International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 11-19
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

CHEMICAL, MINERALOGICAL AND MICROSTRUCTURAL PROPERTIES OF *VILLORITA CYPRINOIDES* (BIVALVIA) SHELLS OF VEMBANAD LAKE, KERALA, INDIA

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ABSTRACT

One among the clam shells, Villorita cyprinoides is largely abundant in Vembanad Lake of Kerala State, India. The shells are utilized for cement, calcium carbide, sand-lime brick production, etc. This paper discusses the chemical, biomineral characteristics and the microstructures of the Villorita cyprinoides shells. XRF, XRD, SEM were applied for analytical and imaging studies. The result of oxides demonstrates that the CaO comprises 54.19%, which is common in organic shells. Other than CaO, the reported other oxides are <1% i.e. SiO_2 (0.73), Al_2O_3 (0.24), Fe_2O_3 (0.30), MgO (0.14), Na_2O (0.61), K_2O (0.03), SrO (0.31) and CuO (0.03). The XRD analysis exhibited that the Villorita cyprinoides shell of Vembanad Lake is formed by aragonite layers. The SEM images reveal homogeneous structures in the umbo and growth line parts of the shell.

KEY WORDS

Vembanad Lake, bivalve, Villorita cyprinoides, XRF, XRD, microstructures.

INTRODUCTION

India has rich molluscan resources all along the coast, in inshore waters, bays, backwaters, and estuaries [1]. In India, bivalve shells largely occurred in the southeast and southwest coasts of India. Bivalves are mostly harvested for meat and lime-based industries utilization. The Kerala State is one of the richest clams producing state in India. Clam resources are most abundant in the Vembanad Lake of the state, with annually estimated landings of 66,000 t, in 2008-09 [2]. In Vembanad Lake, the molluscan bivalve; *Villorita cyprinoides* (Black mollusk) and *Etroplussuratensis* (Pearlspot), the Villorita contribute around 99% of the aggregate fisheries [3]. Several investigations were done on Vembanad Clam shells resources [4]; [5]; [6]; [7; [8]; [9]; [10]; [11]; [12].

Bivalve shells are economically significant; the shells are used for barter, tools, ornaments, etc. The shells are also used by ceramic, cement, and other lime industries [13]. As stated by [14], the majority of marine shells are made up of over 90% calcium carbonate, which can be changed over to various calcium-containing products like, calcium phosphate, and calcium citrate with biomedical and food industries application. Biominerals framed inside living animals' contrast from their mineral partners in that the inorganic crystals have personally become together with a natural grid [15].

Amorphous calcium carbonate (ACC) is an important precursor in biominerals like shells, coral, foraminiferal and urchin spine [16]. Aragonite, vaterite, and calcite are the three polymorphs of calcium carbonate ($CaCO_3$). Aragonite and vaterite are metastable at normal ambient temperatures [17]. Characters of the microstructure, mineralogy, and mien of shell layers have been broadly utilized as a part of a phylogenetic investigation, especially in the Bivalvia [18]; [19]; [20]; [21]. Microstructure in molluscs is portrayed by the morphology of crystal units and their method of introduction and layering pattern. The most widely recognized shell microstructures (spherulitic prismatic, crossed lamellar, nacre and foliated calcite) are known since the Middle Cambrian [22]; [23]. The $CaCO_3$ crystals in shells are arranged in layers with a peculiar pattern to frame complex microstructures. So far, more than 30 distinctive microstructures of bivalve shells have been recorded [24]. The principal objective of this study was to comprehend the biominerals and the microstructure pattern of *Villorita cyprinoides* (bivalve) shell of Vembanad Lake, Kerala, India.

