PRELIMINARY SUBMICROSCOPY OF A VERTEBRAL BONE FRAGMENT FROM A BITINIAN TOMB OF 2ND CENTURY BC IN BURSA, WESTERN TURKEY

BATI ANADOLU, BURSA'DA MÖ 2. YÜZYILA TARİHLENEN BİR BİTİNYA MEZARINDA BULUNAN OMURGANIN MİKROSKOP İNCELEME SONUÇLARININ İLK DEĞERLENDİRMESİ

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ABSTRACT

Despite the availability of large amount of information on human bones, little attention has been given to the environmental conditions of bone weathering and preservation. Secondary hydroxyapatite (carbonated hydroxyapatite for primary bone mineral) as the most well known but intriguing constituent of the bone was determined (SEM) in the pore spaces of a Bitinian (2nd century BC) mans vertebral bone fragment as aggregates together with probable amorphous compounds. Unweathered primary microcrystalline hydroxyapatites of the bone structure were also determined by TEM indicating resistance to weathering. Organic bodies such as the True Slime Moulds of the Phylum Myxomycota were observed feeding on hydroxyapatite fragments, and secondary minute hydroxyapatite aggregates forming on unnamed elongated mycelia. All these features add up to manifest the alterations that primarily occur in post mortem soil-less environments of bones more independently and freely than in soil media, without being masked by the numerous processes the latter would shelter.

ÖZET

İnsan kemikleri hakkında çok fazla bilgi olmasına karşın, kemiklerin ayrışması ve korunmasında çevre koşullarının etkisi üzerine yapılan çalışmalar kısıtlıdır. İkincil hidroksiapatit ve amorf bileşenleri çalışmada örnek olarak kullanılan Bitinya bölgesindeki bir insanının (MÖ 2. yüzyıl) vertablea kemik parcalarında gözenek boşluklarıyla birlikte taramalı electron mikroskobu (SEM) ile belirlendi. Kemik yapısında ayrışmamış birincil mikrokristalin hidroksiapatitler, geçirimli elektron mikroskobu (TEM) ile saptanmıştır. Phylum Myxomycota-True Slime Mould'ları gibi organik canlıların, hidroksiapatit mineral parçacıklarının üzerinde beslendiği gözlemlendi. Çalışmanın bulgularında saptanan tüm bu görünümler, topraklı ortamlardan farklı olarak ölüm sonrası topraksız ortamlarda daha belirgin bir biçimde ortaya çıkmışlardır.

INTRODUCTION

Large amount of information is available on human bones in archaeometry, medicine, and forensic studies indicating the presence of amino acids, collagen and inorganic fractions, namely as the hydroxyapatite (HAP- carbonated hydroxyapatite for primary bone mineral). Seventy percent of the bone is made up of HAP including the compounds of calcium (phosphate, carbonate, chloride, fluoride, hydroxide and citrate). Although may be present in amorphous forms, this primary mineral is predominantly crystalline (Hedges and van Klinken 1992) as platelets or rods, about 8 to 15Å thick, 20 to 40Å wide and 200 to 400Å long. Substitution occurs in the HAP of bone by inter-crystalline exchange and re-crystallisation, via dissolution, with the addition of new electrolytes, influencing the HAP formation in different pathways. Solutions containing phosphate salts initially accelerate the rate of HAP formation by reducing the incongruency of the $CaHPO_4$ dissolution and new crystal formation during which Ca^2 is replaced or electrolytes adsorbed on the crystal surfaces (Smith et al. 1983; Brown and Fulmer 1996).

Secondary HAP, which is of special interest for soil scientists and agronomists studying the dynamics of phosphorous in soils and its availability to plant's may form in a soil-less (the post-mortem conditions of tombs) or soil environment through the weathering of the bone or other phosphate sources. However, little attention has been given to the environmental conditions of bone weathering and preservation (Sobel and Berger 1994), despite the quantities and composition of surviving organic materials in a specimen depending on their burial environment (Garlick 1969).

