The effect of water and mineral content on Ultrasonic parameters in bovine bone scapula, rib and femur

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***Abstract* – In the present investigation, the influence of water and mineral contents on the ultrasonic parameters of bovine bone scapula, rib and femur has been studied. It is found that the water is comparatively high in scapula (12%) and low in femur (10%) when compared to the rib (11%). From the data on density, it is obvious that the deposition of calcium phosphate in the matrix of collagen is more or less homogenous for femur, whereas in the case of scapula and rib, the deposition is inhomogeneous. Among the bones studied, the calcium deposition is comparatively high in femur than rib and scapula, while the magnesium content is low. Study shows the compressional wave velocity in bovine scapula, rib and femur is more or less the same in scapula and rib, but comparatively less in femur. While the values of shear wave velocity in rib and femur are in the close range, but more than scapula. The coefficient of absorption of compressional wave in bovine scapula, rib and femur values lie in a close range with respect to scapula and rib are more when compared to femur. It is found that phosphorous is less than 1%; Si, Fe, Cu, Al, are less than 0.1%, while Ba, Sr, Pb, Zn are very much less than the traces i.e., less than 0.001%. The element magnesium is present in bones studied in the range of 1 to 3%, more in scapula (3%) less in femur (1%) when compared to rib (2%).**

***Index Terms* – calcium phosphate, femur, magnesium, rib, scapula, water content.**

I. INTRODUCTION

Goldman and Hueter [1] published a compilation of the available data, on the velocity and absorption of high frequency sound in mammalian tissues. This publication provides a useful qualitative picture of the frequency dependence and range of values of the important wave propagation parameters. Kessler [2] suggested that the linear frequency dependence of mammalian tissues prevails for about 100 MHz. On of the most important findings in the elucidation of the ultrasonic absorption process in biological media was the observation that protein constituents contribute mostly and that protein solutions exhibit comparable absorption magnitudes and similar frequency dependencies as tissues. After about one decade, measuring techniques has progressed to the point where absorption can be determined in solution of biopolymers to nearly 500 MHz.

Attempts were made to model calcified tissues as a pseudohexagonal elastic structure, using ultrasonic velocity measurements as the means to calculate elastic constants. Lang [3, 4] first postulated the concept of pseudohexagonal elastic structure, giving three examples of dried bovine phalanx, fresh bovine phalanx, and dried bovine femur. Lees and Rollins [5] reported a set of elastic constants of fresh bovine enamel and dentin. Yoon and katz [6] applied the pseudohexagonal concept to the dried human femur. These authors have provided their own argument to support the concept. The pseudohexagonal elastic properties, determined along the axis of long bones are quite different from those in the transverse plane, whether measured or computed from the sonic velocity. It assumes the transverse plane to be isotropic and the sonic velocity in this plane to be independent of the direction. The long bone axis is assumed to be the axis of symmetry. As consequence of the assumption these investigators used relatively large specimens carefully oriented with respect to the long axis of the bone, but arbitrarily taken within the bone. Lang used specimens up to 25mm in dimension, while the specimens of Yoon and Katz were about 5mm thick. Both longitudinal and shear wave velocities were measured. Sonic velocity was calculated by dividing the thickness of the specimen by the transit time of sonic pulse in it. Lang worked at 5 MHz for the longitudinal mode and 2.5 MHz for the shear mode. Yoon and Katz used 1 MHz for both the modes. Lang dried his specimens at 35°C under vacuum, while Yoon and Katz did so at 24°C. Both the authors then allowed the specimens to come to equilibrium with an atmosphere of unknown humidity because they did not know the water content of their specimens.

Sonic velocity can be calculated from the measurement of the transit time for a sonic pulse through a known thickness of the medium. It assumes the sonic path to be along the normal to two parallel plane surfaces. When the material is isotropic and homogeneous, a plane longitudinal wave launched into one surface propagates along the normal to emerge with the same orientation from the second plane surface. If the medium is anisotropic the wave launched at a surface by a transducer results in three waves; none of which is truly longitudinal or transverse, except for special circumstances. In terms of displacement, one component is defined as quasi longitudinal (L) because it has a predominantly longitudinal character. The other two are quasi-transverse (T_1, T_2). Each component will propagate along its own way and with its own phase velocity [7].

