Original article

Study of the feeding effect on recent and ancient bovine bones by nanoparticle-enhanced laser-induced breakdown spectroscopy and chemometrics

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HIGHLIGHTS

• Biosynthesized silver nanoparticles were used to improve the LIBS sensitivity.
• Cattle’s feed, recent, and ancient bovine bone were analyzed via nano-enhanced LIBS.
• PCA validated the spectroscopic data in discriminating bones and fodders.
• EDX and SEM were used also for the validation of the nano-enhanced LIBS results.
• The results were interpreted in view of ancient and recent animal feed strategies.

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ABSTRACT

This study aimed to exploit laser-induced breakdown spectroscopy, enhanced by nanoparticles (NELIBS), as a fast, sensitive and low-cost technique, to correlate the elemental composition of recent and ancient bovine bone with the elemental composition of the fodder that has been fed to the cattle throughout their life. Biosynthesized silver nanoparticles (BS-Ag NPs) were used to enhance the emission intensity of the spectral lines in the LIBS spectra of contemporary and ancient bovine bones and fodder samples. The ancient bones are more than 4600 years old and belong to the 3rd dynasty of the old Egyptian Kingdom. Ag NPs were biosynthesized in a simple and inexpensive manner using potato (Solanum tuberosum) extract. As a validation technique for the NELIBS results, EDX spectra were successfully used, and scanning electron microscopy (SEM) clearly discriminated between recent and ancient bovine bones. Additionally, principal component analysis (PCA), as a multivariate analysis technique, was used to validate the spectroscopic data for the discrimination between different bone types, as well as between different fodders. According to the obtained results, NELIBS spectroscopy combined with PCA can be used as a reliable, accurate, and fast method for the discrimination between different bones and different fodder types as well as for the assessment of the feeding strategies of livestock. The present work demonstrated the potential of NELIBS technique combined with PCA in the interpretation of the influence of feeding regimes on the contemporary and archaeological bone samples.

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Introduction

Livestock provides proteins such as milk and meat, which represent an essential contribution to humanity's food security. Industries depending on livestock and relevant products are among the most important prevailing industries globally. The main goal of animal farms worldwide is to secure food production with reasonable economic policies that are adequate for feeding massive populations. Feeding these animals represents a major challenge in both dairy and meat production farms, especially when green pastures are not easily available year-round, as in Egypt, for example. The production of high-quality forage is problematic in regions suffering from a scarcity of the rain necessary for planting grains and plants usable in feeding livestock [1,2].

Shortage of fodder and inadequate proper nutrients are major problems in the livestock production industry. However, parallel to the rapid growth and development of the animal production industries in Egypt, a similar evolution has occurred in the relevant animal feed industry. For the natural feeding of livestock, clover is considered the most important feed, whereas dry fodder normally consists of hays, grains, grain stalks, straw, dried clover, and dried barley. Additionally, wheat bran and rice bran are essential byproducts that are normally added to dry animal feed. Currently, in Egypt, animal farms depend mainly on artificial fodder produced from mixtures of grains, dried clover, grass, and rice straw in different ratios.

Similarly to the relationship between the elemental composition of human bone and diet [3–5], most elements found in the feed materials of the cattle are fixed in their bones. Consequently, bones can be considered an archive of the dieting system of such animals. In principle, the elemental analysis of bone can determine which feed type an animal depended on when they were alive. Because of differences in crop priorities, cultivation technologies, and available knowledge and experiences, major differences are expected in the dieting strategies of cattle between modern and ancient Egypt. Exploration of fodder cultivation in ancient Egypt is strongly related to the exploitation of dung as fuel. This relationship has been proven by the excavations undertaken by Miller [6] in areas belonging to the Egyptian Old Kingdom, as well as by Moens and Wetterstrom [7] at a site called Kom El-Hisn in the Nile delta. These careful studies revealed that most of the plants remaining in the dung assemblage represent the feed of cattle in these old eras. Such plant remains consist mainly of byproducts of crop processing (e.g., cereal chaff, hay, dry clover, and barley). It is not certain whether clover was provided as natural fodder. However, Charles could not ensure that such seeds eaten by cattle were always fed as fodder.