MATERIAL AND METHODS

2.1. Study area

The Vembanad Lake is situated on the southwest coast of India. It has a length of about 90 km and extends from Alleppey to Azhicode with water spread area of 300 km^2. The Vembanad Lake supports a rich fishery, among clam shells the *Villorita cyprinoides* dominates. Vembanad Lake has a length of about 96 km with a width of 14 km and the depth is 2-7m. The Vembanad Lake is situated between the Latitude 9°30'46'' to 10°11'11''N and the Longitude 76°09'48'' to 76°25'45''E. The study area Vembanad Lake is covered under five topographic sheets numbered 58B/4, 58B/8, 58 C/1, 58C/5 and 58C/6 of the year 1967 produced by the Survey of India which is all in large-scale maps [25]. The area is composed of recent deposits of pure alluvium with prominent paleo strandlines [26]. Vembanad Lake is an ideal habitat for a varied flora and fauna including shrimps and clams [27]. *Meretrix casta*, *Paphiamalabarica*, and *Sunettascripta* are the other clams harvested from the Vembanad Lake [28]. The study area (Vembanad Lake) is shown in Figure 1.

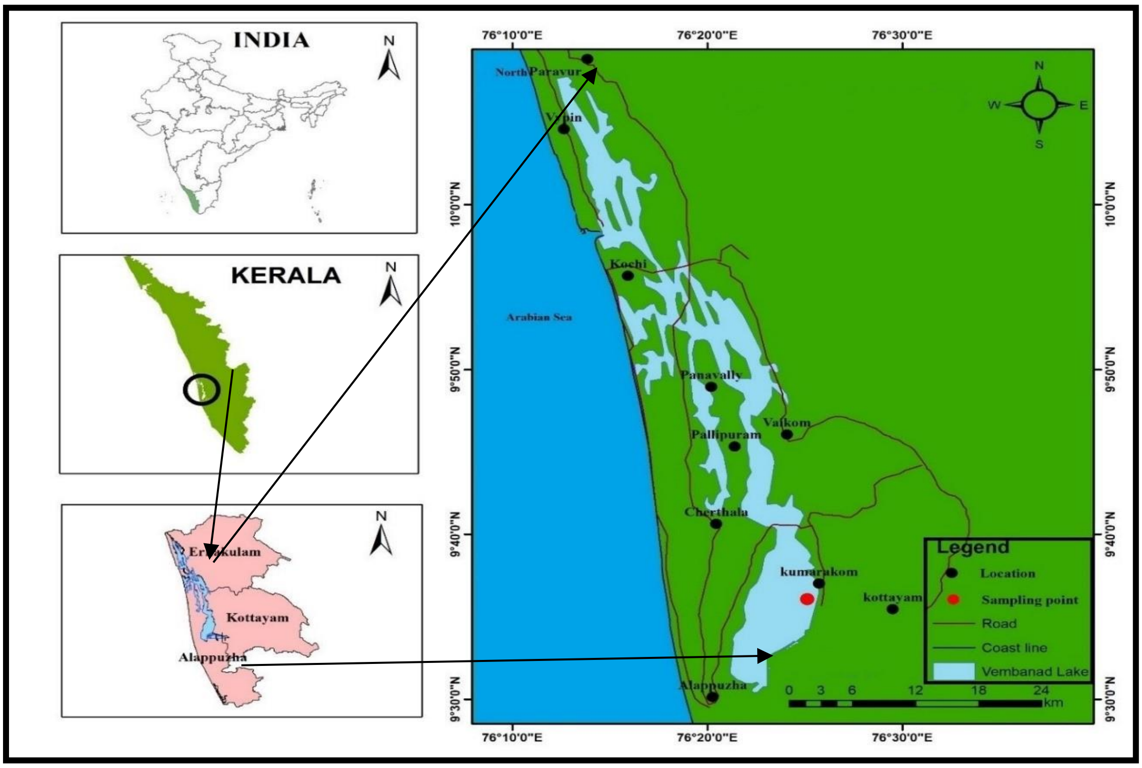

Fig 1.Map showing the study area (Vembanad Lake).

2.2. Sample collection

The bivalve shell *Villorita cyprinoides* (Gray, 1825) were collected along the bank of Vembanad Lake during the month of April 2018. Along the bank, *Villorita cyprinoides* shells were collected by hand digging and picking irrespective of size. The shells were thoroughly washed with lake water at the time of collection then the shells are put into clean plastic bags and packed carefully. Species and morphological characteristics have been confirmed with CMFRI, Thiruvananthapuram, Kerala.

