

Determination on the chemical composition of *Ammonia beccarii* shell using SEM and EDX: Preliminary study of benthic foraminifera capacity in response to anthropogenic metal contamination in coastal areas

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Abstract. The use of single-celled and shelled biota, such as foraminifera that lives as benthic, in coastal environmental monitoring activity is very efficient. Several species of the *Ammonia* have been used as a proxy of various aquatic environmental monitoring activities. Chemical constituents screening in foraminiferal shell is a step ahead to identify the capacity of benthic foraminifera in responding to anthropogenic metal contamination in coastal water areas. The initial hypothesis of this study is the calcite test of *Ammonia beccarii* binds the anthropogenic metal in its shell structure and triggers the deformation test. The normal and abnormal shells of *Ammonia* specimens from Jakarta Bay and Batam waters are used in this study. The Ponar grab was used to sample surface sediment in Jakarta Bay and Batam waters in 2015, and the short core was used to acquire substratum sediment in Jakarta Bay in 2011. The *Ammonia beccarii* shell was analyzed using SEM and EDX detectors (Scanning Electron Microscope and Energy Dispersive X-ray). The shooting was performed three times in each test, i.e. in the first chamber (proloculus), the last chamber and the chamber between the two. The main building blocks of the foraminifera test are oxygen with an average weight range of 42.86 - 58.79% and carbon with an average weight range of 17.69 - 26.32%. There is a tendency for low levels of C and O elements in the abnormal tests.

1. Introduction

Coastal water is a susceptible area to by-products of various human activities in the coastal area and those in the upper land. Anthropogenic flows through the river to a coastal area at various scales that lead to enrichment, hypoxia, anoxia and metal contamination. Accumulation of sediments in coastal areas were medium, particularly to silt-fractioned sediment. Many heavy metals in coastal waters, especially those accumulated in the bottom sediments can be very toxic and may threat the health of marine organisms. For marine organisms that have a hard structure, metal contaminants will have an effect on the biomineralization process. Biomineralization is a biological process that produces materials, such as shells, bone, and teeth, and the processes lead to the formation of these structured organic-inorganic composites [1]. The bioaccumulation of toxicants, such as heavy metals, by living organisms is often a good integrative indicator of exposure, and has been extensively used to assess contamination levels of heavy metals in polluted ecosystems [2].

The use of single-celled and shelled biota such as foraminifera in coastal monitoring activity is very efficient. Benthic forams are very useful proxies for paleoenvironment because specific taxa and assemblages have distinctive distributions relative to proximity to shore, water depth (a complex of variables), productivity (food availability), and dissolved oxygen [3]. Proxies from marine sediments



offer useful data for paleoenvironment interpretations. Planktonic and benthic foraminifera provide detailed analysis of paleoceanographic conditions due to their habitat in surface and deep water reservoirs [4]. According to [5], calcite analysis of foraminifera tests and partition analysis coefficients of minor metals and metal trace potentially reveal the availability of metal traces. *Ammonia beccarii* is benthic foraminifera that exceptionally adapts to the coastal waters environment [6,7]. The use of several species of the *Ammonia* as a proxy of various aquatic environments has been widely studied. [8] have examined the use of the *Ammonia*-*Elphidium* index to track the trace of oxygen depletion from the core samples, [9] observed the impact of Pb metal on *Ammonia parkinsoniana* with an ultrastructural and microanalytical approach, while [10] dan [11] observe calcite content in *Ammonia tepida* in experiment of pH and pCO₂ changes.

In the northern coastal waters of Jakarta Bay, Cirebon and Semarang, *Ammonia beccarii* is a dominant species on benthic foraminiferal communities, which reaches over 60% of the total abundance [12]. This species is endemic in the Jakarta Bay since the 1880s. Core samples study [13] shows peak population occurred in the 1880s and 1990s. Screening of chemical constituents on the foraminiferal test is a step ahead to understand the test capacity in response to anthropogenic metal contamination in coastal areas. The initial hypothesis of this study is foraminiferal calcite test of *Ammonia beccarii* binds anthropogenic metals in its test structure and the increase in anthropogenic metal accumulation in the sediment is one of the triggers of the test deformation.

