

COMPARATIVE STUDY ON CHEMICAL CHARACTERISTICS AND BIOMINERALS IN *MERETRIX CASTA* (CHEMNITZ, 1782) SHELLS OF VELLAR AND YADAYANTHITTU ESTUARIES, SOUTHEAST COAST OF INDIA

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Abstract— X-ray fluorescence and X-ray diffraction are used to determine the chemical characteristics and biominerals in estuarine *Meretrix casta* (Bivalve) shells. Marine bivalve shells are generally rich in calcium content. Variation in chemical composition and biominerals formation in mollusk shells have been used in a range of paleoenvironmental reconstructions. It is necessary to understand the chemical signatures and biomineralization process in the shells. We made an extensive investigation to understand the chemical properties and biomineral process in the *Meretrix casta* (Chemnitz, 1782) shells of Vellar and Yadayanthittu Estuaries, Southeast coast of India. The chemical and mineralogical characteristics of *Meretrix casta* shells of both the estuarine were correlated. The Yadayanthittu Estuary *Meretrix casta* shells consist high amount of CaO (54.47%), whereas Vellar Estuary shells consists 53.43%. Apart from CaO, all other reported oxides aggregate by 1.2735% in Vellar Estuary shells, but Yadayanthittu Estuary shells have 1.5287%, certain oxides (P₂O₅, Sc₂O₃, Re₂O₇ and ZnO) are not reported in Vellar Estuary shells, but these oxides are reported in Yadayanthittu Estuary shells. Both the estuaries have almost similar Ca wt. % (38.20) and (38.95), the other reported elements also not showing much variation (0.8647 wt. %) and (0.1856 wt. %). The XRD image exhibited that both of the estuarine shells are formed by aragonite minerals, however, the minor mineral composition differs each other. The Vellar

Estuary *M. casta* shells minor mineral is hematite (Fe₂O₃) and the Yadayanthittu Estuary *M. casta* shells minor mineral is Selwynite [Na K (Be, Al) Zr₂ (PO₄)₄ 2 H₂O].

Index Terms— Biominerals, Elements, *Meretrix casta* shell, Oxides, Vellar Estuary, Yadayanthittu Estuary, XRF and XRD.

I. INTRODUCTION

Biogeochemical prospecting is a generally accepted technique based on an anomalous distribution in the environment of chemical elements created by organisms [1]. Bivalve shells are mainly consisting of calcium carbonate (CaCO₃) on their hardest part of many common invertebrates such as corals and mollusks. The hard CaCO₃ shell is the result of a mineralization process biologically controlled and genetically programmed [2]. The isostructural calcite group consists of several minerals with the following elements in place of calcium, magnesium, iron, manganese, cobalt, zinc, cadmium, and nickel. The Calcium carbonate (CaCO₃) is an exceptional mineral, which contains the Ca²⁺ and CO₃²⁻ ions, is very common in nature, occurring in egg shells, limestone, marble, seashells, and coral. The CaCO₃ is most desirable for all lime based industries; such as cement, steel, chemical, paint, etc. Plants and animals need calcium carbonate to form their skeletons and shells, so they absorb calcium carbonate from the water where it exists, in mostly dissolved form of calcium hydrogen carbonate Ca(HCO₃)₂. Calcium carbonate

minerals, CaCO_3 , are ubiquitous in biomineralizing systems. They are naturally occurring in marine and fresh water, minerals most commonly as the polymorphs of calcite, aragonite and vaterite nucleated and grown in the exoskeletons and tissues of marine and freshwater organisms ranging from simple bacteria and algae to crustaceans, molluscs, or sponges. Among these, calcite and aragonite are biologically more common than vaterite; besides the calcite (CaCO_3) is generally predominant in marine bivalves as biominerals. The calcium-bearing minerals comprise about 50% of known biominerals [3].

