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Byssus as a Means of Metal Excretion Route and High Metal Levels in Fecal Materials as Metal Retention: An Experimental Laboratory Study Using *Perna Viridis*

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ABSTRACT

The present study aimed at determining the concentrations of Cd, Pb and Zn in fecal materials, byssus and different soft tissues of *Perna viridis* under controlled laboratory conditions during accumulation and depuration tests and to discuss the roles played by byssus and fecal materials in the coastal waters. The present findings indicated that the byssus of *P. viridis* acted as an excretion routes for Cd, Pb and Zn while mussels have a retention mechanism of metals as evidenced in the high metal levels found in the fecal materials during the experimental study. This implied that mussel population played an important role in expediting the metal cycling as well as supposedly nutrient cycling in the coastal ecosystem by rapidly recycling back to the coastal environment in the underlying sediments.

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1. INTRODUCTION

Although there has been studies reporting the employment the mussel byssus as biomonitoring organ of heavy metal pollution [1], [2], a problem can be encountered that 'The metals found in the byssus could be a function of metal absorption rather than metals that had already been assimilated from the soft tissues of mussels'. Hence, the use of byssus as a biomonitoring organ is still questionable.

Pseudofeces production is a kind of selective behaviour in bivalves. The particle selection is an obvious advantage to filter-feeding organisms, not only in sorting organic from inorganic particles, but also in choosing particles of easier digestibility. Although it is generally believed that pseudofeces are produced in bivalves at high total particulate matter concentrations[3], this selective behaviour was also observed at low total particulate matter [4-6].

The digestive processing of ingested particles may affect how bivalves obtain nutrition and accumulate pollutants. This needs to be understood to explain the biological assimilation of food particles in mussels. According to the review done by Decho and Luoma [7], the processing of potential food materials in suspension-feeding mussels involves several steps. At each of these steps, material may be rejected or further processed. According to Bayne and Newell [8], those directly rejected can be considered as pseudofeces. Once food material enters via the siphon, the initial processing involves a physical sorting usually based on particle size, density, or perhaps quality. The gills can act as the particle sieve, generally concentrating the smaller, more organic-rich particles. These are carried to the mouth and stomach. Larger, less desirable particles are rejected as pseudofeces through the incurrent siphon or mantle, often bound by mucus. This has been the principle of retention mechanism of suspended particles by mussels in the coastal waters. In this study, the term 'fecal material' was used to include both the pseudofeces and real assimilated feces since it is difficult to differentiate between the two.

Although byssus and fecal materials had been suggested as ways of metal detoxification [9], the information of these excretory pathways is still lacking in the literature. Hence, the objective of this study was to determine the concentrations of Cd, Pb and Zn in fecal materials, byssus and different soft tissues of *Perna viridis* under controlled laboratory conditions during accumulation and depuration tests and to discuss the roles played by byssus and fecal materials in the coastal waters.

2. MATERIALS AND METHODS

Except for fecal materials, the data of Cd and Zn in the different soft tissues of *P. viridis* in this study were cited from Yap et al [10] while those of Pb was cited from Yap et al [11]. In brief, the individuals of *P. viridis* were collected from a relatively unpolluted area (Pasir Panjang: 02°25′ N, 101°56′ E) of the west coast of Peninsular Malaysia. During the acclimatization period, seawater was changed daily and the mussels were fed with monocultured alga *Isochrysis galbana*. Spawning was observed in most of the mussels after 3 days of acclimatization and they were immediately isolated after spawning. Mussels which had released their spawn were selected for this experiment. Only similar sized (6-7 cm) mussels were chosen for this study. The seawater used was filtered through a plankton net with 200 μm meshes to remove suspended particles.

After the acclimatization period, the mussels were exposed to sublethal concentrations of Cd (measured: 1.210 mg/L; nominal: 1.200 mg/L), Pb (measured: 1.645 mg/L; nominal: 1.500 mg/L) and Zn (measured: 2.950 mg/L; nominal: 3.010 mg/L) and in a separate experimental aquarium. The concentrations of Cd, Pb and Zn chosen in this experimental study were based on the toxicity tests of Cd, Pb and Zn according to Yap et al [12]. Fifteen mussels (shell lengths of mussels: 7-9 cm) were exposed in each glass aquarium. One control was run through during the experimental period simultaneously. Accumulation and depuration studies were conducted in experimental aquaria.