2. Methodology

The *Ammonia beccarii* specimens used in the study were obtained from the Jakarta Bay and Batam waters. The Ponar grab and the short core were used on sampling activities. The Ponar grab was used to sample sediment surface in Jakarta Bay and Batam water in 2015, and the short core was used to acquire substratum sediment in Jakarta Bay in 2011. Substratum sediment of 30 – 35 cm layer was used in further analysis of *Ammonia beccarii* test, the layer originated from 1962 sediment deposition based on Pb₂₁₀ age measurement. ²¹⁰Pb isotope analysis was conducted in BATAN (The national atomic power agency). According to [14] total ²¹⁰Pb activity is determined from the measurement of ²¹⁰Po half-life. Destruction of the sample follows the Sanchez-Cabeza procedure. Both isotopes are chopped with an alpha production spectrometer Canberra with a PIPS (Passivated Implanted Planar Silicon) detector. Supported ²¹⁰Pb was obtained from the measurement of ²²⁶Ra using a Canberra-produced gamma spectrometer with HPGc (High Pure Germaium) detector.

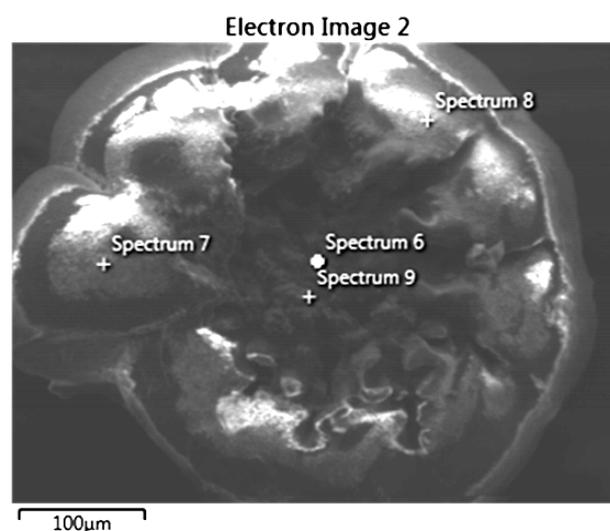


Figure 1. Shooting points of EDX detector on *Ammonia beccarii* test.

Foraminifera was separated from the sediment by washing and oven drying. Specimen was sorted and identified using the Nikon Labophot microscope. Six *Ammonia beccarii* tests were analyzed using SEM (Scanning Electron Microscope) and EDX detectors (Energy Dispersive X-ray). The shooting was performed three to four times for each shell, i.e. in the initial chamber (proloculus), the last chamber and the chamber between the two (Figure 1). EDX analysis in this study used the original samples without coating. X-ray shooting was performed at three different points to obtain information of homogeneous elemental content of the shell. The spectrum was generated by irradiation of the EDX detector at one point (see Figure 1). Each element recorded in the EDX sensor shows the different peak characters.

3. Result

Each x-rays shooting on the test (shell) produces a hint spectrum of elements content on percent weight (wt%) as shown in Table 1. Table 2 shows the average values of each element based on 23 spectra of six *Ammonia beccarii* specimens. Six of the specimens analyzed in the study consisted of three specimens with a normal shell morphology and three shells of abnormal shape. Deformation test which is often found in *Ammonia beccarii* is aberrant form i.e. over size in the last few chambers of the shell (Figure 2B).

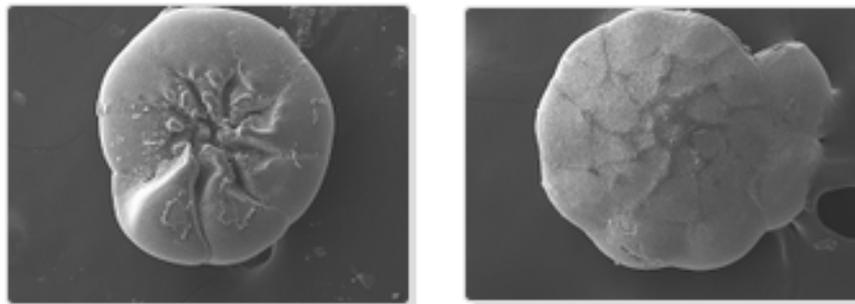
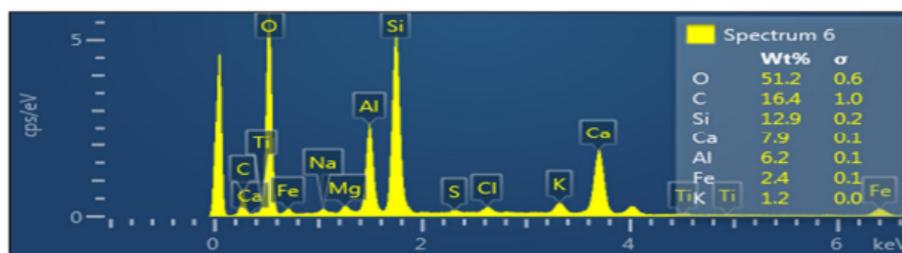


Figure 2. Normal (A) and abnormal (B) test morphology of *Ammonia beccarii* of Batam water.