Additional factors influencing the properties of the bone are attributable to its condition and to the species. It is well known that the elastic properties vary considerably with moisture content [8]. Marino et.al [9] showed a strong dependence of the water content of the bone on humanity. Biltz and Pellegrino [10] showed density, water content and chemical composition to be species specific. Lee et.al. [11] Reported the longitudinal plesio-velocity for various specimens within a single section of adult bovine tibia measured by the pulse echo-method at room temperature of 23°C. The distribution of acoustic properties was determined for the bone sample in its original wet state, when dehydrated, and again in the rehydrated state. It was seen that the plesio-velocity in the bone sample was strongly dependent on the direction of sonic wave propagation as well as the site of origin of each specimen.

Parry and Chivers [12] presented a compilation of the reported values of velocity and attenuation of ultrasound in mammalian tissues, including bones, to give a clear picture of the state of knowledge and enable it to be assessed. Lee et.al. [13] made an attempt to correlate plesio-velocity with physical parameters of the bone by taking compact calcified tissues from a wide variety of species and reported linear dependence of the plesio-velocity on the wet density of three categories of wet mineralized tissue. Pal [14] made an attempt to correlate mechanical properties of human cortical bone with their acoustical properties. The dependence of longitudinal wave velocity on density and mineral content was also seen.

The complex role of various components of bone mineralization is sufficiently exposed by various workers [15, 16, and 17]. A role of magnesium in the ordinary events accompanying bone mineralization has been suggested. In the adult rat, intake for 48 days of abnormal amounts of magnesium, either too much or too little, arrested the growth of the mineralization system of tibia [18]. E Novitskaya et al [19] studied the mechanical properties of demineralized and deproteinized cortical bone as a function of anatomical direction.

So, an attempt is made to study in detail the influence of water and mineral content on ultrasonic parameters in bovine bones scapula, rib and femur.

II. MATERIALS & METHODS

Bovine bone scapula, rib and femur bones were collected from a freshly slaughtered animal within a few hours after death. Fleshy material and bone marrow was removed from the femur leaving only the critical region. Specimen was taken from the mid region of the bones along their axes. After completing the compressional wave velocity, shear wave velocity and absorption coefficient measurements, water content of the bone specimens was determined by finding their masses before and after oven drying for 24 hours at the temperature of 100°C. The specimens were decalcified by treating them with 0.9% nitric acid for 24 hours and then washed in running water for 24 hours. The dimensions – length, breadth, thickness, radius of the specimens were obtained by using vernier caliper and screw gauge.

The density of wet, decalcified and oven dried specimens was measured by taking their masses in air (m_1) and when immersed in water (m_2). Then density (d) is calculated as

$$d = \frac{m_1}{m_1 - m_2} \text{ gm/cm}^3$$

percent water content of bones (W%) was determined by taking masses before and after oven drying and using the formula

$$W\% = \frac{m_1 - m_3}{m_1} x100$$

where m_1 = mass of the bone, m_3= mass of oven dried bone.

For the estimation of calcium in the bone samples, 1 gm. of powder sample was dissolved in conc. HNO_3 and kept for 24 hours. Then the solution was diluted and made up to the mark in 100 ml standard flask. 20 ml of the solution was pipette out and further diluted up to 200 ml. Then the solution was heated to boiling and 25 ml of 6% ammonium solution was added to precipitate ions into calcium oxalate. This is filtered by using Watmann No. 40 filter paper. The precipitate was washed with cold distilled water and transferred into a conical flask. 25 ml of diluted H_2SO_4 was added and titrated against potassium permanganate solution of 0.0098 moralities. By noting the end point the amount of calcium present in the given sample was calculated. Elements present in bovine bones, in traces, were estimated by atomic emission technique. For this purpose, Jewell Ash 3.4 m. Ebert grating spectrograph was used, in which the arc current is 8 amps.

In order to determine the compressional wave velocity, shear wave velocity and absorption coefficient of bovine bone scapula, rib and femur, pulse transmission technique is used [20]. This technique involves the measurement of transit time of ultrasound of a given frequency, through the known thickness of the sample [Fig. 1, Fig. 2]. The transit time of the ultrasound through the bone sample was measured using this technique. The velocity (V) was determined as

$$V = l / t$$

Where 'l' is the travel distance and 't' is the delay time of ultrasonic wave in the experimental sample.

The parameters of pulse propagating in the bone tissue are as follows:

Frequency	:	1 MHz
Pulse length	:	6.5 μ s
Pulse rate	:	400 Hz
Initial delay	:	Zero
Sweep speed (for oscilloscope)	:	1 μ/cm

The velocity of compressional and shear waves was measured in bovine scapula, rib and femur bones in the direction perpendicular to the bone axis (Z-direction).

