International Journal of Physics and Applications.
ISSN 0974-3103 Volume 2, Number 3 (2010), pp. 123--134

http://www.irphouse.com

Estimation of Trace Elements in Various Parts of Human Teeth using External Beam PIXE

R. Venkateswara Rao[1], P. Mary Anupama[2], D. Guru Mahesh[2], A.W. Iqbal[3], Y. Ramakrishna[3] and P. Venkateswarulu[1,*]

[1]Dept. of Engg. Physics, [2]Dept. of Biotechnology, College of Engg., ANITS, Sangivalasa, Visakhapatnam-531162, A.P, India.
[3]Department of Engineering Physics, College of Engineering, Andhra University, Visakhapatnam-530003, A.P., India
**Corresponding Author E-mail: swarulu@gmail.com*

Abstract

The External Proton Induced X-ray Emission (External PIXE) technique was employed to study the elemental profiles and their concentrations on the various parts of human teeth. In the present study, various elements including trace namely P, Ca, V, Mn, Fe, Ni, Cu, Zn, Ba, As, Sr and Pb were estimated on the different parts of human teeth . Among the identified, P and Ca were found to be the major elements whereas all other elements were in trace level. It was noticed that the elemental patterns on the carious region of the teeth are severely affected compared with other portions of teeth. The technique is found very much useful in estimating the trace elements on the different parts of human teeth.

Keywords: External PIXE; human teeth; dental enamel; dentine; cementum; dental caries; trace element.

Introduction

Trace elements play an important role in human teeth as well as health. In recent years, there has been growing interest in understanding the exact role played by trace elements in several disorders and diseases. Though trace elements constitute a minute part of the living tissues, they are important for the vital processes of life. Various diseases of previously unknown etiology have been attributed to an imbalance of trace elements. Both deficiency and excess of trace elements, resulting from exposure to all the natural and man made environment, have been associated with many diseases and disorders including dental caries, tooth decay and erosion etc leading to a wide variety of clinical effects [1]. Because of the fundamental importance of trace elements in a number of biochemical and physiological processes in humans, it is reasonable to postulate that they may influence mechanisms that are responsible for the development of various parts of human teeth such as enamel, dentine, cementum and promoting many disorders and diseases. Teeth are reported to be suitable indicators of trace element exposure for a wide range of elements. Since teeth accumulate a variety of trace elements, it is very interesting to study the elemental characterization in human teeth to evaluate biological processes. The elemental concentrations and their distribution in teeth can provide information about physiology of elements, environmental influence, contamination by metallic amalgams used as restorative materials and dietary habits [2]. In this way, the study of the spatial distribution of trace elements in tissues is involved in many biological functions of living organisms provide the information and probable cause for the imbalance, leading to disorders [3].

Particle induced X-ray emission technique (PIXE) is an analytical technique widely used to estimate the trace elemental concentrations in several types of systems. The use of this technique was first demonstrated by Johansson et al. in 1970 [4]. Several authors have reported that PIXE technique has great potential in the estimation of trace elemental concentrations in biological samples [5-10]. Mangelson and Hill in 1981 and Valkovic in 1980 reviewed the application of PIXE technique in the biomedical field [11-12]. Thus, with the development of PIXE technique, it is possible to study several biomedical problems in nuclear research laboratories. The trace elements absorbed by the body enter the digestion system, pass through gastrointestinal tract and are deposited in the liver by blood stream. From the liver they are carried to different organs for participating in biochemical reactions. Usually, the ions of trace elements act as coordination centers to build up the structure of enzymes or proteins [13]. Thus, when the concentrations of the trace elements in the body differ from the normal values, many clinical and pathological disorders arise [14].

The estimation of the major, minor and trace elements present in biological and biomedical samples by non-destructive analysis are of great importance, because these materials are sometimes too precious to be analyzed destructively. Ion beam techniques have been widely employed in dentistry and their sensitivity for trace elements in various thick tissue specimens was attentively investigated [15-18]. Teeth are not homogeneous in elemental composition as demonstrated in earlier studies [19-21] and consequently thick targets of human teeth were used for analysis. It has been reported that the concentration of Pb in teeth can be used as an index of environmental pollution [22-24]. Lead is preferentially incorporated and stored in calcified tissue such as teeth [25].

