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RESEARCH ARTICLE

ASSESSMENT LEVELS OF TRACE ELEMENTS IN WATER FROM ROMANIA AND THE TOOTH DECAY-A PILOT STUDY

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ABSTRACT

Subject Area: Health Sciences
Introduction: The health of teeth could be considered an indicator for evaluation of the exposure to different environmental contaminants and for appreciation of the nutritional status of individuals. Aim: There is need to evaluate the relationship between the risk assessment levels of trace elements in water and decayed tooth dentin.
Material and methods: First part to determine the evaluation of the levels of fluorine in the drinking water from centralized water supply sources distributed to the population and individual sources in 9 counties of Romania and second part to determine the concentration of fluorine in carious dentin of permanent teeth from 30 male adults from different areas of residence in a pilot study. Determination of mineral composition in water samples and fluoride in dentin of permanent teeth decayed was used an ion chromatography method and to determine the metals in dentin of teeth was used absorption optical emission spectroscopy method with inductively coupled plasma (ICP-OES).
Result: In the water samples with low fluoride concentrations we found low concentrations of trace elements, showing a poor overall mineralization of water which, together with a general lack of fluoride, has a potential negative impact on the health of consumers, including oral-jaw system. Mineral and fluoride levels determined in dentin samples were of a significant negative correlation.
Conclusion: Variability of the concentration of trace elements and fluoride in dentin of permanent decayed teeth from different people in different geographical areas, regardless of the types of teeth (premolars and molars), is of a significant negative correlation. The strong effect of geographical location implies the fact that these communities have unique features that are important determinants of dental fluorine, dependent on the individual characteristics and professional exposure.

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INTRODUCTION

Bio-monitoring of trace elements in human teeth has become an important tool to evaluate an individual's nutritional and environmental status. Variations in the content of trace elements in the teeth have been previously demonstrated. Trace elements can be ingested by humans via different routes, including ingestion in the food and water or by deliberate consumption of soil and by dermal absorption. The presence and/or absence of trace elements in the environment influence their availability to humans (Brown *et al*., 2004). Decay occurs due to the interaction between intrinsic factors related to individual and extrinsic factors, the environmental ones.

Healthy teeth are good indicators for assessing exposure to environmental contaminants and the appreciation of the nutritional status of individuals (Brown *et al*., 2004; Moynihan, 2005). The content of chemicals in the body is directly proportional to their content in the environment, taking into account the correction coefficient and the solubility of the compounds of these elements (Brown *et al*., 2004). Minerals may influence the health of the human body both by default and by excess. For fluorine (essential mineral) human health depends on a delicate balance between excess and deficiency. Fluorine is the only element correlated with resistance to tooth decay. The main way of fluorine intake to the body is fluid, food being the second one. When fluoride is ingested, about 90% of it is absorbed into the bloodstream. Much of it is excreted, but the rest is deposited in bones and teeth (W.H.O. 2003). For many countries, cavities represent a problem of public health. Thus, consumption of fluoridated water and foods prepared with excessive fluoride salt are methods that

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can cause dental fluorosis. Given that there is a linear relationship between the intake of fluorine F (mg F / kg / day) and the prevalence of cavities or dental fluorosis, not only the concentration of fluoride in drinking water must be controlled, but also all other sources of exposure to F must be avoided (WHO. 1986, Martinez- Mier, 2012). Scientific Studies and Expert Committee World Health Organization (W.H.O.) on oral health suggested that fluoride in dentin can be considered a biomarker of cumulative exposure to fluoridation (W.H.O. 2003).

MATERIALS AND METHODS

We investigated the concentration of fluoride and mineral composition in drinking water from centralized sources of water distributed to the population and from individual sources (wells) of N=289 samples of water taken from 9 counties in Transylvania (Romania): Alba, Bihor, Bistrita, Cluj, Maramures, Mures, Salaj, Satu-Mare and Sibiu between 2009-2014. In a pilot study it was determined the concentration of all minerals in dentin of permanent teeth decayed, collected from 30 adults (males) aged 40-60 years, who lived permanently from their birth in three different areas: two areas exposed to lead pollution (workers in non-ferrous metallurgy industry) (CT group and SM group) and a control zone (Cj group). Permanent decayed (premolars and molars) adult teeth were examined for cavities by the dentist. Patients said they had fluoride intake from drinking water, toothpaste and food. These patients have not practiced local fluoridation treatments or administration of fluoride supplements. In accordance with national health legislation, water samples for individual consumption were collected by the beneficiaries from the drinking water distribution network and from individual sources (wells) (Law no. 458/2002). Determination of mineral composition in water samples and fluoride in dentin of permanent teeth decayed was used a ion chromatography method. For determining the metals in dentin of teeth we used absorption optical emission spectroscopy method with inductively coupled plasma (ICP-OES) (Mohamed A. Amr. *et al*., 2010; Semaghiul *et al*., 2004).