The coefficient of absorption (α) of ultrasonic wave was calculated by the relation

$$A = A_o e^{-\alpha x}$$

where A_o = height of the reference pulse

A = height of the pulse with the sample and x=length of the sample between the transducers

The absorption coefficient was determined, in the bones, in the direction perpendicular to bone axis (Z-axis).

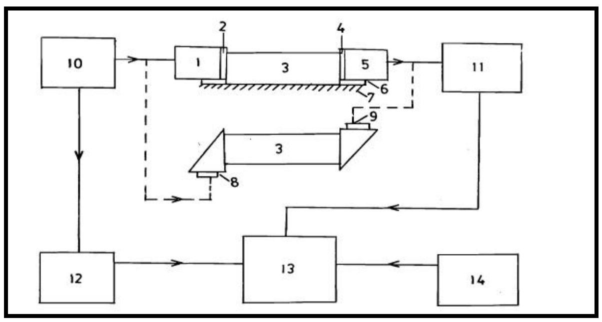

Fig. 1 Block diagram of the experimental set up
(1)Ultrasonic generator housing (2) PZT transmitter (3) Sample (4) PZT receiver (5) Ultrasonic receiver housing (6) Interlocking device holding [1 &2] (7) Table top (8) Wedge type shear wave generator (9) Wedge type shear wave receiver (10) Pulse generator (11) Broad band amplifier (12) Synchronizing generator (13) Oscilloscope (14) Variable delay time marker.

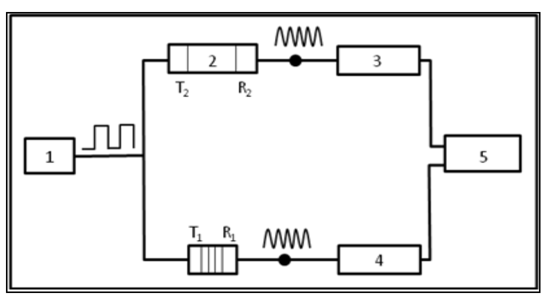

Fig. 2 Compensated Ultrasonic Timer Technique (CUTT)
1: Single shot mode pulse generator (50V, 50W), 2: Sample, 3 & 4: Preamplifiers (0-40dB), 5: Digital universal counter/timer (±0.001 μs).

III. RESULTS

The density of the bone is related to mineral content, water and also the organic composition. From the data on density, it is obvious that the deposition of calcium phosphate in the matrix of collagen is more or less homogenous for femur, whereas in the case of scapula and rib, the deposition is inhomogeneous.

It is found from the data on water content of bovine bones, which is comparatively high in scapula (12%) and low in femur (10%) when compared to the rib (11%). Water plays an important role in influencing the physical properties of biological tissues. On the basis of water content, biological tissues are differentiated as soft and hard [21].

The values of percentage of calcium present in bovine bones, mostly in the form of calcium phosphate, estimated by analytical method. It is evident from the data that there exists considerable variation in calcium content of the same bone along its axis or in different bone samples.

Table I. gives the data on density, water content, mineral content (calcium phosphate) and ultrasonic parameters such as compressional wave velocity, shear wave velocity and absorption coefficient of the bovine bone scapula, rib and femur. From the data it is evident that the density of femur (2.1 ± 0.02 gm/cm^3) is higher than that of scapula (1.824 ± 0.13) and rib (1.83 ± 0.7), but it bears more or less the same value in scapula and rib [22]. Moisture content is found to be high in scapula (13.3 ± 1.3%), low in femur (9.5 ± 0.37%) and in between for rib bone. Calcium phosphate deposition is 47.5% in femur which is slightly more than that of scapula and rib in which the percentage is 42.1 and 41.5 respectively. Further, it can be noticed from Fig.3 to 11that no definite relations exist between the parameters related to the composition of bone (density, water content, mineral content) and the parameters (velocity, absorption) concerned with acoustic wave propagation through the bony material [23]

It is evident from table that the compressional wave velocity in bovine scapula, rib and femur is more or less the same in scapula and rib but comparatively less in femur [24]. Table I. shows the data of shear wave velocity in scapula, rib and femur bones. It is interesting to note that the values of shear wave velocity in rib and femur are in the close range, but more than scapula. Coefficient of absorption of compressional wave in bovine scapula, rib and femur values lie in a close range with respect to scapula and rib are more when compared to femur.

