

A Biomonitoring Study: Trace Metals in *Amusium pleuronectes* Shell from the Coastal Area of Chon Buri Province

Wichian Siriprom^{1*} and Pichet Limsuwan²

ABSTRACT

The objective of the present study was to conduct a preliminary investigation of pollution in the coastal area of Chon Buri province environment with a focus on heavy metals in *Amusium pleuronectes* shell. An energy Dispersive X-ray Fluorescence (EDXRF) spectrometer was used to determine the composition of mineral and levels of the heavy metals, gold (Au), zinc (Zn), lead (Pb), chromium (Cr), iron (Fe) and manganese (Mn) in the *Amusium pleuronectes* shell. The results showed that the highest to lowest accumulated metals are Fe, Mn, Cr, Zn, Pb and Au, respectively. The crystal structure and microstructure of the shells were studied by x-ray diffraction (XRD) technique and IR spectroscopy, respectively. The XRD patterns and IR spectrum revealed that the samples is made of a mixture phase of aragonite and calcite. In addition, the Electron Spin Resonance Spectroscopy (ESR) was used to study the ions in the samples. It was found that the samples consists of Mn²⁺ ions in the calcite lattice of the *Amusium pleuronectes* shell.

Key words: *Amusium pleuronectes*, X-ray diffracton, crystal structure, electron spin resonance

INTRODUCTION

People are becoming more awareness of the complexity of the nature and the delicate balances that exist within the global ecosystem. The discharge of effluents and associated toxic compounds into aquatic systems represents an ongoing environmental problem due to their possible impact on communities in the receiving aquatic water and a potential impact on human health. Especially in highly polluted and industrial areas, such as Chonburi province.

The coastal zone receives a large amount of metal pollution from coastal towns, industrial dumps and rivers. Marine pollution is a global

environmental problem. Different human activities on the land, in the water and in the air contribute to the contamination with potentially toxic substances of seawater, sediments and organisms with potentially toxic substances. Contaminants can be natural substances or artificially produced compounds. After discharge into the sea, contaminants can stay in the water in dissolved form or they can be removed from the water column through sedimentation to the bottom sediments (Abbas Alkarkhi *et al.*, 2008). The coastal zone can be considered as the geographic space of interaction between terrestrial and marine ecosystems that is of great importance for the survival of a large variety of plants, animals and

¹ Faculty of Resources and Environment, Kasetsart University, Sriracha Campus, Chon Buri 20230, Thailand.

² Department of Physics, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

* Corresponding author, e-mail: srcwcs@ku.ac.th, opticslaser@yahoo.com, w.siriprom@gmail.com

marine specie (Maanan, 2008).

Benthic molluscs are the organisms most often used for the biomonitoring of metal contamination. Bivalve molluscs have an ability to accumulate heavy metals to various orders of magnitude with respect to the levels found in their environment (Usero *et al.*, 2005). The levels of heavy metals accumulated by marine molluscs are a function of water quality, but also of seasonal factors, temperature, salinity, diet, spawning and individual variation, among other factors. Moreover, the levels of metals accumulated in some marine organisms may be many orders of magnitude above background concentrations, thus demonstrating the potential of certain species as bio-indicators of heavy metal pollution (Hamed and Emar, 2006).

Sea shells are found as two common mineral forms of CaCO_3 , calcite and aragonite. The crystal structures of calcite and aragonite and their interconversion have been studied. Aragonite shells and aragonite mineral deposits are known to undergo a slow metamorphosis into calcite. Sea shells are composed of 97-99% CaCO_3 (calcite, aragonite, or vaterite) with lesser amounts of MgCO_3 , $(\text{Fe})_2\text{O}_3$, SiO_2 , $\text{Ca}_3\text{P}_2\text{O}_8$, CaSO_4 , protein, and mucopolysaccharides. In addition to these major and minor constituents, traces amount of Sn, Mo, Mn, Cd, Ti, B, Pb, Au, Ag, Ni, Co, Bi, Cu, Sr, Rb, and As have also been found in varying amounts (Linga Raju, 2003).

The electron paramagnetic resonance (EPR) spectra of Mn^{2+} ions have been extensively investigated (Narasimhulu and Lakshmana, 2000; Linga Raju *ed al.*, 2003). However, the free radicals and transition metal ions found in the exoskeletons of marine organisms have not been examined. These paramagnetic metal ions could serve as probes of the microstructure of the sea shells. Spectral investigations of carbonate minerals are very useful in solving the sedimental petrology problem of Mn(II) concentration and its distribution between non-equivalent positions

within the corresponding mineral structure, as produced by the mineral crystallization and evolution. (Narasimhulu and Lakshmana, 2000).

The purpose of this study was to obtain information on the concentration of trace metals *Amusium pleuronectes* shell from coastal area of Chonburi Province. The results reported here will provide valuable information and assessment the potential of using the *Amusium pleuronectes* shell as a biomonitor of heavy metal pollution. Another that the mineral composition of *Amusium pleuronectes* shell were to studied their information on microstructure is one of the most significant relative datasets for clarification of evolutionary trends of shell structure. Thus, the findings may suggest the possibility of using the *Amusium pleuronectes* shell as an alternative biomaterial for bone substitute in managing bone defects.