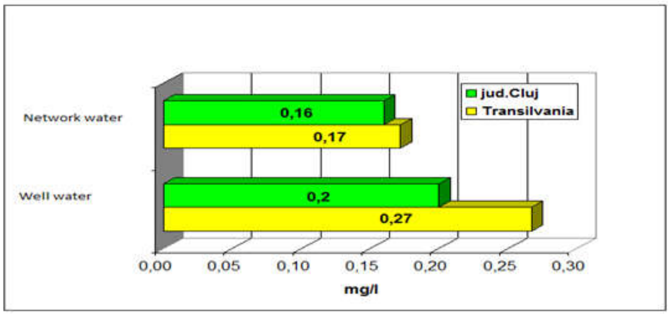

Figure 1. The average values of fluoride samples sources investigated in Transylvania area and Cluj county (mg F/l)

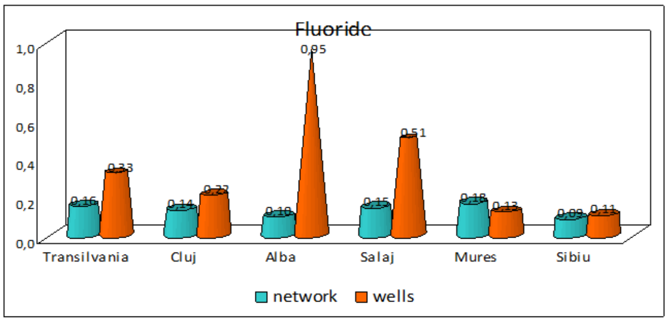

Figure 2. Average concentration of fluoride samples investigated from water drinking sources in Transylvania (mg F/l)

No values over the maximum admissible concentration were registered in Cluj. The average concentrations of fluoride from analyzed water samples are far below the permissible limits maximum permitted quantity. The values of fluoride obtained in the water samples analyzed classify Transylvania as a deficiency area in fluoride (Law no. 458/2002; Creţeanu, 2009; Creţeanu *et al*., 2015). In the water samples with low fluoride concentrations we found also low concentrations of Na, K, Ca and Mg, showing a poor overall mineralization of water which, together with a general lack of fluoride, has a potential negative impact on the health of consumers, including oral-jaw system.

Table 1. The statistical analysis of all the mineral composition of water sources investigated in Transylvania

	Statistical parameters	Ca mg/l	Mg mg/l	Na mg/l	K mg/l
Well water 72 samples	Average	76.88	17.46	258.76	12.09
Transilvania Network water 217 samples	Median	59.48	18.09	234.51	13.32
	SD	22.79	4.08	21.90	3.21
Transilvania N=289	Statistical parameters	Ca mg/l	Mg mg/l	Na mg/l	K mg/l
	Average	34.92	28.82	25.19	66.09
	Median	21.21	25.33	15.00	53.06
	SD	12.85	9.107	12.02	6.49

RESULTS

The results of this study highlight in the analyzed water samples (N=289 samples) from the area of Transylvania show an average value of fluoride of 0.27±0.43 mg F/l in well water and 0.17±0.23 mg F/l in the network water. Among the total investigated samples from Transylvania area, 8 samples (2.86%) have high fluoride levels that exceed the maximum admissible concentration and have a potential risk for dental fluorosis for consumers. The average concentration of fluoride in the water network in Cluj area and Transylvania area is represented in Figure 1.