Table II, reports the data on the elements present, in traces, in bovine scapula, rib and femur bone. It is found that phosphorous is less than 1%; Si, Fe, Cu, Al, are less than 0.1%, while Ba, Sr, Pb, Zn are very much less than the traces i.e., less than 0.001%. The element magnesium is present in bones studied in the range of 1 to 3%, more in scapula (3%) less in femur (1%) when compared to rib (2%).

The magnesium plays a major role in the deposition of apatite in the matrix of proteins present in the hard tissues. Among the bones studied, the calcium deposition is comparatively high in femur than rib and scapula, while the magnesium content is low. It can be concluded that the magnesium regulates the deposition of calcium phosphate in the tissue, the calcium deposition decreases as the concentration of magnesium increases in the tissue. In the adult rat, intake for 48 days of abnormal amounts of magnesium arrested the growth of tibia.

IV. DISCUSSION

In cancellous bone tissues – scapula (3925 ± 350 m/s), rib (3838 ± 413 m/s) ultrasonic compressional wave velocity is the same, but it is relatively more than that of compact bone, the femur (3237 ± 192 m/s). The compressional wave velocity in bovine scapula, rib and femur bones is more than those reported for soft tissues [25] and hard calcified derivatives of integuments [26, 27]. The shear wave velocity in scapula (1588 ± 300 m/s) is less than that of rib (2326 ± 279 m/s) and femur (2134 ± 122 m/s). The shear wave velocity is found to be high when compared to soft tissues and hard calcified derivatives of integument (1250 – 1330 m/s) [28]. The absorption coefficient of ultrasound of a tissue is an important parameter as it is sensitive to the structure and molecular composition of the tissue. The absorption coefficient of ultrasound is higher in scapula (5.13 ± 0.92 cm^{-1}) and rib (5.31 ± 1.47 cm^{-1}) than femur (2.84 ± 1.04 cm^{-1}).

V. CONCLUSION

It can be concluded, in a broad sense, that it is the water which characterizes controls and regulates the ultrasonic parameters of the biological tissues. However, in the past, several relations were proposed between density, water content and mineral content, and ultrasonic velocity by making the measurements of these parameters in different types of cancellous and compact bones of various animals. Further, both molecular composition and structure of the tissue whether it may be a soft tissue (muscle) or hard tissue (bone), have the associated functionality in influencing the ultrasonic propagation properties.

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TABLE I

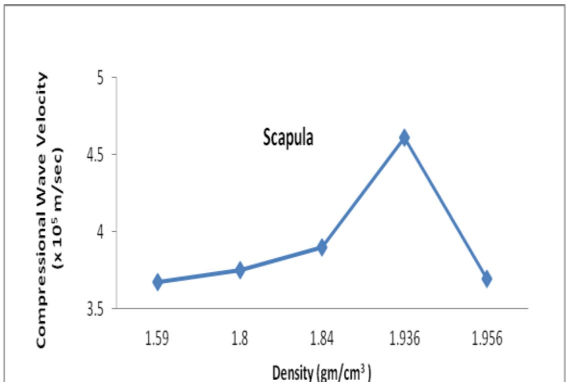
Water content, calcium phosphate and ultrasonic parameters in bovine bones

Identification	Density (gm/cm^2)	Water conetent (%)	Calcium phosphate (%)	Compressional wave velocity (x10^2 cm/sec)	Shear wave velocity (x 10^2 cm/sec)	Absorption Coefficient α (Np/cm)
Scapula						
S1	1.590	14.3	37.5	3677	1405	5.07
S2	1.800	14.5	42.7	3750	1500	4.51
S3	1.840	13.5	44.3	3897	1345	6.41
S4	1.936	13.2	43.6	4609	1432	3.83
S5	1.956	10.8	42.3	3696	2162	5.84
Rib						
R1	1.919	10.6	42.4	3470	2063	4.80
R2	1.916	10.9	42.4	4015	2279	3.32
R3	1.763	11.6	41.8	4559	2541	7.09
R4	1.804	10.9	42.2	3694	2009	4.42
R5	1.760	11.5	38.8	3455	2740	6.94
Femur						
F1	2.128	9.8	48.9	3333	2000	1.87
F2	2.126	9.5	50.5	2875	2000	1.51
F3	2.119	8.9	47.6	3299	2149	2.85
F4	2.080	9.2	46.0	3242	2292	3.72
F5	2.070	9.9	44.6	3438	2076	4.23