Aragonite is an equally important carbonate mineral and the common polymorph of calcite. Polymorphism of minerals implies the same chemical composition, but distinct crystal structure. The marine bivalves have the most mineralogical composition is the aragonite composition [4]. *Vaterite* is the least known polymorph of calcium carbonate and was first described by Vater in 1893 [5]. *Vaterite* occurrence in nature is more widespread than generally assumed. It was first observed in gastropods [6]. As *vaterite* is a relevant mineral in biogenic systems, independent evidence for the stability of *vaterite* over the years comes from biogenic samples such as bivalves, mollusks and other marine organisms [7]-[9]. Biomineralization is the process by which living forms influence the precipitation of mineral materials. The process creates heterogeneous accumulations, composites composed of biologic (or organic) and inorganic compounds, within homogeneous distributions that reflect the environment in which they form [10]. In biomineralization, Calcium carbonate mainly exists as calcite, aragonite, and vaterite, and it is one of the main exoskeletal components in marine invertebrates as biomineral. Bivalve takes place in the extrapallial fluid (EPF), a thin film of liquid between the calcifying shell surface and the mantle epithelium. Mollusk shells, and

it is believed to play key roles in shell biomineralization by their different protein content what they intake [2], [11].

The shell chemistry of marine bivalves and their biomineralization process were studied by several researchers [12]-[21]. In this study, we deliberate the chemical and biomineralogical characteristics of *Meretrix casta* shells of Vellar and Yadayanthittu Estuaries, falls in the Southeast Coast of India. In order to establish the correlation regard to the chemical and biomineralogical characteristics, a comparison study also done within *Meretrix casta* shells of the estuaries.

II. MATERIALS AND METHODS

A. Study area

For the present study, the chosen areas are Vellar Estuary and Yedayanthittu Estuary, which are falling in the Southeast Coast of India. *Meretrix casta*, Bivalve: Veneridae, (Chemnitz, 1782) are richly available in both the estuaries and they play a vital role in food and nutrition, also the shells are used for various lime based applications. *Meretrix casta* shells were collected from the marine zone of Vellar Estuary. The study area located between the Longitudes $79^\circ 46' 01''$ to $79^\circ 46' 31''$ E and Latitudes $11^\circ 29' 13''$ to $11^\circ 29' 54''$ N falls in the toposheet no. 53m/15 of 1:50,000 scale. In the Yadayanthittu Estuary study area (Marakkanam Estuary) falls in the toposheet no. 57P/16 and 66D/4 of 1:50,000 scale, and lies between the Longitudes $79^\circ 56'$ to $80^\circ 00'$ E and Latitudes $12^\circ 12'$ to $12^\circ 15'N$. The Estuary extending three km from a little northeast of Marakkanam Road Bridge is directly connected to the Bay of Bengal at Alamparai Port and develops a lagoon ecosystem. Both the study area maps are shown in Figure 1A & 1B.