Animal bones consist of organic and inorganic components. The major inorganic component is essentially hydroxyapatite (HAP, sometimes called bioapatite), which has the approximate composition Ca_{10}(PO_{4})_{6}(OH)_{2}. HAP gives bone tissue its compressive strength. Moreover, the organic component (type I collagen) gives bone its tensile strength and a certain degree of flexibility. The elemental analysis of bone in archaeological, anthropological and environmental studies has provided a vast amount of information about the relationship between existing elements and dietary habits, culture, customs, health, and diseases in view of the excess or deficiency of certain important elements [11,12]. Hazards of toxicity by exposure to heavy metals (in food and/or water) in different communities, including ancient ones, have also been studied thoroughly via bone elemental analysis [13].

Regarding the elemental analysis of unique archaeological bone samples, it is not advisable to exploit destructive conventional analytical techniques (atomic absorption spectroscopy AAS, inductively coupled plasma-mass spectrometry ICP-MS, wet chemical analysis, etc.). Moreover, such conventional techniques cannot provide spatial analytical information about these samples. A suitable technique for the elemental analysis of archaeological samples that is fast, quasi-nondestructive, and requires no sample preparation is laser-induced breakdown spectroscopy (LIBS). In LIBS, mass removal is negligible (few micrograms). Additionally, LIBS can be used to perform high spatially resolved analysis. The fundamentals and applications of LIBS have been discussed in detail by many authors in numerous textbooks and review papers [14–17]. With robust and compact state-of-the-art lasers and spectrometers, the simplicity of LIBS system equipment furnishes the possibility of in situ (e.g., in museums and in excavation locations) measurements using portable systems.

Normally, a typical nanosecond LIBS system has a limit of detection (LOD) in the range of a few parts per million (ppm) for most elements. To improve the limit of detection of LIBS, many techniques have been proposed, including double-pulse LIBS, resonance - LIBS, microwave-assisted LIBS, and application of magnetic or electrical fields. All these techniques make the LIBS setup more complicated in addition to raising the cost of measurements. Over the past few years, nanoparticle-enhanced LIBS (NELIBS) has emerged as a new approach for enhancing the sensitivity of the conventional LIBS technique. In a typical NELIBS, a thin layer of metallic nanoparticles is deposited onto the target surface, improving the laser-induced plasma due to different mechanisms leading to surface plasmon resonance (SPR) between the laser pulse and the nanoparticles [18,19]. Recently, Poggialini et al. [20] and Abdel-Salam et al. [21] used biosynthesized nanoparticles (BS-NPs) to enhance the sensitivity of LIBS, thus making the technique safer and less costly.

The aim of the present work was to use the NELIBS technique to qualitatively correlate the elemental compositions of recent and ancient bovine bone with the elemental contents of the fodder they ate. Principal component analysis (PCA), as a chemometric technique, was used to validate the spectroscopic data for the discrimination between different bones and different fodders.

Material and methods

Samples

Three contemporary bovine femur samples were obtained from the local market near the slaughterhouse of Sakkara to ensure that the livestock lived in this locality, exposed to approximately the same environmental conditions that dominated older eras [22,23]. The contemporary local Egyptian cattle breed was “Baladi”, which is predominant all over the country. The samples were washed and cleaned thoroughly to remove any surface remains of fat, blood, and meat. Then, small pieces were cut to fit the target holder in the two-dimensional translational stage of the LIBS setup.

The archaeological bovine bone samples were also three of the femur compact tissue, whose denser mineralization effectively reduces any possible diagenetic alterations [24]. These ancient bone samples were obtained from the collections of the Egyptian Museum in Cairo with permission of the Egyptian Ministry of Antiquities. The samples belong to the third dynasty of the Old Kingdom (approximately 2670–2613 BC) and were found in 1974 at an excavation site in the vicinity of the Stepped Pyramid of Djoser at Saqqara, 23 km south of Cairo. Such old bone samples have been analysed without applying any chemical cleaning procedure to preserve the biogenic or diagenic compositional information. All LIBS measurements were performed on the outer surface of the bone samples. For NELIBS measurements, bone samples were...
sprinkled on its outer surface by 500 µL of the BS-Ag NPs (13 mg/L). The samples were left to dry in a clean atmosphere at ambient room temperature for about 1 h before exposing it to the focused laser pulses in the LIBS setup.

