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Biochemical, biomineral and microstructural properties of the present day bivalve *Meretrix meretrix* shells from the Thoothukudi coast, Tamil Nadu, India

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Abstract

Bivalve shells are an affluent source of calcium content and significant for lime-based industries development. Indian coastlines are rich in bivalve shells occurrences. In this study, Meretrix meretrix (Bivalvia, Veneridae) shells of Thoothukudi coast of Tamil Nadu are taken to study the biochemistry, biomineralization and microstructure characteristics of the shell. After the morphological investigation, the shells were made into fine powder for meralogical and chemical analysis. XRF and XRD instruments were used for chemical and mineralogical measurements. Shell microstructure examination carried out using SEM. The XRF results reveal a high percentage of CaO content in the shell along with Fe, Sr and Mo. The XRD exhibits 12 peaks; all the peaks report aragonite minerals. The microstructures are examined in the shell portions of growth lines and umbo. The umbo part exhibits irregular homogeneous microstructures, whereas the growth lines exhibit granular homogeneous structure and prismatic structures. The umbo part consists of pore spaces which signify the growth of the shell is incomplete due to less availability of the extrapallial fluid (EPF). In the growth line part, two parallel sets of linear depression mark are present, which signifies that there is no sufficient nacre to spread uniformly throughout the shell. This study indicates that the study area Meretrix meretrix shells are formed by biogenic aragonite to a greater concentration, which has been proven with XRD and SEM analysis.

Keywords: Bivalve shell, *Meretrix meretrix,* Thoothukudi coast, biominerals and microstructure.

Introduction

There are various types of organisms are living on the earth. For easy understanding and identification, they have been classified into several categories. The primary division is a kingdom, in which animals, plants, bacteria, fungi are categorized. The Animal Kingdom is broadly categorized into two divisions, such as vertebrate and invertebrate. Further invertebrates are classified on the basis of their morphological characters and habits into several phyla, among which Mollusca is one of the important phyla.

The phylum Mollusca is subdivided into three classes such as; Lamellibranchiata, gastropods, and cephalopods. The Lamellibranchiata is also known as Bivalvia or Pelecypods. Clams, scallops, mussels, oysters and shipworms are a few kinds of species of this class. Bivalves have two shells held together by a strong muscle. The shell usually remains open unless the animal senses danger, then the muscle closes the shell tightly for protection. The animals of the class *Lamellibranchia* started their lives from Cambrian and continue to Present day. They possess wide geographical and geological distribution. The living forms are exclusively aquatic (mostly marine and few freshwater forms), with a wide range of adaptability for all depth ranges from shoreline down to the depth of about 6000 m. However, the original name '*bivalvia'* of this group was given by[1].

The animal possesses a calcareous exoskeleton called shell covering its soft parts. This consists of a pair of valves which are generally identical, equal sized but mostly an inequilateral. *Valves* are placed laterally on either side of the animal and referred to as right *valve* and *left valve*.

The two *valves* are mostly articulated along their dorsal margin usually by means of several *teeth* and *sockets* borne by each *valve*. As the two *valves* are identical, a bilateral symmetry is shown by the shell where the symmetry plane is passing in between the two identical *valves* are parallel to the *dorsal* and the *ventral* margin of the shell.

The clam shell occurs extensively along the East Coast of India and few places in the West Coast of India. A few Indian states like Tamil Nadu, Goa, Maharashtra, Gujarat, Group of Andaman and Nicobar Islands and Odisha coasts, etc., have clam shells[2]. The major exploited species are *Meretrix meretrix, Meretrix casta, Katelysia marmorala, K. opima, Villorita cyprinoides, P. latenisulca, Paphia malabarica and P. textile*. Bivalve shells are most abundant in seawater (Seashore) as well as in freshwater (Estuarine or Backwater), living as invertebrates, mainly consists of Calcium Carbonate ($CaCO_3$) with some amount of major, minor elements and trace elements. Trace elemental composition in calcareous organisms have been largely applied for palaeoclimatic study. The hard shells are high in Calcium Carbonate ($CaCO_3$).