Among various analytical techniques, atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS) are becoming more routine, but they are destructive chemical methods involving sample dissolution procedure and their inability for analysis of samples in solid form. The electron probe micro analyzer (EPMA) technique is useful for the analysis of even a mono-mineral grain, but is limited to detection of elements at concentration more than 200 ppm. In this context, multi elemental non-destructive accelerator based external proton induced X-ray emission (external PIXE) technique looks more useful for elemental analysis of samples. It is fast, simultaneous, reliable, quantitative, multi-elemental and non-destructive with an excellent sensitivity in the ppm level and detection limits across a wide range of atomic numbers [26]. On the other hand, the samples can be irradiated as such in air which is extremely useful in analyzing any specific area of the samples. For all of these advantages, external PIXE technique was preferred for the present study for elemental characterization of the various parts on human teeth.

Materials and methods

Sample collection and processing

Human teeth samples were collected from the Anil Neerukonda NRI General Hospital, Sangivalasa, Visakhapatnam, India from individuals in the age group of 21-54. Prior to the teeth extraction, a detailed investigation of dental history and examination were carried out for each individual. Immediately after the extraction, each tooth was kept into oxygenated water (to clean the organic material from its surface) later the samples were acid cleaned and washed with deionized water and then with alcohol. The specimens were sealed in individual plastic containers in deep freezer till the irradiation (at -20^0 C). To avoid the contamination of the samples, proper cares were taken for the collection of specimens and their handling prior to the experimental part and analysis.

Experimental Design and Setup

Considering many factors such as charging effects, normalization and target adjustment, external beam setup was applied in this study. The elemental analyses of the teeth samples were carried out at Institute of Physics, Bhubaneswar which is the unique of its kind in India and was installed by Vijayan et.al, in 2003 [27]. A 3 MeV proton beam induced from the 3 MV tandem type horizontal pelletron accelerator (Model: 9SDH-2, make: National Electrostatics Corporation, Madison, USA), and hit on the human teeth samples which were in air. The proton beam was collimated by a graphite collimator to a beam size of 1-mm diameter. The beam was extracted into air using a Kapton^{TM} foil (8-µm thick) at the exit point of a vacuum scattering chamber [28]. The beam was first focused and centered at the target location inside the scattering chamber and then let through the thin Kapton^{TM} foil placed at the exit port. The Kapton^{TM} foil is used as exit window due to its several special characteristics like low beam-induced background emission, minimal energy loss and resistance to radiation damage. The beam was allowed to travel a few cm in air after which it irradiates the samples. Beam charge measurements were carried out by using a rotating vane chopper designed by Sahu, et.al, in 2003 [29]. The human teeth samples were irradiated with maximum beam current of 15nA. A Si (Li) detector (active area 30 mm^2) having energy resolution of 170 eV at 5.9 keV placed at 90^0 with respect to the beam direction was used to detect characteristic X-rays emitted from the target [27]. The detector has an entrance beryllium window of 8 μm thickness. A 25-μm thick aluminium absorber (with 6% hole) was kept in front of the detector to attenuate the bremsstrahlung background and the dominant low energy X-ray peaks [30].

Analysis of PIXE Data

The X-ray energies were acquired by the Si (Li) detector and collected by the Multi channel buffer on 4096 channel pulse-height analyzer to form energy spectra. Spectra were recorded by using a PC based multi channel analyzer. In the early period, PIXE spectrum analysis was carried out using AXIL software package. Using this software, it is not possible to get automatic quantitative analysis. In the present work, the latest version of GUPIX software package [31-35] has been used to analyze the spectra of human teeth. The concentrations of elements were obtained by analyzing bone standard reference materials NIST SRM-1486. The absolute elemental concentrations could be obtained through normalization to reference standard at a standard error of mean less than ten percent.