MATERIALS AND METHODS

The *Amusium pleuronectes* samples were obtained from the coastal area of Chon Buri Province in the eastern part of Thailand. First of all the soft parts inside the marine mussel samples were removed after opening the bivalve of the shell. The bivalve shell was brushed and washed with distilled water. After ward the bivalve samples were cleaned by soaking in dilute HCl solution for several hours to remove all organic materials. This was followed by washing thoroughly with distilled water. The cleaned sample was ground into fine powder to desired sizes by sieving and labels. The powder samples are denoted as M-SC indicating *Amusium pleuronectes* (Radoated scallop).

The crystal structure was characterized by a powder X-ray diffractometer. Powder diffraction data were recorded at room temperature using a powder diffractometer, Bruker AXS D8 Advance ($\text{CuK}\alpha$ (Ni filtered) with scintillation detector; 2θ range from 20-80° with step size of

0.02°). Infrared analyses were carried out on powder samples with a Fourier transform infrared spectrophotometer, Perkin Elmer model PE2000 (FTIR). All spectra were recorded from 4000-500 cm^{-1} at 4 cm^{-1} resolution. For FTIR measurements, 2 mg sample were dispersed in 300 mg KBr. All mixtures of the compound were pressed into cylindrical pellets of cylindrical shape and carried out at room temperature. The Mn^{2+} ions in the exoskeletons were studied by ESR spectrometer. All ESR spectra of the powder samples were measured on a Bruker E 500 CW ESR spectrometer operating at X-band microwave frequency, using 0.5 mT field modulation amplitude, with a time constant of 0.03 s. The powder samples were pressed into tablets for chemical analysis to measure the trace metals in the shells. The chemical analysis was carried out using An Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer, ED2000 High Resolution.

RESULTS AND DISCUSSION

Figure 1 shows the powder x-ray diffraction patterns of the *Amusium pleuronectes* shell samples. XRD patterns revealed that the crystal structure of *Amusium pleuronectes* shell has a mixture phase of aragonite and calcite, according to the mineral phases of the *Amusium pleuronectes* shell samples Joint Committee on Powder Diffraction Standard (JCPDS) numbers 00-005-0453, and 01-086-2339, respectively.

Figure 2 shows the FT-IR spectrum of the shell samples. A molecular ion, like CO_3^{2-} , exhibits four normal modes of vibration. These modes include a symmetric stretching, ν_1 ; an out-of-plane bending ν_2 ; a doubly degenerate asymmetric stretching ν_3 ; and a doubly degenerate planar bending ν_4 . As the symmetric vibration ν_1 is reported to be infrared inactive. In this spectrum in figure 2, the intense sharp band at 877 cm^{-1} has

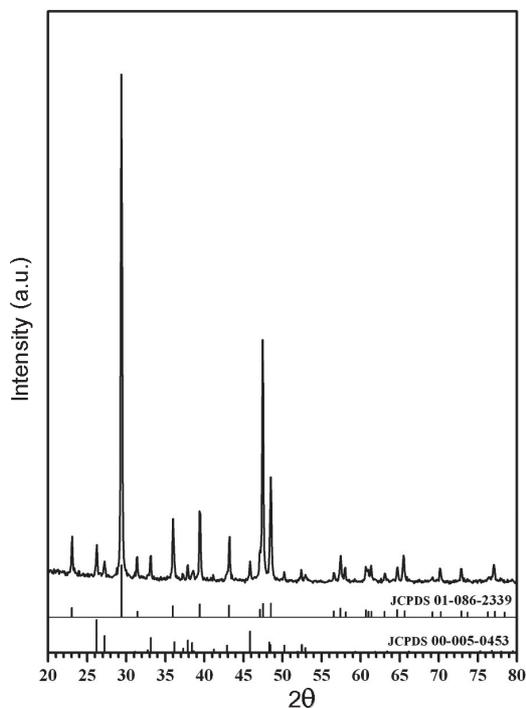


Figure 1 Powder XRD patterns of *Amusium pleuronectes* shell samples.

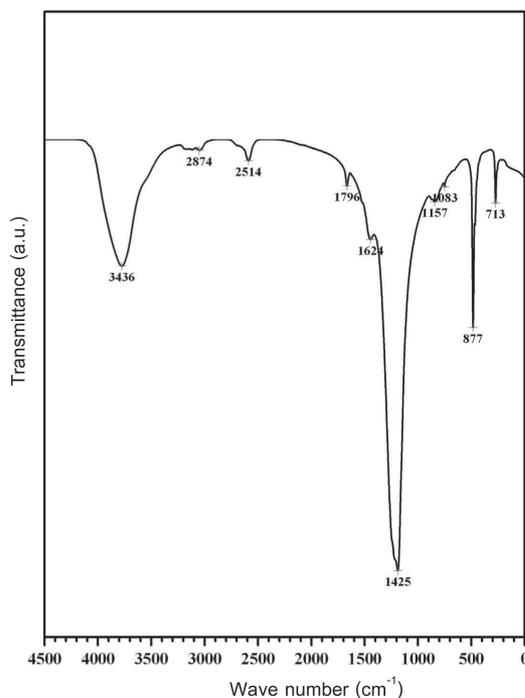


Figure 2 FT-IR spectra of *Amusium pleuronectes* shell samples.