Bivalves consist various forms of $CaCO_3$ in their shells such as aragonite, calcite etc.. The shells may possess completely aragonitic or calcitic or it may consist mixing of aragonite and calcite in an isolate monomineralic layer. Shells are fabricated with a number of layers of different assemblages of calcium carbonate minerals. These assemblages are attributed to shell structures and their regular features are explained. The mineral aragonite occurs as prismatic, nacreous, crossed lamellar, complex crossed lamellar and homogeneous structures. Generally, calcite appears as prismatic or foliated structures. Myostracal layers (calcium carbonate consists lower point of muscle attachment) are typically aragonitic. The ligament and byssus calcined are usually aragonitic hence, it deliberates so as to the key control over of shell mineralogy is genetic. It is studied that environmental influence could alter the primary minerals and construction of shell structures within the super family. A reversal correlation takes place between the ratio of calcite in the shell and the mean temperature of the environment inhabited by the bivalve[3]. Paleontology has fundamentally disseminated on scanning electron microscopy (SEM) for a specific depiction of shell microstructures[4-7]. The prime objective of the present study is to assess the morphological, chemical, mineralogical and microstructural characteristics of the *Meretrix meretrix* shells occurred on the Toothukudi coast of Tamil Nadu, India.

Study area: The Thoothukudi municipal corporation is popularly known as Tuticorin, a port and industrial city in the Thoothukudi District of Tamil Nadu, India. The district is positioned on the southeast fringe of Tamil Nadu. The major part of the people in Thoothukudi District is employed in fishing, salt pans, sea-borne trading, and tourism. The study area latitude and longitude of Thoothukudi is 8º48'10.8" N and 78º8'40.2" E respectively. The district Thoothukudi obtains rainfall from SW and NE monsoons. But the northeast season monsoon is the major contributor to the district. Rivers originated from the Western Ghats of Tamil Nadu flows through the district and finally joins the Bay of Bengal. A few streams developed in the district hillocks area and conjugate straight to the sea.

The only perennial river in the district is the Tamiraparani river with a matured level of growth and on the flow way it receives few vital tributaries such as Karaiyar, Manimuthar, Servalar, Kadana, etc., Sedimentary formations pass through the coastal area comprising of sandy clay, calcareous sand, sandstone, with shell inclusion, laterite, Archaean formation, granite gneisses, charnockite, pegmatite and quartzite. Dug wells and shallow tube wells are prominent in this area. Quaternary sediments exhibits besides the river and the east coast area. They are grouped into fluvial-marine, aeolian and marine. The study location point is shown in a political boundary map (Figure-1).

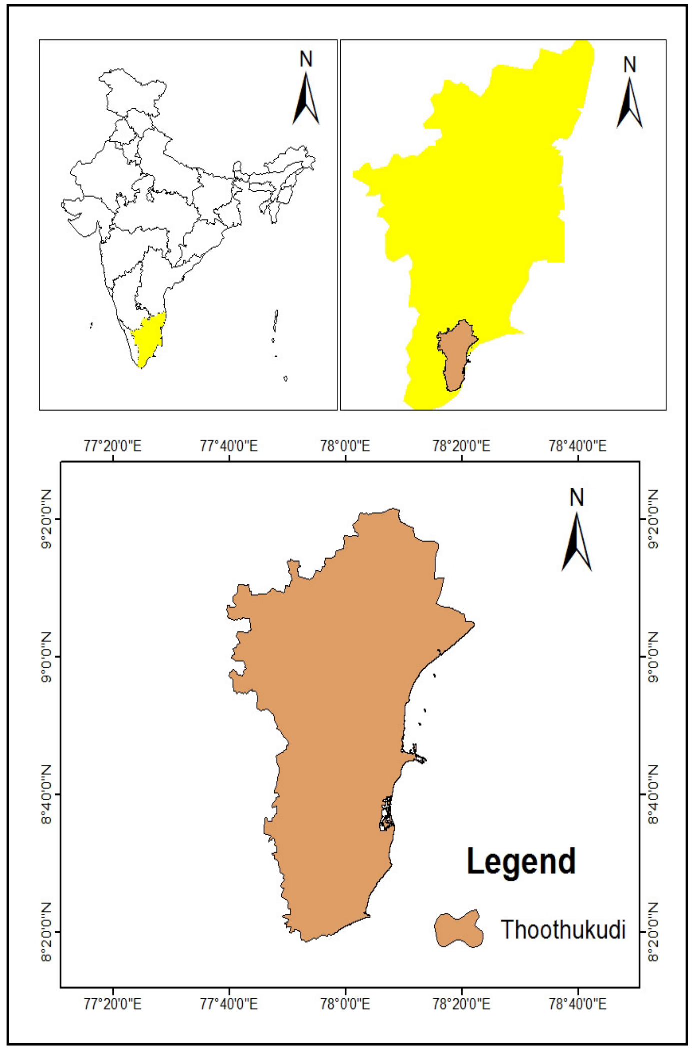

Figure-1: The map showing Thoothukudi District.

