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RESEARCH

Distributions of Cadmium and Lead Levels in the Intertidal Clam *Glauconome Virens*: A Biomonitoring Study

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ABSTRACT

The intertidal clam, *Glauconome virens*, was collected in the northwestern portion of Peninsular Malaysia near Kuala Perlis, Sungai Layar, and Pantai Teluk Air Tawar. This research attempted to determine the cadmium (Cd) and lead (Pb) concentrations in the various *G. virens*'s organs. The Cd concentrations (mg/kg dry weight) in five distinct soft tissues ranged from 0.90 to 1.89, but the concentrations in shells ranged from 0.95 to 1.25. Pb concentrations (mg/kg dry weight) in the five distinct soft tissues ranged from 27.0 to 44.9, whereas shell concentrations ranged from 74.8 to 75.3. In general, the shell had more Pb than the soft tissue components. Future biomonitoring research on this type of clams should concentrate on certain tissues (such as the remainder, shell, and gill) to identify their potential as biomonitoring organs/materials. This biomonitoring study is of paramount importance to establish the intertidal clams as a good biomonitor under the umbrella of International Mussel Watch. Besides, establishment of such biomonitoring baseline data is important for future references and human health risk assessments.

KEYWORDS

Biomonitoring; bivalves; toxic metals; metal distribution.

INTRODUCTION

Biomonitoring study of potentially toxic metals such as cadmium (Cd) and lead (Pb) in intertidal molluscs is not a new idea but this technique is very still significant from many scientific points of view such as a baseline for future references. Numerous scientific investigations have documented the usage of bivalves as heavy metal biomonitors [**1-8**]. According to Goldberg *et. al*. [**9**] and Phillips [**10**], using biomonitor species to scan environmental contamination has proven to be an efficient and informative method. Heavy metals are acquired by filter-feeding bivalves from their food, water, and inorganic particulate matter [**11**]. Therefore, they met the criteria for efficient biomonitors. Being sedentary, widely dispersed, and long-lived organisms are well-established biomonitors for monitoring heavy metal concentrations in coastal environments [**10,12-14**].

The bivalve clam *Glauconome virens* (Family: Glauconomidae) primarily inhabits the tidal-flats of Southeast Asia [**15**]. The animal extends its long syphon out of the mud during filter feeding. The clam is a native bivalve harvested for food in various countries but has not yet been commercially grown [**15**]. In Malaysia, Japan, Hong Kong, and the Philippines, fresh or dried clams are sold [**15**]. Similar to the well-established biomonitor *Perna viridis* in Malaysia [**2-7**], the clam *G. virens* meets the essential criteria for a good biomonitor, including a sedentary lifestyle, the ability to accumulate metals and the ability to provide an assessment of metal bioavailability, and being both tolerant and sensitive to heavy metal exposure [**14**]. Previously, Yap *et. al*. [**7**] reported the concentrations of Cu, Fe, Ni, and Zn) in the various soft tissues and shells of *G. virens*.

Since no studies on the levels of Cd and Pb in *G. virens* from Malaysia have been published, the goal of this study was to determine the concentrations of Cd and Pb in the shells, and different soft tissues (muscle, siphon, mantle, foot, gills, and remainder) of *G. virens* collected from three geographical sites in northern Peninsula Malaysia. Cd and Pb were chosen for this study because they have been extensively documented in the scientific literature as non-essential, potentially toxic metals that could pose dangers to human health [**2-8**].

MATERIALS AND METHODS

Between June and July 2007, sampling was undertaken in Kg. Sg. Berembang of Kuala Perlis (Kperlis), Sungai Layar (Slayar), and Pantai Teluk Air Tawar (TAT) in the northwest of Peninsular Malaysia (**Fig. 1**). Fifty individuals of almost similar shell lengths (5 to 7 cm) of *G. virens* were collected from each of the three sites. In addition to the shells, the soft tissues were dissected into the gill, mantle, foot, muscle, and syphon, with the remainder of the soft tissues together. The soft tissues and shells were dried at 105°C until constant dry weights (dw) were obtained [**16**].

