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Distributions of Ni and Fe in the different tissues of snail *Faunus ater* and bivalve *Psammotaea elongata*

Abstract

Gastropods *Faunus ater* and bivalves *Psammotaea elongata* were sampled from the intertidal waters of Pantai Sri Tujuh, Tumpat (Kelantan), and their different soft tissues and shells were determined for Ni and Fe. The outcomes showed that the shells of the two species had significantly ($P< 0.05$) higher non-essential Ni levels than those in the different soft tissues. The contrary results were found in the essential Fe, where different soft tissues had significantly ($P< 0.05$) higher Fe concentrations than those in the shells of the two molluscs. This phenomenon could be due to the different essentiality of the two different groups of Ni and Fes between the different soft tissues and shells of molluscs. The present finding indicated the shells of *P. elongata* and *F. ater* were storage sites for Ni.

Keywords: intertidal, snails, clams, soft tissues and shells

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Introduction

Previously, Yap et al.[1] reported gastropod *Faunus ater* (Family: Potamididae) and the bivalve *Psammotaea elongata* (Family: Psammobiidae) possessed lower concentrations of non-essential Cd and Pb in the different soft tissues than those in their shells. In contrast, the shells had consistently lower concentrations of essential Zn and Cu compared to the soft tissues (especially the remaining soft tissues). This phenomenon might be attributed to the various strategies and binding affinity of the two different groups of metals between the various soft tissues and shells of molluscs. Yap et al.[2] reported the levels of Cd, Cu, Ni, and Pb within the different tissues *F. ater* collected from four sampling sites in Peninsular Malaysia. However, Fe was not analyzed in their study. Before 1975, Ni was thought to be absent of any important biological functions nonetheless it was later found to have significant high levels in a number of living animals although its role in the biochemical mechanism remained unclear.[3] Depending on the organisms, the Ni accumulation and its metabolism are very crucial for some enzymatic activities.[4] Generally, many of the toxic responses to Ni encompassed interventions with Fe metabolisms and Ni could be bound to nucleic acid in molluscs.[5] Based on some recent literature, Ni had been a focused metal such as in sediments[6,7] mussels,[8–11] snails[12] and plants.[13] Ecological distribution of the *F. ater* and *P. elongata* were reported in territories transcendently in the tropical intertidal regions like Peninsular Malaysia and the Philippines.[14,15] Whereas, the *F. ater* is local to the Philippines, Southeast Asia and East Indies.

These molluscs are potential biomonitors for identifying sources of bioavailable metal contamination in the coastal area.[16–19] The bivalve *P. elongata* had been reported from Mersing, Johor in 1986.[15] Concerning *F. ater*, it was reported from Teluk Dalam, Pulau Perhentian, Malaysia.[14] Coastal molluscs are potential biomonitors because they can accumulate toxic heavy metals directly from sediments, particles and metals dissolved in water.[19] The molluscs are capable to accumulate metals in their tissues which are corresponding to habitat environmental pollution. These metals can be distributed in the different soft tissues of the molluscs.[10] Most interestingly, they are large, abundant, sedentary, easily collected, and they are consumed by humans.[19] One of the well-established biomonitors in this region was the green-lipped mussel, *Perna viridis* where various investigations were accounted for on this species.[8–10,20–22] In any case, information on different molluscs like *P. elongata* and *F. ater* is scant. Since there are limited reports on *P. elongata* and *F. ater* on the levels of Ni and Fe in the literature, the purpose of this study was to determine Fe and Ni concentrations in the shells and different soft tissues of *P. elongata* and *F. ater* collected from Kelantan.