TABLE II

Percentage of elements analyzed in bovine bones

Bone	Mg	P	Si	Fe	Cu	Al	Ba	Sr	Pb	Zn
Scapula	**3**	**<1.0**	**<1.0**	**<1.0**	**<1.0**	**<1.0**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Rib	**2**	**<1.0**	**<1.0**	**<1.0**	**<1.0**	**<1.0**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Femur	**1**	**<1.0**	**<1.0**	**<1.0**	**<1.0**	**<1.0**	**<0.001**	**<0.001**	**<0.001**	**<0.001**



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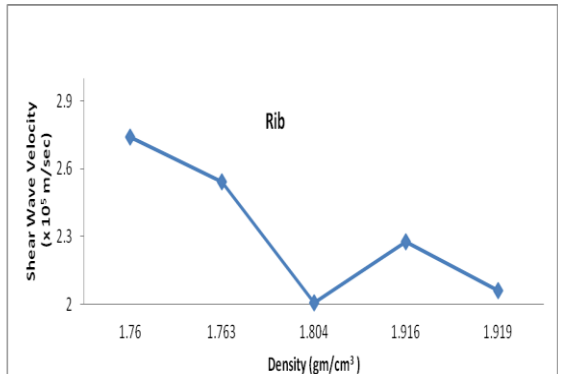

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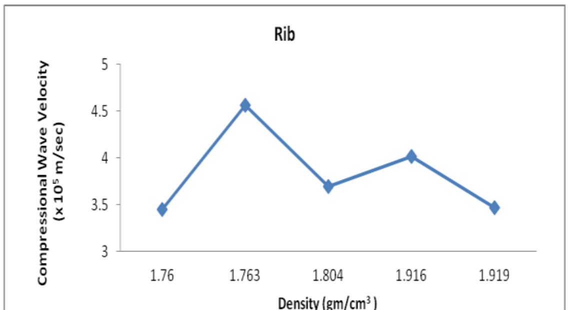

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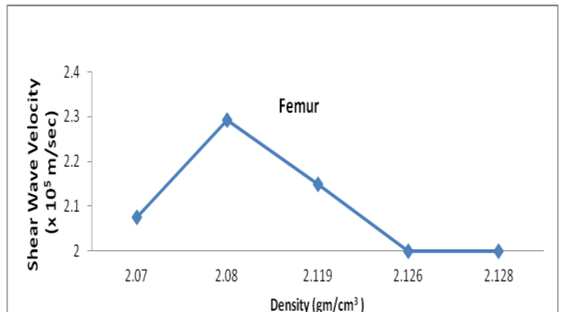

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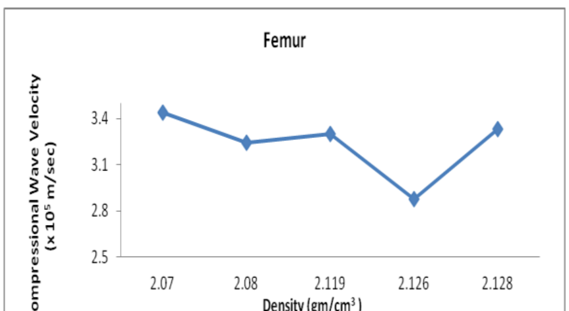

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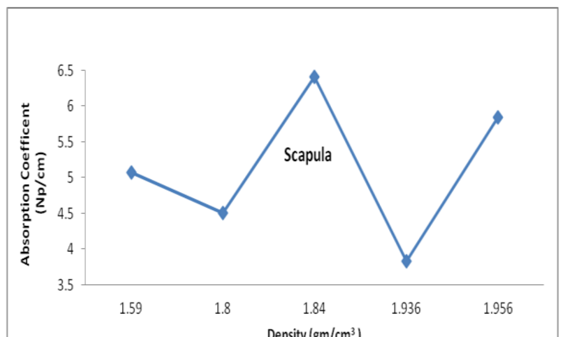
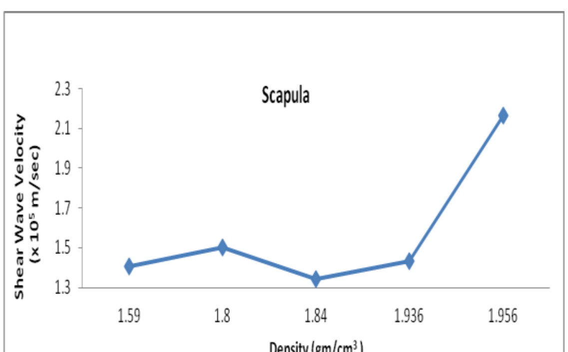