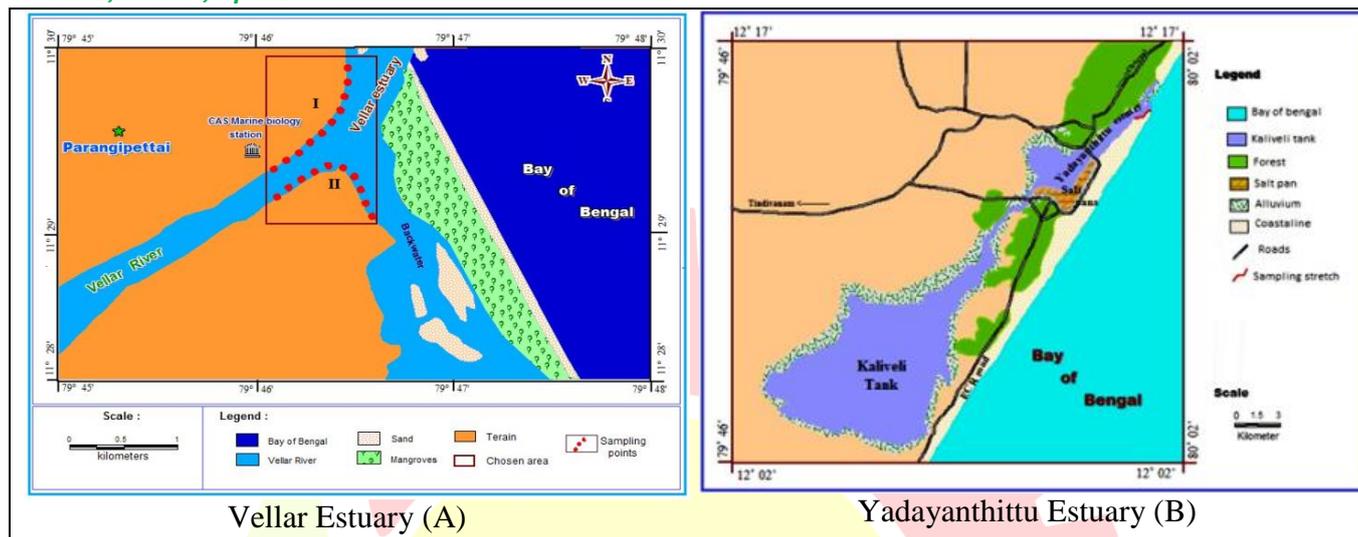


Fig.1. Showing Vellar (A) and Yadayanthittu (B) Estuaries with sampling locations.

B. Sample collection

During the low tide, a larger area is well exposed in near mouth of the river, it is paving easy access to hand picking of bivalves. *Meretrix casta* shell samples were collected in both the estuaries at the time of the low tide period during the months of June and July 2015. The *M. casta* shells were collected within 1×1m squares on the shorelines. In Vellar Estuary, twenty samples were collected for a distance of 2 km. In the Yadayanthittu Estuary, 1x1m squares formed and ten *Meretrix casta* shells were collected along the estuarine shoreline about 1 km distance from the mouth. In the demarcated square, available *Meretrix casta* shells were collected by hand digging and picking. The collected shells were thoroughly washed with backwater at the collection point and placed into clean plastic bags and packed with specific sample numbers.

C. Sample preparation

The shells collected in the Estuaries were washed thoroughly with clean water and dried at room temperature. After, they were crushed and pulverized into very fine powder form with the help of agate mortar and pestle. The powdered samples thoroughly mixed well and

50g powder was taken for chemical and biomineralization analytical purpose.

D. Analytical methods

Some researchers widely used XRF method for major compound analysis and elemental distribution in bivalve shells [22]-[29]. Fewer studies have done on biominerals identification in bivalve shells with the help of XRD method [30]-[39].

The study area *Meretrix casta* shell chemical and mineralogical measurements were done with an integrated XRF and XRD instrument (two techniques integrated into a single instrument), Model: Thermo Scientific-ARL 9900 X-ray Workstation, measurements were done at the R&D Cell, India Cements Ltd. Dalavoi, Tamil Nadu. In order to perform the analysis, samples were dried in an oven at 100°C for 24 hours, after the initial surface free moisture removed at 40°C for 4 hours. The dried samples were ground in an Insmart® ring mill with Tungsten Carbide elements to a fine powder in order to minimize particle size effect on the analysis.

Approximately 9.0g of each resulting powder was mixed with 1ml of Cellulose, which acts as a binding agent, and then pressed into 25mm

diameter disks [40]. Separate disks were made from a sample material. The disks were analyzed on XRF spectrometer using an Rh target X-ray tube operating at 60 KV and LiF 200 in a vacuum path for major oxides and elemental analysis. The X-ray diffraction (XRD) method provides an easy way to distinguish two polymorphs of CaCO_3 , aragonite and calcite. Biominerals identification of *M. casta* shell was studied with the aid of XRD and the results are interpreted with the help of Visual CRYSTAL 6 Analysis and the peak values are interpreted with scintillation and flow counters technique for biomineral identification.

III. RESULT AND DISCUSSION

Chemical and biomineralogical results of *Meretrix casta* shells of Vellar Estuary

A. Major oxides

XRF detected oxides and element concentrations in *Meretrix casta* shell of Vellar Estuary is shown in Table 1. The result of the major oxides indicates that the loss on ignition (LOI) is about 45.30%; the calcium oxide (CaO) concentration is 53.43% of the total chemical compounds. Apart from CaO, all other oxides are reported in very small values. All other reported oxides accumulated to 1.2735%.