Materials and methods

The samplings of *P. elongata* and *F. ater* were conducted at Pantai Sri Tujuh, Kelantan, Peninsular Malaysia on 20 August 2006. About 40-50 individuals of each species were collected from the sampling sites. Identification of both species was based on Anon[15] for *P. elongata*. The samples of *F. ater* were identified with the identification keys of Brandt.[23] All issues of ethics on the two species of molluscs had been fully considered during the course of this study. The clams (*P. elongata*) were carefully dissected into six different soft tissues namely gill, foot, mantle, muscle, siphon and remaining soft tissues (remainder), while the snails (*F. ater*) were dissected into six different soft tissues namely digestive caecum, gill, foot, muscle, operculum, and remaining soft tissues (remainder). The shells and separated soft tissues were then dried in an oven for 72 hours at 105°C to constant dry weights.[24] Approximately 0.5 grams of dried tissues were measured and put into test digestion tubes that had been cleaned with acid. These tubes were then subjected to digestion using 10 mL of concentrated nitric acid (specifically AnalaR grade, BDH 69%). The digestion process took place in a digestion block set at a temperature of 40°C for one hour, followed by digestion at 140°C for three hours.[20] Afterwards, the solution was diluted to a volume of 40 mL using double de-ionized water and filtered through Whatman No. 1 filter papers with funnels of medium speed. The filtered solution was collected in acid-washed pill boxes.

The Fe and Ni content of all samples was determined using a Perkin-Elmer™ flame Atomic Absorption Spectrophotometer Model Analyst 800, operating with an air-acetylene flame. Standard solutions for Fe and Ni were prepared from a 1000ppm stock solution (MERCK Titrisol). The analysis results were expressed in micrograms per gram of dry weight. During the analytical procedures, quality control and quality assurance activities were performed to ensure accuracy and reliability. The method's quality was assessed using a Certified Reference Material (CRM) specifically designed for Dogfish liver-DOLT-3 from the National Research Council Canada. The measured values for Ni and Fe were compared to the certified values to evaluate the method's performance. The recoveries for Ni were found to be satisfactory, with a measured value of 2.77 µg/g dry weight compared to the certified value of 2.75 µg/g dry weight. Similarly, the recoveries for Fe were also evaluated, resulting in a measured value of 1070 µg/g dry weight compared to the certified value of 1484 µg/g dry weight. For statistical analysis, to test which group means did not differ from one another, Tukey HSD's multiple range tests were performed. All the statistical analyses were performed by using Statistical Package for Social Science (SPSS) for Windows package.

Results & discussion

The concentrations of Ni and Fe in shells and different soft tissues of *F. ater* and *P. elongata* collected from Pantai Sri Tujuh, Tumpat (Kelantan), Peninsular Malaysia (Figure 1; Table 1). Overall, all the different soft tissue (except for foot) of the clams had higher levels of Fe when compared to the shell. Shaari et al.[25] reported the Fe concentrations in *P. elongata* collected from five sampling sites in Semerak Lagoon (Kelantan) were 1.47 µg/g in total soft tissue; 85.5 µg/g in stomach; 84.2 µg/g in foot and 80.7 µg/g in ligament. It is found that the shells of *F. ater* and *P. elongata* accumulated highest concentrations of Ni, in comparison to other soft tissues of the molluscs. Based on samples of *F. ater* collected from four intertidal sites in Peninsular Malaysia in 2007, Yap et al.[2] reported the Ni concentrations (µg/g dw) ranged from 24.5-31.5 in the shells, 0.64-10.3 in the DC, 4.82-9.86 in the remainder, 1.62-11.1 in the operculum, 1.70-5.35 in the muscle, 0.11-6.77 in the foot. These Ni levels were comparable to the present mean Ni concentrations (µg/g dw) namely ranged from 28.7 in the shells, 8.56 in the DC, 9.97 in the remainder, 3.13 in the operculum, 3.56 in the muscle, 1.17 in the foot.

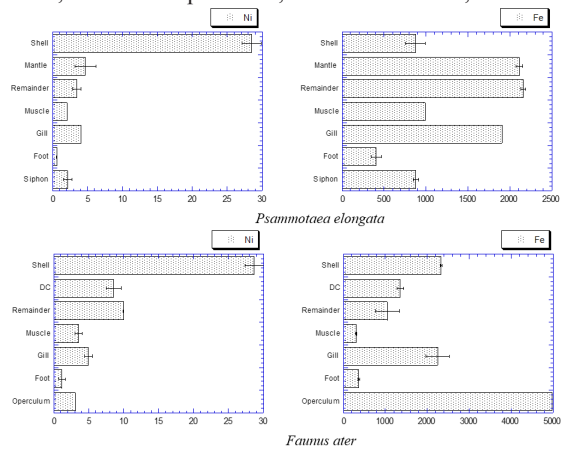

Figure 1 Concentrations (mean ± standard error, µg/g dry weight) of Fe and Ni in the different tissues of *Faunus ater* and *Psammotaea elongata* sampled from Pantai Sri Tujuh.