Fig. 5a



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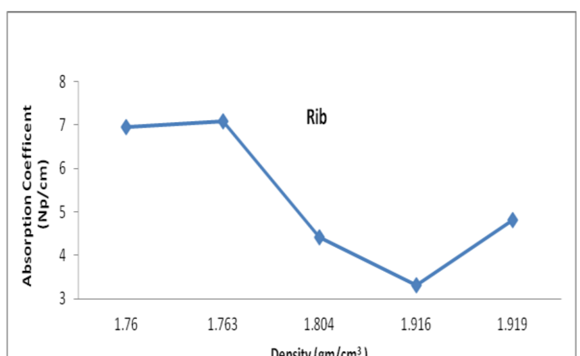
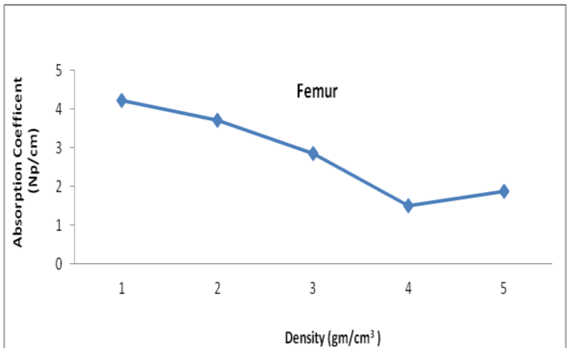


Fig. 5b



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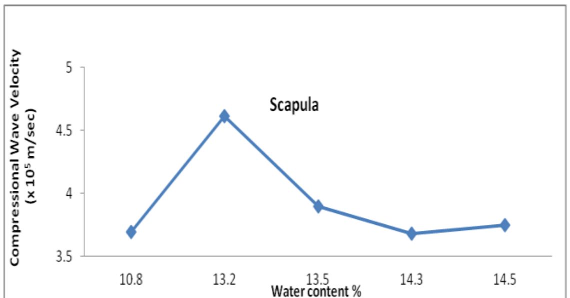
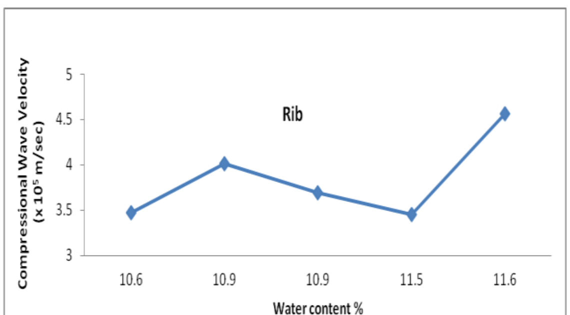


Fig. 6a



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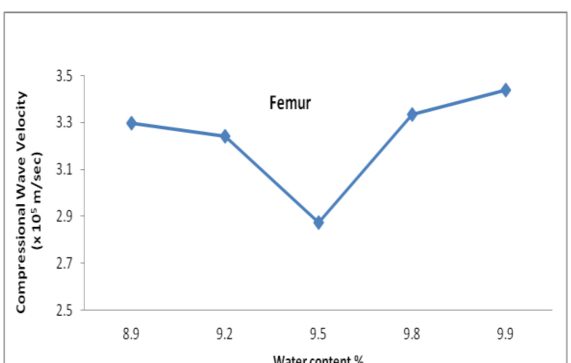

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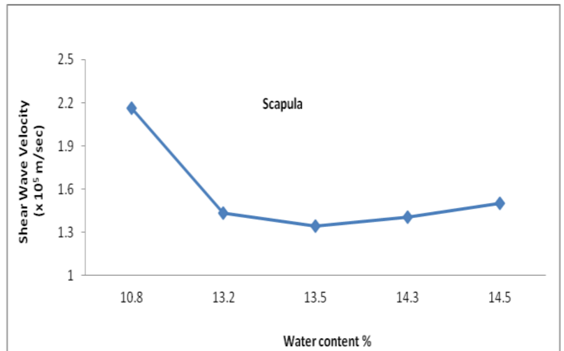

