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Application of PIGE to Determine Fluorine Concentration in Human Teeth : Contribution to Fluorosis Study

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Fluorosis, osteoselerosis, and systemic illness may result when tolerance levels of fluorine intake are exceeded. To avoid these abnormalities, we shall be aware of the mechanisms of their development. For this, quantitative results gathered from different parts of the world are necessary. In this paper, PIGE method is used to determine fluorine concentration in human teeth, collected from different regions of the Algerian Sahara. Both enamel and dentine regions were inspected. The external and internal mappings of the analyzed teeth have shown more variation in the localized fluorine concentration in dentine than in enamel, and the lowest level of fluorine concentration was observed in the frontier crown-root zone. The obtained results reveal relatively high fluorine concentration lying within the limit of fluorosis, according to the standardised grading dental fluorosis.

1. Introduction

In human nutrition fluorine plays a dual role; to prevent tooth decay at a certain level of intake[1,2] and to cause serious damages in bony and dental tissues. When restrained to low level, it can play a role of an inhibitor against certain enzyme systems. But, an excessive ingestion can result in dental fluorosis and bone abnormalities. Skeletal changes and mottled enamel may result when drinking water content of fluorine exceeds few ppm. There is worldwide evidence that fluorine intake above tolerance levels over certain period leads to cumulative effect resulting in systemic illness and osteosclerosis. Several dental fluorosis has been associated with developmental disturbances of ossification. However, bone can be affected at all ages. Clinical inspection constitutes a belated diagnostic and when the harmful effect of fluorine is recognised, no treatment or medicine can be applied. Since fluorine is beneficial at trace amounts, it can not be subtracted from human nutrition. An optimisation of fluorine intake is of a vital importance and this requires deep knowledge of the mechanisms of both of its beneficial and harmful actions on the organism and a sensitive method for its analysis. Conventional methods such as potentiometry are suitable when samples to be analyzed are fluids. These techniques necessitate sample preparations that limit their efficacy and are, usually, destructive. The well known methods for trace elements analysis are the IBA (Ion Beam Analysis) techniques for their advantage to be non destructive and to provide concentrations with high sensitivity. For elements such as Li, Be, B, and F, nuclear reaction analysis with γ-ray emission is recommended. This technique provides quantitative and efficient measurements with sensitivity better than 0.1% for light elements. The γ-ray peaks are generally well isolated and the energy is high enough that correction for absorption is not necessary. No special preparation is needed for the samples. This gives the possibility to analyse the sample without affecting its morphology, allowing fluorine mapping. Having high sensitivity, PIGE is suitable to analyse volatile elements such as fluorine. It permits also the analysis of a great number of samples under same conditions, which result in an accurate comparison. The $^{19}F(p, \alpha\gamma)^{16}O$ reaction has been commonly used for fluorine determination. Many studies have been devoted to tooth analysis. Methods such as PIXE, RBS, and PIGE have been successfully applied to study the cause-effect relationship between fluorine intake and teeth health[2-5] and to determine the role of trace elements such as Al, Fe, Se, Sr, Cu, Mn, and Cd in the mechanisms of inhibition or formation of dental caries in order to develop further progress in understanding dental physiology.[4, 6]

One of the main output of these investigations concerns the concentration variations observed at the enamel surface and at the enamel-dentine junction. Dentine showed less uniformity in fluorine concentration than enamel. Large variations of fluorine concentrations have been observed between the enamel and the dentine part of the teeth.[7] Considering the effect of a single fluoride dose on pre-eruptive enamel mineralization of hamster tooth germs, Lenglet et al found the largest concentration of fluoride at the enamel-dentine junction.[8] Depth profiling measurements of fluorine indicate that it is steeply peaked at the enamel surface.[7, 9] The rate enamel-fluorine/dentine-fluorine may give an indication about the way by which the fluorine enters the tooth since fluorine can be mobilised from bone adjacent to a tooth and affect the formation of enamel.[10] Fluorine can enter the tooth directly through water, food, or other dental products. We expect in this case that the fluorine content of enamel is higher than the dentine fluorine content. Inverse situation may be encountered if fluorine mostly reaches the tooth from the blood. The differences found in the literature can be explained by the dietary habits of the populations considered. The population residing in the south of Algeria may serve as a good example to determine the itinerary of fluorine teeth content since in this geographical area fluorosis is widespread.