Environmental factors that are suggested to influence the rate at which collagen degrades include the composition, pH and hydrology of the matrix; oxygenation; temperature; and changes brought about by soil flora and fauna (Henderson 1987). The protein contents of the bone undergo a relatively slow hydrolysis to peptides, breaking down into amino acids followed by a spontaneous rearrangement of the inorganic crystalline matrix, which weakens the protein-mineral (HAP) bond and leaves the bone susceptible to dissolution by the action of internal and external agents (Henderson 1987). The study we have undertaken is interdisciplinary attempting to determine the formation of crystalline/amorphous P compounds and the HAP orientation on and within the bone using the microscope and submicroscopic methods coupled with XRD to understand the dynamics of P as HAP in a soil-less environment.

MATERIALS AND METHODS

A segment of the backbone (vertebrae) of a Bitinian, Greco-Roman male recovered from a prestigious 2nd century BC tomb near Bursa, located in western Turkey-a tomb of high quality masonry with the bones of a high ranking person lying on the ground with the decayed/weathered artefacts and goods without direct soil contact (Figs. 1-2) was analysed at a Bruker AXS D8 Advance Cu k-alpha x-ray diffractometer as powdered/ground samples of around 20-50 micron size. Scanning electron microscopy (SEM-Carl Zeiss EVO 40) coupled with a Bruker AXS Microanalysis-XFlash 4010 unit was also used in order to reveal the occurrence and formation/chemistry of the HAP and its orientation/relation to the bone microstructure and other minerals. Transmission electron microscopy (TEM-JEOL-JEM 1400) was performed on the ultra thin sections (ultra cuts microtome) of the bone sample in order to determine the crystal morphology, orientation and degradation of the primary carbonated hydroxyapatite.

The soil body overlying the tomb was classified as a Colluvitechnic-Calcaric Regosol (a poorly developed (transported) undifferentiated calcareous clayey soil with low organic matter content -around 1-2% at the surface of the profile-, with a horizon sequence of A1, A2, ACk, Ck, 2Ckm overlying a tomb stone using the WRB soil classification (IUSS Working Group WRB 2006) with degraded macchia vegetation of the mild to xeric Mediterranean climate.

RESULTS

Similar to the earlier works, the bone sample studied contained well crystallized HAP with the full range of peak orders, most likely due to the absence of the long-decomposed/oxidised organic compounds expected to mask the XRD peaks (Fig. 3). Secondary HAP ($Ca_5(PO4)_3OH$) (Deer et al. 1974) together with amorphous phases of calcium phosphate (ACP) aggregates were determined in the large rounded pores of the bone (Fig. 4). The leached solutions (observed during the field study) from the overlying soil and the humid environment created in the tomb have most probably being responsible for the dissolution of some of the primary P minerals (Fig. 5) and development of the secondary hydroxyapatite. The coalescing of the poorly crystalline HAP with amorphous calcium phosphate confirmed the transformation via an internal rearrangement, instead of the prevailing earlier view of the dissolution – re-precipitation process (Fig. 4). The SEM observations also revealed the presence of densely oriented primary fibrous HAP as randomly distributed and interwoven domains acting most likely as reinforcements in the bone matrix along with the minute unweathered primary HAP crystals disclosed by TEM (Fig. 6).

The coalescing pores determined under the SEM with the patchy diffused matrix seem to be the sites of elemental P loss via the probable weathering of the larger primary HAP minerals (Fig. 7). The True Slime Moulds of the Phylum Myxomycota observed on the surface of the bone may have been the repeatedly rejuvenated long-standing generation of scavengers which are still active today. The development of the sporangia of the Myxomycota may well be an indicator of the advanced feeding process taking place on the bone HAP and consequently leading to the liberation and mobilisation of the elements within the cavities of the bone matrix (Fig. 8). This process seems to contribute to the weathering of the bone matrix rich in HAP followed by the development of the secondary (0.25-0.50 μm in size) ACP/HAP minerals and aggregates, confirmed by the EDAX peaks of Ca and P. They are forming on the elongated mycelia-like organic features (determined by EDAX peaks of C) most likely due to the development of the strong bond between the HAP and the protein as stated by Henderson (Henderson 1987) (Fig. 9). Studies on the protein-HAP interfaces together with studies on other minerals transforming to HAP in protein rich environments has been illuminating for the strong relations of the bonded organic-inorganic compounds and of the surface bonding properties of HAP and its predecessor minerals during deposition (Xie et al. 2002; Stayton et al. 2003).