The relationship between characteristic X-ray yield Y (Z) for an element of atomic number Z and its concentration C_z in a given target matrix is

$$Y(Z) = HY1(Z)\, C_z Q \epsilon_z t_z,$$

Where Y1 (Z) is the computed Yield from the database per steradian per unit concentration and per unit integrated beam charge; Q the beam charge; ϵ_z the intrinsic efficiency of the Si (Li) X-ray detector; and t_z the transmission of the X-rays through any absorber placed between the detector and the specimen. The instrumental constant H is the product of the geometric solid angle of the X-ray detector and any systematic normalization factor present in the charge integration system. The instrumental constant H was measured using 3 MeV protons and a wide range of pure single-element standards emitting both K and L X-rays in the energy region 3–26 KeV. Thus the X-ray yield (Y) is converted to elemental concentration via a defined standardization technique involving H value, the theoretical yield, detection efficiency and filter transmission values.

Results and discussion

In the current study the set of various parts on human teeth were exposed to external proton beam and the experiment was carried out in the external PIXE set-up at Institute of Physics (IOP), Bhubaneswar, India. The external PIXE spectra of the dental enamel, cementum and dentine of human teeth are shown in figures 1, 2 and 3 respectively. A total of twelve elements including trace namely P, Ca, V, Mn, Fe, Cu, Ni, Zn, Ba, As, Sr and Pb were estimated in the present study and their respective concentrations are given in Table.1. It was observed from Table.1, the concentration of P ranged between 14.34% and 26.69% whereas Ca ranged between 20.36% and 45.57%. Since the mineral tissue of human teeth consists of hydroxyl-apatite (HAP) crystals $Ca_{10}(PO_4)_6(OH)_2$ as their primary inorganic constituent which contains calcium and phosphorous in it, hence the higher amount of Ca and P is obvious in these dental samples.

