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Analytical Note

Effect of the wavelength on laser induced breakdown spectrometric analysis of archaeological bone☆

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ABSTRACT

The analytical exploitation of the laser induced plasma suffers from its transient behavior due to some nonlinear effects. These phenomena are matrix-dependent and limit the use of LIBS to mostly semi-quantitative precision. The plasma parameters have to be kept as constant as possible during LIBS measurements. Studying archaeological bone samples using LIBS technique could be more difficult since these samples are less tough in their texture than many other solid samples. Thus, the ablation process could change the sample morphological features rapidly resulting in poor reproducibility and statistics. Furthermore archaeological bones are subjected to diagenesis effects due to burial environment and postmortem effects. In the present work comparative analytical study of UV (266 nm) and IR (1064 nm) LIBS for archaeological bone samples belonging to four ancient Egyptian dynasties representing the middle kingdom (1980–1630 BC), 2nd intermediate period (1630–1539/23 BC), Roman–Greek period (30 BC–A.D. 395) and the late period (664–332 BC). Measurements have been performed under identical experimental conditions except the laser wavelength to examine its effects. Elemental fluctuations within the same dynasty were studied for reliable information about each dynasty. The analytical results demonstrated that UV-LIBS gives a more realistic picture for bone elemental composition within the same dynasty, and bone ash could be more suitable as a reference material for bone calibration in the case of UV-LIBS.

1. Introduction

Bone and teeth are calcified tissues where hydroxyapatite is a predominant constituent mineral. Trace elements are commonly found in the mineral phase of these calcified tissues, although some elements could be associated with the organic phase. The elemental composition in bones and teeth varies in concentrations (from several percents to ng per g) depending on the diet intake. In the case of human some elements are essential for bone development. Elements in minor and trace concentrations such as iron, magnesium, zinc, chromium, copper, and manganese are associated with bone development. The bone microstructure is often well-preserved down to the μm scale in fossil specimens recording growth marks and other histological features that are often used for life history reconstructions [1]. The chemical compositions of the mineral phase bioapatite and the protein phase, predominantly collagen, yield important information about palaeobiology and palaeoecology [1]. Elemental analysis of bone's major, minor and trace elements has been used to provide information about the relation between nutrition and diseases, health effect of trace element excess or deficiencies, to investigate exposure to toxic pollutants such as lead (Pb) in historical populations and to explore the source of specific nutritional deficiencies among ancient communities [2,3]. This can be attributed to the fact that once incorporated into the hydroxyapatite structure of the bone a number of elements are known to leach out very slowly [4]. Several factors could influence the concentration of trace elements in bone, especially in buried samples. Factors affecting bone trace element content (in addition to the diet intake) include: remodeling process [5], bone diseases [5–7], and direct exposure from contaminated materials [8,9]. Buried bones are exposed to different environmental conditions that can affect their natural elemental composition. Soil conditions such as temperature, pH, and water exposure can cause changes in the mineral composition of bones leading to exchange of ions with the environment [9]. Archaeological bones from different ancient Egyptian dynasties have been studied before. Discrimination between bones from different dynasties has been also demonstrated [3]. Different dynasties showed different levels of strontium which could be an indication of different dietary habits [3]. Elemental fluctuation within the same dynasty due to individual variations or different individual postmortem effects could lead to an unrealistic picture for the whole dynasty.

The aim of the present work is to study the laser wavelength effect on the elemental fluctuations in the LIBS spectra of archaeological bones within the same dynasty using conventional LIBS arrangement

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at two different excitation wavelengths, namely 1064 nm and 266 nm. Stratigraphic changes of the laser induced plasma will be also demonstrated to examine best conditions for LIBS measurements of archeological bones.

