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Identification of Trace Elements in Bovine Metacarpus Bone by Spectro Chemical Analysis

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*Abstract***.** Bone is highly organized material from the gross macroscopic to the molecular level. Bone is natural composite material, which by weight contains about 60% mineral, 30% matrix and 10% water. Spectro chemical method is described for the determination of major, minor and trace elements in animal bone. In the present study we analyse bovine metacarpus bone sample by spectro chemical analysis using Jerrel ash plane grating spectrograph and we find out major and minor constituents present in it. Analytical methods are described for the determination of major, minor, and trace elements in bone. V, Ca, Mo, Pb, Fe, Zn, Cu, Mg, Al, P are identified in the bovine metacarpus bone as major trace elements and the 16 elements are identified as minor trace elements *i.e*., Os, Sc, Y, K, Mn, La, Cd, Pd, Ru, Na, Sn, Pt, Bi, As, Si and Co. It is interesting to note that very low amount of trace elements have been shown to be essential to the growth of animal bones.

*Key words***:** Bovine metacarpus bone, spectro chemical analysis, Jarrel ash plane grating spectrograph, trace elements, major constituents, minor constituents.

Introduction

Calcium phosphate (CP) materials have been used extensively for bone replacement augmentation due to their similarity to the mineral component of bone. In addition to being non-toxic, they are biocompatible, not recognized as foreign in the body, and most importantly, exhibit bioactive behaviour, being integrated into the tissue by the same process active in remodeling healthy bone. This leads to an intimate physicochemical bond between the CP implants and bone, termed Osseo integration. Frequent request for information from industry, University, and government groups for our bone spectro chemical methods have prompted the preparation of this research paper. The demand for high-quality complete chemical data on bone can only become greater with increased bone use and bone products use, in order to establish reliability and comparability of analytical data some standards and guidelines are required. The analysis of trace heavy elements in animal bone by spectro chemical analysis has been studied although currently, there exists a number of multiple procedures with major use made of neutron activation analysis[1], X-ray fluorescence[2], emission spectroscopy[3], and spark-source mass spectrometry[4]. Spectro chemical Analysis method with its capability for low detection limits, mineral sample requirements and moderately accessible instrumentation, renders it increasingly useful for multiple-element bioassay in various media. This report concerns itself with results of the analysis of selected biological substances for multiple element levels using spectro chemical analysis. Evans and Morrison[5] have evaluated the applicability of SSMS for (Spark- Source Mass Spectrometry) the quantitative determination of trace elements in biological materials, but their study did not match or emphasize the heavy element region or a direct sampling procedure. The mineral phase in bone is a non-stoichiometric Calcium Phosphate arranged in a hexagonal apatite lattice. Bone mineral closely resembles synthetic hydroxyapatite [Ca_{10} $(PO_4)_6$ $(OH)_2$]. Pidapatri *et al.* suggested that about 75% (by weight) of the bone material is deposited in the gaps within the collagen fibrils [6], Bone contains type I collagen, which is arranged in robust fibrils [7,8].

Bone is a natural composite material, which by weight contains about 60% mineral, 30% matrix and 10% water (Fig. 1).

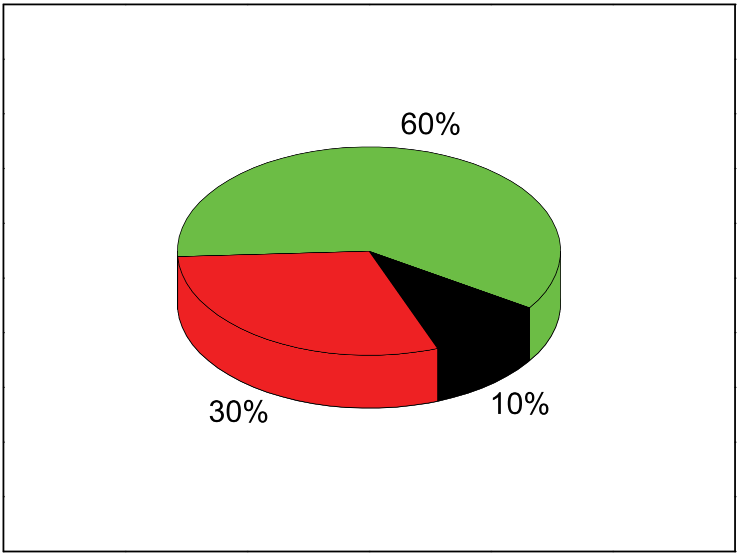

Fig. 1. Pie Chart of bone natural composite material.