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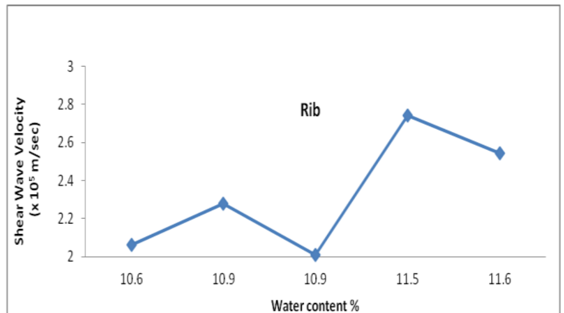

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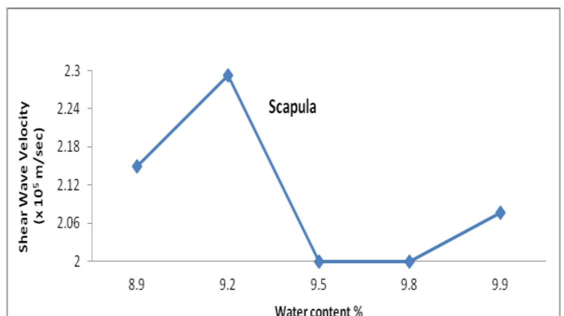

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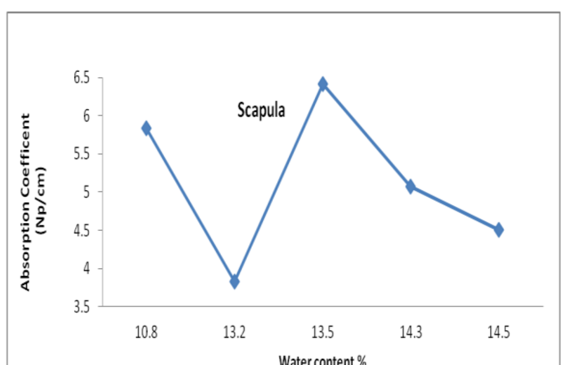

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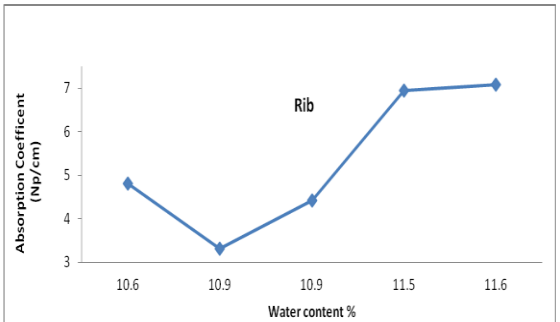

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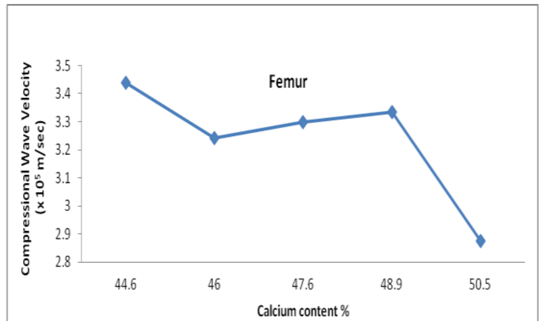

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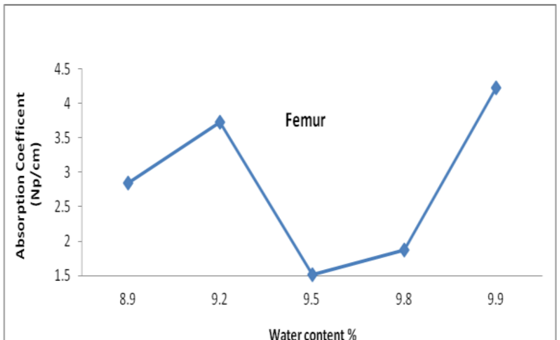

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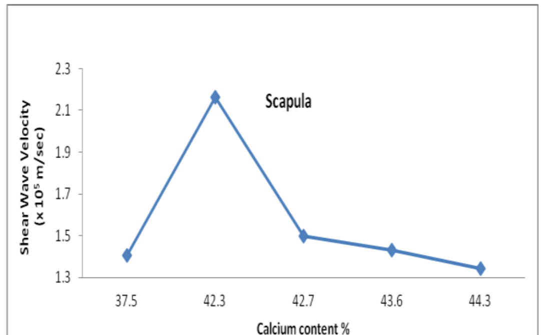