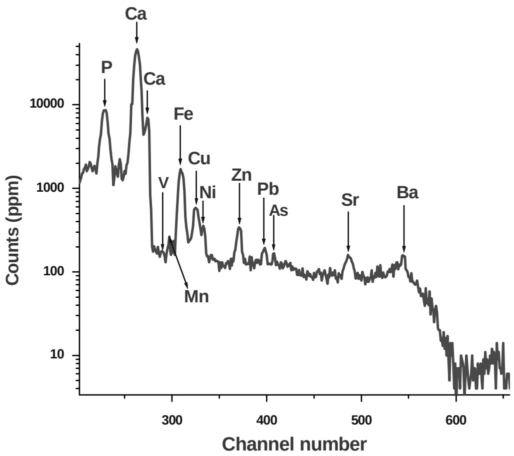

Fig.1. The spectrum of dental enamel of human teeth obtained from external PIXE

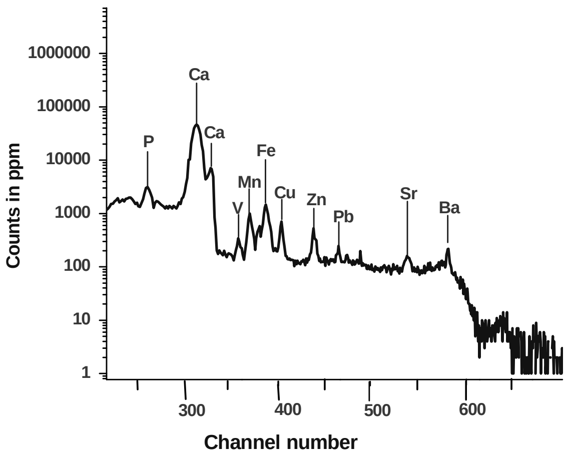

Fig.2. The external beam PIXE spectrum of cementum part of human teeth

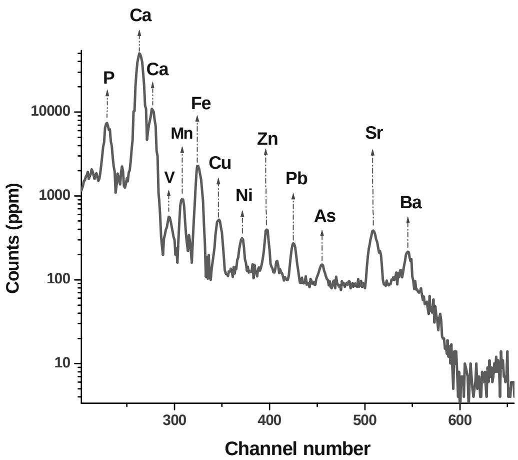

Fig.3. The external PIXE spectrum of dentine region of human teeth

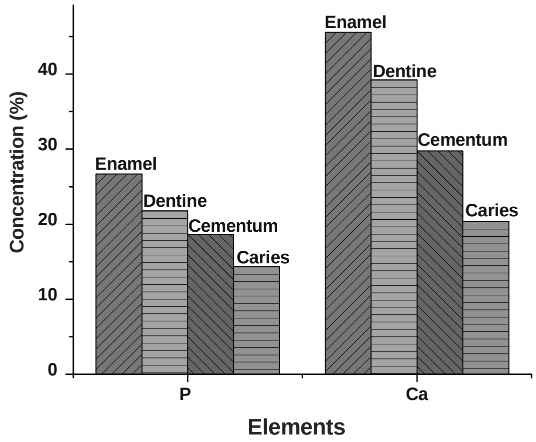

Fig. 4. Major elemental profile of Human teeth

Table. 1. The concentration of elements on the various parts of human teeth (values are in ppm by weight unless specified).

Element	The part of human teeth which is exposed to the external proton beam			
	Enamel	Dentine	Cementum	Dental Caries
P (%)	26.69	21.76	18.65	14.34
Ca (%)	45.57	39.23	29.78	20.36
V	19.98	12.89	15.87	11.45
Mn	10.23	8.93	9.41	8.03
Fe	89.14	76.37	46.59	94.25
Ni	19.85	17.94	18.34	10.68
Cu	11.92	16.73	10.45	28.96
Zn	48.58	40.41	42.92	59.75
As	08.63	11.96	10.28	17.82
Sr	189.62	176.48	205.31	104.87
Ba	34.81	32.93	28.52	21.58
Pb	14.24	10.06	08.95	19.49

To compare the elemental profiles of all exposed parts of human teeth a bar diagram was drawn for major elements and is shown in Fig.4. It is clear from Fig.4 that the concentration of Ca and P are low on caries region compared to dentine and cementum, where as they have highest concentration on enamel. The decrease in concentration of Ca and P on caries is due to presence of contaminants deposited during food intake and due to depletion of hydroxyapatite. On the other hand, the higher concentration of Ca and P in tooth enamel may be attributed to the higher percentage of hydroxyapatite in them because human dental enamel is the hardest tissue in the body with 91-96% of inorganic matter, 1-2% organic material and 3-4% of water in weight [36].

The concentrations of Ca and P by weight percentage should be 39.8 and 18.5 respectively for a stoichiometric hydroxyapatite with a Ca/P weight ratio of 2.15. On this basis, the concentration of Ca in enamel is little bit high and dentine in the teeth samples is nearly close to this value and P concentrations show slightly higher values. While the concentration of Ca in cementum is 29.78%, P concentrations don't show much variation as compared to the theoretical values. On the other hand, Ca and P elemental concentrations are severely depleted in the carious region. Calcium salts provide rigidity to the skeleton and calcium ions play a vital role in many metabolic processes. In the primitive exoskeleton and in shells, rigidity is generally provided by calcium carbonate, but in the vertebrate skeleton it is provided by hydroxyapatite which is embedded in collagen fibrils. But, since the Ca/P ratios in the present study are lower than the standard Ca/P ratio, it may be stated that the excess P concentration is due to some complexes of P other than hydroxyapatite. While Ca and P were present as major elements, all other elements were found in trace level.