We can say that the waters investigated in Transylvania presents a low grade mineralization, both in terms of macro (Ca, Mg, Na, K) and of some microelements (especially F) (Table 1). The results of this study classify this region as a fluoride deficiency area. Eight out of all investigated samples have high fluoride levels that exceed the maximum permitted quantity. From the point of view of the balance between the effect of preventing dental caries and dental fluorosis risk, "the fluoride optimal provision" was determined to be in the range between 0.05 and 0.07 mg F/kg/ day (Popa *et al*., 2006). In the second part-a pilot study included 30 adults (males) aged 40-60 years, who lived permanently since birth in three different areas: two areas exposed to pollution Pb (workers in non-ferrous metal industry) (CT group and SM group) and a control zone (Cj group). Permanent decayed (premolars and molars) adult teeth were examined by the dentist. For this purpose, we have used various techniques, including the use of microprobes. We determined the concentrations of known essential elements, possible essential elements and elements with no known biological role and/or that are potentially toxic (Al, Cd, Ba and Pb) found in permanent teeth (Table 2).

dentin samples from Cj group compared with the CT group had a representative statistical significance ($p<0.05$; $p<0.001$). It is calculated a statistically significant difference among average values Mo and Pb values between SM and CT group ($p<0.001$) (Table 3). The high values of metals in dentin samples found in CT and SM groups may occur by the professional exposure but also due to the existence of fillings with amalgam (Table 4).

Table 2. The analysis of trace elements in permanent decayed (premolars and molars) adult teeth (ppm)

	Cj group	SM group	CT group
	Mean±SD	Mean±SD	Mean±SD
Cd	0.12±0.07	0.15±0.14	0.19±0.12
Cr	3.14±2.21	3.87±0.78	2.94±1.21
Mn	2.70±1.48	4.51±2.97	5.09±3.11
Fe	171.08±88.73	317.21±201.53	280.51±135.68
Cu	8.02±3.91	10.44±3.59	15.36±9.06
Mo	0.64±0.75	0.58±0.16	0.13±0.12
Ni	15.82±6.81	22.66±7.04	24.04±9.84
Ba	4.49±2.58	6.31±2.63	4.87±2.53
Pb	2.94±1.41	1.28±1.38	3.57±1.20
Al	18.14±4.38	16.29±3.45	20.16±7.38
Zn	197.84±47.13	143.42±28.16	219.05±64.64
Hg	0.79±0.50	2.65±2.38	6.65±3.72

Table 3. The statistical analysis of all the mineral composition in dentin (the Student test from 10 samples)

	The significance of differences in mean					
	Cj-SM group		Cj-CT group		SM-CT group	
	t	p	t	p	t	p
Cd	-0.63	0.54	-1.57	0.13	-0.66	0.52
Cr	-0.99	0.34	0.25	0.80	2.05	0.05
Mn	-1.73	0.10	-2.20	0.04	-0.43	0.67
Fe	-2.10	0.05	-2.13	0.05	0.48	0.64
Cu	-1.40	0.18	-2.33	0.04	-1.60	0.14
Mo	0.25	0.81	2.03	0.08	7.15	<0.001
Ni	-2.21	0.04	-2.17	0.05	-0.36	0.72
Ba	-1.57	0.13	0.33	0.74	1.25	0.23
Pb	2.67	0.02	-1.07	0.30	-3.96	0.001
Al	1.05	0.31	-0.75	0.47	-1.50	0.15
Zn	-1.50	0.15	-9.90	<0.001	-3.39	0.003
Hg	-1.53	0.22	-3.81	0.01	-2.07	0,07

Table 4. The statistical analysis of all the mineral composition in dentin (the test correlations "r" Pearson from 30 samples)

Total	Fl	Cd	Cr	Mn	Fe	Cu	Mo	Ni	Ba	Pb	Al	Zn	Mg	Ca	Hg
Fl	1														
Cd	-0.41	1													
Cr	0.01	-0.10	1												
Mn	0.01	-0.11	0.11	1											
Fe	0.02	0.14	0.08	0.46	1										
Cu	-0.25	0.22	-0.16	0.16	0.27	1									
Mo	0.10	-0.24	0.80	-0.17	-0.06	-0.34	1								
Ni	-0.19	0.05	0.48	0.58	0.18	0.30	0.15	1							
Ba	0.07	0.19	0.15	-0.19	0.26	-0.01	0.14	0.02	1						
Pb	-0.24	0.15	-0.61	-0.07	-0.19	0.27	-0.49	-0.25	0.00	1					
Al	-0.23	0.11	-0.21	0.13	0.25	0.49	-0.14	0.12	0.03	0.18	1				
Zn	-0.34	0.16	-0.37	0.01	0.14	0.35	-0.35	-0.24	0.08	0.47	0.62	1			
Mg	-0.14	0.30	-0.02	-0.12	0.12	-0.19	-0.06	0.07	0.02	-0.18	-0.15	-0.32	1		
Ca	-0.06	0.05	-0.11	0.20	0.11	0.20	-0.35	0.28	-0.04	-0.14	-0.21	-0.42	0.32	1	
Hg	-0.34	0.35	0.01	-0.20	0.29	0.53	-0.40	-0.24	0.30	0.25	0.28	0.52	-0.09	-0.03	1