2. Experimental

2.1. Sample and standard preparation. Fluorine concentration determination in human teeth from different regions of the Sahara was carried out. About 20 human teeth were selected with detailed dental histories of the donors. Healthy teeth were obtained after extraction due to periodontal diseases. The considered teeth do not present any visible colour change.

Prior to the analysis, the teeth were first cleaned using a soft tooth brush and washed in distilled water. Then, treated with

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H_2O_2 (10%) and HCl (10%) solutions to remove organic materials and the exterior layer, respectively. Each tooth was rinsed in deionised water, dried in a clean environment, and stored individually in containers. The teeth were cross-sectioned along the vertical plane to permit the analysis of both sides of the teeth; enamel and dentine. To avoid current variations, due to cumulative charges that may be resulted from bad electrical conductivity during the analysis, a thin (50 Å) layer of aluminium was evaporated on the teeth surfaces prior to the analysis.

Hydroxyapatite (HA) was synthesized from calcium and phosphorus precursors by a wet chemical method as reported in Reference 11, and used as standards. A 0.3 M aqueous solution of $(NH_4)_2HPO_4$ was added, drop by drop, to a 0.5 M aqueous solution of $CaCl_2$ at 60 °C. The pH was adjusted to beyond 10 with concentrated NH_4OH and aged for 24 hours under stirring. The resulted precipitated HA was filtered, washed with distilled water, and microwave irradiated for 15 min. The obtained powder was compressed into pellets of 1 mm thickness and then dried in an oven.

2.2. Experimental set up. The $^{19}F(p, \alpha\gamma)^{16}O$ resonant nuclear reaction at a proton energy of 1375 keV was employed to investigate the distribution of the concentration of fluorine along vertical sections of human teeth. The PIGE experiments were carried out by employing the 3.75 MV Van de Graff accelerator. Three sets of diaphragms were used to collimate the beam to a diameter of about 1.5 mm, well defined under different experimental conditions at the sample position. The samples were placed at an angle of 45° with respect to the beam direction and mounted onto the sample holder with the help of a bronze disk frame. The stability of the beam current was checked using a current integrator. The simplified experimental arrangement is given in Figure 1.

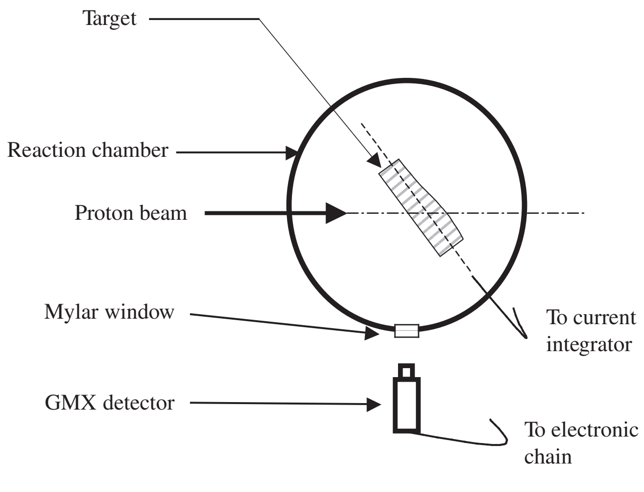

Figure 1. Experimental set-up.