Table 1 Ranges and mean concentrations (µg/g dry weight) in the different tissues of *Faunus ater* (FA) and *Psammotaea elongata* (PE)

FA	Shell	Oper	Foot	Gill	Muscle	Remainder	DC
Ni	28.7 ± 1.29^{g}	3.13 ± 0.01^{a}	1.17 ± 0.46^{a}	4.96 ± 0.57^{af}	3.56 ± 0.50^{a}	9.97 ± 0.01^{e}	8.56 ± 1.09^{ef}
	(27.4-30.0)	(3.10-3.15)	(0.71-1.63)	(4.39-5.53)	(3.05-4.06)	(9.96-9.98)	(7.47-9.65)
Fe	2330 ± 18.7^{c}	4977 ± 2.25^{a}	365 ± 22.2^{be}	2250 ± 281^{c}	301 ± 12.8^{be}	1044 ± 286^{bef}	1357 ± 73.6^{ef}
	(2312-2349)	(4975-4979)	(343-387)	(1969-2531)	(288-314)	(758-1330)	(1282-1430)
PE	**Shell**	**Siphon**	**Foot**	**Gill**	**Muscle**	**Remainder**	**Mantle**
Ni	28.5 ± 1.39g	2.15 ± 0.60a	0.62 ± 0.06a	4.10 ± 0.01a	2.04 ± 0.01a	3.47 ± 0.60a	4.70 ± 1.47a
	(27.1-29.9)	(1.55-2.75)	(0.56-0.68)	(4.09-4.10)	(2.03-2.04)	(2.87-4.07)	(3.23-6.17)
Fe	876 ± 118a	879 ± 29.5a	406 ± 60.6b	1908 ± 1.78c	988 ± 0.04a	2159 ±28.1ce	2115 ± 38.3cf
	(758-993)	(849-908)	(345-466)	(1906-1910)	(988-988)	(2131-2187)	(2077-2153)

Note: Values in brackets are minimum-maximum. The tissues which have common alphabets are not significantly different from one another (Tukey HSD's, multiple range test) (n = 30): Oper = operculum; DC = Digestive Caecum

Generally, the shells of *P. elongata* and *F. ater* showed high metal concentrations. This could be attributed to the significant Ni amount being profoundly amalgamated into the shell crystal structural carbonates that happened as trace amounts.[26] In this study, Ni showed a more prominent Ni affinity to the shell than to the soft tissues of *F. ater* and *P. elongata*. Thus, the shell was the primary tissue for the aggregation of non-essential Ni and acted as a storage site for Ni within the shell crystal structural carbonates. The shells of molluscs employed or proposed as biomonitoring materials for toxic metals had been reported in the green-lipped mussel *Perna viridis,*[22,27] mud-

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flat snail *Telescopium Telescopium,*[1] and mangrove snail *Nerita lineata.*[28] Specifically, nickel (Ni) had been mainly focused upon for the biomonitoring studies in *P. viridis,*[11,29] *T. Telescopium,*[47] and *N. lineata.*[28] Comparable outcomes were accounted for by Bourgoin,[30] Yap et al.[21] and Cravo et al.[31]. They recommended that bivalve shells are superior to soft tissues for checking contamination due to a lower degree of trace metal variation. The essential metals in the different soft tissues are combined metabolically with significant biomolecules playing the vital roles of metalloenzymes or respiratory pigments.[19,32] In the shells, the metal ions might be incorporated into the carbonate crystal lattice by diadochically replacing the calcium ions in the calcite.[33]