The results obtained from the analysis of trace elements in permanent teeth are summarized in Table 2. We found a statistically significant difference in the concentrations of Zn, Pb, Cr, Mn, Fe, Cu, Mo and Ni among permanent teeth in different groups (Table 3 and Table 4). In dentin samples from the CT group, 4 samples were found with high lead values (>4 µg / g). The concentration of Pb in the teeth can be used as an index of environmental pollution and poisoning lead. The levels of Mn, Fe, Cu, Ni and Zn determined in

The average concentrations of fluoride in teeth decayed in the three groups are represented in Figure 3. The average concentration of fluoride in decayed permanent teeth dentin in CT group is statistically significant decreased comparative to the other two groups Cj and SM ($p<0.05$) (Table 3). In our study the levels of minerals and fluoride which were determined in dentin samples of decayed permanent teeth had a significant negative correlation (Table 3).

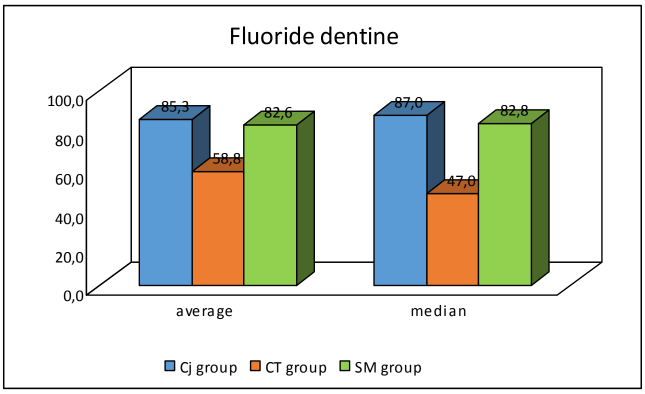

Figure 3. The average concentrations of fluoride dentin from permanent teeth decayed

DISCUSSION

In the analyzed water samples low concentrations of fluoride were highlighted along with low concentrations of Na, K, Ca and Mg, showing a poor degree of global mineralization of water. The general lack of fluoride has a potential negative impact on the health of consumers including dental-maxillary apparatus (Badea *et al*., 2007; Creţeanu *et al*., 2015; Thomson *et al*., 2000). We can say that the investigated waters in Transylvania presents a low degree of mineralization, both in terms of macro-elements (Ca, Mg, Na, K) and of some micronutrients (especially F). The results indicate that the levels of fluoride in drinking water are generally small in terms of legislation that rules the quality of drinking water (Law no. 458/2002). Therefore, most of the drinking water (approximately 95%) investigated in this study had a low level of fluoride. This lack of fluoride in drinking water, which is a major way of intake for humans, can cause problems for human health, especially for teeth and bone structures (Badea *et al*., 2007; Lussi *et al*., 2006; Martinez-Mier, 2012; Moynihan, 2005; Patil *et al*., 2014). Statistically, the concentration of fluoride in drinking water in Romania falls in maximum permitted quantity. Lower concentrations (0.5 mg F/l) correlates with endemic decay and the highest ones (more than 1.5 mg F/l) cause fluorosis, a process in which calcium from hard tissues is replaced with fluorine affecting teeth (dark brown stains) and all human bone tissue (Badea *et al*., 2007; Barlean *et al*., 2013; Popa *et al*., 2006).