2. Methodology

2.1. Samples

The archeological bone samples used in the present study were from four different ancient Egyptian dynasties representing the middle kingdom (1980–1630 BC), 2nd intermediate period (1630–1539/23 BC), the late period (664–332 BC) and the Roman–Greek period (30 BC–AD 395). The archeological samples were authenticated and provided officially by the Egyptian Supreme Council of Antiquities from two different archaeological regions. The Roman–Greek, 2nd intermediate and Late period samples were from Sakkara nearby Cairo, while the middle kingdom and 2nd intermediate period samples were from Aswan in upper Egypt. Bone pellets from each dynasty were prepared by grinding the sample, mixing without binder for 10 min using an automatic mixer machine (Spex Sample Prep, Mixer/Mill 8000 M) and then pressing into a pellet using an automatic press (Spex Sample Prep, X-press 3630), to assure reproducibility, set at 25 tons of pressure for 1 min for each 5 g pellet. Bone ash standard was obtained from the National Institute of Standards and Technologies (NIST 1400). It consists of bone ash that was blended to a high degree of homogeneity. It has certified composition values (Ca 38.18%, P 17.9%, Mg 0.68%, Sr 249 μg/g, Fe 660 μg/g, Pb 9.07 μg/g, K 186 μg/g, Zn 181 μg/g.) and

