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Human enamel analysis in ancient Egyptians and contemporary via LIBS technique

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ABSTRACT
Recent studies have shown that chemical and physical approaches are increasingly being used for dental materials analysis and to develop full details and information on the biological circumstances of Humans groupings, prehistoric diets, and the etiology of different illnesses. As a result, estimates of elements levels in teeth can provide insight into the discovery as well as the pathogenesis of different ailments among ancient and contemporary Egyptians. The level of lead, aluminum, and strontium in enamel of teeth from human Egyptian embalmment dated to the new kingdom (1085 BC) from the Sakkara region, as well as newly extracted sound teeth from the same region's inhabitants, were analyzed. The laser involved breakdown spectroscopy (LIBS) approach was used to estimate the elements. Using the measured LIBS spectra, the elemental content was calculated. Lead, aluminum, and strontium content levels in ancient Egyptian teeth have been found to be higher than they have been in recent years, according to current data. These data suggested that an increase in lead and aluminum levels in ancient Egyptian teeth was due to direct exposure to such elements, which increased contamination during that time duration. Because of the increased strontium content, ancient Egyptian teeth are more calcified than modern teeth.

Keywords: Enamel, Trace Element, LIBS, Ancient Egyptian.

1. INTRODUCTION
The New Kingdom was a pivotal period in Egyptian history. More than a dozen trace elements are definitely important for maintaining health during this time, which runs from 3400 B.C to 2180 B.C (Ghorbal, 2006). Deficiency in certain elements, often known as important trace elements, has negative health consequences. Other elements may have little or no recognized beneficial role, however if present in large enough quantities, they can be toxic. The elemental composition of bodily tissues was used to rebuild ancient populations' diets and to learn about human groups' non biological state and the etiology of different diseases (Sandford, 1992). Mineralized tissue, such as bones and teeth, have been discovered to be excellent "archives" for information on ancient and modern human living patterns, diet, and mobility

(Budded et al., 1998). Bones and teeth have been discovered to preserve much of their biological signature from lengthy periods of living, showing, for example, the uptake of pollutants from surrounding habitats through duration. This refers to the fact that a variety of elements are recognized to leach out very slowly once integrated in the structure of hydroxyapatite of bone and tooth matrix (Vourinen et al., 1996). The use of elemental analysis in anthropology is significant for determining the health effects of trace element shortages or increases in human tissues, as well as assessing the relations among nutrition and disease. Element uptake may be influenced by nutrition, education, customs, and circumferential levels of effective elements in water and soil. Element analysis was used to research toxic contaminants in historical societies, such as lead (Pb) exposures, or to examine the origins of definite nutritional deficiencies between ancient populations (Budded et al., 1998). Enamel has a unique characteristic that distinguishes it from other human tissues: Its composition is preserved prior to tooth eruption, which might give a historical record of trace elements absorbed throughout the early stages of growth (Hillson, 1986).

It is primarily non-invasive and non-destructive, laser triggered breakdown spectroscopy (LIBS) is widely recognized as a promising elemental analysis approach in archaeology. It is obvious that LIBS' sensitivity could be inadequate in some instances to assess ultra-trace amounts of elements, and isotope singularity is virtually difficult to detect. Nevertheless, unlike most other techniques for elemental analysis of teeth, LIBS has the particular feature of preserving spatial information. As a result, determining multi-elemental levels in teeth provides us with complementary knowledge of diets, diagnostics, and the etiology of different diseases on the one hand, as well as inside data about ancient Egyptians' environmental conditions on the other (Anglos, 2001; Radziemski, 1994; Rusak et al., 1997; Sneddon Lee, 1999). The usage of LIBS to examine sustainable minerals and possibly harmful elements inside teeth is documented in this research, and the qualitative elemental composition of the luminous plasma formed during laser ablation of dental tissue was identified. The purpose of this trial is to compare elemental analysis (Pb, Al, and Sr) of human enamel specimens from two various historic ancient Egyptian times to modern living under the same environmental influences and dietary patterns based on the findings.

2. MATERIAL AND METHODS

The study was conducted during two years 2019-2021.

Source of Samples

One hundred human teeth were unearthed at the Sakkara archaeological site near Giza, Egypt, from a recently excavated kingdom. Specimens were taken after agreement of Egyptian antiquities organization free of caries, calculus, or any pathology. Mummies were used to obtain aged specimens of all ancient Egyptian teeth. The mummy's position in the tomb is then categorized, indicating the mummy's age. During the Old Kingdom, the fetus posture was used as an indicator, whereas the middle, newly kingdom, and late periods employed the straight posture.