Before the start of metal exposure, five mussels were collected for the analysis of the background levels of Cd, Pb and Zn. The seawater was changed once for every two days (semi-static) and the mussels were fed with *I. galbana* during the change to new seawater mixed with standard metal solution (nitrate salt). At day 4, aquaria with mussels were rinsed with clean seawater. The clean seawater without metal addition was transferred into the aquarium for the depuration study. Samplings were conducted at days 2 and 4 during the metal accumulation period and after 2 (day 6) and 4 days (day 8) during the depuration period. All samples were stored at -10°C until analysis. The test solution (20 L) was constantly aerated and was held at room temperature (26-29°C), salinity at 18-20 ppt, dissolved oxygen at 6.90-7.40 mg/L (80-90%) and pH 7.55-7.72, during the experimental period.

The total soft tissue of *P. viridis* was carefully removed by deshelling the mussel with a stainless steel knife. The soft tissues of *P. viridis* were dissected into byssus, visceral mass, gill, mantle, gonad, foot and muscle onto clean white tissue paper to drain away excess water. Fresh samples were digested in concentrated nitric acid (Ajax Chemicals, HNO_3 65%, Australia) in a hot-block digester first at low temperature (40°C) for one hour, and then at high temperature (140°C) for at least 3 hours. The samples were completely digested after the acidic solutions were clear (10. Yap et al [10]. The digested samples were then diluted to a certain volume (40 mL) with double distilled water. After filtration, the prepared samples were determined for Cd, Pb and Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data were presented in μg/g wet weight. To avoid possible contamination, all glassware and equipment used were acid-washed. Procedural blanks and quality control samples made from the standard solutions for Cd, Pb and Zn were analyzed once for every five samples in order to check for sample accuracy. Percentages of the metal recoveries were 90-115% for Cd, Pb and Zn.

The concentration factor (CF) was calculated at the end of depuration in comparison with metal level before exposure as follows[11]:-

$$CF = \frac{\text{Metal level}_{\text{end of metal depuration}}}{\text{Metal level}_{\text{pre - exposure of metal}}}$$

3. RESULTS AND DISCUSSION

From Figure 1, it is well shown that the metal concentrations in the fecal materials increased with increasing days of metal exposure (4 days). After 4 days of depuration in the clean seawater, the metal levels in the fecal materials reached the metal levels similar to those in the untreated control mussels. This finding is well indicative of the fact that almost all of the fecal materials are pseudofeces although a certain or negligible percentage might be actual feces originated from metal assimilation and metabolic mechanisms.

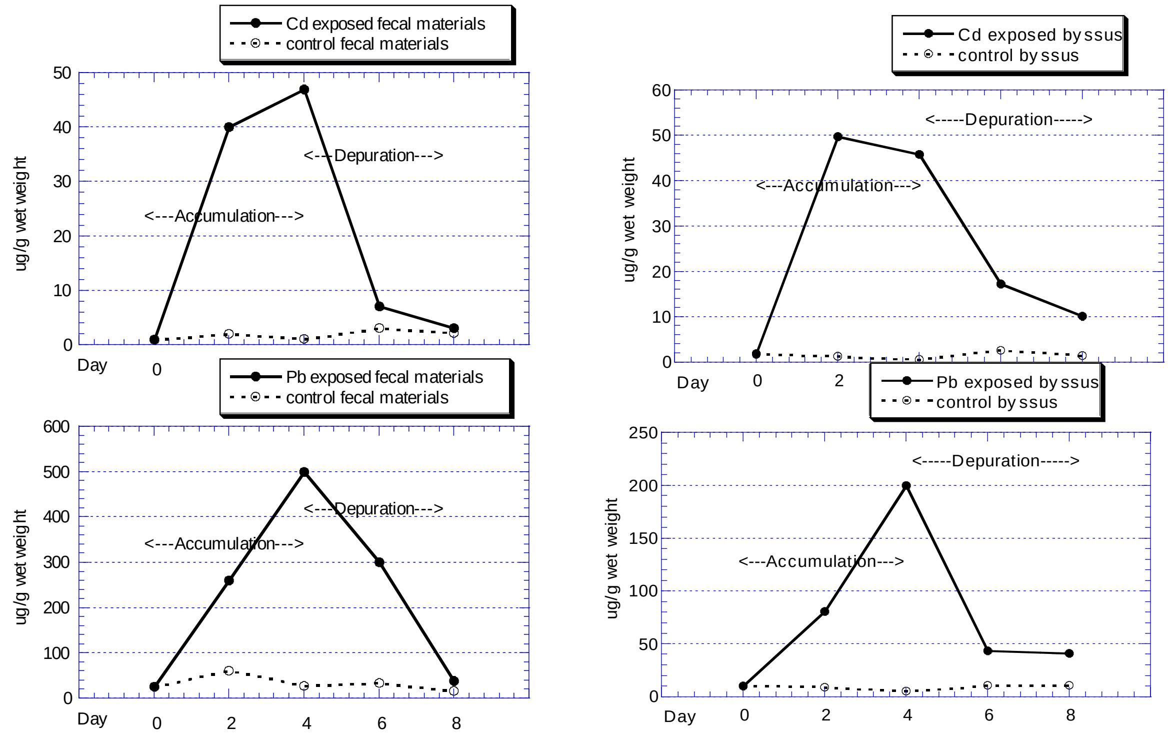

Figure 1. Concentrations of Zn, Cd and Pb in fecal materials and byssus of *Perna viridis* during accumulation and depuration periods under laboratory conditions.