The elements Vanadium and Manganese did not show much variation in their concentrations of all the exposed parts of these human teeth samples. Concentration of Fe in cementum are much less than those present in enamel, dentine and caries regions, which may be attributed to non exposure of cementum to food intake because most of the regular food taken by human beings contain iron(Fe) in them. Moreover, concentration of Fe, As and Pb in caries are higher than those in enamel, dentine and cementum. The accumulation of these elements on carious region may be from the type of food that is rich with these elements.

The external proton beam exposure of various parts on human teeth indicates that there is an excess of copper and deficiency of nickel in carious regions of human teeth compared with unaffected portions of teeth. The observed high concentration of Cu may be due to the transportation and localization of the copper in the caries area during food intake and water taken by the individuals. On the other hand the low concentration of Ni may be due to the fact that Ni is replaced by Ca or P during formation of dental caries on various sections of human teeth. These factors suggest a role for copper and nickel in the formation of dental caries and present potential point for intervention of treatment.

The strontium content of teeth can provide information about the diet of past populations [37]. Natural strontium levels in living bone and teeth are typically 150-200 ppm and in the present study, the concentrations of Sr in the enamel, dentine and cementum are in this range, whereas the carious areas of teeth are low in Sr concentration. An interesting observation has been found that the Ca/Sr ratios are inversely proportional for enamel, dentine and cementum of the teeth samples. However, the negative Ca and Sr correlation may be due to the fact that Ca is substituted by Sr either as a whole or in fractions for the formation of hydroxyapatite; therefore Ca and Sr are negatively correlated. Among the bivalent cations that can replace calcium in calcium hydroxyapatite, Sr has attracted a remarkable interest for its possible biological role. Sr is present in the mineral phase of the teeth, especially at the regions of high metabolic turn-over [38]. The extent to which Sr is subject to diagenetic alteration is a matter of debate, though it is widely believed to be one of the least affected elements.

Pb can be toxic for humans and animals at some concentrations and it is a serious public health issue worldwide. Significant Pb contamination of ambient air, food, and wastewater provides exposure which will result in Pb accumulation in the teeth. Blood lead is an effective biomarker for lead exposure, for although lead also accumulates in bone, teeth and other tissues, the effects of lead on the human nervous system correlate with blood lead levels [39]. In the present study, it was observed that a few ppm of Pb is present in the human teeth samples whereas Pb concentration is high on the carious parts. This may be attributed to the accumulation of Pb directly from the food or water taken by the individuals. Lead toxicity directly and indirectly alters many aspects of bone cell function. Retention and mobilization of Pb in teeth occurs by the same mechanisms involved in regulating calcium influx and efflux, namely calcitonin and other hormones that influence calcium metabolism [40].

Divalent cat ions such as Sr and Pb particularly interact and replace isovalent calcium sites in the hydroxyapatite in teeth matrix to form Sr- or Pb-substituted hydroxyapatite of dental tissue resulting in a permanent record that can indicate the past exposure [41-42]. Hence, some parts of the total Sr or Pb measured in the teeth may be from the Sr and Pb-substituted hydroxyapatite. The estimated Sr or Pb concentration may be associated with Sr or Pb substituted hydroxyapatite present in teeth. The Zn concentrations in carious areas are considerably less than the remaining parts of the tooth sample. Since Sr and Zn play major role in bone growth by increasing osteoblasts and decrease the number and the activity of osteoblasts, hence the carious areas showed depletion in Zn and Sr concentrations. Zn is naturally present in bone [43] and stimulates bone growth and bone mineralization [44-45]. Zn has a direct effect on osteoblastic cells in vitro [46] and a potent inhibitory effect on osteoclatic bone resorption [47]. But, the clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral changes [48]. In conditions of bone resorption and tissue catabolism, zinc is released and may be reutilized to some extent. Zinc is also involved in the general metabolism of humans so that relations may exist between dietary intake, diseases or occupational exposure and the bone mineral composition.