Approximately 0.50 g of dried tissue was weighed and inserted into acid-washed test digestion tubes. The tube for digestion was supplied with 10 millilitres of concentrated nitric acid (AnalaR grade, 69% BDH). They were digested in a hot-block digestor at a low temperature (40°C) for 1 hour, followed by a high temperature (140°C) for 3 hours [**7**]. The samples were then diluted to a level of 40 mL using double-distilled water. After filtering, Cd and Pb were measured using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model Analyser 800.

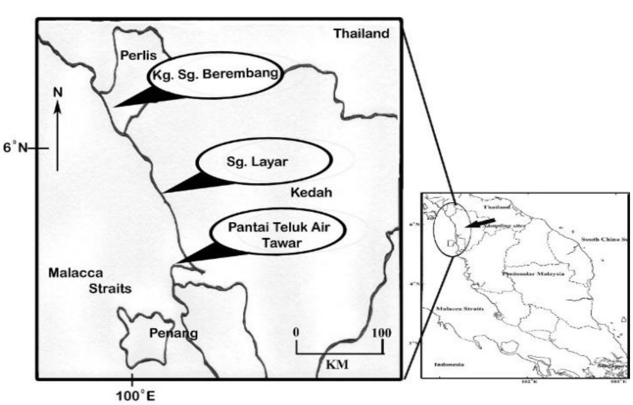

Fig. 1. Map showing sampling sites for *Glauconome virens* from Kg. Sg. Berembang of Kuala Perlis (Kperlis), Sungai Layar (Slayar) and Pantai Teluk Air Tawar (TAT) [7].

Using 1000 mg/L stock solutions, standard solutions for Cd and Pb were prepared (MERCK Titrisol). To prevent possible contamination, all used glassware and equipment were acid-washed, and the results were checked against a blank to ensure their accuracy. Every 5 to 10 samples were evaluated for accuracy using quality control samples produced from Cd and Pb reference solutions. Using a Certified Reference Material (CRM) for Dogfish liver-DOLT-3 (National Research Council Canada), the quality of the procedure was tested, and good recoveries were observed (103% for Cd; 78.7% for Pb).

For statistical analysis, the Post-hoc test (Student–Newman–Keuls) was employed to evaluate if there was a significant difference ($P<0.05$) between the metal concentrations in the various clam tissues.

RESULTS

The overall distributions of Cd and Pb concentrations in the various tissues of *G. virens* obtained from the three sampling sites are shown in **Table 1**.

Table 1. Overall distributions of Cd and Pb concentrations (mg/kg dry weight) in the different parts of *Glauconome virens* collected from Kg. Sg. Berembang of Kuala Perlis (Kperlis), Sungai Layar (Slayar) and Pantai Teluk Air Tawar (TAT). N = 3.

Cd	**Gill**	**Muscle**	**Siphon**	**Foot**	**Mantle**	**REM**	**Shell**
Min	1.28	1.50	0.97	1.06	0.90	1.09	0.95
Max	1.86	1.89	1.41	1.37	1.48	1.44	1.21
Mean	1.57^{b}	1.74^{a}	1.22^{d}	1.23^{d}	1.16^{e}	1.31^{c}	1.09^{e}
Median	1.57	1.82	1.28	1.27	1.10	1.39	1.12
SD	0.29	0.21	0.23	0.16	0.29	0.19	0.13
SE	0.17	0.12	0.13	0.09	0.17	0.11	0.08
Skewness	0.00	-0.62	-0.45	-0.40	0.36	-0.65	-0.36
Kurtosis	-1.50	-1.50	-1.50	-1.50	-1.50	-1.50	-1.50
Pb	**Gill**	**Muscle**	**Siphon**	**Foot**	**Mantle**	**REM**	**Shell**
Min	31.9	27.2	27.0	29.8	33.9	39.9	74.8
Max	40.4	34.5	42.4	37.1	40.1	44.9	75.3
Mean	37.3^{c}	31.1^{d}	36.2^{cd}	33.9^{d}	37.4^{c}	42.4^{b}	75.1a
Median	39.6	31.5	39.1	34.7	38.2	42.3	75.1
SD	4.69	3.67	8.11	3.72	3.18	2.50	0.25
SE	2.71	2.12	4.68	2.15	1.83	1.44	0.15
Skewness	-0.68	-0.21	-0.58	-0.39	-0.43	0.05	-0.24
Kurtosis	-1.50	-1.50	-1.50	-1.50	-1.50	-1.50	-1.50