On the other hand, byssus had a similar pattern of accumulation and depuration of metals with those in the fecal materials, except for it could never reach the metal levels similar to those in the untreated control mussel byssus. This phenomenon is expected since some portions of Cd, Pb and Zn were accumulated and stored or fixed in the different tissues of *P. viridis* and therefore, not all metals are eliminated from the mussel tissues. The rapid loss during the initial depuration period is due to the fact that the mussel byssus acted as an excretion route of waste metals [13] after involvement in the metabolic mechanisms although the involvement of adsorption of metals onto the byssal threads surfaces cannot be ruled out.

The levels of Cd, Pb and Zn in byssus and fecal materials of *P. viridis* were higher than in the total soft tissues of the mussels (Tables 1, 2 and 3). These elevated levels of Cd, Pb and Zn found in the byssus and fecal materials indicated that the elimination of these metals from the soft tissues during both periods of accumulation and depuration. During both periods of accumulation and depuration, byssus and feces were most sensitive to Cd and Zn as higher levels of these metals were accumulated in byssus and feces. This indicated that byssus and feces acted as short-term compartments for Cd and Zn elimination.

From Table 1, the significantly ($P< 0.05$) higher CF values (ranging from 19.05-31.82) in the total soft tissues and six different soft tissues, than those for byssus (4.04) and fecal materials (2.75), indicated that most of Cd was tightly bound to the metallothionein. Similarly, from Table 2, the significantly ($P< 0.05$) higher CF values (ranging from 26.04-100.2) in the total soft tissues, gill, visceral mass, mantle, and muscle, than those for byssus (4.62), fecal materials (1.37), gonad (3.26) and foot (5.75) indicated that most of Pb was also tightly bound to the metallothionein. The lower CF values for gonad and foot could be due to Pb was not tightly bound to these two tissues.

The CF values for Cd in Table 1, at the end of 4-days depuration, followed the order: fecal materials< byssus< foot< muscle< mantle< gonad< gill< total soft tissues< visceral mass. This indicated that fecal materials retained the least Cd when compared to byssus and other soft tissues. Similarly for Pb (Table 2), the CF values followed the order: fecal materials< gonad< byssus< foot< muscle< mantle< total soft tissues< visceral mass< gill.

Table 1. The concentrations (µg/g wet weight) of Cd in different soft tissues and concentrations factors (CF) in *Perna viridis* during accumulation of Cd 1.21 mg/L and depuration (Mussel shell lengths ranged from 7 to 9 cm).

Tissues/parts		Accumulation		Depuration		CF
	Day 0 (Ci)	Day 2	Day 4	Day 6	Day 8 (Ce_8)	Day 8 Ce_8/Ci
Soft tissues						
Byssus	2.50	49.75	45.75	17.27	10.09	4.04
Gill	0.84	23.45	28.45	24.00	22.04	26.24
Visceral mass	0.74	29.34	39.52	27.00	23.55	31.82
Mantle	0.51	12.55	18.10	14.44	12.4	24.31
Gonad	0.70	16.86	24.33	21.00	18.00	25.71
Foot	0.63	11.89	19.35	17.00	12.00	19.05
Muscle	0.29	5.90	10.21	7.65	6.31	21.76
Total soft tissues	0.80	27.2	36.8	26.0	22.0	27.5
Fecal materials	1.09	40.0	47.0	7.00	3.00	2.75

Note: Except for fecal materials, all the data in the Table 1 were cited from Yap et al[10].

Table 2. The concentrations (µg/g wet weight) of Pb in different soft tissues and concentrations factors (CF) in *Perna viridis* during accumulation to Pb 2.95 mg/L and depuration (Mussel shell lengths ranged from 7 to 9 cm).