The Phytolith of the wheat glume is of an undefined primitive species which is still present in Anatolia, and is most probably a remnant of the tomb accessories/donations of the funeral ceremony (Fig. 10). The rhombohedric calcite crystals found around the wheat glume appear to be slightly dissolved secondary crystals, and they are likely formed on the bone and were subsequently subjected to slight dissolution perhaps by percolating waters from the overlying soil. The existence of the same wheat species in Turkey since the 2nd century BC, may document the consistency of the climatic conditions from that period to the present.

CONCLUSIONS

The changes/alterations of the 'P' states from primary to secondary, determined in the bones studied revealed that HAP is a relatively soluble mineral. However, despite the loss/weathering/dissolution of the primary HAP in the course of history, the sharp peaks of the mineral determined by XRD are most probably of secondary origin (the individual HAP minerals and their aggregates with ACP) as has also been observed visually. The leached soil solutions should be responsible for this morphological-wise subtle, but micromorphological-wise evident phenomenon. The fibrous HAP seems to be consistent against the dissolution by retaining its interwoven morphology in the bone matrix at scanning electron microscopy magnifications, whereas the ultra thin sections of TEM with higher magnification revealed the co-existence of the platelets and fibres of HAP in the bone matrix at much smaller sizes, probably indicating higher resistance to hydrous dissolution. The probable feeding process of the Mycomycetes (The True Slime Moulds) on the HAP grain – although a well known fact at post mortem environments- seems to indicate the consumption of P as a nutritive source by the organisms taking part in the bone weathering and consequently contributing P as a plant nutrient to the soil environment, following their life cycle. The partly dissolved surfaces of the calcite crystals determined around the wheat glume may also manifest the leaching processes from the overlying soil. However, this phenomenon requires further elaboration by the use of higher resolution microscopy and other relevant equipment on bones in the soil as well as soil-less environments. The wheat glume lying on the bone, of contemporary morphological similarities, may indicate the somewhat similar climatic conditions of the area to that of the 2nd century BC.

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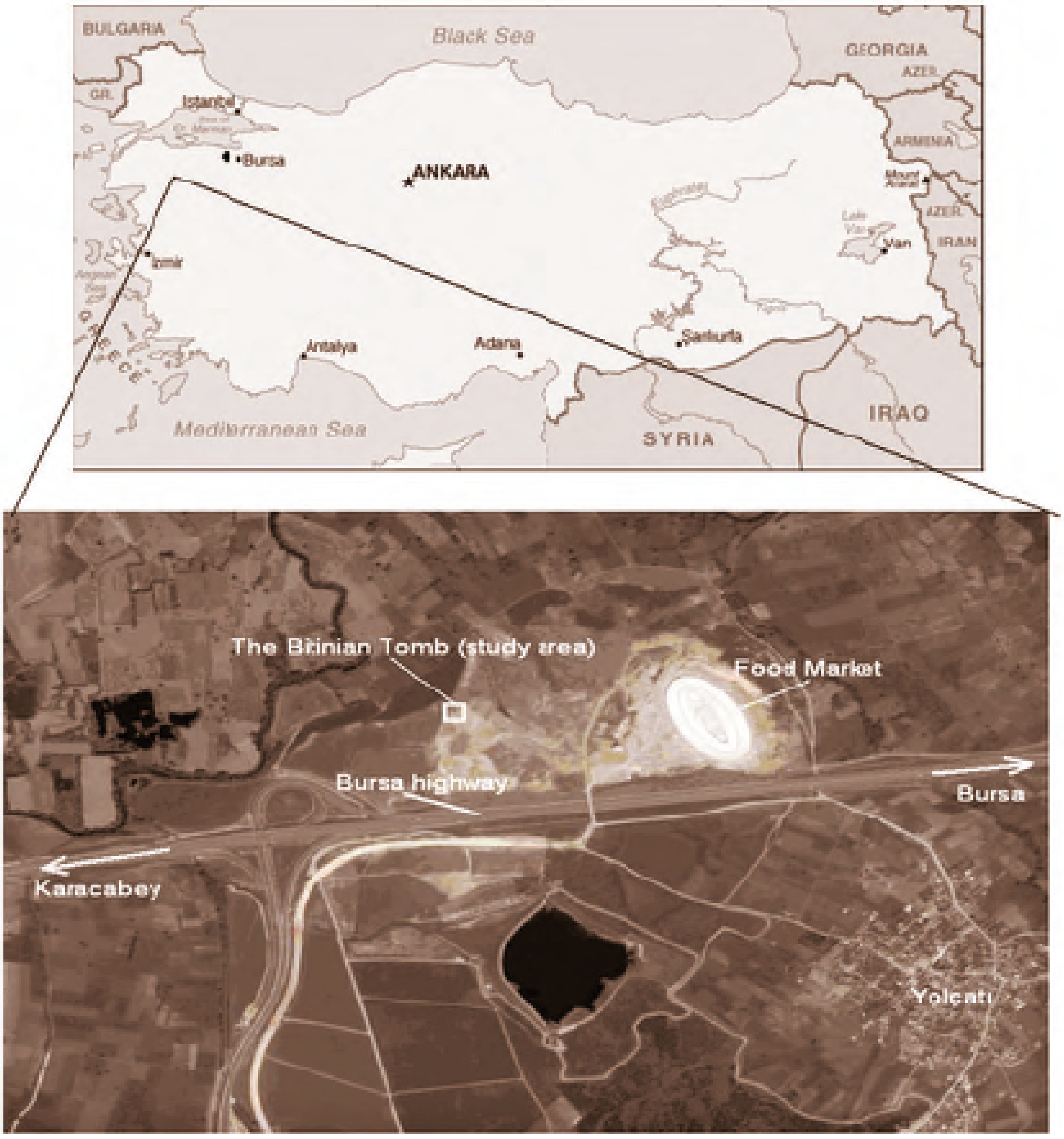