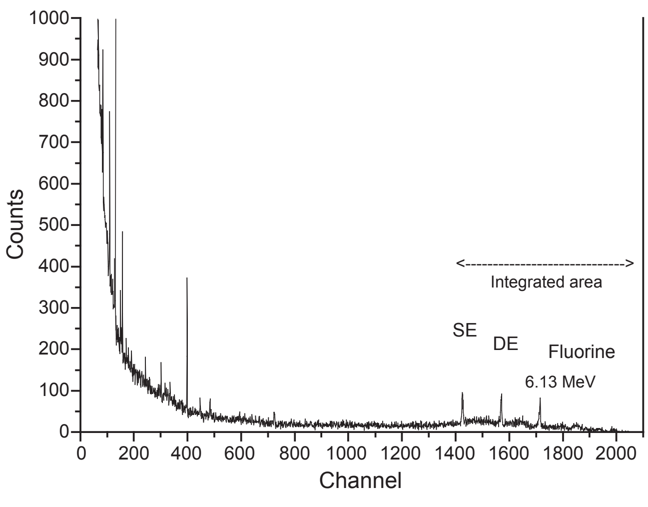

Figure 2. Typical γ-ray spectrum obtained for a molar tooth selected from R01 group.

The emitted γ-rays were detected via 6 μm Mylar entrance window, at 55° with respect to the beam axis by a high purity germanium detector (GMX) of 1.90 keV resolution at 1.33 MeV (^{60}Co). Energy calibration was performed using the well known resonances of the $^{19}F(p, \alpha\gamma)^{16}O$ and $^{27}Al(p, \gamma)^{28}Si$ reactions.

An accurate determination of fluorine depends on the correct identification of the interfering resonances and γ-ray, and on minimizing the interferences. The background of the measured γ-ray yield was mainly due to natural background radiation, competing reactions and noise in the electronics. The γ-ray energies of interest are 6.13, 6.92, and 7.12 MeV. The 6.13 MeV γ-ray was used. The natural background occurs mainly below 3 MeV, much below the considered energy range of measurements. As it is shown in Figure 2, in the high energy region where single and double escape peaks are observed besides the full energy peak corresponding to the 6.13 MeV, the background yield is very low and no interference for this range of energy is known at proton energies lower than 3 MeV. The competing intensity of the background is negligible and the measurement can be made over the whole region, permitting to sum up the counting rate over a large region reducing the standard deviation.

The beam intensity (≈ 50 nA) was chosen in such a way to limit the effect of desorption under beam impact. All the measurements were made under the same conditions for a counting charge of 1×10^5 μC. The cross section of the reaction being high (300 mb), the measurements can be considered as accurate and precise. The calculated uncertainties are statistical, mostly of the order of 8%.

Fluorine concentration is determined by comparison to the standard, using a simple expression:

$$C_{FS} = \frac{C_{ST} S_S Y_{FS}}{S_{ST} Y_{ST}},$$

where Y_{FS} and Y_{ST} correspond to γ-ray yields measured by detecting the prompt γ-ray emitted respectively from the sample and the standard, S_S and S_{ST} are the stopping powers, calculated at the energy corresponding to a half of the yield obtained at the incident energy. For accurate elemental determinations the standard spectra were taken in the same geometry as the sample and chosen to have quite similar properties to allow an extrapolation in determining the relative stopping powers. Using this method, correction for absorption is not necessary.

3. Results and Discussion

The results presented here concern a set of twenty persons originating from Algerian desert. None manifested clinical symptoms of fluorosis (dental lesions or brown discoloration).

An example of a typical γ-ray spectrum obtained from a molar enamel, using $^{19}F(p, \alpha\gamma)^{16}O$ reaction at 1375 keV is shown in Figure 2. The 1375 keV resonance is chosen for its high cross section, permitting to reduce the beam intensity on the samples. Fluorine concentration in teeth samples were determined by direct comparison of the ratio of their γ-ray yields, measured from the areas covering all the peaks appearing at energies 6.13, 6.92, and 7.12 MeV, with those from the standards. The fluorine content in the standard is chosen of the order of that expected in the sample to assure identical environments for the measurements.