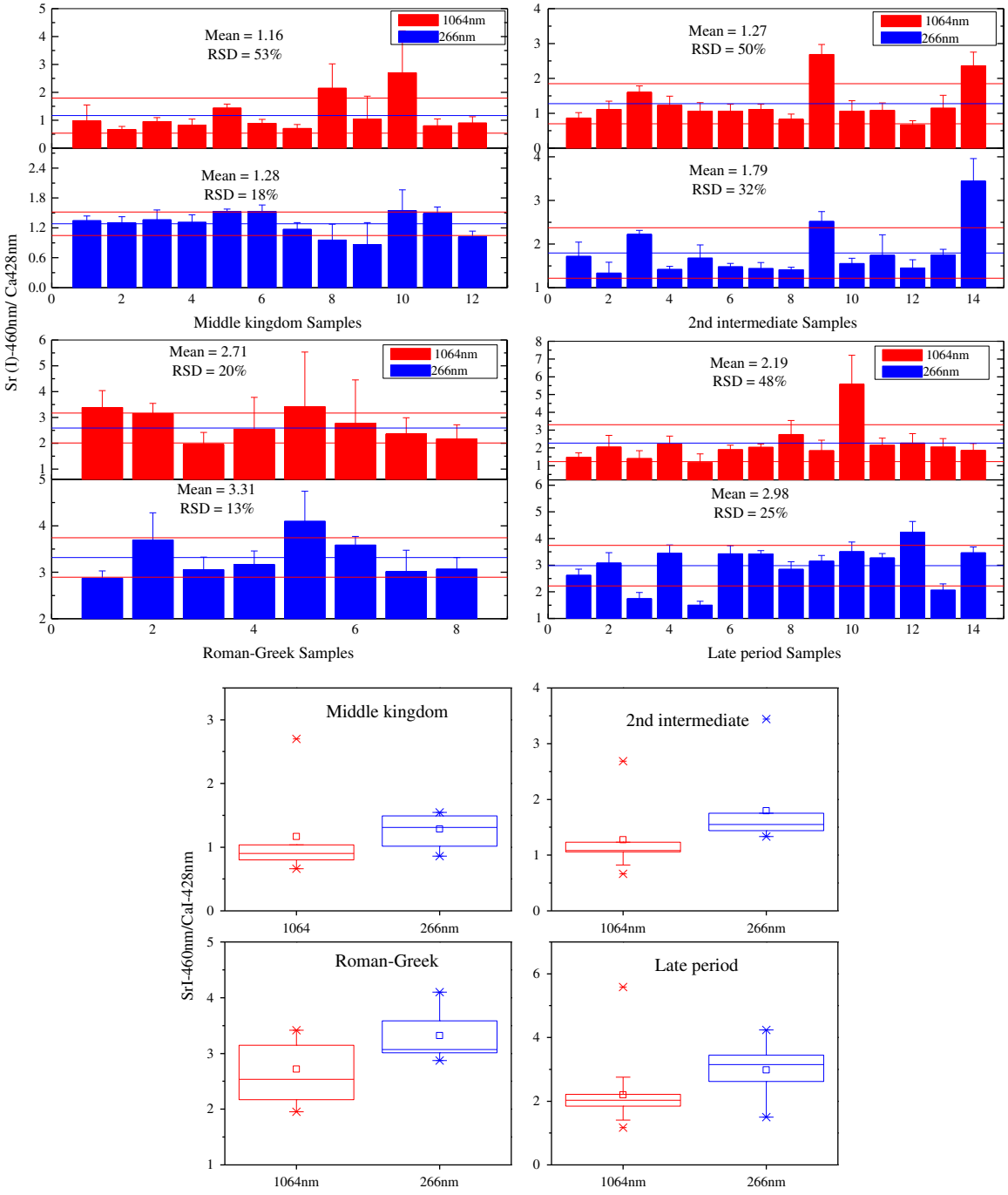

Fig. 1. Elemental fluctuation of (Sr) within the same dynasty.

non-certified concentrations (Si 0.13%, Na 0.6%, Al, Ar, Cd 0.03%, Cu 2.3%, F 1250, Mn 17, Se 0.08%). Bone ash pellet was prepared without binder in the same way as mentioned previously. Bone measurements were done for cortical compact surface of bone. Samples were measured under ambient conditions without any alternation of their original state.

2.2. LIBS arrangements

Two LIBS experimental systems based on an RT100 design (Applied Spectra, Inc) with different laser excitation wavelengths (1064 nm, 266 nm) were used throughout the measurements. The experimental conditions (laser energy, spot size, repetition rate, irradiance etc.) for the two systems were kept as close as possible to investigate the wavelength effect. In the 1st setup the laser source was a Q-switched Nd:YAG laser (New wave, Minilase II, USA), operating at its fundamental wavelength ($\lambda = 1064$ nm), with a pulse duration of 6–8 ns. The pulse energy was set to 22 mJ and the repetition rate to 5 Hz. The laser beam was tightly focused vertically onto the target surface using an objective lens (LmH-5x-1064, Thorlab, USA). The samples were mounted on an X–Y–Z motorized translational stage. Detailed description of the instrumentations is given elsewhere [10]. In the 2nd system, the laser was (New wave, Tempest-10Hz, USA) operating at its 4th harmonic ($\lambda = 266$ nm), with a pulse duration of 3–5 ns. The pulse energy was set to 14 mJ and the repetition rate to 5 Hz. The laser beam was tightly focused vertically onto the sample surface using an objective lens (LMV-5x-UVB, Thorlab, USA). LIBS spectra were collected from 5 replicates from fresh