Contemporary individuals

In the village of Sakkara, one hundred sound permanent molars were taken from alive people. The teeth excised for prosthesis or orthodontics (Badrachin hospital-health ministry) was also free of caries diseases.

Archaeological contexts

Sakkara is located on the Nile's western bank, 28 kilometers south of Cairo. Sakkara's name originates from the word Sokar, an ancient symbol of the dead. It's an open book that reflects ancient Egyptian civilization. If we wish to learn about the history of this place, we can learn about the ancient kingdom, the middle kingdoms, the new kingdom, the Roman and Greek periods, as well as the Islamic and Coptic periods. The mummified teeth used in this research came from the new kingdom.

The dental enamel's structure

The elemental analysis will take conducted on dental enamel that was chosen as a suitable location on the tooth. This is due to the tooth's hard structure that allows it to be preserved prior to tooth eruption. The covering of the teeth is dental enamel that varies in thickness from roughly 2.5mm at the tip of the unworn cusps to a feather edge where it stops at the tooth's neck. The toughest human tissue is dental enamel, which is composed of 95% hydroxyapatite, 4% water, and 1% organic matter. Hydroxyapatite is a mineralized compound having the chemicals formula Ca_{10} $(PO4)_6$ $(OH)_2$. Pollutants of trace elements intrude into the crystal lattice of dental enamel.

In dental enamel, considerable trace elements are found in concentrations ranging from below the part-billion regime to the regime of percent (Samek et al., 2000). The actual concentrations frequently reveal details about reduction in or illness states, as well as if toxicity or impurities have happened. Al, Ba, K, Li, Mg, Mn, Na, Pb, and Sr were the most popular trace elements. Some of these, including Al, Sr, and Pb, are regarded to be potentially toxic elements to search for, and will be discussed further in our findings.

Analytical Method

The radiance from a pulsated, fixed frequency laser is focused in LIBS to initiate luminous micro plasma from analyte, resulting in high power laser densities. The plasma composition is roughly reflective of the elements composition of the analyst. Figure (1) shows a schematic diagram of the LIBS experimental setup that was employed. To generate the plasma, a 10cm focal length quartz lens focused a laser beam with pulse energy of 100mJ on the teeth specimens. A Q-switched Nd: YAG laser (surelite I, continuum, USA) working at 1064 nm (pulse duration of 7 ns) and a recurrence rate of 0.1 Hz-10 Hz was used to help develop the plasma. A power meter was used to measure the pulsation energy from shot to shot (Nova 978, Ophir Optronics Ltd., USA). To combine the emitted light from the plasma plume and feed it to a portable Echelle spectrometer with a 0.17m focal length, a one-meter-long fused-silica optical fiber (600 m diameter) placed on a micro xyz-translation phase is employed (Mechelle 7500, Multichannel instruments, Sweden). Echelle grating spectrometers, which are designed to work at high orders and angles of occurrence and diffraction, can enhance high resolution in a further dense size and envelop a considerably wider spectrum range than traditional grating spectrometers. The Mechelle 7500 has a fixed spectral resolution (CSR) of 7500, which is identical to 4 pixels FWHM, and can display a single spectrum with a wavelength range of 200-1000nm. The scattered light was determined using a gateable, intensified CCD camera (DiCAM-Pro- 12 bit, UV provides, 43000 channels, PCO Computer Optics, Germany) linked to the spectrometer. The spectrometer camera system's total linear dispersion varies from 0.006 (at 200 nm) to 0.033 nm/pixel (at 1000 nm). The intensifier's high voltage was optically actuated to evade electrical involvement and jitters. Mechelle software (Multichannel instruments, Stockholm, Sweden) and GRAMS/32 version 5.1 Spectroscopic Results Analysis Software were used for Echelle spectra display, control, processing, and analysis (Galactic Industries, Salem, NH, USA). To enhance our LIBS resolution and sensitivity, as well as to decrease measurement irregularities and issues caused by the sample, we improved a number of experimental variables (Lindblom, 1999).