been assigned to the ν_2 mode, the out-of-plane deformation band, which is infrared active. The broad band with center at 1425 cm^{-1} was assigned to the ν_3 mode of the CO_3^{2-} ion and is conformably of the CaCO_3 material. The band at 713 cm^{-1} in this layer corresponds to the ν_4 in-plane deformation. The band at 1083 cm^{-1} corresponds to the ν_1 fundamental mode of the CO_3^{2-} molecular ion in the aragonite group. The bands observed at 1796 and 2514 cm^{-1} are in good agreement with the bands observed for the CO_3^{2-} ion in CaCO_3 lattices. The water region also appears as a broad band, indicating a significantly different chemical environment of the water molecules in the sample. The bands at 1624 cm^{-1} and 3436 cm^{-1} were attributed to water molecules, which the broad band apparently due to asymmetric stretching and the O-H-O bending vibrations.

The average concentrations of trace element (Ca, Sc, Cr, Mn, Fe, Zn, Br, Sr, Au and Pb) in the shell samples are given in Table 1. The concentrations of metals in the *Amusium pleuronectes* samples decreased in the order $\text{Ca} > \text{Sr} > \text{Sc} > \text{Fe} > \text{Mn} > \text{Cr} > \text{Zn} > \text{Pb} > \text{Au} > \text{Br}$.

The ESR spectrum of the *Amusium pleuronectes* shell samples is shown in Figure 3; it is well known that the ESR spectra corresponds to Mn^{2+} in carbonate minerals. The six main lines arise from the coupling of the electron magnetic moment, $S = 5/2$, with the manganese nuclear

moment, $I = 5/2$, for the $M_s = -1/2 \leftrightarrow +1/2$ transitions. The other transitions, such as $M_s = +1/2 \leftrightarrow -3/2$ are very anisotropic and often difficult to observe with powdered samples. The weaker pairs of lines between the main lines are the so-called forbidden lines in which both electron and nuclear spin states change. As we shall see the EPR data are consistent with Mn^{2+} substituted for Ca^{2+}

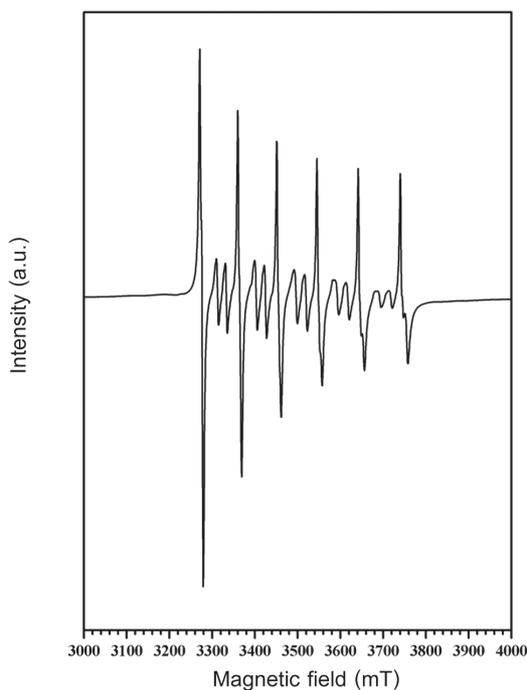


Figure 3 X-Band ESR spectra of *Amusium pleuronectes* shell samples.

Table 1 Metal concentrations in the *Amusium pleuronectes* shell samples.

Species/element	<i>Amusium pleuronectes</i>	unit
Ca	26.7824	Wt %
Sc	0.2799	Wt %
Cr	68	ppm
Mn	124	ppm
Fe	124	ppm
Zn	65	ppm
Br	8	ppm
Sr	0.3626	Wt %
Au	12	ppm
Pb	22	ppm

in the calcite lattice of the *Amusium pleuronectes* samples (Narasimhulu and Lakshmana, 2000) .

CONCLUSION

The *Amusium pleuronectes* shell samples obtained from the coastal area of Chon Buri Province, the eastern part of Thailand consisted mainly of CaCO₃. The calcite phase was not the only type of calcium carbonate crystal to make up the shell structure in the mussel. The calcified part of the shell consisted of two types of calcium carbonate crystals, either aragonite or a calcite mixture. The information obtained on trace elements in the *Amusium pleuronectes* shell samples showed a significant relative quantity between heavy metal element of heavy metal elements in the shell. The *Amusium pleuronectes* shell could be used as an indicator of all metal elements (Ca, Sc, Cr, Mn, Fe, Zn, Br, Sr, Au and Pb) in the marine environment. Furthermore, the ESR spectroscopy can be profitably used to examine certain trace elements and radicals in calcified material and to obtain information concerning structural organization. Then biomineralized can provide unusual metal ion environments in which to study crystal field effects on transition metal ions.

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