



## Chemical characterization of oyster shells of the species *Crassostrea mangle* and *Crassostrea* *gigas*

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### 1. Introduction

The elemental composition of mollusk shells is determined by environmental factors, but is also controlled by animal biology [1]. Trace elements in bivalve shells can record the physical and chemical properties of seawater during its formation, so tracking this elemental signature can provide a wealth of environmental information, such as temperature, salinity, pH, nutrient and oxygen availability. This information prevails during growth and is preserved in the shell in the form of elemental variation [2]. Knowledge of these factors has sparked considerable interest in using bivalve mollusk shells as indicators of seawater quality and composition in paleoenvironmental studies [3]. The shell of bivalves is formed by incremental growth, resulting in a record that can be sampled even at an intra-annual resolution [4]. The ability to quantitatively track patterns of climatic seasonality through the geological record can be the key to understanding past, present and future climatic processes, especially when considering average values throughout the animal's growth period. In the biomineralization process, bivalve species build their shell in isotopic equilibrium with seawater. In this way, the ratios of stable isotopes, such as oxygen, for example, can reflect the physical and chemical conditions of the water in which the organisms grew [4].

The study of oyster shells, used as an indicator of the environmental conditions of the place where they developed, proves to be a possible effective tool for understanding both, natural and anthropic alteration processes. Oyster shell fossils have been used to provide data on paleotemperature on geological and seasonal scales, due to their occurrence in Triassic to Quaternary sediments, due to their relatively high resistance to postmortem changes [4].

In this study, two species of oysters were analyzed, *Crassostrea mangle* and *Crassostrea gigas*, originating respectively from Cananéia, SP and Florianópolis, SC and the concentrations of the elements Br, Ca, Ce, Co, Cr, Eu, Hf, La, Lu, Na, Nd, Sc, Sm and Th, were determined using Instrumental Neutron Activation Analysis (INAA) to verify the differences in the shell composition related to the growing environment. Also, termo-gravimetric characterizations were performed using DTA-TG (Differential and Gravimetric Thermal Analysis) and crystallographic analyses using DRX (X-Ray Diffraction).

## 2. Methodology

### 2.1 Sample Preparation

Samples from two regions and two species were analyzed, namely Cananeia-SP oysters of the *C. mangle* species and Florianópolis-SC *C. gigas* species. The *C. mangle* oyster shell samples from Cananeia-SP were acquired from a local oyster farming facility, while the samples from Florianópolis were provided by the Federal University of Santa Catarina (UFSC).

The removal of surface contaminants was carried out with a micro grinder, using cutting discs reinforced with fiberglass, a mounted silicon carbide tip and sandpaper. After removing surface contaminants, the samples were washed in 4% hydrochloric acid (HCl), in this process each sample was immersed in the acid solution for one minute to remove the surface layer susceptible to possible contaminants. After the acid treatment, the shell was washed with (Milli Q® water) and then the drying procedure was carried out in an oven at 100 °C.

The samples were crushed manually in a porcelain mortar, after grinding, the samples were sieved in an aluminum sieve with a nylon mesh, to obtain a fine and homogeneous material with a particle size of 100 mesh and then, the samples were stored in polyethylene bags.

### 2.2. Instrumental Neutron Activation Analysis (INAA)

For determination by INAA, about 0.100 g of the powder shell samples were irradiated for 20 seconds, in the short scheme irradiation and for 8 h, in the long scheme, in a thermal neutron flux of  $1.0 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$  in the nuclear research reactor IEA-R1 at IPEN-CNEN/SP. Primary standards and biological certified reference material were simultaneously irradiated with the samples for standardization and quality control purposes.

Gamma ray measurements were performed using a GC2018 Canberra HPGe detector coupled to a Canberra DSA-1000 multichannel analyzer. Gamma ray spectra were collected and processed using a Canberra Genie 2000 version 3.1 spectroscopy software. Element content calculations were carried out using a Microsoft Excel spreadsheet.

### 2.3. X-Ray Diffraction (DRX)

The X-Ray Diffraction was utilized to determine the crystalline structures of the oysters' shell in fine powder form. For this characterization it was used a SmartLab Rigaku diffractometer (Cu X-Ray

generator tube and a linear detector Dtex Ultra 250) in a 20°-70° 2 $\theta$  range, measurement speed of 2°/min. The results were refined by Rietveld's method using software PROFEX.

#### 2.4. X- Differential and Gravimetric Thermal Analysis (DTA-TG)

The samples were submitted to DTA-TG analysis by a STD 2960 from TA Instruments. The mass of samples was around 27 mg with 100 mL nitrogen flux.