Materials and methods

Sample collection: Shell samples obtained by handpicking and digging along the coast of Thoothukudi, near CMFRI. Marine shells were identified in the field, based on their morphological features. Among the shells, the Meretrix meretrix shells were collected. The exact sample location coordinates point is 8º46'23" N Latitude and 78º9'36" E Longitude. The collected specimens washed thoroughly with water and carefully packed for analysis.

Sample preparation: The collected specimens washed with potable water and dried for 48 hours. Later, they crushed with iron mortar and ground with agate mortar and pestle. From the finely powdered sample, a 50g powder was separately taken for analytical studies. Process of crushing of the specimens to make it into powder form is shown in Figure-2.

Figure-2: Crushing of samples using iron mortar and agate mortar and pestle.

Analytical methods: X-ray Fluorescence (XRF): X-ray Fluorescence (XRF) is a quite known technique for analysing the geological materials because of its preciseness, correctness, adaptability, computerization, sensitivity, and selectivity. It is one of the most rapid and relevant methods of geochemical analysis for obtaining the qualitative and quantitative elemental analysis. X-ray Fluorescence measurements applied by several researchers towards analysing major compound and elemental dissemination in Mollusca as well as in bivalve shells[8-15]. Major element concentrations are determined to adopt the X-ray fluorescence spectrometry method following the procedures given by Calvert[16]. The XRF measures the chemical constituents of the materials as element magnitude in total counts or counts per second (cps), which are comparable to the chemical accumulation.

While, element severity mainly reckon on the element concentration, they subjective to the energy achievement of the X-ray source, the count time, and the physical properties of the sediment[17]. For XRF measurement, we employed Horiba XGT micro XRF analyser (Model: XGT 2700 X-ray analytical microscope). HORIBA's XGT systems provide energy dispersive X-ray fluorescence (EDXRF) analysis. High intensity X-ray beams with diameters ranging from 3 mm down to a unique 10 µm are used in the system to obtain full qualitative and quantitative elemental analysis. The system uses an Rh-target X-ray tube with tube voltage of 50kV and current 1mA. The fluorescent X-ray beams are detected by a silicon based SDD detector. The specifications of the instrument is given in the Table-1.

Table-1: The specifications of the Horiba XGT-2700 micro XRF analyser.

X-ray source	Rh target / Tube voltage 50kV / Tube current 1mA
Fluorescent X-ray detector	Eltier cooled Silicon Drift Detector (SDD)
Transmitted X-ray detector	Nai (Tl) scintilltor
X-ray guide tube	Mono capillary 10µm / 100µm with no filter
Detectable elements	Sodium to Uranium (Atomic numbers 11 to 92)
Samples	Metal plates, powders and coatings
CCD camera	Magnification 30 and 100 approx.
Signal processing	Digital pulse processor

The X-ray diffractometer used for the analysis of the sample is a product of PANalytical Company distributed by Spectris Technologies Pvt. Ltd., (Model: PANalytical X'Pert³ Powder model). The specifications of X'Pert^3 Powder model is given in Table-2.

Table-2: System Specifications of PANalytical X'Pert³ Powder model.

X-ray source	Generator: 3 kW generator supporting all current and future X-ray Tubes. Standard sealed X-ray tube: Cu, Co radiation
Detector	Direct optical encoding positioning system (DOPS) for lifetime goniometer accuracy curvature radius: 240mm maximum usable range (depending on accessories) 40°< 2θ <220°
Goniometric table	Vertical Goniometer (theta-theta)
Sample holders	Fixed, with or without sample rotation
Dimensions	1972 (h) x 1370 (w) x 1131 (d)mm
Weight	1100kg
Computer	Windows© 8 OS
Software	Match 3- for search and matching of minerals as per their peaks
Database used	COD- Crystallographic Open Database

X-ray diffractometer: In the collected *Meretrix meretrix* shells mineralogical measurements were made using XRD instrument (model Rigaku mini flex II desktop x-ray diffractometer) at (CLRI), Tamil Nadu. The details on analytical parameters and conditions are given in Table-3.