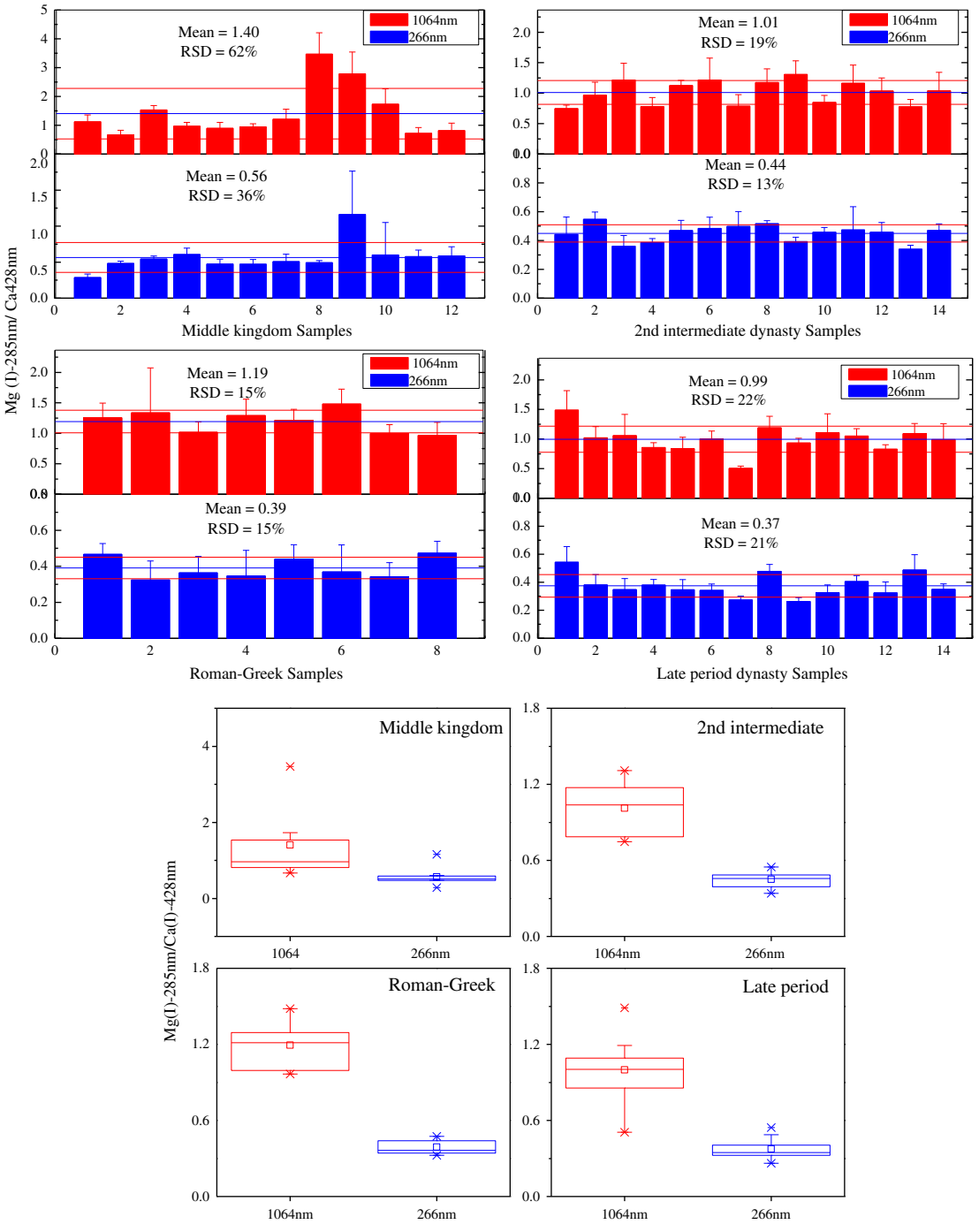

Fig. 2. Elemental fluctuation of (Mg) within the same dynasty.

spots each, with 20 accumulative shots. Depth profile measurements were recorded through 100 single shots at the same spot for 3 fresh spots in each sample.

3. Results and discussion

3.1. Elemental fluctuation within same dynasty

Figs. 1 and 2 represent the integral intensity of elements Sr and Mg in different dynasties. The variation of intensities and relative standard deviation values (RSD) were compared within the same dynasty. Data were presented using a statistical box chart. The box is determined by the 25th and 75th percentiles (interquartile range). The whiskers are determined by the 5th and 95th percentiles. Additional values are represented including the minimum, median, mean and maximum. Lower RSD means lower variability while high percentage means high variability from individual to another in the same dynasty. The percentage RSD values compared at different excitation wavelengths are shown in Table 1.

Generally 266 nm excitation wavelength shows lower percentage RSD values. Studies have shown that the wavelength is one of the most critical parameters affecting the nanosecond laser ablation behavior and plasma formation. It was reported that mass ablation rate at UV wavelength of 266 nm is more than one order of magnitude higher compared to the infrared wavelength of 1064 nm [11,12]. Among the reasons for this behavior is the fact that UV wavelengths offer higher photon energies (4.66 eV for 266 nm) for bond breaking and ionization than that of the infrared wavelengths (1.16 eV for 1064 nm). Short wavelength ablation minimizes also fractionation effects, which can cause the ratios of the elements in a plume to differ from the stoichiometry of the sample. This reduces effectively the difficulties associated with calibration, reproducibility, and accuracy of the analysis. Another suggested reason is that there is stronger plasma shielding at the infrared wavelength, leaving less total laser energy available to interact with the target. There are 3 factors affecting LIBS signal for buried archeological bones: i) biogenic effect, namely the concentration of the elements which is preserved in bone during life from diet or toxicity; ii) postmortem or diageneic effects due to burial conditions, which may lead to bacterial degradation, bone decomposition, leaching of elements from bone to surrounding or diffusion of elements from surrounding soil to bones that lead to pronounced changes in bone physical properties; iii) laser wavelength dependent ablation. Factors ii) and iii) are related since bone physical properties are nearly the same except in some cases like osteoporosis that results in decreasing bone density.