Fresh samples of barley, grass silage (clover) and artificial recent feed were obtained from the farms of the Departments of Animal Production at the Faculty of Agriculture, Cairo University. Forty grams of each sample type was milled and homogenized carefully in a clean mixer. A hydraulic press was used to produce tablets measuring 15 mm in diameter and 4 mm in thickness (each tablet was produced under 25 tons of pressure for 1 min) from each fodder type to be used in the LIBS measurements.

**LIBS instrumentation**

In LIBS high power laser pulses are focused onto the surface of the target. Focusing such a tremendous amount of energy on a tiny volume lead to melting and evaporation of few micrograms of the target material. With further heating of the material, vapor, atoms are excited, then ionization takes place and at the end, a collection of ions and swirling electrons forms the so-called plasma plume at very high temperature (>6000 K). As the plasma cools down, it gets rid of the previously absorbed energy in the form of optical radiation emission. The emitted light is collected and spectrally analyzed to give the characteristic spectral lines of the elements in the plasma plume, and consequently in the target material in case of stoichiometric ablation. The obtained spectrum provides qualitative information about the elemental structure of the target. To obtain quantitative results, suitable calibration using authenticated samples should be performed. At the early times of the laser-induced plasma plume evolution, the emission is very bright due to the overwhelming continuum emission that masks most of the characteristic spectral lines. To get rid of the continuum emission effect, the detector is triggered after a certain delay time after firing of the laser, and the time window during which the detector is sensitive is called the gate width.

To reduce the effects of the experimental fluctuations, namely the mass ablation, the plasma temperature, and the electrons density, the obtained spectra are normalized to the intensity of a spectral line of an element existing in the target material and considered as an internal standard. The line chosen for normalization should be free of self-absorption, well resolved, and its intensity is near the average of most other spectral lines [25].

The LIBS experimental setup used in the present work, described in detail elsewhere [26], includes a Q-switched Nd:YAG laser (Brilliant Eazy, Quantel, France) operating at its fundamental wavelength (λ = 1064 nm), producing laser pulses, each is of 5 ns duration and 50 mJ energy at a repetition rate of 10 Hz. A plano-convex fused silica lens with a 10 cm focal length was used to focus the laser beam onto the target surface, where the focal spot size was 86.54 µm. An X-Y micrometric translational stage was used to mount and move the sample in front of the focusing lens to obtain a fresh sample spot for each laser pulse. For dispersion and detection of the light emitted from the laser-induced plasma plume, an echelle spectrometer (Mechelle 7500, Multichannel, Sweden) coupled to a gateable ICCD camera, DiCAM-Pro (PCO, computer optics-Germany), was used. The ICCD is UV-enhanced, and the spectroscopic system covers the spectral range from 200 nm to 700 nm. The delay time and gate width of the ICCD camera were set to 1.5 µs and 3 µs, respectively. The LIBS++ software program [27] was used for spectra display, processing, and analysis.

**Biosynthesis and characterization of NPs**

A Milli-Q water purification system provided deionized water for the preparation of all reactant solutions. All glassware used was washed in aqua regia (HCl: HNO₃ = 3:1 (v/v)) followed by rinsing with deionized water. The required AgNO₃ and NaOH were provided by Sigma-Aldrich, St. Louis, Missouri, USA. Potatoes (Solanum tuberosum), for the preparation of the silver nanoparticles, were purchased from a supermarket near Cairo University. The Ag NPs were biosynthesized in a simple manner using potato extract following the method described elsewhere [21]. The estimated equivalent-circumference average diameter of the produced silver NPs was 15 ± 2 nm according to TEM measurements and UV–Vis spectroscopic analysis [21].

**PCA of LIBS spectra**

Principal component analysis (PCA) is an efficient statistical multivariate analytical method. In PCA, the dimensionality of spectra is reduced to extract the most crucial spectral feature variables by correlating the input data. The resulting new variables, normally called principal components (PCs), are calculated as linear combinations of the original variables. In the present work, the measured data were analysed statistically via PCA using commercial software (Origin Lab 2017). PCA was employed to examine the variations in the LIBS spectral data from ancient and recent bone samples and different types of animal feed.