Table-3: Analytical parameters and conditions used for XRD determination.

Parameter	Condition
XG	Cu/30 kV/15 Ma
Goniometer	
Duration time/ Scan speed	4°/min
Step/Sampling Step	0.005°
Scan range	5-80°
Measurement axis	2Theta/Theta
K-beta filter	-
Incident monochromator	-
Receiving monochromator	-
Counter	-
Attachment	-
Incident slit	-
Vertical divergence slit	-
Receiving slit No.1	-
Receiving slit No.2	-

Bivalve microstructure study with sem: For the microstructural study, the shell specimens were mechanically cleaned and the targeted area of the shell was marked. In this study, the hard parts of umbo and growth line microstructure were identified. A small portion of the shell was sliced with rock cutting machine. The sliced part underwent for gold coating. The gold coated specimen was analyzed by SEM. The analysis was performed at the Manufacturing Engineering Department of Annamalai University. The SEM examination was performed with SEM Eds and Ebsd; model: Joel-Jsm-66101v with Inca (Dispersive Analytical System). The condition of the Scanning Electron Microscope is mentioned in Table-4.

Results and discussion

Compounds: The result of the chemical compounds reveals that the loss of the sample (LOI) is 43.37%, which is common in organic shells. Generally, the loss comprises of carbon dioxide, inorganic matter, moist, etc. The CO_2 was deliberated to assure the base, and the amount thus resolute, subtracted from the total LOI, gave a legitimate but a fairly accurate estimation of organic matter plus water. Distribution of CaO in the study area shell show to a great extent. The concentration of CaO is 56.19 % of the total oxide compound and SrO concentration is 0.44%. Table-5 shows oxides and elements concentration of *Meretrix meretrix* shells of Thoothukudi coast.

Table-4: The specification of Scanning Electron Microscope (SEM).

Model	JSM-6610LV
Make	JEOL India PVT. LTD.
Resolution range	3.0nm (30 kV), 8.0nm (3 kV) & 15nm (1 kV)
Magnified range	*5 to *300,000
Electron gun	Factory Pre-Centered Filament
Condenser lens	Zoom Condenser Lens
Objective lens	Conical Objective Lens
Specimen stage	Eucentric Specimen Stage
X-Y	125nm – 110nm
Z	5mm to 8mm
Rotation	360^0
Tilt	-10^0 to $+90^0$
Vacuum system: High vacuum model Low vacuum model	 DP*1, RP*1 DP*1, RP*1

Table-5: Oxides and elemental concentration of *Meretrix meretrix* shells of Thoothukudi coast, identified through XRF.

Compound	m/m%	Major Element	Weight %
CaO	56.19	Ca	71.17
SrO	0.44	Sr	0.35
LOI	43.37	O	28.47

Elements: The elemental concentrations in *Meretrix meretrix* shells of Thoothukudi coast is shown in Table-5. From the reported elements, Ca reported at a high level as a weight percentage of 71.17. The remaining elements, Sr and O are just on a reporting level not showing any signs. The element Ca is most abundant in the earth‘s crust and essential for plants and animals.

XRD: Biomineral study of *Meretrix meretrix* shell was made with XRD. The XRD patterning represents 12 peaks (Table-6). All the 12 peaks represent aragonite mineral (Figure-3). The

Figure-3, illustrates the XRD peaks; the green coloured peaks stand for aragonitic mineral indicating three axes namely alpha, beta and gamma. All the 12 peaks endorsed that the study area *M. meretrix* species, shells are entirely fabricated by aragonite. The Thuthookudi coast *meretrix meretrix* shells are chiefly composed of aragonite biominerals which indicates that the configuration of shell mineralogy is purely genetic.

Table-6: XRD peaks detail of the study area *Meretrix meretrix* shell.