Groups of 2–3 persons were considered for each region, selected according to their nutrition habits. For a good accuracy of fluorine concentration determination, each analysis was repeated several times. Average fluorine concentration values obtained are summarised in Table 1.

TABLE 1: Fluorine concentration in the enamel, dentine and crown-root junction of teeth samples collected from the south and north of Algeria

Region		Average fluorine concentration (mg/g)			
		Enamel		Crown-Root junction	Dentine
		Surface	Interior		
South	R01	4300	3700	2900	3200
	R02	3700	3600	2900	3500
	T01	4500	3500	3000	3300
	B01	1300	1030	840	1000
	B02	4000	3900	2900	3500
	B03	3700	3090	2900	3100
	B04	1500	–	1100	500
North	Algiers	1030	–	–	–
		740	–	–	–
	Oran	750	–	–	–

B, R, and T denote the southern regions and the given values correspond to an average taken over a group of 2-3 persons.

TABLE 2: Contents of calcium, magnesium, sulphates, and fluorides in drinking water in southern Algeria

Region	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	$SO4^{2-}$ (mg/L)	F^- (mg/L)
B01	152	31	674	0.23
B02	100	57	501	1,40
B03	120	52	410	1.85
R01	72	30	655	2.49
R02	140	263	710	1.73
T01	300	197	530	2.79

TABLE 3: Average fluorine concentration mapping obtained for both surface and interior sides of a molar tooth, taken from R01 group

Tooth region	Concentration (μg/g)	
	Surface	Interior
Crown	4490	3740*
	4020	2600
Frontier	2900	2500
Root	5890	3080*
	4300	4100
	2900	2520

* From up (crown) to down (root) as shown in Figure 3.

A comparison is made with the fluorine levels found in northern regions. The results reveal high fluorine concentrations in the southern regions, except for two groups (B01, B04) where values ≤ 1500 μg/g are found for the outer layer and 1000 μg/g in the internal regions of the teeth. Mostly, the fluorine concentration fluctuates between 3700 and 4500 μg/g along the outer regions of the teeth, while the concentration value in the internal regions is found to lie between 3090 and 3900 μg/g.

The main concentration values lie within the limits of fluorosis, according to the standardised grading dental fluorosis. But, these are still not too high as could be expected by clinical findings, even if neither lesions, nor discolorations were observed. It is clear that the set of 20 teeth analysed here is not suitable for conclusion of a statistical quality. However, it may give insights in studying the mechanisms of fluorosis. Excess of fluorine may not be directly correlated to fluorosis since no clinical symptoms were observed on analyzed samples. The role of other elements may be crucial in its development.

From the northern regions, considered in the present study, less fluorine concentration is observed. The average value lies in the range reported for low fluorine levels in environmental areas.[12]

The highest fluorine concentration recorded in the southern regions is associated with the environment area. The hot climate, characteristic of these regions, leads to high level fluorine intake via drinking water and fluorine rich soil chemistry results in foodstuffs containing high levels of dissolved fluorides. This agrees well with the fact that fluorine contents found in enamel are higher than those obtained for dentine regions. Clinical studies have shown that fluoride intake rapidly enters mineralized tissues (bone and developing teeth). The factors controlling the incorporation into dental structures have been reviewed by Weidemann.[13] It is reported that fluoride content in adult teeth reaches about 2700 μg/g in areas with fluoride concentration in the drinking water of 3 mg/L.[13]

Fluoride levels in fresh water vary according to geographical location and proximity to emission sources. Surface water concentrations generally range from 0.01 to 0.3 mg/L. Fluoride levels higher than 25 mg/L are found in areas where the natural rock is rich in fluoride and close to industrial outfalls.[14]

In the south of Algeria where endemic fluorosis is recognized, subterranean sources are used to supply the population with drinking water. To assess information on the relationship between the appearance of fluorosis and the concentration of fluorine in drinking water, many studies have been undertaken. The obtained results show that in these regions, water is characterized by high levels of fluoride content, often associated with excessive hardness and mineralization.[15]