Conclusion

The present investigation is an attempt towards the specific identification of trace elemental role on various parts of human teeth and their effect on caries regions. In the current study, a total of twelve elements including trace namely P, Ca, V, Mn, Fe, Ni, Cu, Zn, Ba, As, Sr and Pb were identified. Among the estimated, Ca and P were found to be the major elements whereas all other elements were found in trace level. It was observed that the respective concentrations of elements namely P, Ca, Fe, Zn and Pb in enamel are more than those in dentine and cementum. From the nondestructive elemental analysis on different parts of the teeth, it was clear that the elemental patterns in the carious part of the teeth are severely affected. The possible absorption of trace elements from food intake was observed. Dental healthy status may be corelated to trace element concentrations and the presence of dental caries. This study, suggest the role of trace elements on the human teeth and in the formation of dental caries and presents the potential point for intervention of treatment. Hence this non-destructive external PIXE technique is perfectly suited for the trace elemental analysis on various parts of teeth and dental caries of human being.

Acknowledgements

The authors are very much grateful to the dentists of Anil Neerukonda NRI General Hospital, sangivalasa for providing the human teeth samples. The authors thank the authorities of the Institute of Physics (IOP), Bhubaneswar for providing external proton beam facility from Tandem type pelletron accelerator. The authors also highly appreciate the technical support rendered by Dr.T.R.Rautray and the other staff of the IOP during the sample irradiation. The authors are grateful to the authorities of ANITS Engineering College, sangivalasa, Visakhapatnam for giving necessary permissions.

References

[1] Brown, C.J., Chenery, S.R.N., Smith, B., Mason, C., Tomkins, A., Roberts, G.J., Sserunjogi, L., Tiberindwa, J.V., 2004, “Environmental influences on the trace element content of teeth implications for disease and nutritional status”, Archives of Oral Biology, 49, pp. 705-717.

[2] Anjos, M.J., Barroso, R.C., Perez, C.A., Braz, D., Moreira, S., Dias, K.R.H.C., Lopes, R.T., 2004, “Elemental mapping of teeth using μSRXRF”, Nucl. Instrum. Meth. B 213, pp. 569– 573.

[3] Bhuloka Reddy, S., John Charles, M., Ravi Kumar, M., Seetharami Reddy, B., Anjaneyulu, Ch., Naga Raju, G.J., Sundareswar, B., Vijayan, V., 2002, “Trace elemental analysis of adenoma and carcinoma thyroid by PIXE method”, Nucl. Instr. and Meth. B 196, pp. 333-339.

[4] Johansson, T.B., Akselsson, R., Johansson, S.A.E., 1970, “X-ray analysis: Elemental trace analysis at the 10^{-12} g level”, Nucl. Instr. and Meth. 84, pp. 141-143.

[5] Umbarger, C.J., Bearse, R.C., Close, D.A., Malanify, J.J., 1973, Adv. X-ray Anal. 16, pp.102.

[6] Campbell, J.L., Orr, B.H., Herman, A.W., McNelles, L.A., Thomson, J.A., Cook, W.B., 1975, Anal. Chem. 47, pp.1542.

[7] Iyengar, G.V., 1989, “Elemental analysis of Biological Systems”, CRC Press, Florida, Vol. 1.

[8] Rath, A.K., Vijayan, V., Ramamurthy, V.S., 1996, Ind. J. Phys. B 70, pp. 361.

[9] Vijayan, V., Behera, S.N., 2000, Int. J. PIXE 9, pp. 417.

[10] Bhuloka Reddy, S., Venkateswarlu, P., Bhaskara Rao, K., Seshagiri Rao, V., Ramachandra Rao, K., Ramakrishna, Y., Naga Raju, G.J., 2001, Proc. AP Akad. Sci. 5, pp.185.

[11] Mangelson, N.F., Hill, M.W., 1981, Nucl. Instr. and Meth. 181, pp. 243.

[12] Valkovic, V., 1980, “Analysis of Biological Material for Trace elements Using X-ray Spectrometry”, CRC Press, Boca Raton, USA.

[13] Lindh, U., 1995, Nucl. Instr. and Meth. B 104, pp. 285.