Fig. 1 - Location map and site of the excavated Bitinian tomb and overlying soil.

Fig. 2 - Colluvitechnic-Calcaric Regosol (a poorly developed undifferentiated soil profile overlying a tomb stone- the Technic qualifier of the recent WRB soil classification) deposited on the tomb of the recovered bone.

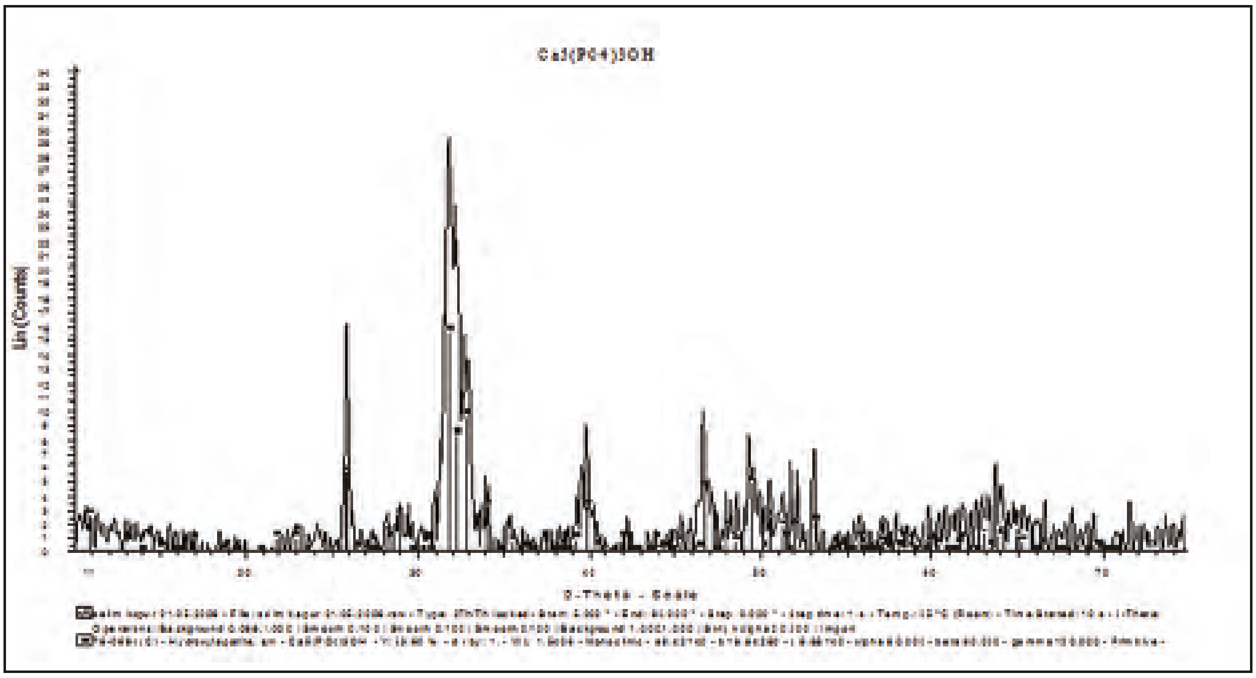

Fig. 3 - HAP mineral (XRD) in Bitinian bone.

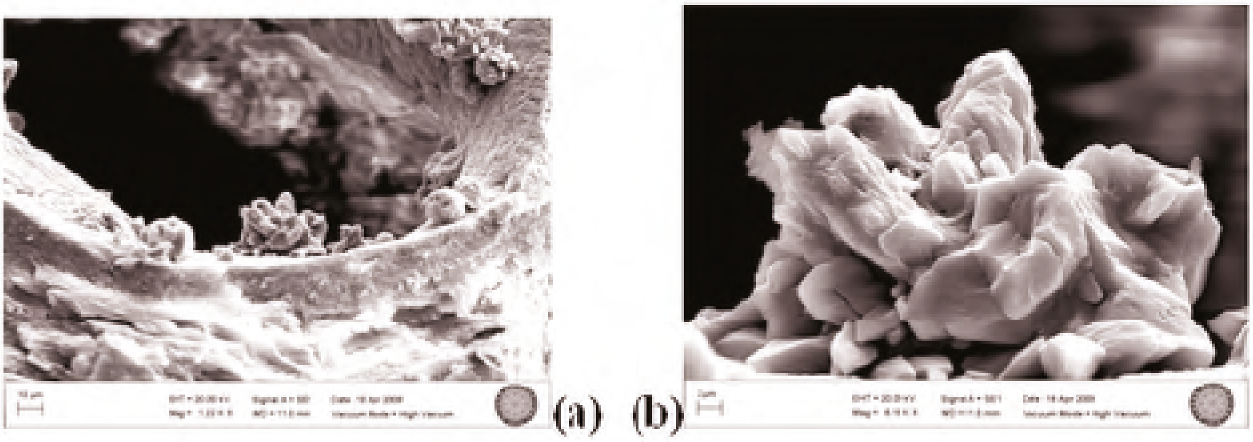

Fig. 4 - (a, b) HAP and ACP aggregate in pore (SEM).