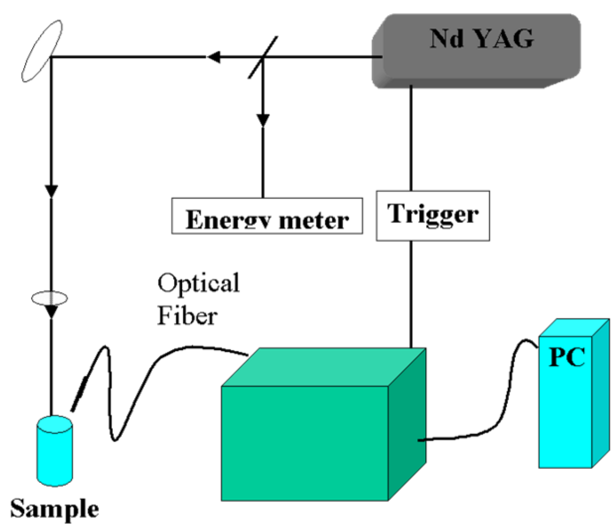


Figure 1 Schematic diagram of LIBS setup.

Heterogeneity

The laser was adjusted to single shot mode to increase data reproducibility and evade issues with electronic trembling. The Nd: YAG laser beam was then directed at a 90o angle onto the sample surface. The high-energy 1064 nm wavelength was reflected 99 % by a dichroic mirror with a diameter of 25 mm. As seen in figure (1), this mirror is placed just prior the laser-focusing lens. Because it produces plasma with an 800-meter spot diameter, the focal point has been positioned 5 mm beneath the sample's surface. It also reduces particle and aerosol breakdown on the sample's surface. Otherwise, the usage of a micro xyz-translation phase as a holder for fused-silica optical fiber allows for the greatest intensity of recorded plasma plume emission light. Twenty laser pulses have been used to clean the specimen surface and remove surface oxides and impurities prior to spectral collection for every recent specimen to confirm that the recorded spectrum accurately matched the specimen composition.

To boost LIBS accuracy, spectra from various laser shots must be moderated to decrease statistical inaccuracy caused by laser shot-to-shot fluctuation. Furthermore, we discovered that accumulating consecutive recorded spectra during exposure of period 10000 ns, every 2500 ns delayed from the laser pulse, can improve data reproducibility. For the ICCD camera, this retard time and exposure window time (gate time) values resulted in spectra with less background and main line signals which did not saturate the detector. For each specimen surface, 20 shots were fired and saved in independent files with the average calculated and saved as the library spectrum.

The peak intensity, full at width half maximum FWHM, and center wavelength of every line, and the background emission continuum, are also detected for every measured spectrum. GRAMS/32 version 5.2 (Thermo Galactic, USA.) and LIPS++ data analysis program (LIPS++ software for spectral analysis 1999-2001 treated laser spectroscopy laboratory @IFAM-CNR, Pisa, Italy) were used to preprocess the averaged spectra data on a Pentium III PC under Windows conditions (Microsoft office Excel 2003). The averaged spectra's peak tables (lists of wavelengths and mintensities) were rolled up in GRAMS/32 and forwarded for data estimation (Ciucci et al., 1999).

3. RESULTS AND DISCUSSION

The applicability of laser induced breakdown (LIBS) to the analysis of remarkable toxic elements in enamel is discussed here.

Qualitative analysis

Figure 2 displays a typical qualitative LIBS spectrum generated by the LIPS++ program for a human tooth enamel specimen. The mean of 20 single spectra collected with a 2.5 second delay and a 10 second gate width yielded this spectrum. The emission lines of Al, Ba, K, and Li, as well as the UV-visible emission lines of calcium as a main element, are apparent in the panoramic Echelle spectra in the spectral range 200-800nm. The Nd: YAG laser had energy of 100mJ at a wavelength of 1064 nm, and the plasma emissions were collected with a delay of 2.5 s and a gate width of 10s.nMg, Mn, Na, Pb, and Sr were found as trace elements in the tooth enamel specimen. The obtained spectrum indicates the employed spectroscopic system's wide spectral range and high resolution.

