Journal of Animal and Veterinary Advances 3 (7): 445-447, 2004


# Mineral Composition of the Cockle (*Anadara granosa*) Shells, Hard Clamp (*Meretrix meretrix*) Shells and Corals (*Porites spp.*): A Comparative Study

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**Abstract:** The study was conducted to determine the composition of mineral content of cockle (*Anadara granosa*) shells, hard clam (*Meretrix meretrix*) shells and corals exoskeleton (*Porites spp.*) at three different levels of depths (5m, 10m and 15m). Three samples of corals exoskeleton (*Porites spp.*) collected from the sea at three different levels of depths (5m, 10m and 15m depths), the cockle (*Anadara granosa*) shells and the hard clam (*Meretrix meretrix*) shells were evaluated to determine the content of 12 macro- and micro-elements [Calcium(Ca), Carbon (C), Magnesium (Mg), Sodium (Na), Phosphorus (P), Potassium (K), Ferum (Fe), Copper (Cu), Nickel (Ni), Zinc (Zn), Boron (B) and Silicon (Si)]. For convenience and ease of reference, Ca and C were combined into one unit (Calcium Carbonate, CaC) while Mg, Na, P and K was evaluated individually, and Fe, Cu, Ni, Zn, B and Si were evaluated as one group (others). Analysis of the elements content was done using an Inductively Coupled Plasma, Auto Analyzer, an Atomic Absorption Spectrophotometer and Carbon Analyzer. Results in this study revealed that the composition of CaC, Mg and Na in the cockle (*Anadara granosa*) shells, hard clam (*Meretrix meretrix*) shells and the corals (*Porites spp*) at three different level of depths were similar with the value of more than 97% of the total elements content. Whereas the percentage of P, K and others constituents form the remaining of the elements.

**Key words:** Corals (*Porites spp*), cockle (*Anarada granosa*) shells, hard clam (*Meretrix meretrix*) shells, macro- and micro-elements

## Introduction

*Porites spp.* also known as coral can be found in tropical and subtropical waters of low turbidity, low terrestrial runoff and low level of suspended sediment. *A. granosa* or commonly known as blood cockle is an important bivalve found in muddy areas. The red pigment in *A. granosa* is composed of haemoglobin similar to that in human blood. Hard clam (*Meretrix meretrix*) can be found in sandy mud bottoms.

Coral exoskeleton has been successfully used in reconstructed bony defects in cranial surgery (Marchac *et al.*, 1994 and Vuola *et al.*, 2000). The success has been due to the present of calcium carbonate in the coral. The content of calcium carbonate is undoubtedly one of the essential components in any forms of bone implantation. The minerals content of coral were as follows: Ca >97%, Mg 0.05-0.2%, Na <1% and P <0.05% (Demers *et al.*, 2002). However, the harvesting of coral from the seabed may disturb its population and may come to a level of extinction if no effort is taken to curtail this activity. Therefore an alternative material must be ventured as a substitute to coral. The marine lives that could fulfill this dream are the cockle and hard clam shells. There is no information available to our knowledge in the literature on the mineral composition of cockle and hard clam shells. Thus the main objective of the study was to determine and compare the composition of certain elements present in the cockle (*A. granosa*) and hard clam (*M. meretrix)* shells. In addition the mineral compositions of corals (*Porites* spp.) at three different depths were also studied and the comparison was made.

## Materials and Methods

**Source of Samples:** The cockles and hard clams were collected from several stalls in the wet market located in, Selangor, Peninsular Malaysia. The three coral samples at different level of depths were provided by Kolej Universiti Sains dan Teknologi Malaysia (KUSTEM), Mengabang, Telipot, Kuala Terengganu, Malaysia. A total of 15 samples comprising five samples with three replications for each sample were analysed to determine the composition of mineral contents.

**Sample Preparation and Analysis:** The content of the cockles and hard clams were removed and the shells were washed thoroughly in distilled water and scrubbed free of dirt. All the samples were then dried in the oven at 50°C for three days.