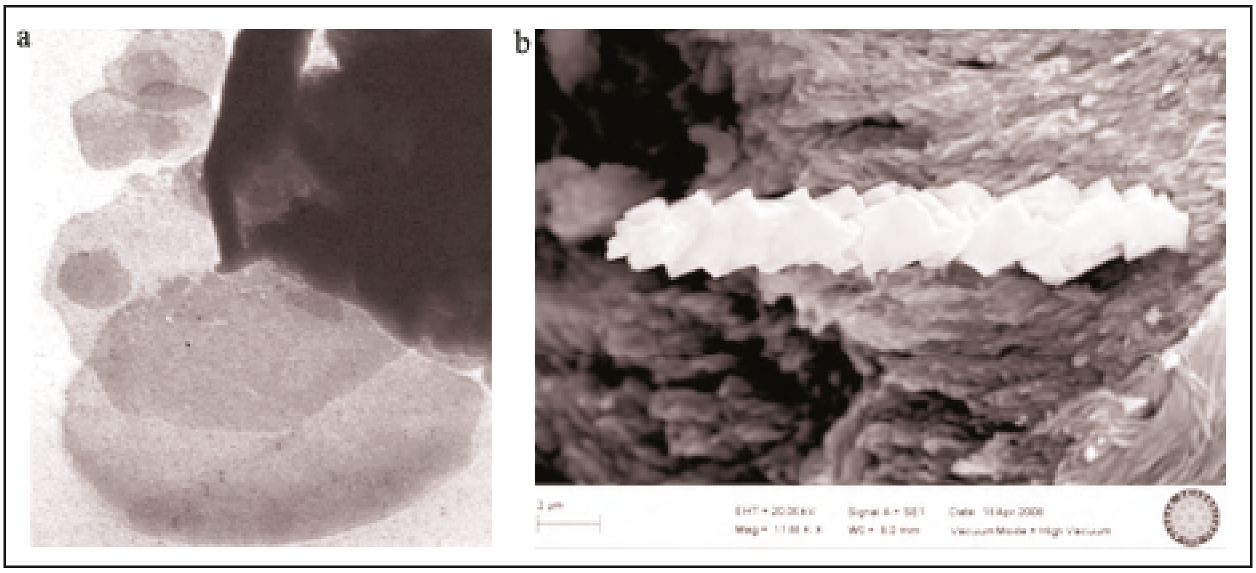

Fig. 5 - (a) Partly degraded/weathered hexagonal primary HAP minerals in bone (TEM), (b) coated/encrusted secondary (pseudo-hexagonal) calcite in bone matrix.

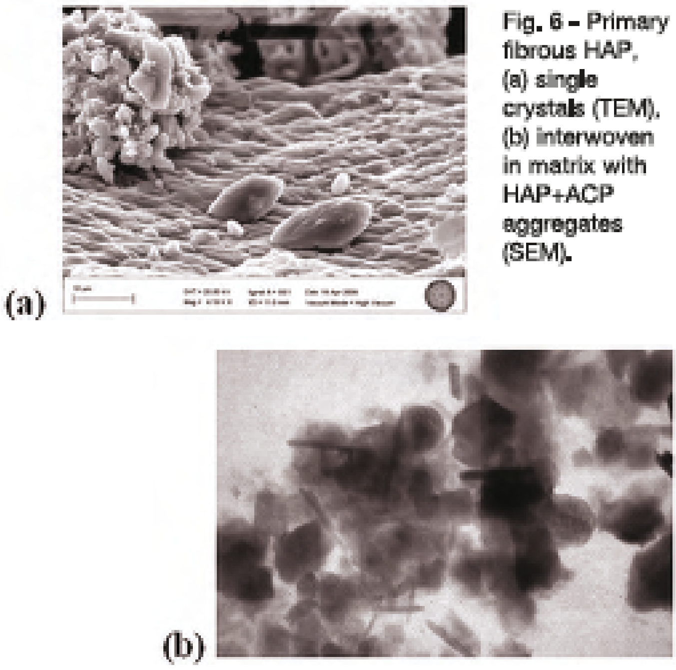

Fig. 6 – Primary fibrous HAP, (a) single crystals (TEM), (b) interwoven in matrix with HAP+ACP aggregates (SEM).

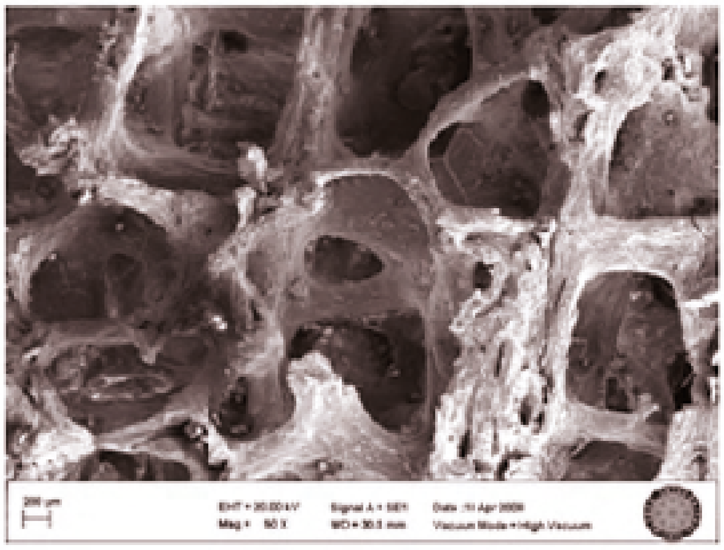

Fig. 7 - The coalescing pores with the patchy diffused matrix (SEM).