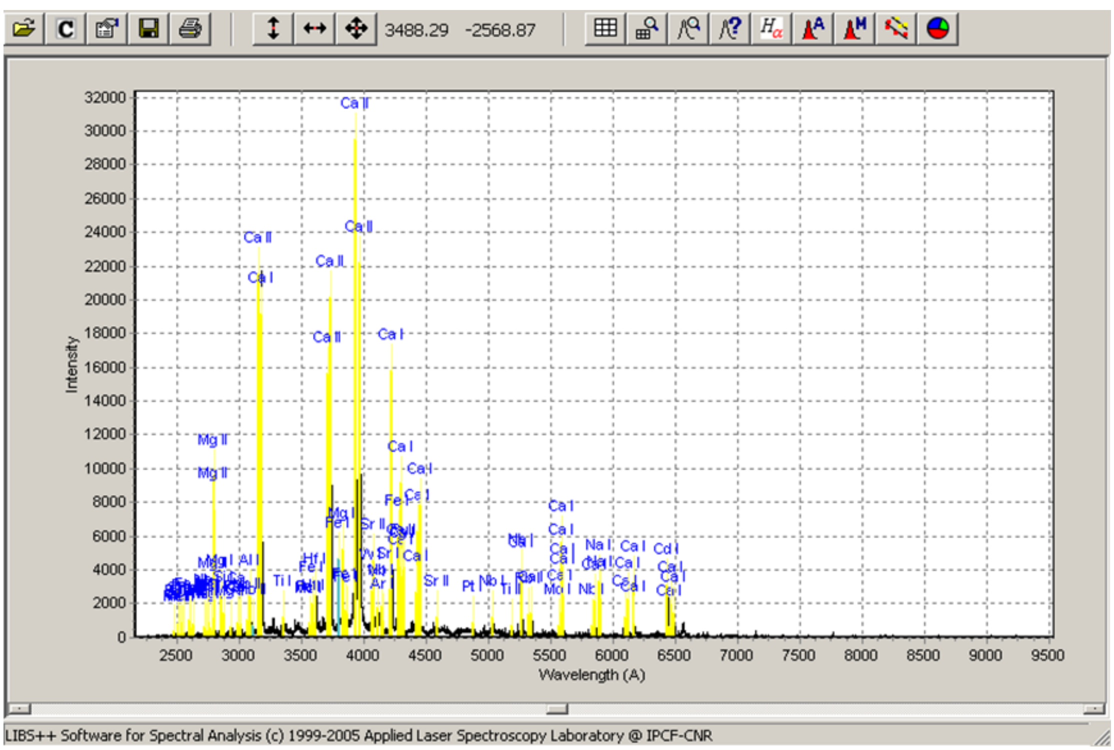


Figure 2 The elemental composition of enamel is represented by a typical qualitative LIBS spectrum. At a wavelength of 1064 nm, the Nd: YAG laser produced 100mJ of energy, plasma emissions are accumulated with a 2.5-second delay and a 10-second gate width.

Quantitative analysis

Due to its significance in terms of clinical, biological, and environmental exposure, Pb, Sr, and Al were selected for quantitative elemental analysis. We used the calibration curves obtained by Samek et al., (2001) by generating fake artificial reference specimens CaCO3 to resample the dental enamel (hydroxyapatite Ca10(PO4)6(OH)2 utilizing LIBS technology under test settings identical to ours in order to get quantitative calibration data. These were manufactured from pressed CaCO3 pellets spiked with known Al, Sr, and Pb concentrations (as the basic matrix material). To decrease the number of individual specimens and permit cross-calibration, Al, Sr, and Pb were putted to the pellets at the same time. The proportionate element levels were regulated within the range of 100-10000 ppm, proportionate to the matrix's Ca content, as cleared in Table (1). The elemental levels of Al, Pb, and Sr in the 200 dental specimens were determined by using the generated calibration curves and their accompanying calibration equations (100 for newly kingdom and 100 for contemporary). In histogram Figs (3-5), the mean concentrations of every element for every group (100 teeth) are then displayed as a column. A comparison of the obtained concentrations for every element in the two group categories is described separately in these histograms, as follows;

Table 1 Al/Pb/Sr weight relative to Ca weight in sample pellets (from Samek et al., 2000)

	Element concentration relative to Ca (%)						
Sample No.	1	2	3	4	5	6	7
Al	0.024	0.037	0.057	0.118	0.9	0.50	1.00
Pb	0.028	0.034	0.067	0.112	0.22	0.49	0.97
Sr	0.025	0.030	0.065	0.124	0.25	0.02	1.00

Lead analysis

Figure 3 displays the lead percentage difference in enamel, which gradually decreased from the time of the ancient kingdom to the modern day. These findings showed that lead concentrations increased more in the ancient Egyptian new kingdom than in the contemporary area. Our findings corroborate those of Attramadal and Jonsen (1978), who discovered that Bronze Age teeth had a greater lead concentration. They got these findings from archaeological burial sites that point to ancient people using glazed pots with high lead concentration. Furthermore, our findings are consistent with those of Reitznerova et al., (2000), who discovered that the lead concentration in Bronze Age teeth is comparable to that of contemporary teeth. These results might be interpreted as follows: - First, the ancient Egyptians used lead in a range of applications, including small sculptures loaded for net fishing by Petrie (1917; 1920).