2-theta (deg)	d (ang.)	Height (cps)	Int. I(cps·deg)	FWHM (deg)	Size	Phase name
26.386(5)	3.3750 (6)	616(91)	163(3)	0.204(7)	418(15)	Aragonite, (1,1,1)
27.408(6)	3.2514 (7)	331(66)	94(2)	0.213(11)	401(20)	Aragonite, (0,2,1)
33.257(10)	2.6917 (8)	571(87)	137(3)	0.155(15)	559(53)	Aragonite, (0,1,2)
36.246(18)	2.4763(12)	233(56)	74(2)	0.258(13)	339(17)	Aragonite, (2,0,0)
38.018(19)	2.3649(11)	199(51)	83(3)	0.327(19)	268(16)	Aragonite, (1,1,2)
38.644(17)	2.3280(10)	191(50)	69(2)	0.294(15)	299(15)	Aragonite, (1,3,0)
41.34(3)	2.1824(17)	51(26)	11.4(12)	0.20(3)	434(61)	Aragonite, (2,1,1)
48.548(18)	1.8737(7)	204(52)	61(2)	0.274(13)	332(15)	Aragonite, (0,4,1)
50.320(18)	1.8118(6)	166(47)	42.3(17)	0.212(16)	432(32)	Aragonite, (1,3,2)
52.534(14)	1.7405(4)	263(59)	70(2)	0.202(16)	457(37)	Aragonite, (1,1,3)
53.082(15)	1.7238(4)	158(46)	38(2)	0.18(2)	508(57)	Aragonite, (2,3,1)
66.104(16)	1.4123(3)	38(23)	8.8(8)	0.17(3)	571(85)	Aragonite, (2,2,3)