Note: Means with different letters are significantly different at $P<0.05$.
Min = minimum; Max = maximum; SE = standard error; SD = standard deviation; REM= remaining soft tissues.

Cd concentrations (mg/kg dry weight) in five distinct soft tissues ranged from 0.90 to 1.89, whereas shell values ranged from 0.95 to 1.25. The Cd concentrations (mg/kg dry weight) ranged from 1.28-1.86 for gill, 1.50-1.89 for muscle, 0.97-1.41 for siphon, 1.06-1.37 for foot, 0.90-1.48 for mantle, 1.09-1.44 for remainder, and 0.95-1.21 for shell. Overall, the Cd mean order of different tissues follows: muscle> gill> remainder> foot> siphon> mantle> shell. Based on **Fig. 2**, the Cd levels were found to be highest in the TAT site in gills, muscle, siphon, mantle and remainder, even though they are not significantly ($P> 0.05$). Higher. However, foot and shells in TAT showed the reverse trend where Cd levels in both tissues were the lowest.

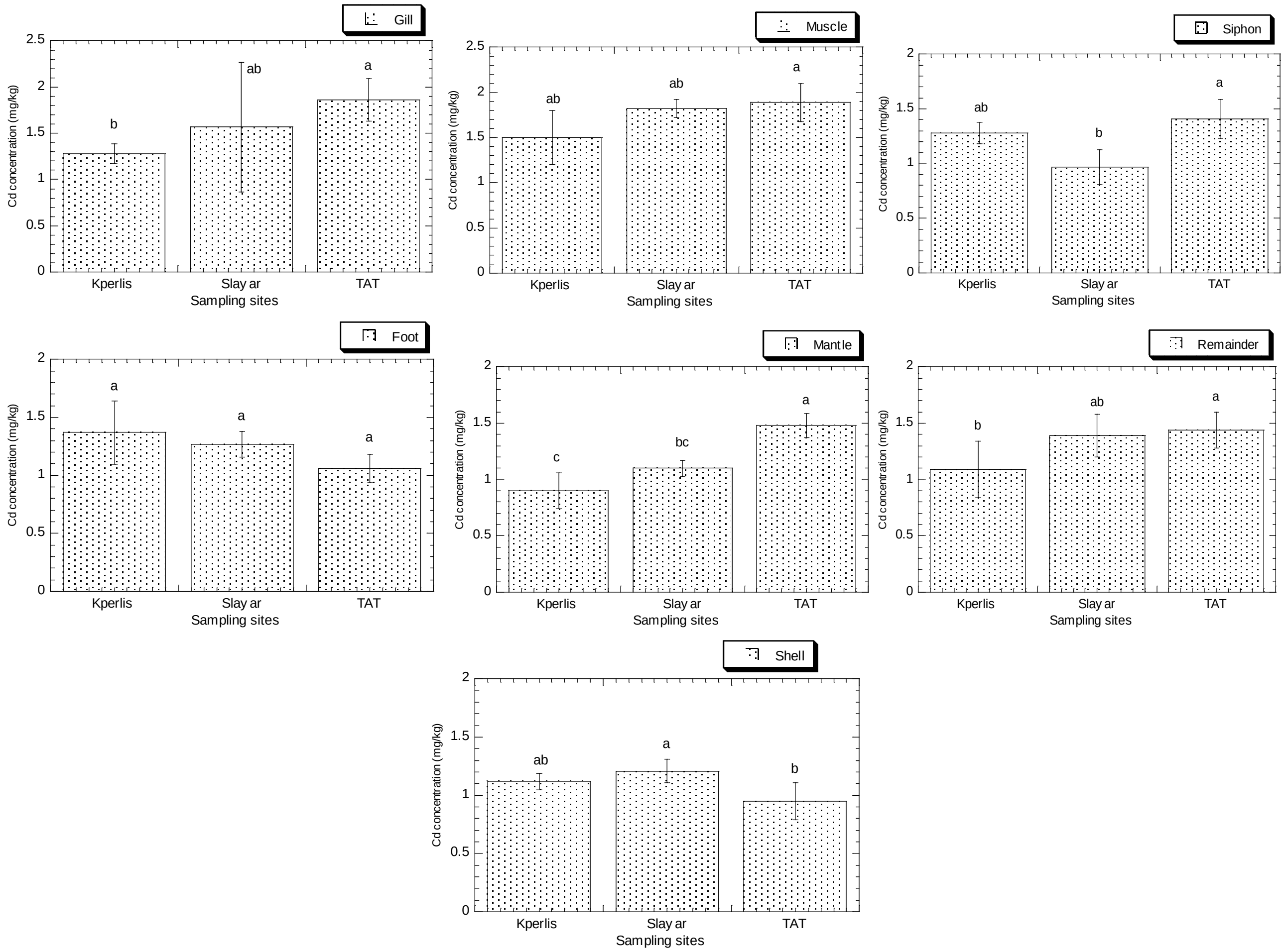

Fig. 2. Concentrations (mean ± standard error, mg/kg dry weight) of Cd in the different parts of clam *Glauconome virens* from Kg. Sg. Berembang of Kuala Perlis (Kperlis), Sungai Layar (Slayar) and Pantai Teluk Air Tawar (TAT). Note: Means with different letters are significantly different at P<0.05.

Pb concentrations (mg/kg dry weight) in five distinct soft tissues ranged from 27.0 to 44.9, whereas shell concentrations ranged from 74.8 to 75.3. The Pb concentrations (mg/kg dry weight) ranged from 31.9-40.4 for gill, 27.2-34.5 for muscle, 27.0-42.4 for siphon, 29.8-37.1 for foot, 33.9-40.1 for mantle, 39.9-44.0 for remainder, and 74.8-75.3 for shell. Overall, the Pb mean order of different tissues follows: shell> remainder> mantle> gill> siphon> foot> muscle. Based on **Fig. 3**, the Pb levels were highest in the TAT site in gills, muscles, siphons, and mantles, even though they are not significantly (P> 0.05) higher. Pb levels in the foot and remainders to found to be the highest in Slayar.