TABLE 1: Oxides and elements concentration in *Meretrix casta* shells of Vellar Estuary

Sl. No	Compound	Formula	m/m%	Std. Err	Element	Weight %	Std. Err
1.	Calcium oxide	CaO	53.43	0.25	Ca	38.20	0.18
2.	Sodium oxide	Na_2O	0.593	0.030	Na	0.440	0.022
3.	Silicon oxide	SiO_2	0.202	0.010	Si	0.0945	0.0047
4.	Strontium oxide	SrO	0.135	0.007	Sr	0.114	0.006
5.	Iron oxide	Fe_2O_3	0.0850	0.0042	Fe	0.0594	0.0030
6.	Magnesium oxide	MgO	0.0698	0.0035	Mg	0.0421	0.0021
7.	Sulphur oxide	SO_3	0.0654	0.0033	Sx	0.0262	0.0013
8.	Aluminum oxide	Al_2O_3	0.0576	0.0029	Al	0.0305	0.0015
9.	Chlorine	Cl	0.0231	0.0012	Cl	0.0231	0.0012
10.	Potassium oxide	K_2O	0.0166	0.0008	K	0.0138	0.0007
11.	Cadmium oxide	CdO	0.0098	0.0039	Cd	0.0086	0.0034
12.	Zinc oxide	ZrO_2	0.0043	0.0009	Zr	0.0032	0.0007
13.	Titanium oxide	TiO_2	0.0040	0.0011	Ti	0.0024	0.0007
14.	Manganese oxide	MnO	0.0025	0.0010	Mn	0.0019	0.0008
15.	Bromine	Br	0.0019	0.0003	Br	0.0019	0.0003
16.	Phosphorus oxide	P	0.0018	0.0002	P	0.0018	0.0002
17.	Nicol oxide	NiO	0.0017	0.0008	Ni	0.0013	0.0006

B. Major elements

The XRF finding demonstrates that the *M. casta* shells of Vellar Estuary consist several elements within it (Ref. Table 1). Among the elements, Ca reported in high level as a weight percentage of 38.20. All other reported elements are shown insignificant values, perhaps reflecting the low absorption capacity in shells. Bivalve shells form the majority of Ca element, but the other elements are changing in time to time. They may contain small inclusions of foreign matter [41].

C. Biomineralization

The X-ray diffraction (XRD) method provides an easy way to distinguish two polymorphs of CaCO_3 , aragonite and calcite. Biomineral identification in *Meretrix casta* shells of the Vellar Estuary has been done with the aid of XRD and the results are interpreted with the help of *Visual CRYSTAL 6* Analysis. The *Visual CRYSTAL 6* image detects 29 peaks, among the 29 peaks 28 peaks were determined from the scan results, and 2 phase's index also identified. The output results show that the mineralogical composition CaCO_3 is 93.32 wt. % and Fe_2O_3 are in 6.68 wt. %. The XRD image of Vellar Estuary is shown in Figure 2.

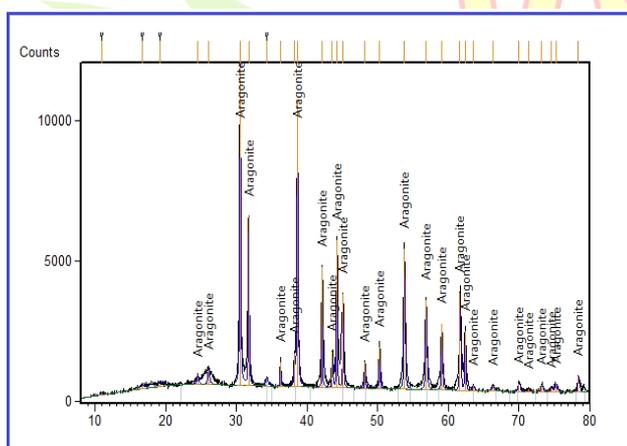


Fig.2.XRD images showing the aragonite peaks in the *Meretrix casta* shells of Vellar Estuary.