Using UV-LIBS diminishes the differences in sample ablation due to difference in physical properties. LIBS signal then will be samples' elemental concentration dependent on either biogenic or diagenic sources. In fact, separating the biogenic signal from diagenic is not an easy task. There are many studies trying to give answers for this problem. In our case the bones from a certain dynasty were subjected to same burial conditions for nearly same period therefore it can be assumed that the diagenic effect will be nearly equal. Using UV-LIBS provides a more realistic picture of the samples' elemental concentrations within the same dynasty.

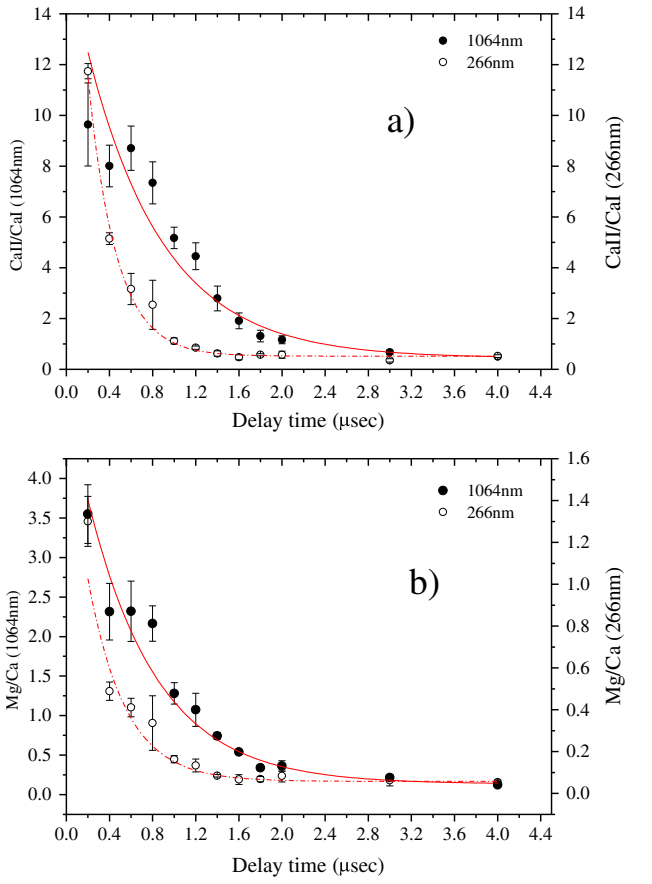

Fig. 3. Time evolution of the plasma line intensities for a) CaII/CaI and b) Mg/Ca in bone pellet at laser excitation wavelengths of1064 nm and 266 nm.

The results show that the highest mean values for Mg were in the middle kingdom and for Sr, they were in the Roman–Greek and late periods respectively. It has been reported that consequences of magnesium deficiency are the acceleration of bone loss, decreased trabecular volume and damage of bone microarchitecture. The high Sr intake leads to defective bone formation and mineralization [13,14].

3.2. Time resolved measurements

Fig. 3 shows the time evolution of plasma integral line intensities for calcium and magnesium in a bone pellet from the middle kingdom dynasty. In the case of UV-LIBS, plasma decay is faster and the emission intensity is lower. It is known that the initiation of the plasma and its properties depend on laser wavelength. Plasma formation requires vaporization of the sample surface as a first step. For longer wavelength inverse Bremsstrahlung absorption dominates while photoionization is the predominant in the case of shorter wavelength [15].

Table 1
Relative standard deviation (RSD %) for Sr and Mg in different dynasties compared at different laser excitation wavelengths.