DISCUSSION

Analyzing the distributions of metals in the various organs of *G. virens* was more informative than the analysis of total soft tissues in *G. virens*. The present results indicated that different soft tissues of the clams had different levels of Cd and Pb accumulation, agreeing with many such studies in intertidal molluscs in Malaysia [**17**]. This could be due to different tissues possessing different physiological functions and regulations of metals that affect metal sequestration of the essential Cd and Pb [**18**].

According to Rainbow [**19**], an aquatic invertebrate's non-essential accumulation pattern of metals can be classified into accumulation with and without some excretion. If there is an accumulation without excretion, the metal will still be in a form that can be metabolised upon intake and must be expelled or detoxified to keep the body's level below the harmful impact threshold. The majority of accumulating metal may be in detoxified form. Such a pattern might be the explanation for the Cd that *G. virens* took in from the solution [**20**], with freshly picked up Cd (or Pb) being added to the existing total body store without excretion by the clam [**21**]. It is hypothesised that a large portion of the Cd (or Pb) that the clams accumulated was bound to metallothionein in the cytoplasm of the organ primarily employed to store accumulated Cd [**22,23**]. There is indirect evidence that Cd (or Pb) from metallothionein may be deposited in insoluble form in lysosomal residual bodies in certain instances of high Cd (or Pb) exposure [**23,24**]. There is a chance that any Cd-rich (or Pb-rich) cell inclusions will be expelled if the cells carrying these lysosomal leftover bodies line a tract with external access, providing a chance to determine the final accumulation pattern. The non-essential nickel in molluscs is treated with the same conceptual framework [**25**].