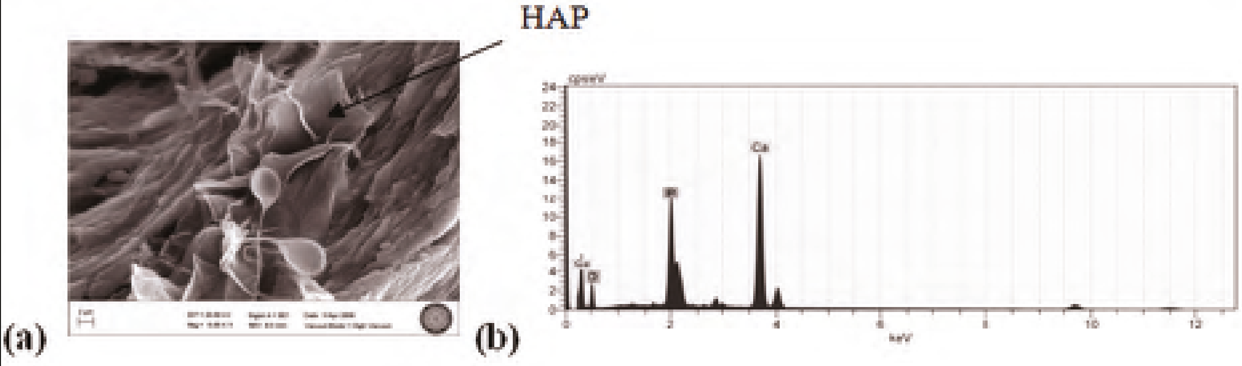

Fig. 8 - (a) Myxomycetes on HAP mineral and (b) chemical composition of the HAP mineral under the net of the Myxomycetes.