**Results and discussion**

Fig. 1 compares the LIBS and NELIBS spectra for ancient (upper) and recent (lower) bovine femur bone samples. The displayed spectra represent the averages of 50 LIBS and NELIBS spectra for the ancient and the recent bone samples. Both sample types show a remarkable enhancement in the spectral line intensity in the case of NELIBS. The reasons behind this enhancement have been explained in detail by Dell’Aglio et al. [19], who showed that the main differences between nano-enhanced LIBS and conventional LIBS are the different ablation and excitation processes that affect the characteristics of the laser-produced plasma. The field enhance-
ment in LIBS produced by the nanoparticles deposited onto an insulating surface, bones in the present case, may be due to surface plasmon resonance (SPR), when the laser is in resonance with the local surface plasmon (LSP), or due to the effect of the high laser irradiance ($>1 \text{ GW/cm}^2$) on the NPs. In the first case, nanoparticle surface electron oscillation enhances the electromagnetic field and produces strong localized heating on the sample surface. In the second case, breakdown occurs in the NPs themselves, and the evolved plasma can be transferred to the part of the sample in contact with such nanoparticles [18]. In fact, the laser wavelength used in the present measurements (1064 nm) was not in resonance with the absorption peak (420 nm) of the NPs used [21]. Hence, the direct interaction of the laser with the NPs is the effect producing the LIBS intensity enhancement. In view of the different plasma production mechanisms that occur in the case of LIBS and NELIBS, it might be appropriate to follow different optimization regimes of the detection systems for conventional and nano-enhanced LIBS. However, the optimized values for the delay time and gate width were very close to each other for both LIBS and NELIBS measurements; therefore, the spectra collected in both cases in the present work were measured using the same values for these experimental parameters. As is clearly shown in Fig. 2, burial effects appear in the presence of spectral lines of silicon and titanium in the emission spectra of the archaeological bone samples, but not in the spectra of the recent bone. This diagenetic effect is mainly due to the diffusion of such elements from the soil into the bones buried for thousands of years. However, the intensity of the spectral lines of Fe, Ca, Mg, and Na is not as strong in the spectra of the contemporary bone as in the spectra of the ancient bone due to differences in the nutritional regimes, as will be demonstrated. From now on, all presented spectral data pertain to NELIBS unless otherwise mentioned.

Estimation of the bone hardness via the assessment of the ratio of the ionic to atomic spectral line intensity for calcium and magnesium in LIBS spectra has been previously used successfully [5].

The bar graph in Fig. 3 shows the spectroscopic estimation of the surface hardness of the investigated samples via the ionic-to-atomic intensity ratios of magnesium spectral lines at 279.5 and 285.2 nm. The loss of tensile strength and degradation of the mineral phase in archaeological samples occurs as a pronounced decrease in surface hardness (ionic to atomic intensity ratio) due to the loss of organic components. This degradation is, of course, more evident in the case of NELIBS, which improves the spectral line intensity.

To validate the LIBS results, EDX spectra were obtained for ancient and recent bones, as depicted in Fig. 4. The most impressing feature is the strong carbon line in the spectrum of the contemporary bone, which nearly disappeared in the spectrum of the ancient bone. This, of course, is in very good agreement with the LIBS and NELIBS spectra shown in Fig. 1, where the carbon line at 247.8 nm and the CN band at 388.3 nm appear only in the spectra of the contemporary bone. In contrast, silicon appears clearly only in the spectrum of the ancient bone. In the same figure, the micrographs of both bone types demonstrate the porous and rough surface of the ancient bone compared with the surface of the recent bone.

Principal component analysis (PCA), as a multivariate statistical approach, was used to discriminate between archaeological and fresh bones. Fifty spectra from each sample type were used, and the entire range of each spectrum (200–750 nm) was included. Fig. 5 shows that only two principal components are needed for a clear discrimination between archaeological and recent bones. Ancient bone samples data accumulated on the negative PC1 side, while most of the data of the recent bone accumulated on the positive PC1 side of the plot. PC1 and PC2 account for 90.3% of the data variance with PC1 = 82.5% and PC2 = 7.8%. Hence, the PCA shows a clear qualitative spectroscopic divergence between the ancient and recent bone samples, which distinguishes them according to their age.