2.3. Sample preparation

The collected shells were soaked into a bucket of clean water for one night then it is cleaned with a hand brush and dried under the sun for a day. The dried shells were crushed and pulverized into a fine powder with the help of iron and agate mortars. The 230-size mesh was used for sieving the powdered samples. The power samples completely blended and 20g powder was taken for chemical analysis. For microstructure examination, the umbo and growth line portions were cut into small pieces, after gold coating it was examined using Scanning Electron Microscope.

2.4. Chemical Analysis/Analytical method

2.4.1.X-Ray Fluorescence (XRF)

X-Ray Fluorescence methods were widely used by some of the researchers for analysing oxides and elements distribution in bivalve shells [29]; [30]; [31]; [32]; [33]. Bruker model S4 Pioneer sequential wavelength-dispersive X-ray spectrometer equipped with a goniometer (which holds seven analysing crystals) with 4 kW Rh X-ray tube and 60 samples automatic loading system available at National Centre for Earth Science Studies, Thiruvananthapuram, is used to measure different peaks and background counts for the major and trace elements. The supporting software used for computer analyses is able to take care of dead time correction, background, line overlap corrections and matrix effects giving the output directly as the concentration in weight percent or in ppm after converting the counts into concentration with the help of calibration curve.

2.4.2.X-ray diffraction (XRD)

X-ray diffraction (XRD) is a powerful non-destructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects [34]. Studies on the biominerals identification in bivalve shells are also done by researchers with the help of X-Ray Diffraction method [35]; [36]; [37]. The X-Ray diffraction patterns of the samples in this present study are obtained on Rigaku Mini Flex Desktop X-Ray Diffractometer. The diffractograms were recorded in terms of 2ϴ in the range 5°-80° with a scanning speed of 4° per minute. The source is copper having a wavelength of 1.5418Å. The Tube voltage is 30kv and tube current is 15ma. The interpretation is done by PDXL.

2.4.3. Scanning electron microscope (SEM)

The scanning electron microscope (SEM) is probably the most frequently used auxiliary instrument in shell microstructure studies. The majority of studies focus on the examination of surface textures [38]; [39]; [40]; [41]; [42]; [43]; [44]; [45]. In this study, JEOL JSM-6610LV scanning microscope, a JOEL India Pvt. Ltd.,was used to take SEM images, which is having a resolution of 3.0nm (30kV), 8.0nm (3kV) and 15nm (1kV), the magnification is from x5 to x300,000 and the accelerating voltage is 0.3kV to 30kV.

RESULTS AND DISCUSSION

3.1. Oxides

Oxide distributions in the *Villorita cyprinoides* shell of the study area were measured with XRF. The XRF measurement results are shown in Table 1. The result indicates that the CaO concentration is higher and constitutes about 54.19%. All other oxides were observed at low values and they constitute about 2.39%. A higher level of CaO is common in marine and backwater shells, however, the concentration of MgO (0.14%) in *V. cyprinoides* shell was very less which indicates that the dolomitization process is completely absent in this shell. The loss on ignition (LOI) is about 43.24%. LOI encompasses the gases, impurities like inorganic matter, and water, etc., such impurities are prevalent in organic shells.

3.2. Elements

The elemental concentration (wt.%) of *Villorita cyprinoides* shells of Vembanad Lake (Table 1) reveals that the element Ca (38.72) is very high when related to other elements. All other reported elements are just on a reported level not showing any significance, perhaps reflecting the low absorption capacity in shells, bivalve shells from time to time may contain small inclusions of foreign matter. Biochemical composition is a useful indicator of the nutritional value of edible molluscs [46]; [47]; [48].