Table 1. The spectrum generated by irradiation of the EDX detector at one shooting point of *Ammonia beccarii*.



Element	k Ratio	Wt%	Wt% Sigma
C	0.00439	16.36	0.95
O	0.01748	51.19	0.62
Na	0.00021	0.44	0.04
Mg	0.00039	0.56	0.04
Al	0.00541	6.22	0.10
Si	0.01246	12.88	0.17
S	0.00019	0.18	0.03
Cl	0.00058	0.53	0.03
K	0.00142	1.16	0.04
Ca	0.00997	7.94	0.12
Ti	0.00021	0.18	0.04
Fe	0.00275	2.36	0.08
Total:		100.00	

Ammonia beccarii test consists of 16 elements (Table 2). The main building element of the foraminifera shell is oxygen on an average range of 42.86 - 58.79 Wt% and carbon on an average range of 17.69 - 26.32 Wt%. Micro elements of the test were contained in the average above 1Wt% such as Si, K, Fe, Mg, Cl and Na (Table 2). Ten elements identified from all specimen's test were macroeconomic elements (Na, Mg, S, Cl, Ca, K, and P), microessential element (Fe), Si and Al.

Table 2. The element content of the *Ammonia beccarii* shell in the average value (% wt).

No.	Element	Station					
		FB10 N	FB 10 AN	TJ 15 N	TJ 15 AN	Batam N	Batam AN
1	C	22.5	17.69	26.32	22.28	21.2	20.35
2	O	56.3	42.86	58.79	44.7	53.2	52.04
3	Na	0.23	0.56	0.21	0.26	0.11	0.46
4	Mg	0.33	0.08	3.96	0.24	0.07	2.9
5	Al	1.84	0.44	0.28	0.18	0.9	2.74
6	Si	3.59	1.02	0.32	0.36	1.12	3.73
7	S	0.07	0.22	0.02	0.02	0.07	0.14
8	Cl	0.15	4.12	0.3	0.09	0.05	0.24
9	Ca	13.76	16.59	9.98	31	22.5	16.44
10	Fe	0.66	6.82	0.3	0.03	0.17	0.48
11	K	0.29	8.56	0	0	0	0.36
12	Ti	0.04	0.24	0	0	0	0
13	P	0	0.04	0	0	0.05	0
14	Cu	0	0.25	0	0	0	0
15	Mn	0	0.14	0	0	0	0
16	Zn	0	0	0	0	0	0.07

Abnormal specimens originated from Jakarta Bay core sediment of 31 – 35 layer dated 1962, consists of higher elements (15) including Ti metal in which obviously was anthropogenic element. Ti metals were highly detected in abnormal shells. Element Ti is not detected in the Jakarta Bay specimens sampled in 2015 nor from Batam waters (Table 2). The Jakarta Bay's specimens sampled in 2015 were also the lowest detected elements content (Table 2).

There is a tendency of low levels C and O elements in abnormal shell although the Wt% gradient between the specimens of Jakarta Bay and Batam shows the difference. Gradation differences in the content of both elements in the Jakarta Bay are sharper than the test specimens derived from the Batam waters. The similar trend is shown by Na content, which lower in the normal shell than in abnormal specimens in both research site.

The elements content in each specimen was analyzed using one-way cluster based on the Bray-Curtis coefficient of similarity (Figure 3). The dendrogram reveals two groups of specimens at the 0,85 similarity; (1) normal test specimens of Jakarta Bay dated 1962 and 2015, and both abnormal and normal specimens of Batam waters specimens and (2) abnormal test specimens of Jakarta Bay in another group. The Bray-Curtis coefficient of similarity detected similar characters between abnormal test specimen from Jakarta Bay both from the sediment layer dated 1962 and sampled 2015.