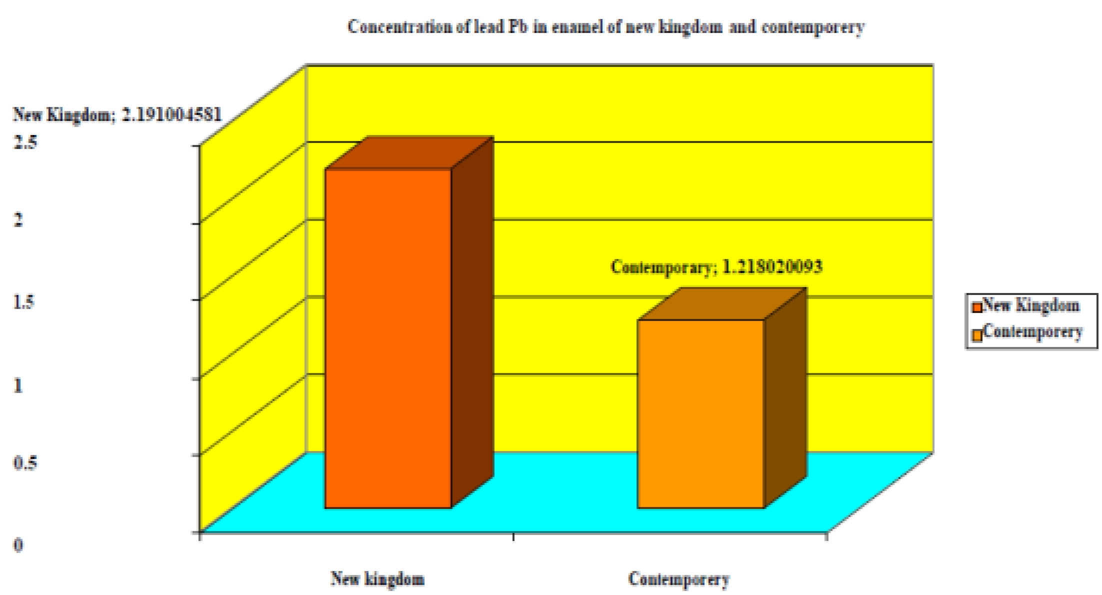


Figure 3 Displays the Pb percentage difference in enamel, which diminishes from the new kingdom to the contemporary day

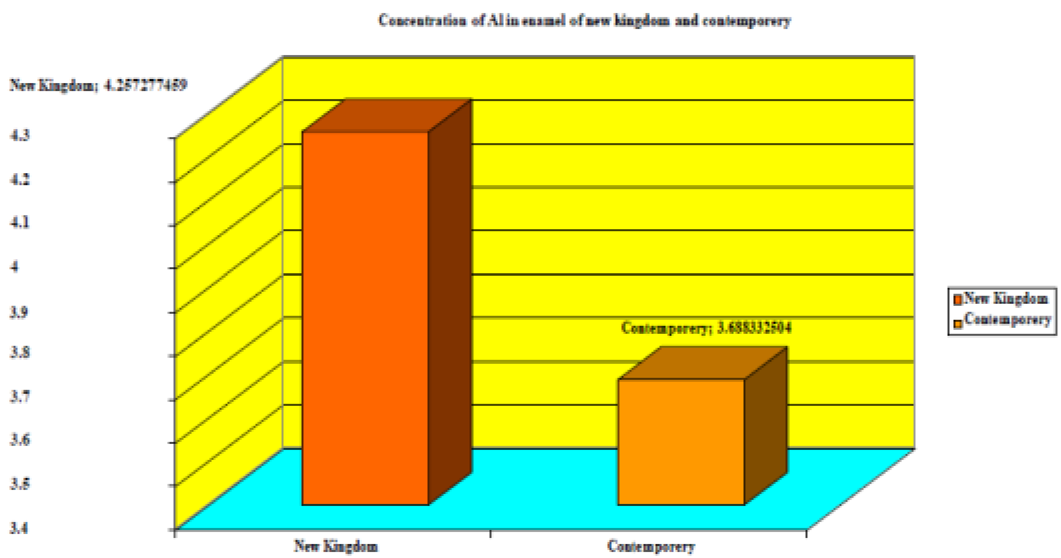
Lead oxides were employed in ancient times for painting in 3 ways, including rings, necklaces, and specific coatings on the inner surfaces of dishes and pots.

1. Some Greek and Roman walls were painted with red lead oxide Barthoux (1962).
2. Red lead oxide found on painted wall suggested being in late period Barthoux (1962).