[14] Naga Raju, G.J. , Sarita, P., Ravi Kumar, M., Ramana Murty, G.A.V., Seetharami Reddy, B., Lakshminarayana, S., Vijayan, V., Rama Lakshmi, P.V.B., Satyanarayana, G., Bhuloka Reddy, S., 2006, “Trace elemental correlation study in malignant and normal breast tissue by PIXE technique”, Nucl. Instr. and Meth. B 247, pp.361-367.

[15] Bennun, L., Greaves, E.D., Blostein, J.J., 2002, “New procedures for intensity and detection limit determination in spectral trace analysis: application for trace mercury by TXRF”, X-Ray Spectrom. 31, pp. 289-295.

[16] Beck, L., 2005, “Improvement in detection limits by using helium ions for particle induced x-ray emission”, X-Ray Spectrom. 34, pp. 393-399.

[17] Ryan, C.G., Etschmann, B.E., Vogt, S., Maser, J., Harland, C.L., van Achterbergh, E., Legnini, D., 2005, “Nuclear microprobe – synchrotron synergy: Towards integrated quantitative real-time elemental imaging using PIXE and SXRF”. Nucl. Instr. and Meth. B, 231, pp. 183-188.

[18] Pallon, J., Garmer, M., Auzelyte, V., Elfman, M., Kristiansson, P., Malmqvist, K., Nilsson, C., Shariff, A., Wegden, M., 2005, “Optimization of PIXE-sensitivity for detection of Ti in thin human skin sections”, Nucl. Instr. and Meth. B, 231, pp. 274-279.

[19] Evans, R.D., Richner, P., Outridge, P.M., 1995, “Micro-spatial variations of heavy metals in the teeth of walrus as determined by laser ablation ICP-MS: the potential for reconstructing a history of metal exposure”, Arch. Environ. Contam. Toxicol. 28, pp. 55-60.

[20] Carvalho, M.L., Casaca, C., Pinheiro, T., Marques, J.P., Chevalier, P., Cunha, A.S., 2000, “Analysis of human teeth and bones from the chalcolithic period by X-ray spectrometry”, Nucl. Instr. and Meth. B, 168, pp. 559-565.

[21] Carvalho, M.L., Marques, J.P., Marques, A.F., Casaca, C., 2004, “Synchrotron microprobe determination of the elemental distribution in human teeth of the Neolithic period”, X-Ray Spectrom. 33, pp. 55-60.

[22] Frank, R.M., Sergentini-Maier, M.L., Turlot, J.C., Leroy, M.J.F., 1990, “Comparison of lead levels in human permanent teeth from Strasbourg, Mexico City and rural zones of Alsace”, J. Dent. Res. 69, pp. 90-93.

[23] Bercovitz, K., Laufer, D., 1992, “Systemic lead absorption in human tooth roots”, Arch. Oral Biol. 37, pp. 385-387.

[24] Gulson, B., Wilson, D., 1994, “History of lead exposure in children revealed from isotopic analyses of teeth”, Arch. Environ. Health. 49, pp. 279-283.

[25] Begerow, J., Freier, I., Turfeld, M., Kramer, U., Dunemann, L., 1994, “Internal lead and cadmium exposure in 6-year-old children from western and eastern Germany”, Int. Arch. Occup. Environ. Health 66, pp. 243-248.

[26] Nayak, P.K., Das, D., Vijayan, V., Singh, P., Chakravortty, V., 2004, “PIXE and EDXRF studies on banded iron formations from eastern India”, Nucl. Instrm. Meth. B 215, pp. 252–261.