Bone is also living tissue, with about 15% of its weight being due to the cellular content[9]. In order to better understand and mimic the properties of bone, much work has gone into finding the trace elements present in the bone, major elements and minor elements. At the molecular level, bone has three helices are wound in to a super helix, 3000 Å long 15 Å diameter and a molecular weight of 300, 000 Da [10]. Analytically on a dry weight basis, bone consists roughly 65-70% of the inorganic crystals of the Calcium Phosphate salt, apatite and 30-35% of organic matrix of which collagen makes up the major fraction (95-99%)[11]. Another important aspect which has large remained explored and their importance poorly understood is the trace element of bovine meta- carpus bone. Several workers[12,13] investigated the presence of many elements in the body. It is obvious that physical and electrical properties of bone will be drastically affected by the presence or absence of some or all of the ions. The material aspect of bone is rather neglected. This is probably because of the fact that the degradation of material is thought to drastically effect its behaviour[14]. So estimation of trace elements in bovine bone is essential to analyse bone status. Little is known about the relation between bone composition and structural properties of bone. In therapeutic application detail knowledge of the trace elements present in bone may be useful in future. Although, estimation of trace elements in bone by different methods have been described earlier[4,12-14,19], the estimation of trace elements in bovine metacarpus bone have not been studied to our knowledge. So we conducted this study.

Materials and Methods

Fresh bovine bones namely meta carpus were collected from slaughterhouses in different places of Andhra Pradesh State in India, *i.e.*, Hyderabad, Mahabub Nagar, Nalgonda, Khammam, Anantapur, Dharmavaram, Kadiri, Kadapa, Rayachoty, Puganur and Palamaner as shown in Fig. 2.

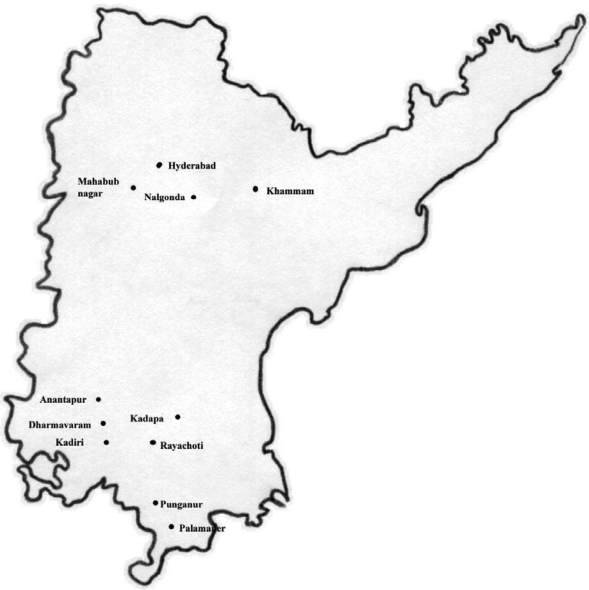

Fig. 2. Different places selected in Andhra Pradesh State in India for the collection of bone samples.

Fleshly material present on the bone was removed and sun dried. Small pieces were cut from mid region of the bone, and grinded in mortar. Then this powder was shifted in to ball mill (model No: Retsch PM 200 Germany) to achieve fine powder of 45-micron particle size.

Meta Carpus Bone

Classification: Long bones.

Location: The metacarpus is located between the distal row of the carpus (knee) and the phalanges (Digits).