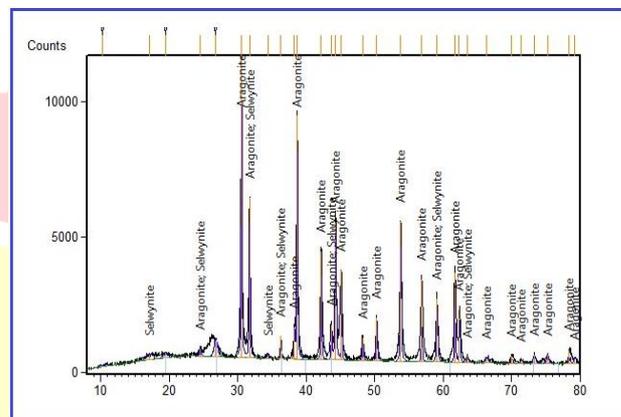


Fig.3. XRD images showing the aragonite peaks in the *Meretrix casta* shells of Yadayanthittu Estuary.

Comparison of chemical characteristics and biominerals of *Meretrix casta* shells of Vellar and Yadayanthittu Estuaries

We did a comparison study of chemical and biomineral characterization of *Meretrix casta* shells of Vellar and Yadayanthittu Estuaries. Both the estuaries are fallen in the Southeast Coast of India and the distance between the estuaries is almost 95 km. Both the estuaries are having anthropogenic effects. The Vellar Estuary is highly involved in the fishing activity and the Yadayanthittu Estuary is actively involved in tourism recreation include boat trips. Keeping this a view, a comparative study made to understand the chemical and mineralogical alterations in *Meretrix casta* shells between the estuaries.

A. Major oxides

The shells of Vellar Estuary consist 53.43% of CaO whereas, in the Yadayanthittu Estuary shells consists 54.47% of CaO. About 1.04% of CaO is higher in Yadayanthittu Estuary, which indicates that the shells are richer in calcium content. While comparing the other

reported oxides, the Vellar Estuary shell aggregate of 1.2735%, however, the

TABLE 2: XRF detected oxide values of *M. casta* shells of Vellar and Yadayanthittu Estuaries

Sl. No.	Oxides detected by XRF	Vellar Estuary (m/m %)	Yadayanthittu Estuary (m/m %)
1	CaO	53.43	54.47
2	Na ₂ O	0.593	0.697
3	SiO ₂	0.202	0.241
4	SrO	0.135	0.184
5	Fe ₂ O ₃	0.0850	0.116
6	MgO	0.0698	0.0661
7	SO ₃	0.0654	0.0664
8	Al ₂ O ₃	0.0576	0.0587
9	Cl	0.0231	0.0504
10	K ₂ O	0.0166	0.0037
11	CdO	0.0098	0.0135
12	ZrO ₂	0.0043	0.0041
13	TiO ₂	0.0040	0.0038
14	Br	0.0019	0.0012
15	NiO	0.0017	0.0025
16	MnO	0.0025	--
17	P ₂ O ₅	---	0.0104
18	P	0.0018	--
19	ZnO	--	0.0018
20	Sc ₂ O ₃	--	0.0050
21	Re ₂ O ₇	--	0.0031

Apart from CaO%, all other reported oxides are little more (0.255%) in Yadayanthittu Estuary. The loss on ignition (LOI) values shows slight variations, the Vellar Estuary shells loss is 45.30%, the Yadayanthittu Estuary shells loss is (44.0%) and the difference is 1.3%. Because of low LOI the CaO content is more in Yadayanthittu Estuary shells. The elements P₂O₅, ZnO, Sc₂O₃, and Re₂O₇ are not detected by XRF in the *M. casta* shells of Vellar Estuary. But these oxides are reported in

Yadayanthittu Estuary shell aggregate of 1.5287%, both are having nearly same values.