Table 1. Oxide and Elemental concentration of *Villorita cyprinoides* shells of Vembanad Lake

Oxides (%)	m/m%	Element	Weight%
SiO_2 (%)	0.73	Si	0.341202
Al_2O_3	0.24	Al	0.127032
Fe_2O_3	0.3	Fe	0.20982
CaO	54.19	Ca	38.72959
MgO	0.14	Mg	0.08442
Na_2O	0.61	Na	0.452559
K_2O	0.03	K	0.024903
SrO	0.31	Sr	0.36735
CuO	0.03	Cu	0.037554
LOI	43.24		

3.3. Biomineralization

Biomineralization is a process where organisms form minerals. The control applied by numerous living beings over mineral development recognizes these procedures from abiotic mineralization [49]. It is critical to look at the connection between mineralogy and basic gathering in shells considering the organic procedures [50]. The Fig. 2 exhibits, XRD image of *Villorita cyprinoides* of the study area. The XRD detects 21 peaks, among the detected 21 peaks, 2 phases (major and minor) index were identified. The major phase is predominantly aragonite (95.60%), XRD has also detected a minor phase (4.39%) which is reported as an unknown mineral. The mineral name could not be identified due to very low intensity. According to the XRD results, the *Villorita cyprinoides* shell of the study area is mainly composed of aragonite biomineral with 95.60%. Aragonite can occur in a low-pressure environment, like the earth's surface [51]; [52]. The bivalve shells are mainly made of calcium carbonate ($CaCO_3$), calcite and aragonite are the regular structures, however, there are unstable amorphous [53].