3. Yellow lead oxide on painted wall suggested being 400 B.C. Laurie (1926) Secondly, in the period from middle ages till the eighteen dynasties the most of lead products were extracted only from Egypt, not form other countries like Syria until building the Egyptian Emperor so that according to review of literature, lead was used from oldest Ages (before dynasties) till late period with viability of high level in old kingdom with little decrease new kingdom. - Thirdly, Recently ages lead is mostly used only in area of industrial application. So that lead pollution at industrial cities may give high record in environment therefore affect human being. On the other hand, in country side area (like Sakkara), the effect of lead pollution is minimized and gives a little record as we found in our measurements. This is because Sakkara is away from the source of lead pollution like industries and high traffic ways (Aufderheide, 1989)

Aluminum analysis

Figure 4 Shows a histogram for the Aluminum percentage variation from the new kingdom to contemporary. This figure reveals that the concentrations of Aluminum in enamel of Egyptians of new kingdom (1085 BC) are slightly higher than that of contemporary. These results may be explained due to increasing of direct exposure to Aluminum oxide in ancient time with primitive fabrications of ancient ceramic and using Alum in different fields like water purification (Casarett and Doull, 1991).



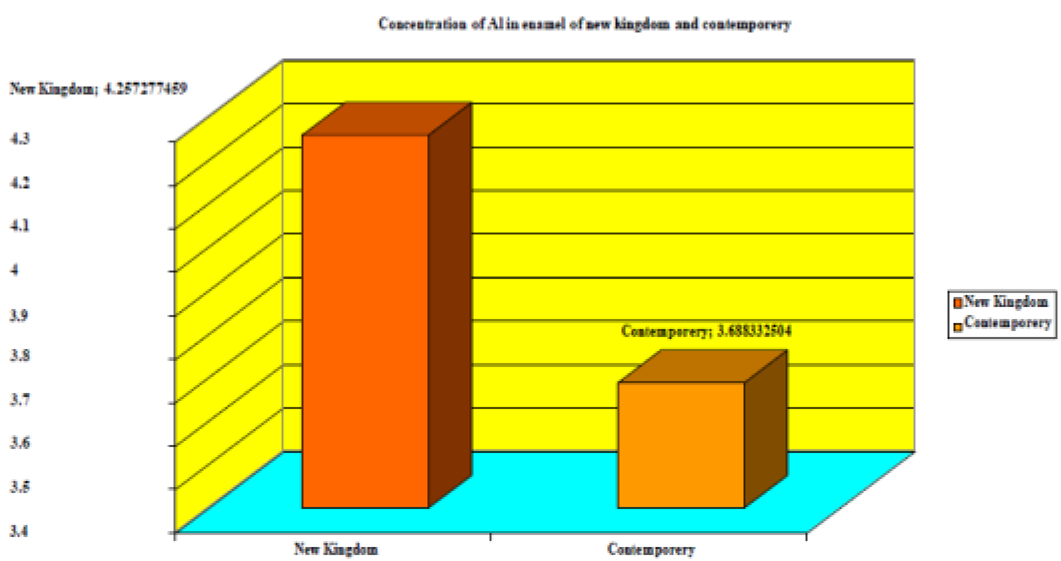


Figure 4 Shows a histogram for the Aluminum percentage variation from the new kingdom to the contemporary.

Medicine and clothing color fixation, as well as tomb drawing. Aluminum was not recognized as a pure metal by the Ancient Egyptians, but rather as a compound in various materials utilized in various fabrications like Alum (Aluminum sulphate) (Casarett and Doull, 1991). On the other side, environmental safeguards have been taken in recent years to limit aluminum contamination. The normal population's daily aluminum intake ranges from 9 to 36 milligrams per day (Casarett and Doull, 1991). As a result, the prevalence of aluminum in recent years has been erratic, that fits with the findings of Samek et al., (2000), who found a high concentration of Al in specific bands of dental pastes accessible in markets today.

Strontium analysis

A histogram showing the percentage of Strontium decreasing from the old kingdom to the recent times is shown in Figure 5 and 6. This figure depicts the rise in Strontium in the outer surface of enamel in the new kingdom over the contemporary one. The ancient Egyptian diet, according to our research and a survey of the literature, consisted of more grains as well as less meat than contemporary diet. The ancient Egyptians enjoyed eating pulses such as beans, chickpeas, entails, and green peas, as attested by countless tombs. These meals contain Strontium (Sarry El- Din and Hawass, 2000). Strontium concentration affects tooth form and is linked to calcium concentration, according to Schraer et al., (1962) (direct proportional). Strontium has effects on tooth formation, according to the researchers.