Content average in calcium, magnesium, sulphates, and fluorides found in drinking water in southern Algerian regions is of about 190 ± 31 mg/L for Ca^{2+}, 120 ± 24 mg/L for Mg^{2+}, 520 ± 50 mg/L for SO_4^{2-}, and 1.6 ± 0.3 mg/L for F^-.[16] However, according to certain studies, values as high as 5.82 mg/L were found for fluorides in the eastern zones of the south.[17,18] In Table 2, the reported values correspond to regions that are close to those considered in the present work. Nevertheless, lower values of the order of 0.5–0.9 mg/L F^- are obtained for waters provided by deep underground sources.

Additional fraction of fluoride may come from foodstuffs. Tea and date, particularly rich in fluoride, constitute daily nourishment in these regions. Fluorides content in dates can reach 14 to 23 mg/kg and in tea leaves 50 to 125 mg/kg. Date is sufficient to provide daily fluoride intake of 0.5 mg.[17,18] Summarized results of studies aimed to assess the levels of fluoride in some vegetables, cultivated locally, give: potatoes 14.3 ppm, tomatoes 9 ppm, watermelon 12 ppm, and tea leaves 31 ppm.

In spite of the high fluoride content, certain regions are not concerned with dental fluorosis. This is attributed to the high magnesium levels found in the corresponding regions, due to the high adsorption rate of fluorites on the formed $Mg(OH)_2$ sites.[19]

In northern regions, drinking water is supplied by surface waters. Fluoride content found varies from 0.15 to 0.3 mg/L.[20]

Table 3 gives a detailed mapping (as shown in Figure 3) of a molar tooth collected from R01 zone. Significant variations either for enamel or dentine were found for the whole analyzed teeth. However, it should be pointed out that the fluorine con-

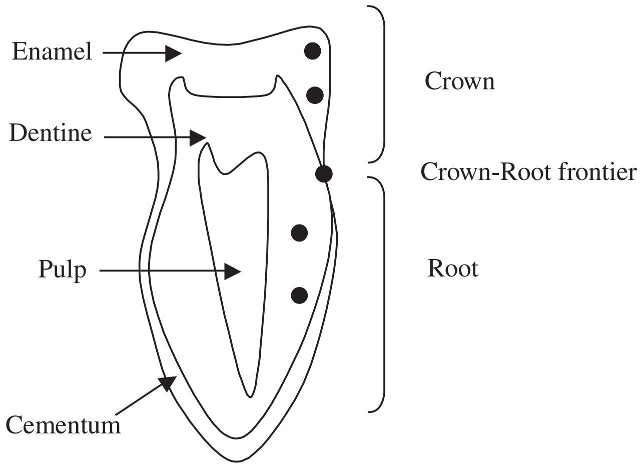

Figure 3. Schematic diagram showing the anatomy of a cross-sectioned tooth along vertical plan. The dark areas correspond to the analyzed regions (beam impacts).

centration is found to decrease from up (crown region) to down (root region) over the scanned tooth. This suggests, once again, that fluorine is provided mostly by water. In the considered regions, dental products are not widely used.

The most remarkable finding of this study is the lowest level of fluorine concentration observed in the frontier crown-root zone.

4. Conclusion

The fluorine intake in teeth of adults residing in the south of Algeria was studied using PIGE method for teeth analysis. High concentration levels were found compared to northern regions. These findings may be explained by a consumption of high amount of water as a consequence of hot climate.

Tea, well known to be rich in fluoride, constitutes the main source of drinks in these regions and may be considered as an important provision for fluorine intake.

These results are obtained for the selected specimens that were extracted from questioned persons about their age, oral hygiene and consumption habits. At time being, no conclusion can be drawn regarding these factors, more dental tissues have to be analysed.

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