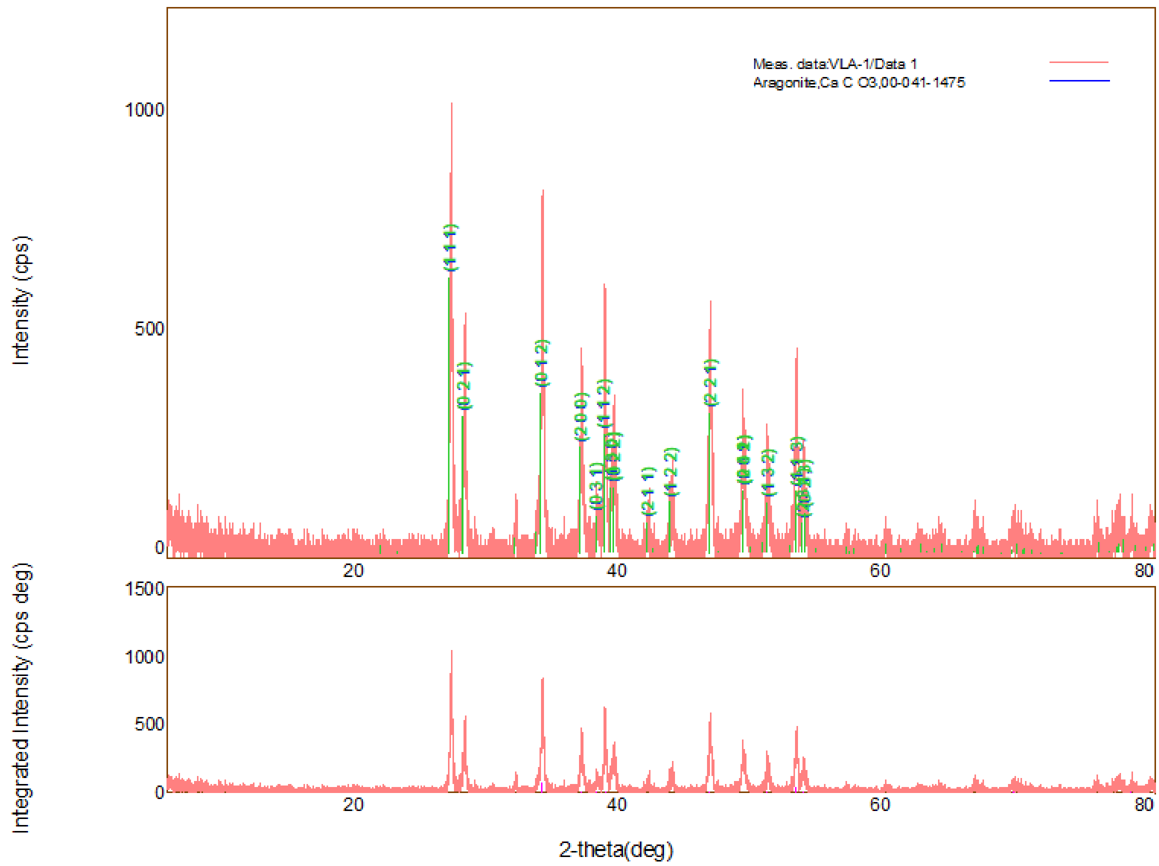

Fig. 2. XRD peaks obtained from *Villorita cyprinoides* shells of Vembanad Lake.

3.4. Shell Microstructure observed with SEM

As the shell grows, crystallites are laid down into inner, intermediate, and outer layers of calcium carbonate. Each of these layers has its own structural arrangement of aragonite or calcite, called microstructure. The microstructure is independent of mineralogy, though certain microstructures are associated with a particular polymorph [54].

Venerid shells indicate five fundamental microstructures; composite prismatic, crossed lamellar, homogeneous and complex structures. [55], represented the microstructural properties of mollusc shells from thin sections, as in ordinary light we see no structure at all but pointed out it has an arrangement of the crystallographic axes. Structural characteristics vary between species of the molluscan shell as well as within the same species [56].

The study area shell, *Villorita cyprinoides* umbo, and growth line parts were investigated with SEM. The SEM images are shown in Figures 3a-3b. The Sem image of outer part of umbo composed of aragonite granules in irregular shape and size (Fig. 3a and 3b). The granules size difference observed evidently in the scale bar = 5µm. *V. cyprinoides* exhibits homogeneous microstructure with no other specific structures. The homogenous microstructure shows no clear structural arrangement and is made up of irregularly shaped units [57]. [58] describes it as “aggregations of more or less equidimensional, irregularly shaped crystallites lacking clear first-order structural arrangement except for possible accretion banding”. In other words, when a shell structure has no other identifiable elements than minute granules, it is homogeneous.

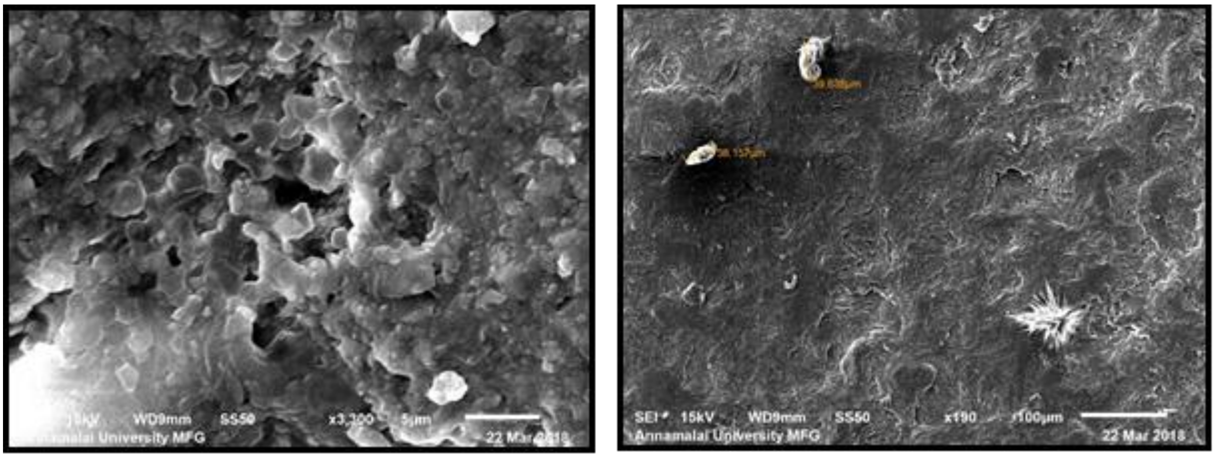

Fig 3 (a) and (b). Homogeneous structure is shown by the Umbo region of *Villorita cyprinoides* shellat different scale bars.