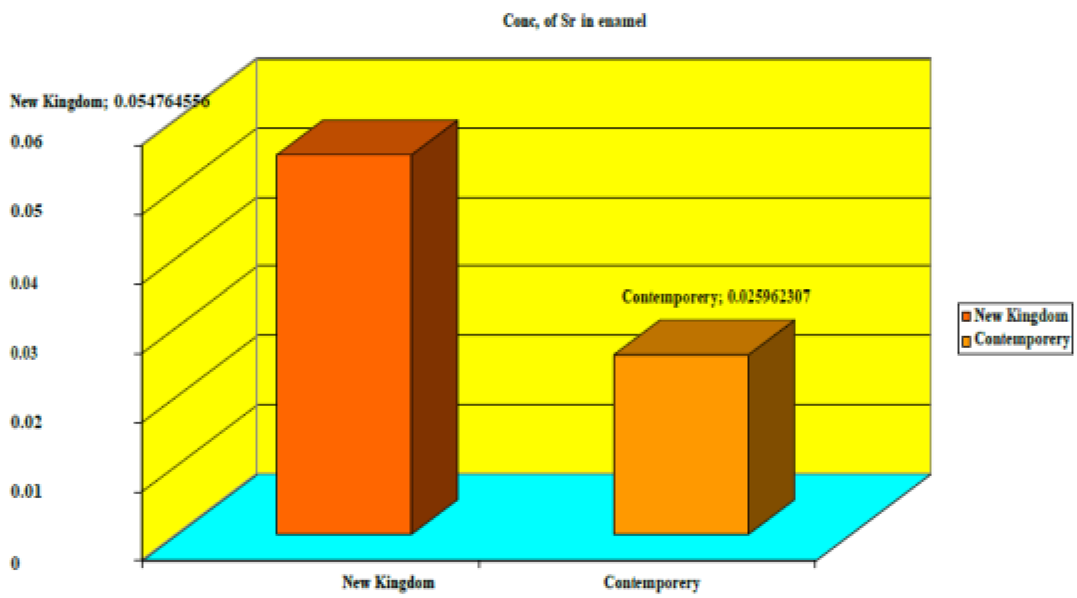


Figure 5 Displays a histogram of the Strontium percentage as it decreases from the new kingdom to the contemporary.

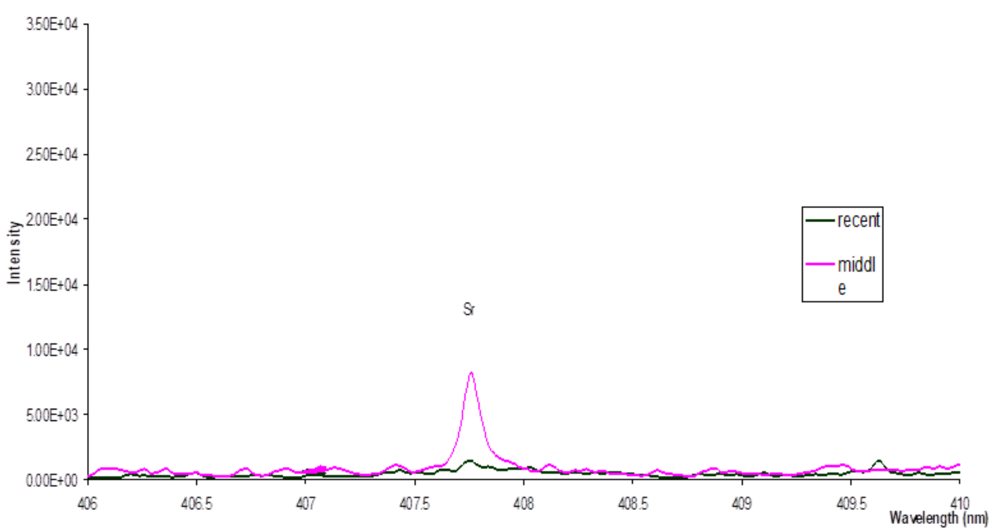


Figure 6 Superimposed Sr spectra new kingdom and contemporary

During tooth development, strontium may modify a cell that is important for the production of enamel and dentine matrices. The ancient Egyptians' teeth became harder and more resistant to decay as a result of this. Figure 5 displays a histogram of the reduction in Sr percentage from the old kingdom to the modern era. Sarry El-Din et al. discovered that newer teeth have higher levels of caries (2002) Figure 6 and Table 1 shows the weight of Al/Pb/Sr in comparison to the weight of Ca in specimen pellets (Samek et al., 2000).