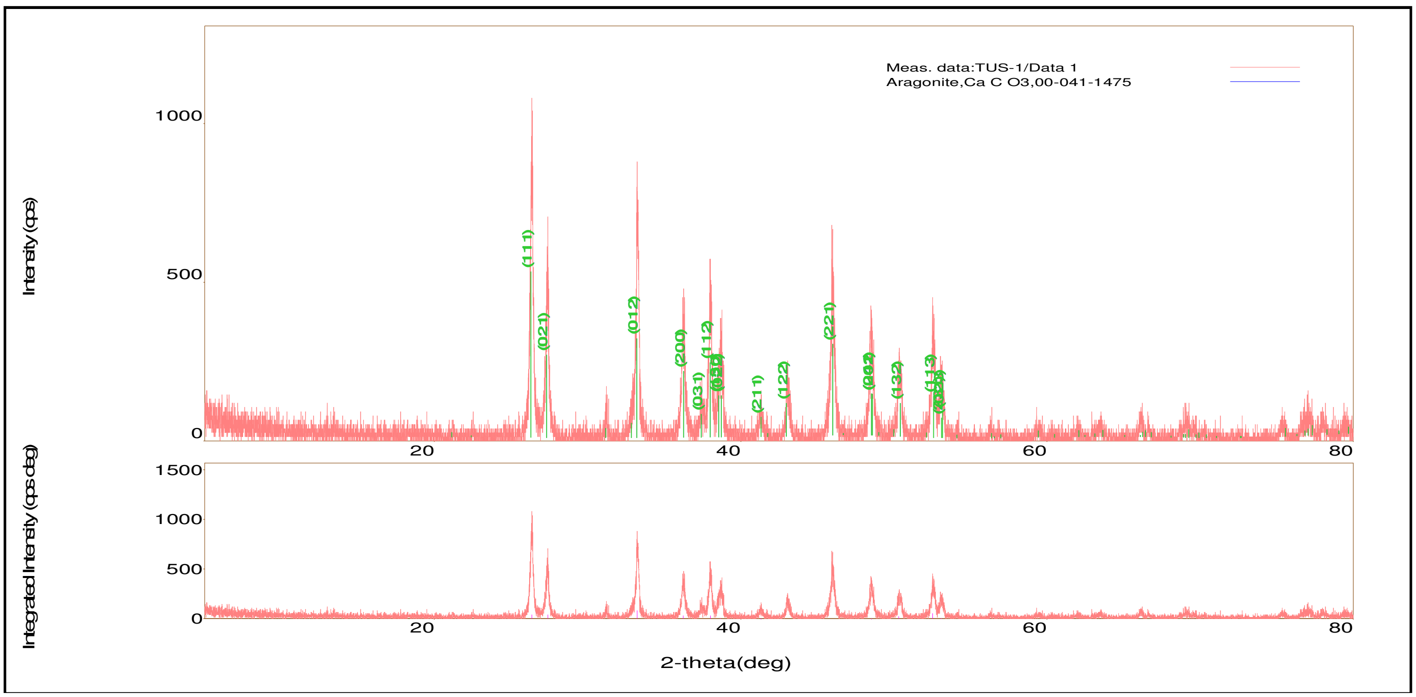

Figure-3: Illustration showing the XRD peaks of *M.meretrix* shells of the area.

In a considerable quantity of mollusca shells, the $CaCO_3$ configuration is generally aragonite[18]. Only in certain shells like *pecten maximus* contain calcite and aragonite minerals[19]. Biomineralization in bivalve shells involves with the epf, lamella layer[20]. Inner shell layer precipitated by central epf while the exterior by outward EPF). Elements from environs get to the calcification. Usually, ions penetrate to molluscs principally over the mien, even though it penetrate through the gut or by direct uptake over the outer mantle[21]. The soluble proteins have shown that of carbonate is deposited[23].

Although the mineral and the inner structure of mollusc shells are principally controlled by the biological process, the environmental chemistry also affects the shell constituents. In the case of bivalves, there are only a few studies that report the influence of environmental parameters on the shell mineralogy. In Mytilus, increasing water temperature increases the aragonite/calcite proportion in the shells and salinity reduces such proportion[24]. Variations in the Mg/Ca ratio of seawater (molar Mg/Ca>2=aragonite seas, <2=calcite seas)[25].

The main discussion is on whether secular changes in certain chemical components of marine waters may have induced changes in the polymorph from which the skeleton is made[26]. Sandberg[27] originally proposed that ancient oolites shifted from being calcitic to aragonitic in the Mesozoic due to an increase in the Mg/Ca ratio. Present day marine waters have an Mg/Ca molar ratio of 5.2, close to the point in which inorganic carbonates are composed only of aragonite, any proportion of calcite (even high-Mg) being excluded[26]. Temperature is a key environmental variable and has a significant effect on biomineralization processes and growth in bivalve shells[28]. Generally, the temperature has a positive linear effect on shell growth, and can also result in changes in mineral composition. For example, shells built of the aragonite polymorph of calcium carbonate are more susceptible to dissolution at low temperatures, which may lead to a thinning of the shells, and could explain why shells in cold-water bivalves are characterised by an increase in the whole-shell calcite: aragonite ratio due to an increase in calcite, the less soluble but mechanically weaker polymorph of calcium carbonate[29].

Microstructures: The study area shell *Meretrix meretrix* microstructures were studied mainly through sections orientation. In this study, two parts of the *Meretrix meretrix* shell were examined; one is on the growth lines part and the other one for the umbo part. Two SEM images were taken for each part with different magnifications. The shell microstructures were recognized and examined carefully with previous SEM studies.

Microstructure of growth lines: The *Meretrix meretrix* growth line part of the shell exhibits crossed acicular structure. It is clearly shown in Figure-4A and B. Which shows some fractures with cross acicular homogenous microstructure. Here, elongated crystallites are oriented in several directions.