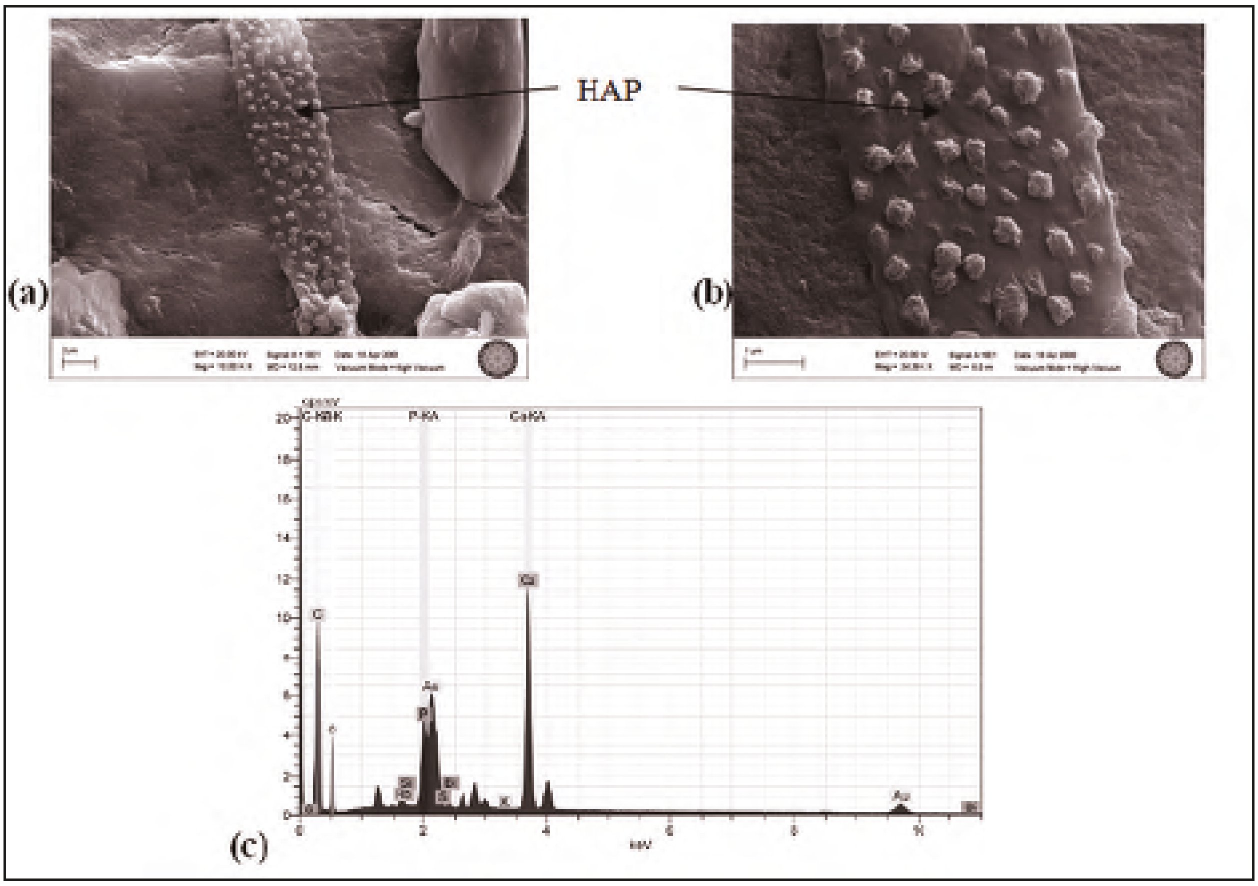

Fig. 9 – (a,b) Elongated organisms with HAP aggregate formations on surface (c) chemical