The Fe levels were significantly lower in the molluscs' shells than in the soft tissues. Similar outcomes were found even in the gastropod *Littoraria scabra,*[34] shellfish,[35] mussels *Mytilus edulis*[36,37] and other molluscs.[38] The biomineralized magnetite ($FeO.Fe_2O_3$) that has been steadily studied in the literature[39–42] and found in the gumboot chiton (*Cryptochiton stelleri*)[42] may be the cause of the Fe accumulation in the molluscs' shells. By mistake, Cardoso et al.[42] found magnetic on the shell of the freshwater bivalve golden mussel (*Limnoperna fortunei*). They claimed to have discovered iron hydroxy/oxide nanoparticle aggregates that were about spherical in the aragonite and nacreous layers. It is assumed that magnetic nanoparticles might be spread throughout the nacreous layer given that the aragonite layer makes up more than 97% of the shell of the *L fortunei* and given the anticipated size of magnetic nanoparticles. The participation of hemocytes in the biomineralization of magnetite ($FeO.Fe_2O_3$) in the shells may provide an explanation for the Fe buildup in the shells.[43,44] This might mean that some or all of the calcium that reaches the mantle comes as calcium carbonate ($CaCO_3$), potentially in the amorphous condition.[55] Ivanina et al.[43,44] claimed that $CaCO_3$ nucleates and develops inside certain motile hemocyte cells before being transferred to the mantle. The amorphous state is metastable and possesses an excess of energy from a thermodynamic perspective. The extrapallial area is where these sub-micrometer-sized aggregates are then introduced to the inner sides of the developing shell. It transforms into crystalline phases (calcite and aragonite) inside the shell formations there, as predicted.[43–45]

The metal distribution in the different soft tissues of *F. ater* and *P. elongata* could be clarified by three points. Initially, different distribution is because of differences in the contact surfaces of each organ or part of the soft tissues.[20] Also, the variable affinities of metals to the binding sites of metallothioneins that was found in each of the organs. Lastly, different rates of accumulation and excretion of metals in each tissue were the results of internal metal regulation.[29,46] Moreover, the essential Fe which is found in high levels in the different soft tissues of molluscs could be because of consolidation in blood pigments (Cu) and proteolytic compounds (Zn). It is realized that the concentrations of essential Fe in molluscs can be directed by homeostatic mechanisms.[40,47] The regulation of essential Zn has been reported by several authors.[48] The current investigation showed that the remainder consistently had higher Fe levels (particularly in *P. elongata*) than the other tissues. The current finding showed that the soft tissues of molluscs addressed a significant supply of Fe. The shells could address a sink for Ni by eliminating a moderately higher level of this metal from the surrounding medium. This concurred with Langston et al.[48] that shells are an expected sink for metals because of their ability to eliminate bioavailable metals from the environment.

The Ni level in the snail operculum was significantly ($P< 0.05$) lower when contrasted with the shells, however, the Fe level in the snail operculum was essentially ($P< 0.05$) higher when contrasted with the shells. Yap et al.[46] revealed that the substantial metal levels of the operculum of *F. ater* showed no significant differences with those of shells ($P> 0.05$) for Cu and Zn which could be because of the normal design of the shell and the operculum as both comprised of calcified tissues.[49] The incorporation of Ni happened in the shells which could be because of the deposition of unnecessary non-essential Ni.[21] This clarified why the operculum, which was not a detoxifying site of the Ni, had a lower Ni level as compared to the shell. The variable affinities of metals to the binding sites of the metallothioneins in the different soft tissues of molluscs[46,50] could cause the different metal levels found in the different tissues of molluscs. Additionally, the functions of a particular organ in the molluscs could likewise be related to the substantial metal accumulation in the various tissues.[50] The raised Ni levels in the shell of *F. ater* and *P. elongata* could be clarified based on calcification in molluscs happening inside the extrapallial liquid, which is discharged by the mantle.

The arrangement of the extrapallial liquid may be subjected to a seawater-changing environment because of the impact of mantle metabolic action.[51,52] Furthermore, Ni was not really amalgamated into shell crystal structural carbonates yet they can likewise be adsorbed onto the skeletal natural framework.[53] Therefore, shells could provide a more practical sign of environmental metal contamination since molluscs shells can display less variability of metal levels due to the independence of seasonal changes. The shells can accumulate metal contents over the life of the molluscs.[31] Besides, they also act as a storage site for the unwanted chemical species including Ni based on the present finding.[21]

Conclusion

The various degrees of metals in the various tissues of *P. elongata* and *F. ater* showed that they have a distinctive capacity for metal accumulation. Ni was found higher in shells for the two species, demonstrating the shells of gastropods and bivalves can be a monitoring tool for these non-essential Ni. As opposed to Ni, the different soft tissues are potential biomonitors for Fe.

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None.

Conflicts of interest

The author declares there is no conflict of interest.

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