The coral samples were first washed with distilled water and trimmed into small pieces with electric cutter before being placed in a Blendor (240V) for blending process until they turned into powder form. The dried shells of cockles and hard clams were similarly processed as above. The powder form shells were then shieved twice at

850 $\mu$m after which they were packed into respective McCartney bottles and sterilized for one hour in the oven at 105°C. The dry-sterilized samples were then subjected to biochemical test.

For carbon (C) detection, approximately 1 – 2 g samples were taken directly from the prepared samples. Carbon content was detected by using Carbon Analyzer. For detection of other elements (Ca, Mg, Fe, Cu, Ni, Zn, B, Si, Na, P and K) the prepared samples initially gone through the dry ashing process. Approximately 1 – 2 g samples were weighed in porcelain dish and placed in a muffle furnace. The temperature was increased gradually to 300°C and maintained for one hour until smoke disappeared. The temperature was further increased to 500°C and maintained for five hours until a whitish or grayish ash formed, after which the samples were taken out and leaved them to be cooled at room temperature. Once the ash was cooled, by using a pipette the ashes were moisturized with 2 ml distilled water. They were then added with 2 ml of concentrated hydrochloric acid and steamed on a hot plate. Subsequently, 10 ml of nitric acid were added in which they were then dissolved with water for one hour in a water bath at a temperature of 100°C. These dissolved materials were then placed in a 100 ml volumetric beaker. The porcelain dish was rinsed several times to ensure all dissolved materials were totally collected into the volumetric beaker in which distilled water was topped up till 100 ml mark. These solutions were then shake and filtered through no.2 filter paper.

Once all the above procedure completed, the aliquot was then utilized for the analysis of Ca, Mg, Fe, Cu, Ni, Zn, and Si by using Inductively Coupled Plasma (ICP) machine. The elements content of P and K were obtained through an Auto Analyzer machine while the Atomic Absorption Spectrophotometer machine was used for analysing the Na.

Five groups of samples comprising three samples of coral (5m, 10m and 15m depths), hard clam shells and cockle shells each with 3 replications were arranged in a Completely Randomized Design. The mineral content was expressed in parts per million (ppm). However, they were expressed as percentages in this study for convenience in making comparisons with other studies associated with the animal bone content. The Ca and C elements were combined together because their compositions in animal bone studied by several authors were generally expressed as one unit. Whereas the elements of Fe, Cu, Ni, Zn, B and Si were combined together and termed as 'others' based on the fact that their compositions in the samples were too small to be counted individually.

**Statistical Analysis**: All data were analysed using Statistical Analysis System (SAS).

## Results

The composition of CaC, Mg, and Na were not significantly different in all the samples (Table 1). The results indicated that the content of these elements were almost similar in all the three samples of coral, hard clam and cockle shells. The content of CaC in all the samples ranged from 97.6 % – 98.7% (Fig. 1). Even though the composition of these two elements were not significantly different among the cockle shells, hard clam shells and corals at three different level of depths, the highest values (98.7%) was in hard clam and cockle shells as compared to the corals at three different level of depths.

Comparatively small amount of Mg and Na were detected in cockle and hard clam shells (Table 1). The content of these two elements, however, showed different trend in the samples evaluated. They were higher in the coral samples but lower in hard clam and cockle shells. The Mg content ranged from 0.20 % – 0.52 % (Fig. 2) in which the highest value (0.52%) was observed in coral at 15m depth at 10m depth, followed by coral at 5m depth (0.35%), clam shells (0.32%) and cockle shells (0.20%). The Na content was slightly higher than the Mg content and ranged from 0.72% – 0.96 % (Fig. 3). The maximum value was detected in coral at 5m depth (0.96%) followed by coral at 10m depth (0.90%), cockle shells (0.87%), coral at 15m depth (0.83%) and hard clam shells (0.72%).

However, the content of the remaining elements (P, K and Others) in each samples were observed to have significant different among the samples (Table 1). The value of P ranged from 0.02% - 0.05% with the highest value was observed in hard clam shells (0.05%) and the least value was in cockle shells (0.02%) (Fig. 4). The P content in the coral at three different levels of depths was not significantly different. The content of K in coral at 10m depth and coral at 15m depth was significantly higher than that of the clam and cockle shells (Fig.5). For the others (Fe, Cu, Ni, Zn, B and Si) elements, the content in each sample was not significantly different except for coral at 15m depth which was significantly higher (0.8%) than the other samples.