**LIBS and animal feed**

Among the most important farm animals, cattle could be considered multipurpose animals, facilitating agricultural tasks in
the field, in addition to providing milk and meat. In the countryside, animal production farms are an essential source of wealth. The feeding of farm animals in ancient Egypt was dependent on natural plants, such as barley, clover, and legumes. However, the current feeding of farm animals relies mainly on artificial feed (with different mixed components). In the present work, LIBS was also used to analyse different types of animal food, namely, feed, barley, and clover, to correlate their elemental composition to that of the ancient and the recent bovine bones. Fig. 6 shows typical LIBS spectra of samples of feed, barley, and clover, with labeled spectral lines of the major and minor elements. Clover normally shows high digestibility, with relatively higher protein contents compared with those of other herbs provided to animals in pastures in ancient Egypt [28]. The abundance of plant types recognized as fodder vegetation, such as barley and legumes in samples found in the excavations of Kom el-Hisn (in the northwest Nile Delta), could be proof of the cultivation of such plants for use in feeding animals. Research has also ascertained the use of dung as fuel during these older eras based on charred plant remains [6]. In 2003, Crawford’s excavations at Tell el-Maskhuta (in the eastern part of the Nile Delta) indicated that clover represented 19% of the total number of seeds discovered at the site. This led Crawford to identify clover as an economic crop in addition to barley and emmer wheat. Crawford interpreted the excavated collections of charred plants as the probable use of most such plants to feed farm animals, along with grazing on the edges of waterways. In addition, Crawford mentioned that clover was mostly provided as a supplement to natural fodders either as a crop or a wild plant [9].

PCA has been utilized to obtain more information about spectral changes in LIBS data. In fact PCA has been used by many researchers in food studies [29–31]. In the present work, PCA analysis was
performed over the entire recorded spectral range (200–700 nm) in the LIBS spectra obtained from the samples under investigation. For each sample type, 50 spectra were used to construct the corresponding PCA model.

LIBS data pertaining to samples of clover, barley, and feed were used to plot the two principal components, as shown in Fig. 7 (a)–(c). The figure clearly shows that in all three score plots, the ancient and recent bone data are clustered together, whereas they are well separated from the feed, as expected. In Fig. 7a, the total variance is 91.1%, where the first principal component (PC1) accounts for 65.8% of the variance and the second principal component (PC2) accounts for 25.3% of the variance. The clover scores are clustered between the upper positive PC2 and the lower negative PC2. The score plot, in this case, did not elucidate the greater importance of clover as part of the diet of cows in ancient Egypt than in recent times.

Similar results were obtained for the PCA score plot of barley (Fig. 7b), with an overall variance of 92.9%. The PC1 variance was 75.9%, and the PC2 variance was 17%. This PCA result, of course, does not reflect the fact that barley was used as a major component of the cow diet in ancient Egypt. However, barley also represents one of the components of recent artificial feed.

Fig. 7c depicts the PCA results for feed. The total variance was 89.4%, with 58.4% associated with PC1 and 31.0% associated with PC2. The feed data points cluster almost equally between the upper positive and the lower negative PC2 areas. Accordingly, it is clear that artificial feed components include, also, many of the components fed to cattle naturally in ancient Egypt.

The results presented in Fig. 7 demonstrate that PCA is not decisive in detecting similarities and dissimilarities between the two bone types and any of the three dieting systems. However, PCA, in this case, can be considered just as a supporting indicator of the correlation between any of the bone types and any of the fodders that were already clearly attributed by the LIBS data. This might be due to the combined effect of the metabolism of the cattle and the effect of ageing of the bones, which might hinder the correlation between the elemental composition of clover, barley, and feed and the elemental composition of the bones. Consequently, a direct comparison of the elements most strongly assimilated from the three dieting systems in the LIBS spectra, combined with the PCA results might provide a more reliable distinction.