### 3. Results and Discussion

From the mineralogy results, it is observed the predominance of calcite, which appears in a minimum proportion of 95.69%, in the samples where characteristic peaks of the aragonite phase appeared, its mass percentage was less than 1% (the minimum percentage that can be quantified by conventional X-ray diffraction technique). As expected in the thermal analyses, the mass losses of the samples are in accordance with the stoichiometry of CaCO<sub>3</sub>, due to the transformation of carbonate to CO<sub>2</sub> into Ca oxide. It was possible to observe that the onset temperatures of these samples were practically the same, indicating a physical and chemical homogeneity of the shells.

The results obtained demonstrate how the oyster shells of the *C. mangle* and *C. gigas* are capable of storing information about the environment in which they live, and it was possible to observe a significant elemental variation between the studied environments, Cananeia SP, and Florianópolis SC. A greater variation in the concentrations of the elements was observed for Ce, Co, La, Mg, Mn, Na. In the Florianópolis samples, the elements Br, Mn, Ce, Co, Eu, Na showed higher concentrations than in Cananeia-SP, which may result from the levels of these elements in the seawater from which these samples were collected. The Cananeia samples showed higher concentrations for the elements Cr, Mg, and Sm. The remaining elements exhibited a similar behavior in both locations with Table 1 showing the element concentration and variations between the species of the oysters.

Table 1 Concentration the elements in mg/kg<sup>-1</sup> and values maximum and minimum for oyster shells of the *C.mangle* and *C.gigas*

Element Concentration [mg/kg <sup>-1</sup> ]	Max	Min	Max	Min
	Shells <i>C. mangle</i>		Shells <i>C. gigas</i>	
<b>Br</b>	9.48	3.66	9.27	1.37
<b>Ca [%]</b>	42.6	34.2	42.6	36.5
<b>Ce</b>	0.99	0.29	1.58	0.04
<b>Co</b>	0.053	0.018	0.609	0.089
<b>Cr</b>	1.6	0.26	1.00	0.07
<b>Eu</b>	0.008	0.005	0.166	0.017
<b>Mg</b>	864.3	272	104.6	27.6
<b>Mn</b>	40	7.5	47.9	10.6
<b>Na [%]</b>	0.381	0.193	0.707	0.132
<b>Nd</b>	2.23	0.13	1.18	0.45
<b>La</b>	0.7	0.15	0.157	0.027
<b>Sc</b>	0.1247	0.0012	0.0724	0.0102
<b>Sm</b>	0.7	0.007	0.043	0.007

To verify if the elemental composition may reflect the grown environment of the shell formation, a cluster analysis was applied and the result is shown in Figure 1. The two groups formed, one composed exclusively by Cananéia samples and the other, exclusively by Florianópolis samples, confirmed this hypothesis.

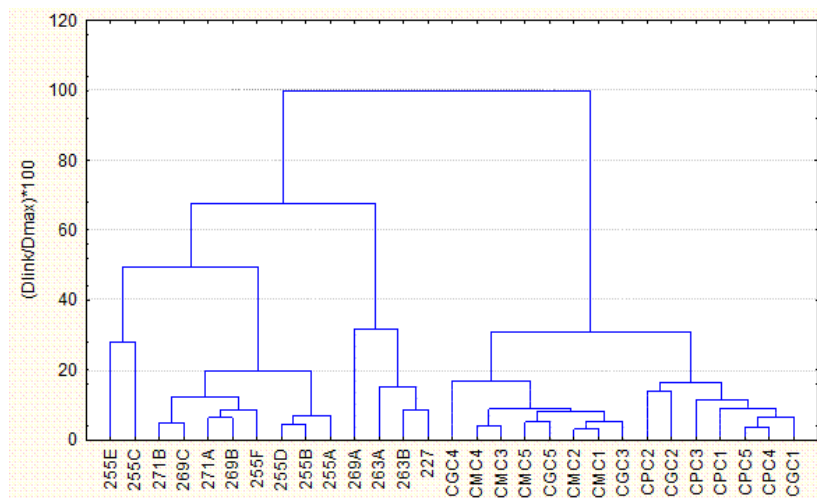


Figure 1: Cluster analysis in relation to the shell growth environment

#### 4. Conclusions

The results obtained showed that oyster shells carry an elementary identity, which can be used to provide information about the environment in which they were found, information that can be used to infer environmental changes. The carbonate formations of shells can be used for research in studies such as quality indicators, seawater composition and paleoenvironmental studies.

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