The net accumulation of a non-essential Cd (and Pb) in the presence of some excretion of the accumulated metal in detoxified form is known as accumulation with some excretion. The clams that ingested Cd (or Pb) from the diet seemed to handle it physiologically, similarly to how the necessary metals did, detoxifying it in the ventral caeca cells of invertebrates [**19**].

According to the present investigation, the gills and muscles had higher levels of Cd. As revealed by TAT tissue samples, the gills showed elevated levels of Cd. Because their intertidal habitat was in direct contact with ambient seawater, this may explain why the gills of *G. virens* from the TAT contained more significant levels of environmental Cd [**6,26**] in the habitat seawater. Previously, Yap et al. [6] reported that the mussel gill was a suitable biomonitoring organ for ambient concentrations of Cd, Cu, Pb and Zn in coastal wetland water.

The high levels of Cd in bivalves' muscles have been reported in the literature. For example, Yap et. al. [**27**] (2003) reported the mean value of bioconcentration factor of 21.8 in the Cd muscles of *P. viridis* compared to 1.26 in Zn muscles after the experimental depuration of the pre-exposed Cd mussels. This indicated that the non-essential Cd in the bivalves' muscles are tightly bound to metallothionein and not quickly mobilized. According to Bebianno and Langston [**28**] and Bebianno and Serafim [**29**], metal accumulation in muscle may be caused by metallothionein induction. The Cd could also be related to soluble cytosolic and insoluble particulate cell fractions in the bivalve *Laternula elliptica* from a naturally Cu-elevated environment [**30**].

Based on muscle, the TAT was found to have the highest Cd level, indicating the highest bioavailability of Cd in TAT when compared to Kperlis and Slayar. Intriguingly, the clam population from the TAT site had the highest amounts of Cd and Pb in most tissues compared to populations from the other two sites. This revealed greater Cd and Pb bioavailability at TAT [**4,5**]. Increased water and solid waste pollution have been a potential coastal threat at TAT [**31**].

In contrast, the highest concentrations of the non-essential element Pb were found in the shells but not in Cd. Some trace metals are integrated into the shells of clams by substituting calcium ions in the shell's crystalline lattices, or they are associated with the shell's organic matrix [**2,32**]. Many such reports agreed with the present findings with high levels of Pb in the shells of mussel *Perna viridis* [**2**] and other molluscs shells [**18**].

The distribution of Cd and Pb in the clam *G. virens* reported by the present study may be helpful for biomonitoring investigations in Malaysia. Future research should concentrate on the tissues or parts of *G. virens* with substantial metal concentrations. To establish the species of clam as a viable biomonitor, however, additional ecotoxicological and genetic research is necessary [**17**].