The content of each element in the respective samples are illustrated in Figs 6, 7, 8, 9 and 10. For all the samples, the content of CaC was the highest with a value of over 97% and followed by Na, Mg, K and P. Other elements (Fe, Cu, Ni, Zn, B and Si) were present with a small quantity in each sample.

Table 1: Composition of mineral elements in five samples

| | Element Content (%) | | | | | |
|---|---|---|---|---|---|---|
| Samples | CaC | Mg | Na | P | K | Others |
| Coral 5 | 98.33[a] | 0.35[a] | 0.96[a] | 0.02[bc] | 0.13[b] | 0.21[b] |
| Coral 10 | 98.07[a] | 0.52[a] | 0.90[a] | 0.03[ab] | 0.18[a] | 0.30[b] |
| Coral 15 | 97.64[a] | 0.52[a] | 0.83[a] | 0.04[ab] | 0.17[ab] | 0.80[a] |
| Clam shells | 98.70[a] | 0.32[a] | 0.72[a] | 0.05[a] | 0.05[c] | 0.17[b] |
| Cockle shells | 98.68[a] | 0.20[a] | 0.87[a] | 0.02[c] | 0.04[c] | 0.20[b] |

Means with the same letters in the same column are not significantly different ($\alpha = 0.05$) using Tukey. Others = Elements Fe, Cu, Ni, Zc, B, Si.

## Discussion

The mineral composition of cockle (*Anadara granosa)* shells and hard clam (*Meretrix meretrix*) shells were evaluated in this study to look at their potential as an alternative to coral exoskeleton as bone substitute. In addition, the mineral compositions of the coral exoskeletons *(Porites* spp.) at three different levels of depths were studied and the comparison was made.

The use of coral as a bone substitute has been documented in the literature (Vuola *et al.,* 2000; Marchac and Sandar, 1994). The substitution is made possible due partly to the presence of several elements such as Ca, C, Mg, Na, P, K, Fe, Cu, Ni, Zn, B and Si. As a comparison to the coral samples at three different depths, the composition of CaC and Mg were also present in the cockle shells and hard clam shells at a value of 97% and 0.3% respectively. These findings were similar to the findings reported by Demers *et al.* (2002). Inevitably, the contents of Na, P and K were at par with Demer's study. Thus, these findings could throw some light on human and animal bone graftings.

Coral exoskeleton has been successfully used to fill the aseptic defects eg. after trauma, tumor resection or removal of bone grafts from the iliac crest (Guillemin *et al.*, 1987). They had also observed the positive use of coral in grafting large bone defect, loose pseudarthrosis, bone lengthenings, spine fusions and maxillofacial reconstructions. Since the contents of these elements in cockle and clam shells were similar with that of the coral, the similar defect could also be repaired if cockle and clam shells were to be used as substitutive materials for bone grafting.

The content of elements P, K and others in the five samples evaluated were observed to be significantly different among the samples. The results were slightly higher than that reported by Demers *et al.*, (2002) on coral exoskeleton. This discrepancy might be due to the location of the sample collected which influenced by the several factors including the environmental factor.

This study revealed that the contents of CaC, Mg, Na and P in natural corals collected at the three different depths (5m, 10m and 15m depths) were similar. However, the K content was observed to be increased as the depth increased.

The results of this study showed that the cockle and hard clam shells contained similar composition of CaC, Mg and Na as compared to that of the coral. This finding could suggest the possibility of using cockle and hard clam shells as an alternative biomaterials for bone substitute in managing bone diseases.

However, further studies concerning the availability of these elements in cockle and hard clam shells from various places should be conducted in order to achieve more accurate result. Furthermore, biological evaluation in animal studies need to be carried out to evaluate the suitability of cockle and hard clam shells as biomaterial for bone substitute.

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