Tissues/parts		Accumulation		Depuration		CF
	Day 0 (Ci)	Day 2	Day 4	Day 6	Day 8 (Ce_8)	Day 8 Ce_8/Ci
Soft tissues						
Byssus	8.84	81.00	200.0	43.35	40.82	4.62
Gill	4.17	900.0	1200.0	699.21	418.0	100.2
Visceral mass	1.30	211.35	231.73	111.57	105.0	80.8
Mantle	1.59	152.83	173.83	127.74	74	46.5
Gonad	9.51	50.25	55.61	45.0	31.0	3.26
Foot	6.03	60.47	81.94	52.49	34.66	5.75
Muscle	0.96	27.67	46.58	26.59	25.0	26.04
Total soft tissues	1.50	216.0	240.0	144.0	104.0	69.3
Fecal materials	27.9	261.0	500.0	300.0	38.31	1.37

Note: Except for fecal materials, all the data in the Table 2 were cited from Yap et al[12].

Table 3. The concentrations (µg/g wet weight) of Zn in different soft tissues and concentrations factors (CF) in *Perna viridis* during accumulation to Zn 1.95 mg/L and depuration (Mussel shell lengths ranged from 7 to 9 cm).

Tissues/parts		Accumulation		Depuration		CF
	Day 0 (Ci)	Day 2	Day 4	Day 6	Day 8 (Ce_8)	Day 8 Ce_8/Ci
Soft tissues						
Byssus	43.68	91.74	193.69	120.04	59.99	1.37
Gill	25.70	44.27	51.83	28.07	26.48	1.03
Visceral mass	26.00	32.19	44.32	30.13	27.00	1.04
Mantle	12.80	32.38	37.12	16.08	15.26	1.19
Gonad	17.50	26.39	35.87	25.36	22.67	1.30
Foot	16.72	23.44	28.82	20.02	17.18	1.03
Muscle	16.30	26.01	33.89	23.73	20.57	1.26
Total soft tissues	22.0	38.0	50.0	28.0	24.0	1.09
Fecal materials	43.0	389.0	441.0	130.00	48.00	1.12

Note: Except for fecal materials, all the data in the Table 1 were cited from Yap et al[10].

At the end of 4-days depuration, the fecal materials, byssus, total soft tissues and different parts of soft tissues almost reach the Zn levels before exposure (Table 3), with CF values ranging from 1.03-1.37. To explain this phenomenon, gill, visceral mass, mantle, gonad, foot and muscle almost had similar partial regulative mechanism for Zn, similarly to total soft tissues. This is due to the fact that Zn 'accumulated' during accumulation periods in the different soft tissues were not tightly bound to metallothionein but easily mobilized [10], [14], as they were rapidly released after 4-days depuration. Once entering the cytosol of *P. viridis*, some of the Zn was quickly eliminated from the soft tissues and defecation was also believed as one of the processes [15], [16]. Therefore, the Cd and Pb were most likely stored in membrane-bound granules or metallothionein and thus the rate of depuration was lower than Zn.

On the other hand, the low CF values of Cd, Pb and Zn for byssus could be attributable to these metals that were all released from the different soft tissues, although they were assimilated from the soft tissues. For fecal materials, the almost-reached-initial-metal-level was due to the Cd, Pb and Zn had not gone processes of assimilation. This was well indicative of retention mechanism of metals by the mussels.

The present accumulation and depuration tests also showed that byssus and different soft tissues played an important role in the biological regulation of Cd, Pb and Zn. This result indicated that metals accumulated in the soft tissues of *P. viridis* were secreted through the feces. The fecal materials from the present study comprised of pseudofeces (without biological processes) and feces (after biological assimilation). Nicholson [17] suggested that metal detoxification in *P. viridis* occurred through efficient exocytosis of metal-rich residual bodies (tertiary lysosomes) through the feces.

The production of fecal materials of mussels was highly influenced by organic fraction of seston and higher total particulate matter values [18]. According to Nizzoli et al [19], mussel farming *Mytilus galloprovincialis* induced intense biodeposition of organic matter to the underlying sediments, which stimulated sediment oxygen demand, and inorganic nitrogen and phosphorus regeneration rates compared to the nearby control station. Consequently, the net effect of the mussel farming on phytoplankton dynamics, may be to increase phytoplankton turnover and overall production, rather than to limit phytoplankton biomass. This was due to mussel population exerted an intense grazing pressure on the phytoplankton, the ingested organic nutrients were rapidly recycled back to the water column by the mussels and the underlying sediments, where they would fuel further phytoplankton growth[19]. Based on the above literature, the high metal levels found in the fecal materials in *P. viridis* from the present study indicated that mussels have a retention mechanism of metals.

4. CONCLUSION

The present findings indicated that the byssus of *P. viridis* acted as an excretion routes for Cd, Pb and Zn. The high metal levels found in the fecal materials of *P. viridis* showed that mussels have a retention mechanism of metals in the tropical intertidal waters in which this could enhance and expedite the metal cycling as well as the supposedly nutrient cycling in the coastal ecosystem.

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