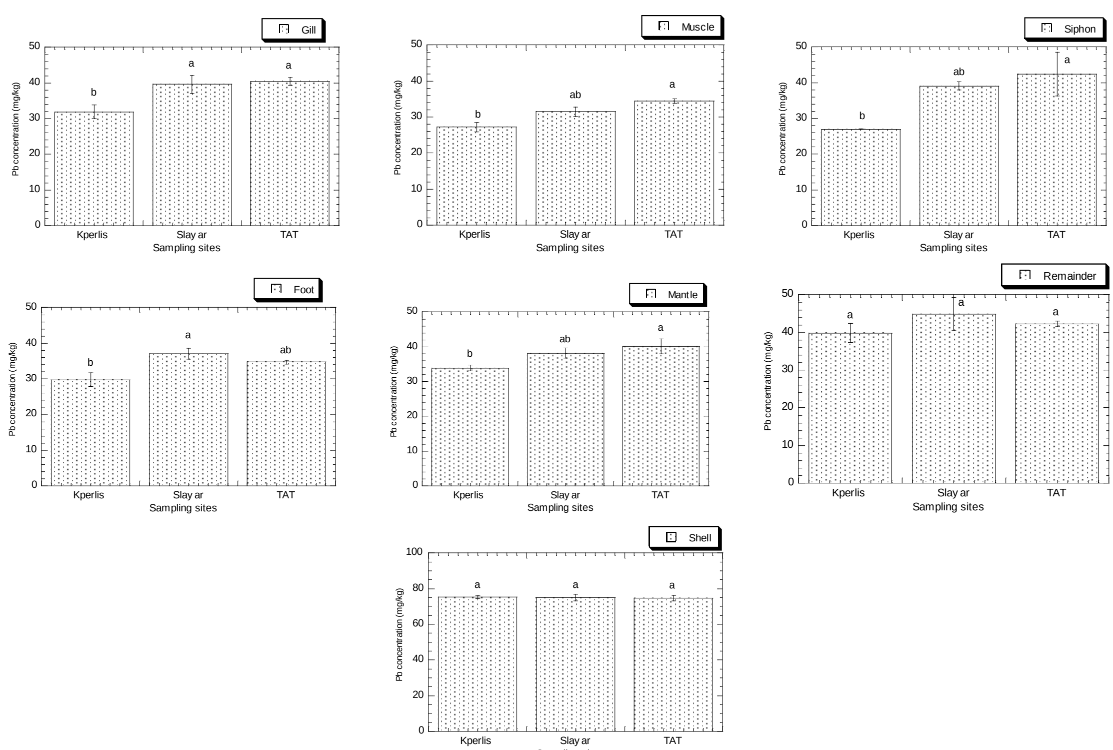

Fig. 3. Concentrations (mean ± standard error, mg/kg dry weight) of Pb in the different parts of clam *Glauconome virens* from Kg. Sg. Berembang of Kuala Perlis (Kperlis), Sungai Layar (Slayar) and Pantai Teluk Air Tawar (TAT). Means with different letters are significantly different at P<0.05.

CONCLUSION

According to the present results, the soft tissues generally had high quantities of Cd and Pb. On the other hand, the highest Pb concentrations were typically found in shells. Cd and Pb were substantially accumulated in the gills. This study demonstrated that particular metals target distinct *G. virens* tissues. Consequently, if *G. virens* is to be developed as a biomonitor, the current data should serve as a reference point for future research.

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CONFLICTS OF INTEREST

There are no conflicts to declare.

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Chee Kong Yap is working as a full professor in Universiti Putra Malaysia (UPM) since 2021. Prof Yap is an academician for more than 18 years in UPM and 23 years as a researcher. Prof Yap has supervised more than 80 undergraduates and 30 postgraduate students in the fields of ecotoxicology, environmental biology, environmental sciences, water quality and ecotoxicological genetics. Prof Yap has published more than 330 papers in refereed academic journals, 5 books (three of them published in NOVA Science Publishers, USA) and 32 book chapters. Until November 2022, 218 of them have now been indexed in *Elsevier's Scopus* with an H-index of 32 (> 3234 citations). Prof Yap has also been invited in honorary as Editorial Board members for more than 30 international academic journals. Prof Yap has been an invited visiting researcher at the National Institute of Environmental Studies, Tsukuba (Japan). Internationally, Prof Yap has been an invited visiting professor at Nihon University (Japan) and Hokkaido University (Japan), and an invited visiting researcher at Kobe University (Japan) and Kobe College (Japan). He has one accepted patent in Phytoremediation method. Starting June 2022, Prof Yap has been appointed as an Adjunct Professor at the INTI International University Malaysia.