The shell growth lines in the SEM image display pores homogeneous layers (Fig. 3a and 3b). The growth line outer shell exhibits numerous uneven sized pores, with aragonitic layer overlapping (Fig. 3b). The openings of shell tubes have more or less hexagonal shape with the good and bad resemblances. The pores size ranges from 1.184 to 1.83 nm in a scale bar of 2 nm. In most cases, the pores are build during primary shell growth, the pores of the bivalves alone show a secondary origin. These pores are 'etched' into the final shell by dissolving the calcareous shell material by chemical and mechanical process or by the erosion of calcified layer [59]; [60]; [61]. In each case, the inside of the pores is loaded with a monocellular or multicellular mantle process, the capacity of which is yet to despite much speculation. The reason that these porous layers can be seen at a magnification of 5 nm and below may be because pores can penetrate all shell layers except the periostracum which the outermost organic layer [62]. Highly porous and oriented units in Fig. 4 (a and b) indicate that the shell units built of loosely packed nanoparticles. The low packing density and weak unit boundaries may attribute for the low hardness and easy initiate the cracks in this layer.

Watabe in 1988, found that organic molecules are common in all modern mollusc shells, making upto ten percent of the weight of the calcified layers of the shell. Different taxonomic groups have different microstructures composed by varied morphological and structural arrangements, derived from polymorphic phases of calcium carbonate. Environmental pressures select more efficient structures and manufacturing processes [63].

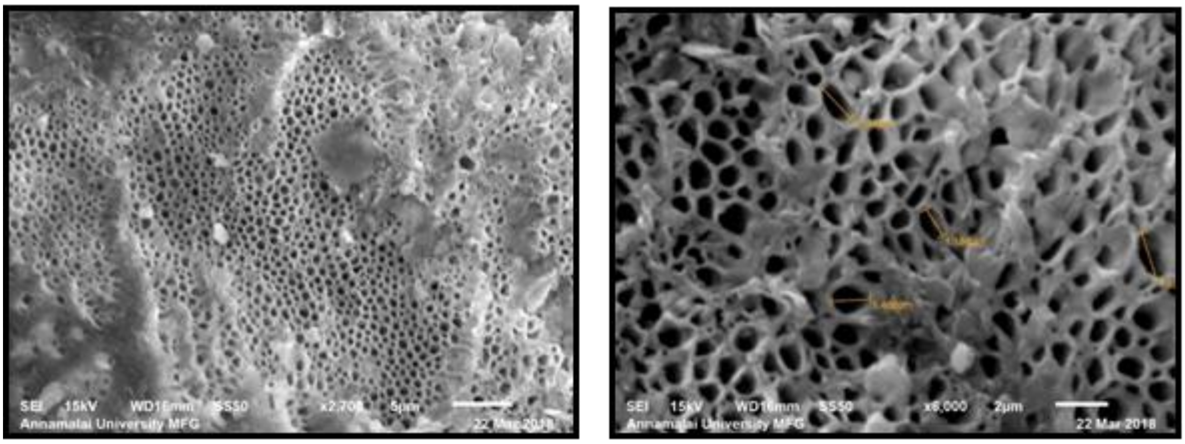

Fig 4 (a) and (b). Porous homogeneous structure reveals in growth line part of *Villorita cyprinoides*.

CONCLUSION

The present study has deliberated the chemistry, biominerals, and microstructures of the *Villorita cyprinoides* of Vembanad Lake. From the overall studies, the *Villorita cyprinoides* shells are chemically abundant with calcium carbonate (54.19%), mineralogically consisting of aragonite mineral furthermore, the umbo and growth line part of the shell have homogeneous microstructure. The Vembanad Lake *Villorita cyprinoides* shells formed only by aragonite polymer, calcite and vaterite not discovered, which shows that amid ACC crystallization, kindle the transition process from ACC to aragonite, but suppress calcite and vaterite. The aragonite has much stronger binding ability than calcite.

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Received:04.08.18, Accepted: 07.09.18, Published:01.10.2018

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