composition of the aggregates.

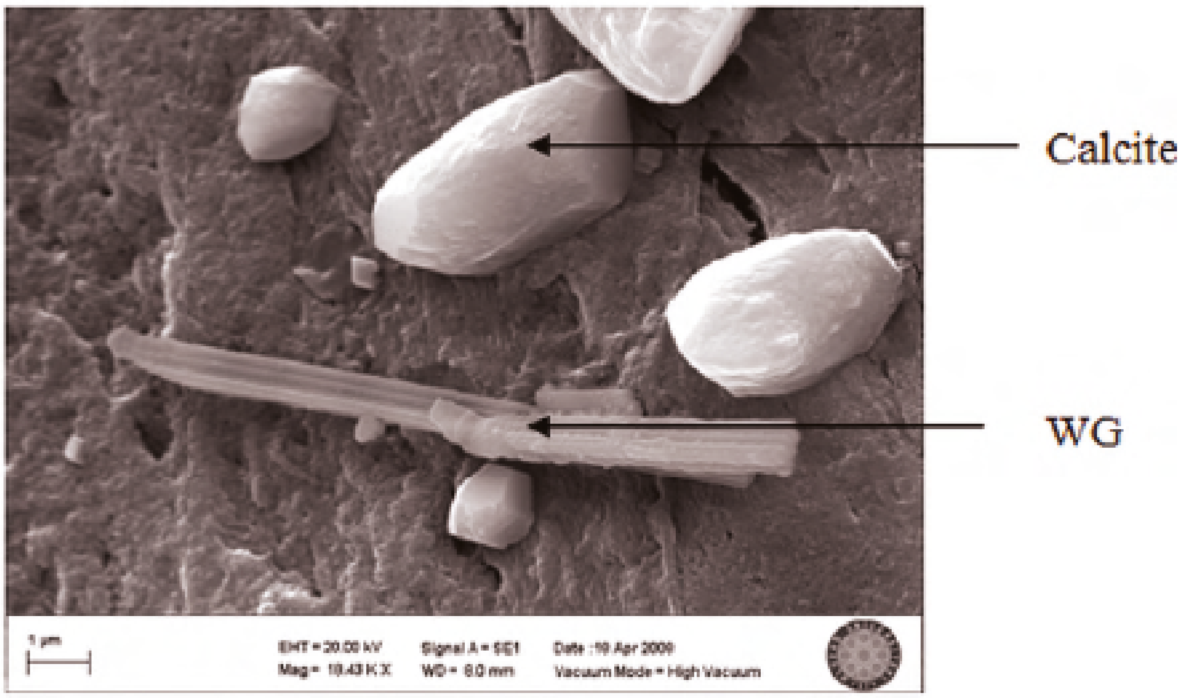

Fig. 10 – The wheat glume (WG) remnant on the bone (SEM).