4. CONCLUSION

Elemental analysis for lead, aluminum, and strontium in tooth enamel of 200 teeth specimens dating from the New Kingdom to the contemporary day was performed using LIBS technology. The differences in concentration of the examined trace elements lead, aluminum, and strontium in enamel of ancient Egyptian and contemporary teeth, it is argued, reflects disparities in their environmental influences, notably the diets of both populations.

Ethical approval

Ethical approve cleared by ethic committee of *National Institute of laser Enhanced Science (NILES), Cairo University (*Ethic No. Cu-NILES/8/21).

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Declaration of conflicting interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Anglos D. Laser-induced breakdown Spectroscopy in Art and archaeology. Appl spectrosc 2001; 55(6):186A-205A.
2. Attramadal A, Jonsen J. Heavy trace elements in ancient Norwegians. Acta Odontol scand 1978; 36: 97-101.
3. Aufderheide AC. Chemical analysis in skeletal remains. In reconstruction of life from the skeleton. Aln R.liss Inc., 1989; 237-260.
4. Barthoux J. Les fards, pommades et couleurs dans l'antiquit, in congres internat de Geog. Le Carie avril, 1962; IV: 257- 258.
5. Budded P, Mongomery J, Cox A, Barreiro B. And Thomas R, The distribution of lead within an ancient and modern human teeth. Sci Total Environ 1998; 220: 21-36.
6. Casarett and Doull, Toxicology: the basic science of poisons. 4th edition, Pergamon Press, 1991; 662.
7. Chraer H, Posner A, Schraer R. and Zipkin I. Effect of fluoride on human bone "crystallinity" in the growing rate. J Biochem biophys Acta 1962
8. Ciucci A, Corsi M, Palleschi V, Rastelli S, Salvetti A. and Tognoni E. New procedure for quantitative elemental analysis by laser induced plasma spectroscopy. Appl Spectrosc 1999; 53; (8) 964-969.
9. Ghorbal MS. History of Egyptian civilization" Dar El Maaref, pp.92-108 (In Arabic).36 Science Echoes 2006; 7: 28-38 37
10. Hillson S (1986): Diet and dental diseases. World archeology vol.212:pp147-162.
11. Laurie A. Ancient pigments and their identification in works of arts, in Archaeologia, LXIV, 1926; 318-319.
12. Lindblom P, New compact Echelle spectrographs with multichannel time-resolved recording capabilities. Anal Chim Acta 1999; 380: 353-361.
13. Petrie W, Prehistoric Egypt, Britch school of Egyptian Archeology in Egypt. London university college, Gower street. 1917; 27.
14. Petrie W. Object of daily use, s Britch school of Egyptian Archeology in Egypt. London university college, Gower street. 1920; p. 29.
15. Radziemski LJ. Review of selected analytical applications of laser plasmas and laser ablation, 1987-1994", Microchem. J1994; (50): 218-234.
16. Reitznerova E, Amarasiri D, Kopcakova M. and Barnes R, Determination of some trace elements in human tooth enamel. Fresenivs S. Anal Chem 2000; 367: 748-54.37
17. Rusak DA, Castle BC, Smith BW, Winefordner JD, "Fundamentals and applications of laser-induced breakdown spectroscopy. Crit Rev Anal Chem 1997; 27: 257-290.

18. Samek O, Beddows D.C.S, Telle H.H, Kaiser J, Liska M, Caceres J.O, Gonzales A, Urena Quantitative laser-induced breakdown spectroscopy analysis of calcified tissue samples", Spectrochimica Acta Part B, 2001; (56): 865-875.
19. Samek O, Liska M, Kaiser J, Beddows Telle H.H, and Kukhlevsky S. Clinical application of laser-induced breakdown spectroscopy to the analysis of teeth and dental materials, J Clin Laser Med Surg 2000; (18): 281-289.
20. Sandford MK, A reconsideration of trace element analysis of prehistoric bone" In SR Saunders and MA Katzeberg (eds): Skeletal Biology of Past Peoples: Research methods. New York: Wiley-Liss, 1992; pp. 79-103.
21. Sarry El- Din A, Hawass Z, Teeth pathology in an ancient Egyptian sample from Giza- old kingdom. Egypt dent j 2000; 40: 1533-1544.
22. Sneddon J, Lee YI. Novel and recent applications of elemental determination by laser-induced breakdown spectrometry. Anal Lett 1999; 32: 2143-2162.
23. Vourinen H, Pihlman S, Musslo H, Tapper and Varrela T. Trace and heavy metal analyses of a skeletal population representing the town people in Turku (Åbo), Finland in the 16th–17th centuries: with special reference to gender, age and social background Sci. Total Environ. 1996; 177: 145-160.