Excitation wavelength	RSD %							
	Middle kingdom		2nd intermediate		Roman–Greek		Late period	
	Sr	Mg	Sr	Mg	Sr	Mg	Sr	Mg
1064 nm	53	62	50	19	20	15	48	22
266 nm	18	36	32	13	13	15	25	21

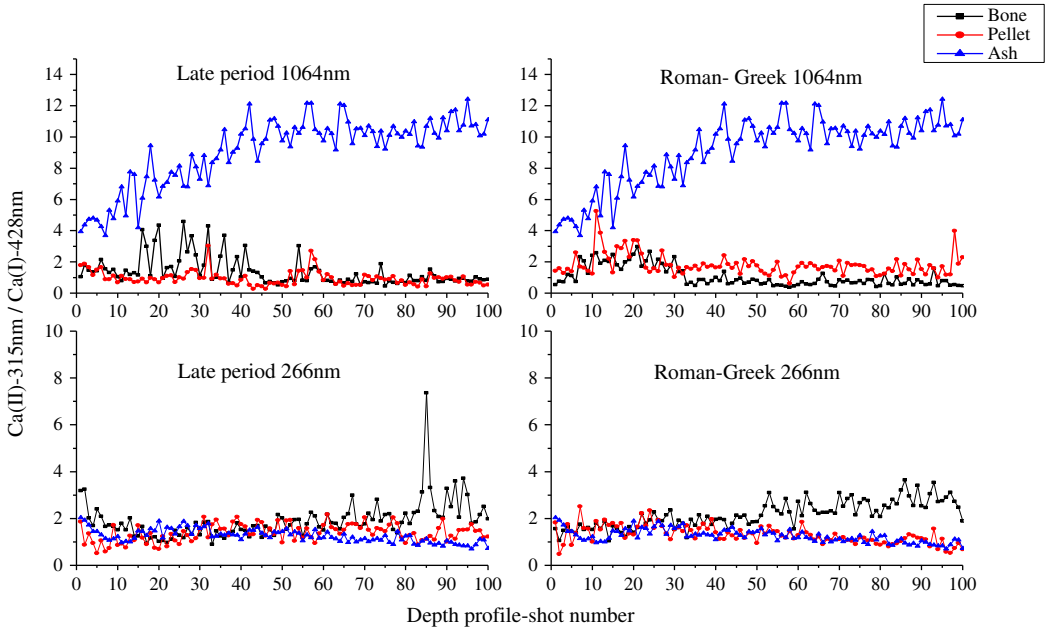

Fig. 4. Different bone dynasties' LIBS depth profile measurements Ca (II)-315 nm/Ca(I)-428 nm ionic to atomic line ratio for two laser excitation wavelengths λ = 1064 nm and 266 nm comparing bone pellet and bone ash standard.

3.3. Depth profile in archaeological bones

Fig. 4 shows 100 shot depth profiling for ionic to atomic line ratio of Ca. The figure represents a bone sample, bone pellet for the same sample, and pure bone ash standard pellet. Depth profiling was compared for two LIBS excitation wavelengths at 1064 nm and 266 nm. The analytical results demonstrated that UV-LIBS shows a stable plasma stoichiometry (CaII/CaI) with depth for bones from different dynasties and it was similar to plasma stoichiometry of homogenous bone pellet and bone ash standard. For laser wavelength at 1064 nm the intensity ratio of ionic to atomic calcium lines shows different plasma stoichiometries with depth. Tracking plasma stability with depth is important to ensure that the changes in LIBS signal with depth are due to change in elemental concentrations relevant to diagenesis and not due to changes in ablation [10]. It is clear that the differences in the physical properties between the three samples play an important role in the obtained depth profiling results. Surface reflectivity determines the fraction of the laser energy that can be absorbed by a sample, and consequently it affects the ablation rate of the material. Shorter optical penetration depth takes place with UV-wavelengths, providing more laser energy per unit volume for ablation [10,16].

From these results using bone ash standard for tracking change in elemental concentration is more reliable than using UV-LIBS since plasma stoichiometry will be stable with depth and we can accumulate more number of shots. In order to track the strontium concentration with depth, Fig. 5 shows the depth profile for Sr(I)/Ca(I) for 100 single