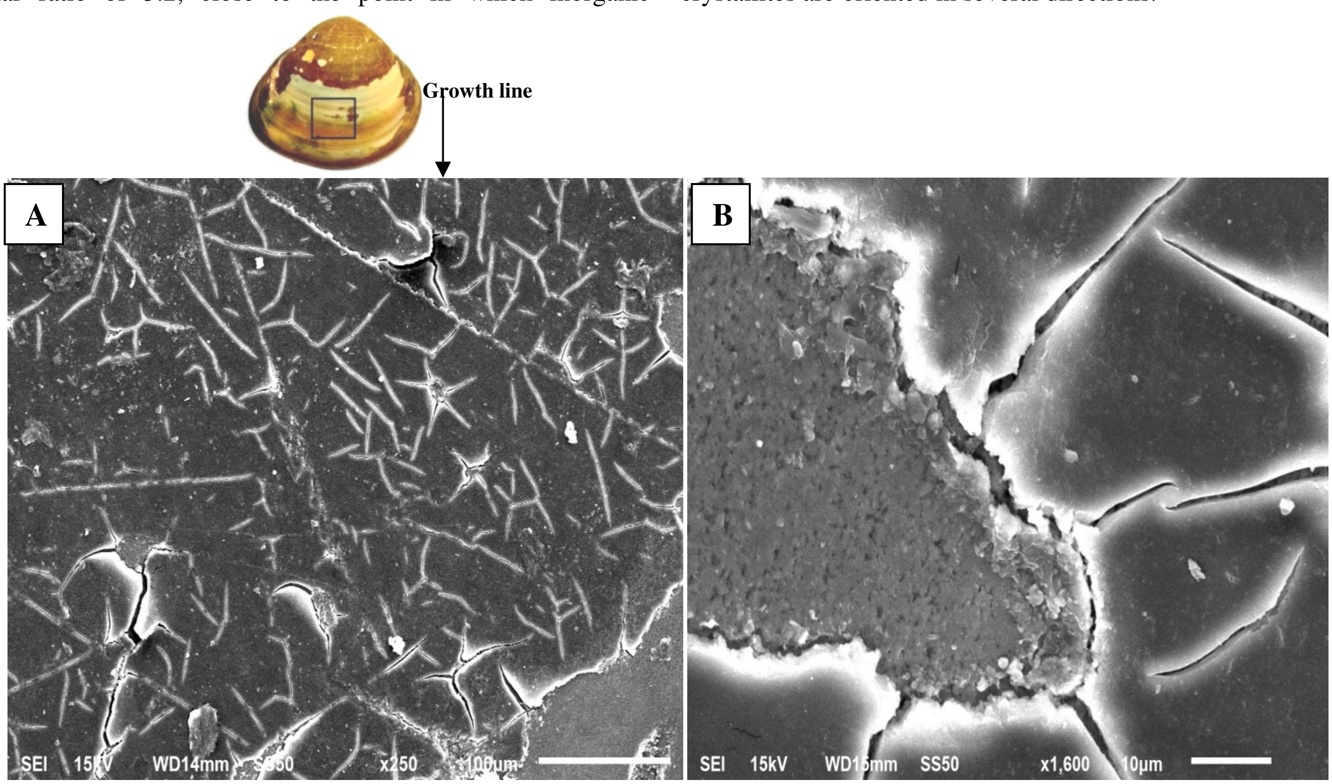

Figure-4A: The image of the growth line part is viewed in the magnification of X 250 (the scale bar of 100µm) and the Figure-4B viewed with the magnification of X 1600 (the scale bar of 10µm).

The permeable nature homogeneous layer is seen in the growth line part *of M.meretrix* shell. The image (Figure-4A) illustrates cluster of small to medium sized pores and cracks in the aragonitic layer. Uneven minute openings specifies the preliminary phase of calcium formation. Figure-4B demonstrates that the thin sheet has no specific pattern. The aragonite texture is not consistent and spreads in disorder manner. Elongated cleavages are seen with different widths ranging between 0.96 and 2.835 µm.

Microstructure of umbo: The *Meretrix meretrix* umbo part of the shell exhibits irregular homogenous structure. It is clearly shown in Figure-5. Here, the shell is composed of complex irregular elements ranging from 0.8 to 4µm.

The Figure-5A and B, displays fractured homogeneous aragonitic layer in the umbo part of the shell. Noted fractures indicate the weak development in the shell is due to poor segregations of mantle fluids. The magnified image (x1000) reveals the desperation (~1.020 – 2.163µm) in the aragonitic shell layer.

