 mg/kg bw/day, given by EPA was calculated on the basis of results obtained in a study, conducted among children, consuming fluoride via drinking water, where dental fluorosis was used as critical effect (EPA, 2002).



Fig. 1. Sampling locations: fluorotic region of Vranjska Banja and control region of Valjevo.

Table 1
Basic characteristics of the population under study.

Gender	Number of participants	Valjevo		Vranjska Banja	
		Number of participants	Number of participants (%)	Number of participants	Number of participants (%)
Boys	33	17	50.0	16	38.1
Girls	43	17	50.0	26	61.9
Total	76	34	100.0	42	100.0
Pearson Chi-Square			6.12, $p = 0.12$		

p -Value was obtained by Pearson Chi-Square statistical analysis, value of $p < 0.05$ was considered significant.

Table 2
Estimated total daily intake of fluoride.

Pathway of exposure	DI (kg)	Region		Fluoride DI (mg)	Fluoride DI (mg)		
		Valjevo				Vranjska Banja	
		Fluoride content, $\bar{x} \pm Sd$ (mg/kg)	Fluoride DI (mg)			Fluoride content $\bar{x} \pm Sd$ (mg/kg)	Fluoride DI (mg)
Water	1	0.10 \pm 0.01	0.1	11.00 \pm 1.69	11		
Bean	0.006	9.46 \pm 0.28	0.059	18.95 \pm 0.65	0.119		
Potato	0.244	12.42 \pm 0.17	3.205	17.91 \pm 1.44	4.363		
			$\Sigma = 3.364$		$\Sigma = 15.482$		

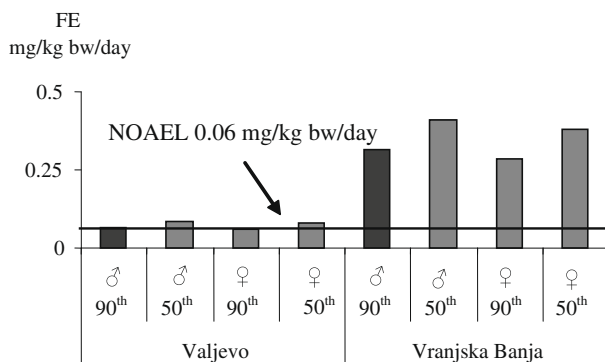


Fig. 2. Fluoride exposure assessment.

The prevalence of dental fluorosis (scores 1, 3 and 5) was 16.7% in Vranjska Banja, while in Valjevo region dental fluorosis was not observed. According to the CFI, region of Vranjska Banja is classified as endemic fluorotic area (Table 3).

Results of statistical analysis confirmed positive and significant correlation between fluoride levels in all examined samples and

fluorosis level ($r = 0.61$; $p = 0.000017$) (Table 4). This relationship could be evaluated by means of binary logistic regression analysis.

Thus, probability for fluorosis occurrence could be predicted using the following equation:

$$\text{Fluorosis occurrence (\%)} = (34.852 \times C_{\text{water}} - 12.644 \times C_{\text{potato}} - 9.362 \times C_{\text{bean}} - 7.673) \times 100$$

$$\text{Chi-Square}(3) = 33.033; \quad p < 0.001.$$

4. Discussion

Fluoride is ubiquitous in the environment, and the amount of fluoride present naturally in non-controlled fluoridated drinking water is highly variable, being dependent upon the individual geographical environment from which the water is obtained (Kahama et al., 1997; WHO, 2002). Fluoride is an element of major health concern and it is absorbed into the organism as a result of environmental or industrial exposure (Hać et al., 1997). Notably, the total intake of fluoride by individuals consuming fluoridated or non-fluoridated drinking water may not be markedly different, due to the intake of significant amounts of fluoride from food as well (US DHHS, 1991; Burt, 1992). Exposure to all sources of fluoride is important for the estimation of daily intake and consequently for the assessment of its adverse health effects. However, there is a narrow range between the intakes associated with its beneficial and adverse health effects (Erdal and Buchanan, 2005). In certain areas worldwide in which the concentration of fluoride in the surrounding environment may be exceedingly high and/or where diets are composed of foodstuffs rich in fluoride, estimated intakes of fluoride in adults as high as 27 mg daily have been reported (Liu, 1995; Cao et al., 1996; Karthikeyan et al., 1996). Thus, the monitoring of fluoride in water, potato and bean, in this study, provides information on intake of the element, and subsequent fluorosis occurrence in two different Serbian regions.

Based upon the studies conducted by Dean (1942) the "optimum" level of fluoride in drinking water, associated with the maximum level of dental caries protection and minimum level of dental fluorosis, was considered to be approximately 1 ppm (Grobler et al., 2001). Also, WHO Expert Committee (WHO, 2002) recommends levels of fluoride in drinking water that are

Table 3
Dental fluorosis score in Valjevo and Vranjska Banja.