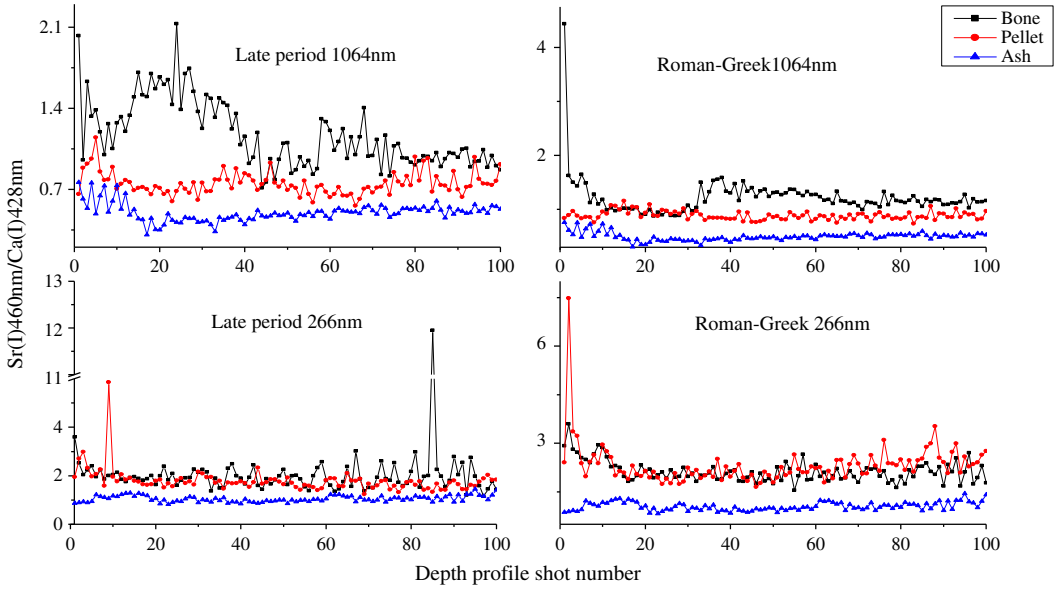

Fig. 5. Different dynasties' LIBS depth profile tracking Sr concentration for two laser excitation wavelengths λ = 1064 nm and 266 nm comparing bone, bone pellet and bone ash standard.

shots for bone, bone pellets and standard bone ash samples. Depth profile was compared for the two LIBS excitation wavelengths at 1064 nm and 266 nm.

Since bone represents the main reservoir for calcium and strontium (99%), their elemental concentrations in bone would indicate the quantities in which they were initially consumed. Strontium to calcium ratio (Sr/Ca) could give a picture for the palaeodiet. Human display values of (Sr/Ca) depend on the proportion of meat and plant resources in their diet. Specifically, an increased proportion of plant foods would increase the Sr/Ca ratio; on the other hand an increased proportion of meat would lower such ratio in human tissues [17]. Buried bones suffer from post-mortem effects where it becomes difficult to discriminate between the signal coming from biogenesis origin i.e. (paleodiet or paleonviroment) and that of diagenic origin from burial environment [18]. Sr/Ca depth profile could be an indication for diagenesis. Bone pellets' Sr/Ca depth profile should be stable with no variation since pellets have homogenous distributions of elements. In case of real bones if there is elemental diffusion of elements from the soil this could be reflected on the depth profile by a change in concentration gradient from outside to inside the cortical bone surface. Fig. 5 shows that for 1064 nm laser excitation, bone samples from the late period and Roman–Greek dynasties show variation in strontium depth profile as it was high then it depletes, while their pellets remain stable; this could let us conclude that these Sr levels are due to diagenic effect and uptake, but the results of the 266 nm laser excitation show stability in Sr for bone samples as pellets for the same samples. It could be concluded that the variation in the case of 1064 nm excitation is due to the interaction of laser beam with the samples and its physical properties so using 1064 nm LIBS with such samples could give unreliable results about the diet and diagenesis and using 266 nm excitation should be more suitable.

4. Conclusions

- The UV-LIBS study for elemental fluctuation within the same dynasty is important to have a reliable picture of health conditions and dietary habits during these ancient eras.
- Depth profile measurements for bone samples using two laser excitation wavelengths show that UV-LIBS depicts more stable plasma properties which was very close to the bone ash standard reference sample used for calibration.

Depth profiling for strontium concentration relative to calcium can be taken as an indicator for the diagenesis effect from the surrounding postmortem environment.

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