Fig. 10a

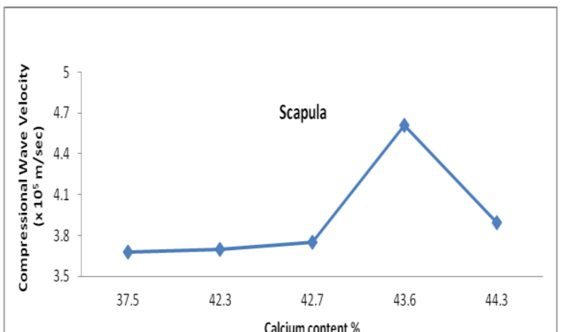

Fig. 9a

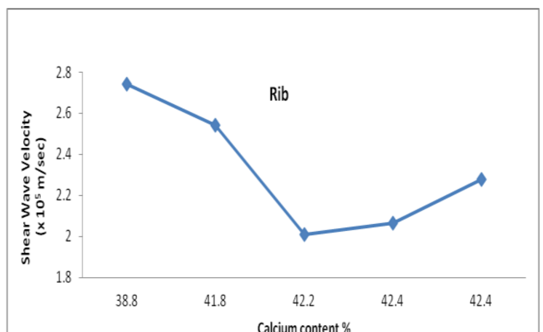

Fig. 10b

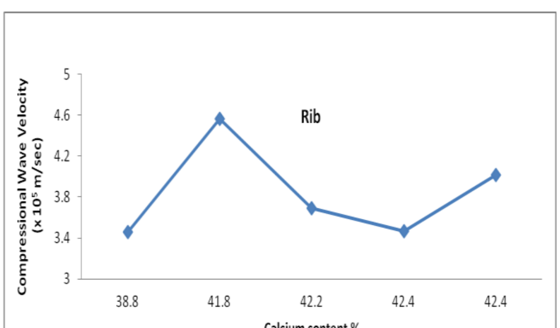

Fig. 9b

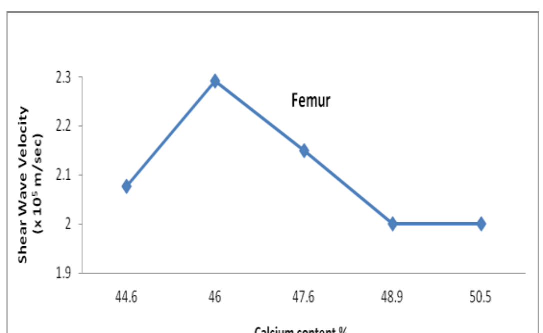

Fig. 10c

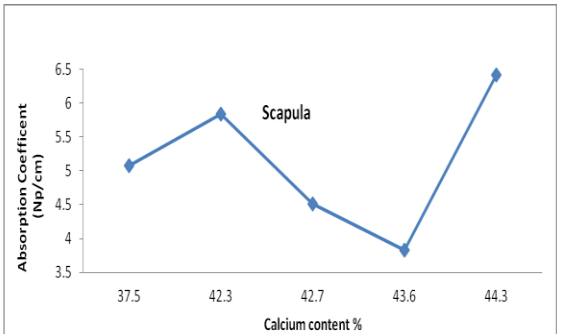

Fig. 11a

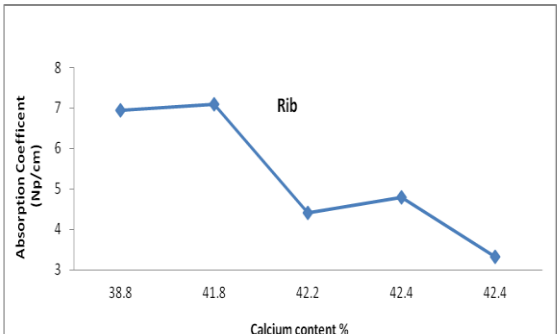

Fig. 11b

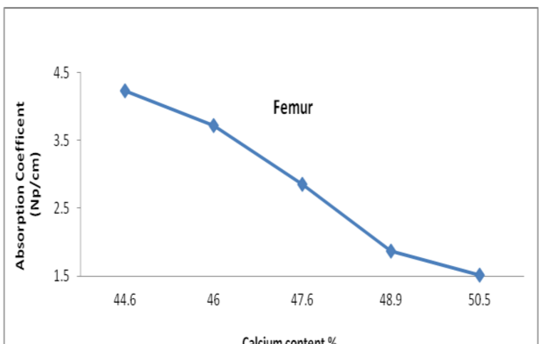

Fig. 11c