Dental fluorosis score	Valjevo		Vranjska Banja	
	Number of participants	%	Number of participants	%
0 = normal	34	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	42	100
Pearson Chi-Square		36.12, $p = 0.001^*$		
CFI	-	1.429		

p -value was obtained by Pearson Chi-Square statistical analysis.

* Value of $p < 0.05$ was considered significant.

Table 4
Correlation between fluorosis level and fluoride content in Vranjska Banja municipality.

	Water		Potato		Bean		All examined samples	
	R	p	R	p	R	p	R	p
Fluorosis level	0.62	0.000012	0.55	0.000162	0.80	0.000000	0.61	0.000017

p-Value was obtained by Pearson Chi-Square statistical analysis, value of $p < 0.05$ was considered significant; R – correlation coefficient.

considered useful for the prevention of dental caries, in a range from 0.5 to 1.2 ppm. In our study significant difference in fluoride content in water, potato and bean, consumed by 12-year-old schoolchildren in two investigated municipalities, was shown (Tables 1 and 2, Fig. 1), although there are some limitations related to the methodology used. Firstly, FAO database does not consider 12-year-old population, but only adults. Secondly, we made a presumption that water, potato and bean are the only source of fluoride. This approach rises from the consumption rate of all three items. Additionally, in calculation procedure body weight values for 50th and 90th percentiles are included, implying “the worst scenario” at 50th percentile, and “the best scenario” at 90th percentile (Fig. 2) (Fisher et al., 2001). Other values used for the calculation were fixed (daily intake of food and average fluoride content in food), so we speculated with the worst and the best scenarios on the base of body weight differences only.

In our study, calculated daily intake based on fluoride content in water, bean and potato in Vranjska Banja, region reach in natural occurring fluoride, is six to seven times higher than value of 2 mg/day that was estimated as worldwide average for fluoride intake in children and adolescents (WHO, 2002). Results of fluoride exposure in Vranjska Banja that are in a range between 0.28 and 0.31 mg/kg bw/day (Fig. 2), and further confirmed by the prevalence of dental fluorosis (Tables 3 and 4), are similar to the results of the study given below. Although, in a study of Erdal and Buchanan (2005), daily intake rate of fluoride had been measured from various sources such as food, beverages, fluoridated drinking water, cow's milk, toothpaste and fluoride supplements, daily fluoride intake in fluoridated areas was estimated at 0.20 and 0.11 mg/kg bw/day.

Examination on the prevalence of dental fluorosis conducted by WHO confirmed high association with the concentration of fluoride in water and foodstuffs, with a positive dose–response relationship (WHO, 2002). The occurrence of endemic fluorosis has been well documented in various case reports and surveys of individuals residing in certain areas of the world (e.g., India, China, northern, eastern, central and southern Africa), where the intake of fluoride may be inordinately high as a result of the often significant consumption of drinking water containing substantial amounts of naturally occurring fluoride, the preparation of foodstuffs in water containing increased fluoride and/or the consumption of specific foodstuffs naturally rich in fluoride (Haimanot et al., 1987; Krishnamachari, 1987; Liu, 1995; Zhang and Cao, 1996; Liang et al., 1997; Cao et al., 2001, 2003, 2004, 2005, 2006; WHO, 2002; Jackson et al., 2002; Martinez-Mier et al., 2003; Malinowska et al., 2008). In our study, the occurrence of dental fluorosis (score of 1 and higher) was related primarily to the fluoride level in the water: none of the children had evidence of fluorosis in the area with low fluoride concentration in comparison with prevalence of 16.7% among the children living in the fluorotic area (Table 3). According to Dean, the CFI score of 1.429 found in the high fluoride area of Vranjska Banja, represents public health problem (Table 3) (Budipramana et al., 2002). Furthermore, correlation analysis between all samples and dental fluorosis level among schoolchildren showed statistical significance (Table 4). Due to application of binary logistic regression model the calculation of probability of dental fluorosis occurrence could be done, based on the data of fluoride

contents. Estimated FEs in presented scenarios (50th and 90th percentile) are at the level or slightly higher than NOAEL value reported by EPA (0.06 mg/kg bw/day) in non-fluorotic region. Ketley and Lennon (2001) reported that “optimal” range of fluoride intake in young children has been quoted as 0.05–0.07 mg/kg bw/day (Ketley and Lennon, 2001). In other study, a dose of 0.07 mg/kg bw/day was considered to be the upper limit of fluoride exposure in terms of fluorosis risks (Casarin et al., 2007). However, in Vranjska Banja region fluoride exposure in both scenarios is 4–5 times higher than NOAEL value, and consequently may indicate a risk for occurrence of dental fluorosis.

In conclusion, the principal findings from this study provide evidence that there is positive and statistically significant correlation between daily intake of fluoride and dental fluorosis level in the fluorotic municipality. Based on assessment of fluoride intake and its impact on dental health, mathematical expression for the quantification of this relationship is given, and could be used for the prediction of dental fluorosis occurrence.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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