Certainly, the use of a large number of samples in this study would improve both the analytical and statistical results. However, dealing with archaeological samples limits the possibility of increasing the sample numbers, since this is related to the availability of such rare ancient objects in museums.
In the present work, LIBS was used to analyse different types of animal fodder, namely, artificial feed, barley, and clover, to correlate their elemental composition to the elemental composition of contemporary and ancient bovine bones. To enhance the LIBS analytical sensitivity, a NELIBS approach was used by sprinkling biosynthesized silver nanoparticles onto the bovine bone and fodder sample surface before analysis. The spectroscopic assessment of the bone surface hardness indicated the loss of tensile strength and degradation of the mineral phase due to the loss of the organic components in the ancient calcified tissue compared with that of contemporary bone. The LIBS results were validated by obtaining EDX spectra and SEM micrographs of the same bone samples.

A statistical analysis of the obtained LIBS spectra via the PCA technique revealed a highly pronounced discrimination between contemporary and ancient bone samples. However, PCA could not discriminate decisively between different fodders because, for example, the fresh fodder provided to cattle in ancient Egypt features many components, such as clover and barley, compared with the artificial dry fodder. In addition, the similarities between types of fodder and contemporary or ancient bone are not clear based on the obtained PCA results. Hence, a direct analysis of the LIBS spectra, in addition to the PCA analysis results, could be more trustworthy in the discrimination between different fodder types and in correlating them to the proper bone type. Moreover, the spectrochemical analytical data depicted in the present work demonstrates the presence of numerous elements in common in bone and fodders. This, of course, is relevant to the feeding strategy of the cows and their health along the lifetime, in general. In addition, it should be mentioned that this study is also beneficial for human beings health that depends on farm animals as one of their major food resources.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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References

[1] Naik PK, Swain BK, Singh NP. Production and utilisation of hydroponics fodder. Indian J Anim Nutr 2015;32:1–9.
[2] Soder KJ, Heins BJ, Chester-Jones H, Hafla AN, Ruhano MD. Evaluation of fodder production systems for organic dairy farms. Prof Animal Sci (PAS) 2018;34:75–83.
[3] Price TD, Kavanagh M. Bone composition and the reconstruction of diet: examples from the Midwestern United States. Midcont J Arch 1982;7:62–79.
[4] Fabig A, Herrmann B. Trace elements in buried human bones: intra-population variability of Sr/Ca and Ba/Ca ratios – diet or diagenesis? Naturwissenschaften 2002;89(3):115–9.
[5] Kasem MA, Russo RE, Harith MA. Influence of biological degradation and environmental effects on the interpretation of archeological bone samples with laser-induced breakdown spectroscopy. J Anal At Spectrom 2011;26:1733–5.
[6] Miller N. The use of dung as fuel: an ethnographic example and an archaeological application. Palaeorient 1984;10:71–9.
[7] Moens M, Wetterstrom W. The agricultural economy of an old Kingdom town in Egypt’s West Delta: insights from the plant remains. J Near East Stud 1988;47:159–73.
[8] Malleson C. Archaebotanical investigations at tell el-retaba. 2nd intermediate period – 18th dynasty cemetery and settlements. Egypt Levant 2016;26:129–43.
[9] Crawford, P., Weeds as indicators of land-use strategies in ancient Egypt. In: Neumann, K.; Butler, A.; Kahleheber, S. (Eds.), Food, fodder and fields. Progress in African Archaebotany. Kluwer, London; 2003. p. 107–21.
[10] Charles M. Fodder from dung: the recognition and interpretation of dung-derived plant material from archaeological sites. Environ Archaeol 1998;1:111–22.
[11] Nielsen-Marsh CM, Hedges REM. Patterns diagenesis in bone I: the effects of site environment. J Archaeol Sci 2000;27:1139–50.

Fig. 7. PCA analysis of the NELIBS spectra of ancient and recent bovine bone with clover (a), barley (b) and feed (c).

Conclusions

In the present work, LIBS was used to analyse different types of animal fodder, namely, artificial feed, barley, and clover, to correlate their elemental composition to the elemental composition of contemporary and ancient bovine bones. To enhance the LIBS analytical sensitivity, a NELIBS approach was used by sprinkling biosynthesized silver nanoparticles onto the bovine bone and fodder sample surface before analysis. The spectroscopic assessment of the bone surface hardness indicated the loss of tensile strength and degradation of the mineral phase due to the loss of the organic components in the ancient calcified tissue compared with that of contemporary bone. The LIBS results were validated by obtaining EDX spectra and SEM micrographs of the same bone samples.