Description: The metacarpus bones vary in number with the species of animals. The metacarpus is composed of a shaft and two extremities. The shaft has two surfaces, anterior and posterior. The anterior surface is convex, while the posterior surface is somewhat flattened, giving the bone an oval cross section. The shaft is slightly curved interiorly, giving it a bowed appearance. The metacarpus bone of bovine is shown in Fig.3.

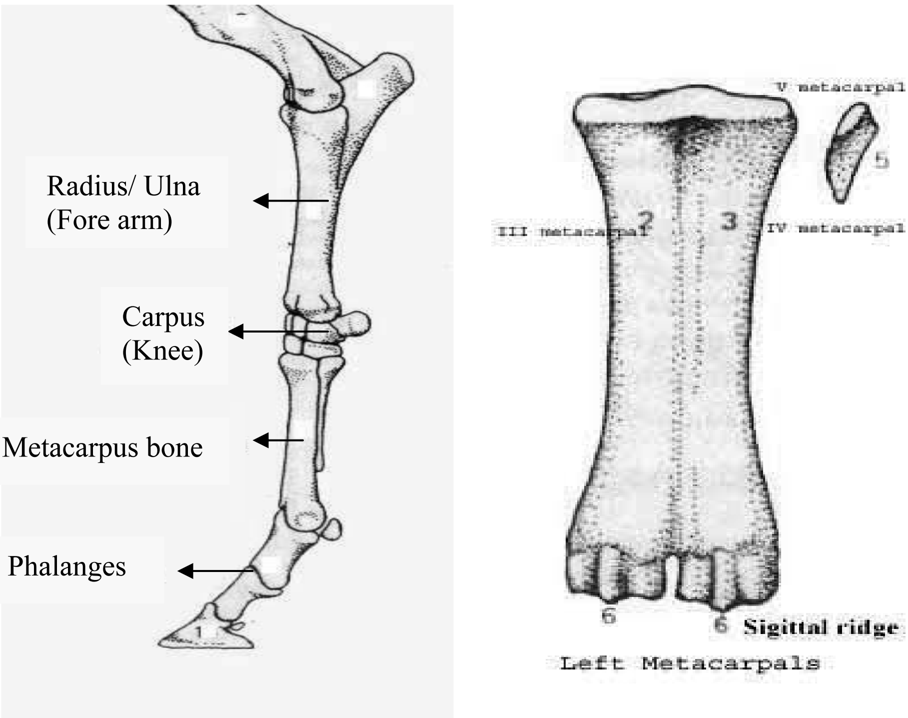

Fig. 3. Bones of the Pectoral limb- bovine.

To analyse the bovine metacarpus bone sample by spectro chemical analysis we used Jerrel Ash 1.5 M plane grating spectro graph (model no. 19-300 USA). The electrodes were drilled with a fine drill bid, one of them is filled with the R.U powder, (RaiseUltima), while the other filled with sample under investigation. The electrode containing the given sample is placed in the arc stand and arc is struck between the electrodes and spectrum was recorded. During the exposure the current strength was varied so as to make the requisite excitation potential for various elements present in the sample. The R.U powder (RaiseUltima) spectrum was recorded in juxta position. Similarly iron spectrum was recorded in juxta position (upper) of the sample, so that spectrum of the sample was in between R.U powder and iron spectrum as shown in Fig. 4.

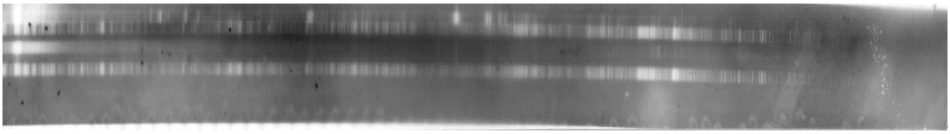

Fig. 4. Grating spectro graph of sample of Rock and bovine metacarpus bone.

The wavelengths of any line in the sample spectrum is found out, and by comparing the iron spectrum with R.U powder (RaiseUltima) spectrum whether a particular element present in the sample or not. The intensity variation will enable us to determine the major and minor components of the elements.

Results and Discussion

After spectro chemical analysis of bovine metacarpus bone by using Jerrel-Ash plane grating spectro graph the major and minor elements are tabulated in Table 1.