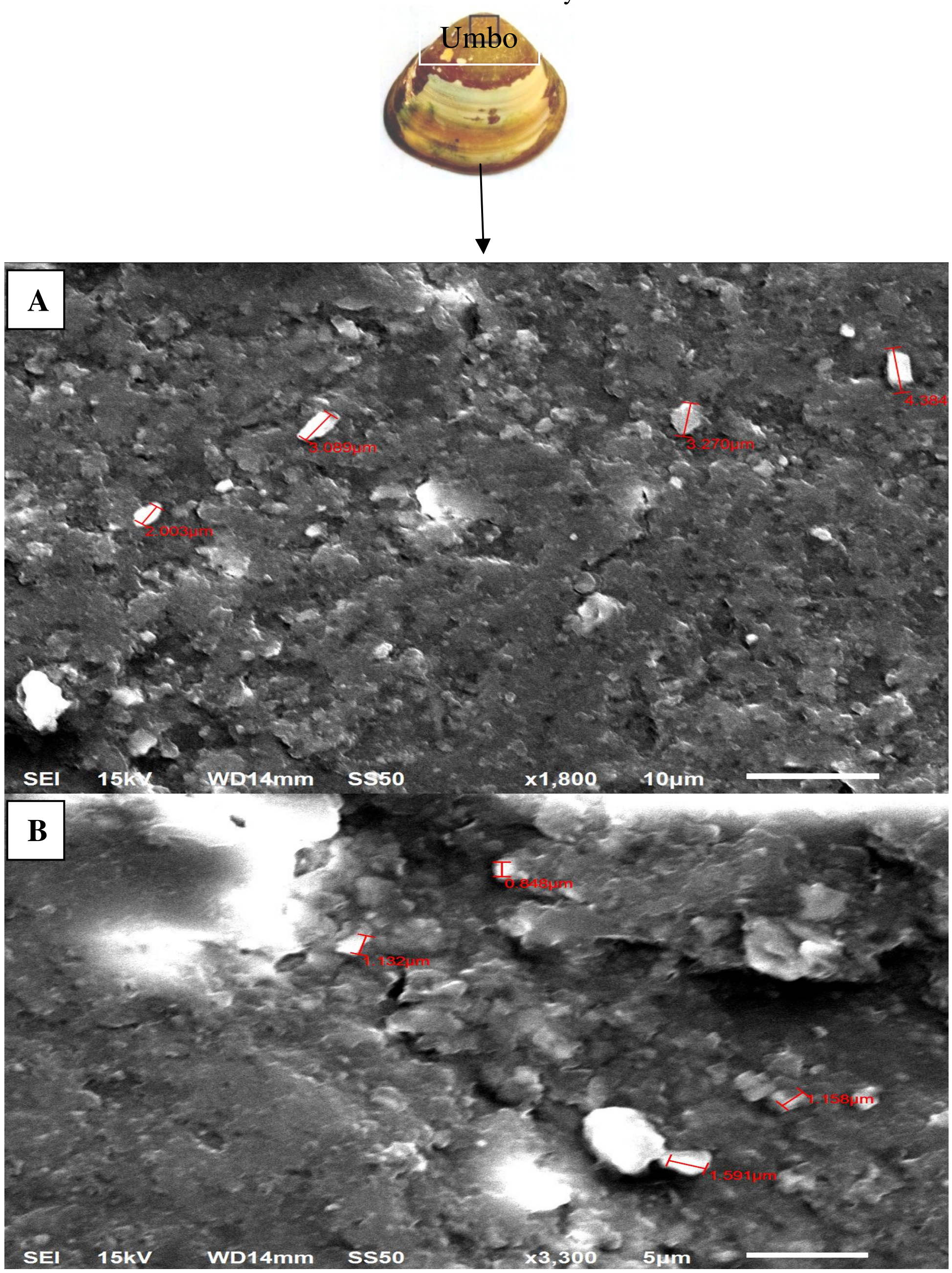

Figure-5A: shows the SEM images of the umbo part. The image is viewed with the magnification of X 1800 (at the scale bar of 10 µm) and the Figure-5B the same umbo part was viewed with the magnification of X 3300 (at the scale bar of 5µm). Both the images are showing the irregular homogenous microstructure.

Conclusion

The XRF investigation reveals that the study area *M. meretrix* shells have CaO content of 56.19%, SrO 0.44% and LOI 43.37%. Elements presented in the shells are Ca (71.17 wt%), Sr (0.35wt%), and O (28.47 wt%). The XRD measurement exhibits that the study area shells are wholly composed of aragonite biomineral. The SEM examination shows that the aragonite minerals formed as fractured cross acicular and irregular homogenous structures. Fractured and porous nature in microstructure generally indicates that the shells are immature or juvenile, which signifies that there is no sufficient nacre to spread uniformly throughout the shell for which depression lines are formed.

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