[27] Vijayan, V., Choudhury, R.K., Mallick, B., Sahu, S., Choudhury, S.K., Lenka, H.P., Rautray, T.R., Nayak, P.K., 2003, "External particle induced X-ray emission", Current Science 85, pp. 772-777.
[28] Prasad, A.S., 1995, "Zinc: an overview", Nutrition 11 (1), pp. 93-99.
[29] S. Sahu, S.K. Choudhury, B. Mallick, T.R. Rautray, V. Vijayan and R.K. Choudhury, (2003). Design of a Rotating Vane Chopper for External PIXE Analysis, Proc. Ind. Particle Accel. Conf., Indore, India, 695.
[30] Choudhury, R.K., Vijayan, V., Mohanty, N.C., 2003, "Scientific study of metallic compositions of Orissa state museum specimens", Orissa Review 59, pp. 48.
[31] Maxwell, J.A., Campbell, J.L., Teesdale, W.J., 1989, "The Guelph PIXE software package", Nucl. Instrum. Methods B 43, pp. 218–230.
[32] Maxwell, J.A., Campbell, J.L., Teesdale, W.J., 1995, "The Guelph PIXE software package II", Nucl. Instrum. Methods B 95, pp. 407–421.
[33] Campbell, J.L., Hopmann, T.L., Maxwell, J.A., Nezedly, Z., 2000, "The Guelph PIXE software package III: Alternative proton database", Nucl. Instrum. Meth. B 170, pp. 193-204.
[34] Rautray, T.R., Vijayan, V., Panigrahi, S., 2007, "Analysis of Indian Pigment Gallstones", Nucl. Instr. Meth. B 255, pp. 409-415.
[35] SrinivasaRao, K., Mary Anupama, P., GuruMahesh, D., Venkateswara Rao, R., Rautray, T.R., Venkateswarlu, P., 2010, "Trace elemental analysis of dental caries in human teeth by External PIXE", International Journal of Applied Biology and Pharmaceutical Technology [IJABPT] 1, 1, pp. 68-78.
[36] Gwinnett, A.J., 1992, "Structure and composition of enamel", Oper. Dent.5, pp. 10-17.
[37] Gilbert, R.I., 1985, "The Analysis of Prehistoric Diets. eds", Gilbert R.I. and Mielke J.H. Academic Press, Orlando, pp. 339.
[38] Blake, G.M., Zivanovic, M.A., McEwan, A.J., Ackery, D.M., 1986, "Sr-89 therapy: strontium kinetics in disseminated carcinoma of the prostate", Eur. J. Nucl. Med. 12, pp. 447–454.
[39] Younes, M., 1995, "The role of biomarkers in derivation of WHO guidance values for air pollutants", Toxicol. Lett. 77, pp. 189-190.
[40] Pounds, J.G., Long, G.J., Rosen, J.F., 1991, "Cellular and molecular toxicity of lead in bone", Env. Health. Persp, 91, pp. 17-32.
[41] Fergusson, J.E., Purchase, N.G., 1987, " The analysis levels of Pb in human teeth: a review", Environ. Pollut. 46, pp. 11-44.
[42] Sharon, I. M., 1988, "The significance of teeth in pollution detection", Perspect. Biol. Med. 32, pp. 124-131.
[43] Jallot, E., Irigaray, J.L., Oudadesse, H., Brun, V., Weber, G., Frayssinet, P., 1999, " Resorption Kinetics of four hydroxyapatite-based ceramics by PIXE and neutron activation analysis", Eur. Phys. J. Ap. 6, pp. 205-215.
[44] Yamaguchi, M., Inamoto, K., Suketa, Y., 1986, "Effect of essential trace metals on bone metabolism in weanling rats: comparison with zinc and other metals' actions", Res. Exp. Med. 186(5), pp. 337–342.

[45] Yamaguchi, M., Oishi, H., Suketa, Y., 1987, “Stimulatory effect of zinc on bone formation in tissue culture”, Biochem. Pharmacol.36 (22), pp. 4007-4012.
[46] Hashizume, M., Yamaguchi, M., 1993, “Stimulatory effect of beta-alanyl-L histidinato zinc on cell proliferation is dependent on protein synthesis in osteoblastic MC3T3-E1 cells”, Mol. Cell Biochem. 122(1), pp. 59-64.
[47] Kishi, S., Yamaguchi, M., 1994, “Inhibitory effect of zinc compounds on osteoclast-like cell formation in mouse marrow cultures”, Biochem. Pharmacol. 48(6), pp. 1225-1230.
[48] Hambidge, K.M., 1987, “Zinc, In: Trace elements in human and animal nutrition”. W. Mertz, ed., Academic Press Inc., Orlando, Florida 1, pp. 1-137.