This result are important to the practicing dentists in the area of Transylvania for prophylaxis of tooth decay and dental fluorosis taking into account all sources of fluoride, including the water. The most significant contribution of fluoride for the body is made from water, being between 2/3 and 3/4 of the fluoride daily intake as compounds with high solubility coefficient that are easily assimilated by the body in proportion of 97%. The level of exposure for an individual via drinking water is directly proportional to the level of water fluoride and the daily amount of consumed water (Martinez-Mier, 2012; Rojas *et al*., 1999). Fluoride has been shown to reduce dose dependent molar crowns formed in the dentin and to increase the risk of caries. Fluoride uptake is probably related to the tubular structure of dentin amount of dentinal fluid and metabolic activity of the dentin. This finding supports the possibility that fluoride ions may have an odontoblas-mediated effect on dentin formation (Sinikka, 1995). Daily increased consumption of mineral water in addition to the public water supply could have implications for the safety of fluoride supplementation (Jayalakshmi *et al*., 2011). Water fluoridation was considered likely to have a beneficial effect, but the range could be anywhere from a substantial benefit to a slight risk to children's teeth with a narrow margin between achieving the maximal beneficial effects of fluoride in caries prevention and the adverse effects of dental fluorosis (McDonagh *et al*., 2000). Our study has shown that fluoride level found in carious dentin of permanent teeth taken from the CT area is significantly lower (58.76 ± 19.81 µg/l) comparing to the concentration of fluoride found in Cj and SM groups. In our study we have investigated the importance of the location of trace elements in the teeth, with a focus on Mo, Cr, Zn, Hg and Pb. Our results indicate that trace elements are systematically distributed within the dentine of teeth caries (Budd *et al*., 2000). Fluoride concentration of dentin has been suggested as a biomarker for cumulative fluoride exposures. Fluoride concentration of the dentin of permanent teeth of lifelong residents is correlated with the fluoride concentration of the community water supply and consumption of high-fluoride containing foods (Cruz *et al*., 2008). Yet no study has compared lifetime systemic fluoride intake from multiple sources with dentin fluoride concentration, an analysis that is necessary to determine if dentin fluoride might serve as a valid biomarker of fluoride exposures.

This study quantifies exposures to fluoride in drinking water sources and relates them to the concentration of fluoride in dentin of permanent teeth. The lack of definitive conclusions could be explained by the presence of many factors (fluoride in water, table salt, fluoride supplements, mouthwash, toothpaste for children) that might influence the storage of fluoride in dentin (Rojas-Sánchez *et al*., 1999). The results of several investigations in humans and rats indicate that Mo when present in food and drinking water may be responsible for caries reduction. Other studies suggest that similar interactions may exist between F and Mn, Al and F (Mohamed A. Amr *et al*. (2010). We found a statistically significant difference in the concentrations of Zn, Pb, Cr, Mn, Fe, Cu, Mo, Ni, and Ba among permanent teeth in different groups. In dentin samples were found with high lead values (>4 µg / g). The concentration of Pb in the teeth can be used as an index of environmental pollution (Mohamed A. Amr. *et al*. 2010). This element is preferentially incorporated and stored in calcified tissues, such as the teeth. A Pb concentration above 4 mg/kg in the teeth has been suggested as being indicative of Pb toxicity (Mohamed A. Amr. *et al*., 2010). According to these observations, we conclude that the trace elements are positively associated with caries. Geographic location can explain the variation of the concentrations of fluoride in dentin. The setting up of the three groups included in our study was on the basis of some criteria: gender (males), age (40-60 years), lead exposure (industrial areas), social and cultural origin, medical and dietary conditions that lead to a variation of total fluoride ingestion between communities.

Conclusion

This study revealed the presence of low levels of fluoride in drinking water network in Transylvania. The values of fluoride obtained in the analyzed water samples classify Transylvania as a deficiency area with an increased frequency of tooth decay among the population. The water sources with the highest risk for both deficiency and excess of fluoride are represented by individual wells. For this reason, the surveillance of fluoride levels in all sources of drinking water is recommended in order to apply effectively other methods of prophylaxis by the consumers according to the indications of dentists. The association between fluoride water exposures and dentin fluoride was weak in this study, particularly for permanent teeth. The variance in dentin fluoride seems to indicate that primary molar dentin may be a promising biomarker for fluoride exposure. This study suggests that a major source of variability in dentin fluoride is the community itself, independent of water fluoridation status. Future studies will need to account for community residency status, which will require a large sample to separate individual and community level contributions to dentin fluoride. It was suggested that the effect of Mo on dental caries is due to a higher F-retention. The concentration of Pb in the teeth can be used as an index of environmental pollution and poisoning lead According to these observations, we conclude that the trace elements are positively associated with caries. Multivariate analysis revealed three of the variables independently correlated with the development of carious lesions: concentration of fluoride in the water, poor overall mineralization of water and the levels of minerals increase in dentin of teeth from the exposure people. The sample size prevent us to fully test the effects of some variables upon minerals and fluoride in dentin. The strong effect of the geographical location implies that these communities have unique characteristics which are important determinants of minerals and fluoride in dentin, regardless of the individual characteristics. The presence and/or absence of trace elements in the environment influence their availability to humans.

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