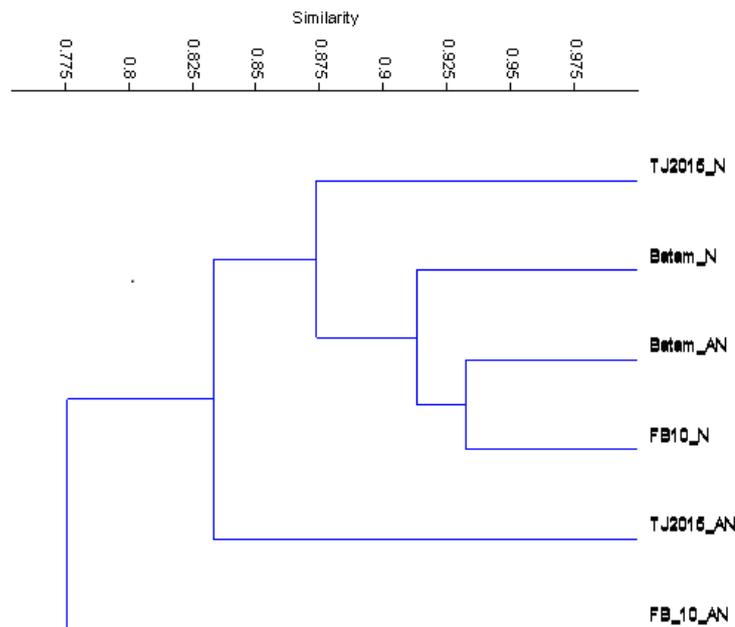


Figure 3. One-way cluster based on the Bray-Curtis coefficient of similarity of each specimen element content.

4. Discussion

The main element of the foraminiferal shell, particularly in hyaline calcareous is CaCO_3 . Indeed the elements of O, C and Ca are the highest elements contained on *Ammonia beccarii* test that might also be the same in most of calcareous foraminifera. The O content on the normal test ranged from 53.2 – 58.79, and less than 52.04 on the abnormal test. The C content on the normal test is also higher than on the abnormal test. There is no clear trend revealed the Ca content in the normal and/or abnormal test since the abnormal shells of Jakarta bay specimens tend to have a higher Ca content than the normal shells. However, abnormal shells of Batam specimens tend to have lower Ca content than the normal shells. Therefore, it is necessary to do further research to better understand the content of Ca in benthic foraminiferal shell in biological and environmental aspects.

Foraminiferal calcitic shell contains chemical information of the environment change, in which these unicellular organisms live. For example, at higher ambient seawater temperatures, more Mg is incorporated in the calcite crystal lattice [5]. Calcite crystal lattice is a crystal on calcite matrix. The binding mechanism of the contaminant element in the foraminiferal test may be similar to the mechanism of REE (Rare Earth Elements) in the foraminiferal test examined by [15]. The REE is bound in a calcite matrix known as REE lattice; REE is associated with an abundant FeMn authigenic (OM) through the adsorption phase by the surface of the test after the organism is dead (commonly referred to as REE coating) and the REE associates with an alumina sylvite detritus (called the detrital REE) which generally fills the chamber within the shell after being buried in the sediment [15].

Eight surface cores in the northeastern Pacific showed that REE in planktonic and benthic foraminifera was useful as a proxy for the biogenic productivity of surface water and mix layer. As an alternative, REE in benthic foraminiferal test is useful as a carbon-flux proxy in the surface of sediment waters. This proxy will become increasingly coarser towards the deeper layers, except for the sediment undergoing anoxic diagenesis, which stabilizes Fe carbonate covering the mark of the primary REE. However, the distribution of REE in foraminifera is very clear when anoxic conditions occur [16]. To understand the relationship between the environment and the chemistry of foraminiferal calcite, a physiological understanding of the calcification pathway is necessary. This, in turn, improves calcite-based proxy-relationships as used in paleoceanographic reconstructions.

5. Conclusions

The research indicated that the signature of metal elements on the shell of *Ammonia beccarii* shows that benthic foraminifera has the capacity to respond to anthropogenic contaminants. Ti metals were found in *Ammonia beccarii* shells derived from sedimentary layers in 1962. Shell deformation is the other respond of benthic foraminifera to environmental changes. This preliminary research showed that the abnormal shells of *Ammonia beccarii* tend to contain lower C and O elements.

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