TABLE 3: The occurrence of elements (wt. %) in *M. casta* shells in the Vellar and Yadayanthittu Estuaries

Sl. No	Elements detected by XRF	Vellar Estuary (wt. %)	Yadayanthittu Estuary (wt. %)
1	Ca	38.20	38.95
2	Na	0.440	0.517
3	Si	0.0945	0.113
4	Sr	0.114	0.156
5	Fe	0.0594	0.0813
6	Mg	0.0421	0.0399
7	Sx	0.0262	0.0266
8	Al	0.0305	0.0311
9	Cl	0.0231	0.0504
10	K	0.0138	0.0031
11	Cd	0.0086	0.0118
12	Zr	0.0032	0.0030
13	Ti	0.0024	0.0023
14	Br	0.0019	0.0012
15	Ni	0.0013	0.0020
16	Mn	0.0019	---
17	P	0.0018	---
18	Px	---	0.0045
19	Zn	---	0.0014
20	Sc	---	0.0033
21	Re	---	0.0024

Yadayanthittu Estuary shells. Likewise, the elements MnO and P, are not detected in the *M. casta* shells of Yadayanthittu Estuary, but the two oxides are reported in Vellar Estuary shells. Both the estuarine *M. casta* shells major oxide values are shown in Table 2.

B. Major elements

M. casta shells elemental concentrations (weight %) compared between both the estuaries. Regarding Ca wt. %, both of the

estuarine shells are having almost similar values (38.95wt. %) and (38.20wt. %) with a very little variation of 0.75wt. %. While correlating all other reported elements, Yadayanthittu Estuary shells show a bit higher values than the Vellar Estuary shell, and the values are 1.0503wt. % and 0.8647wt. %, respectively. The difference between them is 0.1856wt. %, which is negligible. The elemental concentrations of both the estuarine *M. casta* shells are tabulated in Table 3 for the comparison.

C. Biomineralization

The XRD, *Visual CRYSTAL 6* image results exhibited that both the estuarine *M. casta* shells are formed by aragonite mineral CaCO_3 (Figure 2 & 3). Vellar Estuary shells consist 93.32wt. % of aragonite whereas, in Yadayanthittu Estuary shells consists 98.0wt.%, about 4.68%, higher than the Vellar Estuary shells. While comparing the minor biomineral composition, the Vellar Estuary shells contain hematite, Fe_2O_3 with 6.68wt. %. On the other hand, the Yadayanthittu Estuary contains rare mineral varieties of Selwynite [$\text{Na K (Be, Al) Zr}_2(\text{PO}_4)_4 \cdot 2 \text{H}_2\text{O}$] with 2wt. % (see Figure 3). Both the estuaries *M. casta* shells are having aragonite biomineral as a major mineral, which is more common in all marine bivalves. But the minor mineral of both the estuaries is differing. The mineral constitution in backwater also one of the factors for minerals present in the shells. Yadayanthittu Estuary shells comprise 98.0wt. % of aragonite CaCO_3 because of this the CaO% is higher in this estuary shells than the Vellar shells.

IV. CONCLUSION

We made an extensive investigation of the chemical and biomineralogical properties of *Meretrix casta* shells of Vellar and Yadayanthittu Estuaries. From the detailed investigation, we conclude with the following points.

- The Vellar Estuary *Meretrix casta* shells consist with CaO of 53.43% and Ca of 38.20wt. %.
- The Yadayanthittu Estuary *Meretrix casta* shells consist 54.47% of CaO with Ca of 38.95wt. %.
- The oxides P_2O_5 , ZnO, Sc_2O_3 and Re_2O_7 are not reported by XRF in the *Meretrix casta* shells of Vellar Estuary.
- The oxides MnO and P are not reported by XRF in the *Meretrix casta* shells of Yadayanthittu Estuary.
- The Vellar shells formed by Aragonite as a major mineral with 93.32 wt.% and Hematite as a minor mineral with 6.68wt. %.
- The Yadayanthittu shells also formed by Aragonite with 98.0wt. % along with the rare mineral of Selwynite with 2.0wt. %.

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