Table 1. Major and minor constituents present in bovine metacarpus bone.

Major constituents	Minor constituents
Vanadium (V)	Osmium (Os)
Calcium (Ca)	Scandium (Sc)
Molybdenum (Mo)	Yttrium (Y)
Lead (Pb)	Potassium (K)
Iron (Fe)	Manganese (Mn)
Zinc (Zn)	Lanthanum (La)
Copper (Cu)	Cadmium (Cd)
Magnesium (Mg)	Palladium (Pd)
Aluminium (Al)	Ruthenium (Ru)
Phosphorous (P)	Sodium (Na)
	Tin (Sn)
	Platinum (Pt)
	Bismuth (Bi)
	Arsenic (As)
	Silicon (Si)
	Cobalt (Co)
	Strontium (Sr)

However, the recent experimentation suggests that dead bone also possesses a variety of Physical properties[15]. Therefore it seems quite pertinent to explore and correlate the presence of trace elements to the overall bone behaviour.

Trace metals are thought to play an important role in the synthesis, cross –linking, calcification and diseases of connective tissues[16-18]. Bone disease analysis could be possible through this estimation of elements in bovine metacarpus bone by spectro chemical analysis. Several investigators[19,20] observed regularity in the appearance of certain metals Cu, Fe, and Zn. These and possibly other ions may be structurally incorporated in the collagenous matrices. These are not merely passive cellular products, but are themselves active substrates for initiating the growth and repair of connective tissues[21,22]. Spadaro and Becker[23] found that Pb, Si, Sr, and V found in relatively reduced concentrations in bone mineral, and seems to be absent from demineralized bone to relatively low limits. But in bovine metacarpus bone we found these elements. Therefore, part of the content of these metals in bone is bound to the mineral, either as substituted or interstitial ions. In contrast, part of Cu and most of the Fe remained with the decalcified organic matrix, with little or none remaining with the bone mineral as suggested by Behari[14]. This indicates that these ions are relatively strongly bound to the bone collagen and may therefore be functionally important to it. The actual average concentrations of Cu and Fe in the organic matrix in terms of dry mineralized bone were found to be 2.7 ± 0.4 ppm and 8.4 ± 3.7 ppm respectively. Zn was also detected in bone samples. Rai and Behari[24] detected Mg, Pb, Cu and Fe as trace elements in rat bones. They found the presence of Cu in the range of 5-50 ppm and Fe, Mg and Pb in the range of 50-500 ppm. The results of Spadaro and Becker[23] confirming thereby that these are ions functionally important. The experiment is repeated several times with different bone samples of bovine metacarpus bones and an excellent reproducibility is observed. The study of identification of trace elements in bovine metacarpus bone by spectro chemical analysis may provide the means for quantitative diagnosis of bone status. In addition better understanding of major and minor trace elements present in the bone, may help one to improve techniques related to electrical stimulation and fallow up of healing process in fracture bone. The paper constitutes a step towards the application of bone composition and its diagnosis.

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تحديد وتعريف العناصر المكونة لعظم مشط اليد للبقر بالطرق الكيميائية

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المستخلص. تعتبر العظام مكونة من مواد طبيعية منتظمة وتحتوي على ٦٠٪ مواد معدنية طبيعية، و٣٠٪ مواد مختلفة أخرى، بالإضافة إلى ١٠٪ من الماء. عادة ماتستخدم طريقة الطيف التحليلي الكيميائي لتحديد العناصر المكونة للعظام. في هذا المقال تم تحديد العناصر التي تدخل في تكوين عظام حيوانية من عظمة مشط اليد عند البقر وبطريقة التحليل الكيميائي. ومن العناصر التي وجدت بكميات عالية هي V, و Ca و Mo و Pb وFe وZn وCu وMg وAl وP ، أما العناصر الأخرى التي وجدت بكميات قليلة فهي Os, Sc, Y, K, Mn, La, Cd, Pd, Ru, Na, Sn, Pt, Bi, As, Si and Co.