THE POTENTIALS OF *PERNA VIRIDIS* SHELLS AS BIOMONITORING TOOLS FOR CADMIUM CONTAMINATION IN COASTAL AREA OF PENINSULAR MALAYSIA

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Abstract. This study evaluates the potential of *Perna viridis* shells as reliable biomonitoring materials for cadmium (Cd) contamination in coastal ecosystems across 16 sites in Peninsular Malaysia. Mussels were sampled from diverse environments, including industrial, aquaculture, and recreational areas, providing a comprehensive assessment of environmental Cd exposure. Each mussel's shells and soft tissues were analyzed separately, with shells showing consistent, significant positive correlations with bioavailable and organic-oxidizable sediment-bound Cd fractions. These correlations were stronger in shell components, particularly in the periostracum, compared to soft tissues, suggesting that shells provide a more stable, long-term record of environmental Cd contamination. Multiple linear regression analysis further identified bioavailable Cd fractions as significant predictors of Cd accumulation in shells, indicating that shell-based monitoring can reliably reflect ambient Cd levels in sediments. Surface sediment samples were also analyzed, confirming the environmental Cd distribution across sites. Findings highlight that *P. viridis* shells are better than soft tissues in monitoring chronic Cd contamination, offering a durable, cost-effective solution for coastal pollution assessment. This approach aligns with the United Nations Sustainable Development Goals, by supporting sustainable marine conservation and pollution management practices.
Keywords: *metal accumulation, pollution assessment, coastal monitoring, shell biomonitors, marine contamination*

Introduction

Heavy metal contamination, particularly cadmium (Cd), is a critical global concern in coastal ecosystems with high industrial, agricultural, and aquaculture activities (Gardiner, 1982; Luoma, 1983; Reolid et al., 2024). Due to accumulation in sediments and water Cd persists in marine environments and poses risks to aquatic organisms and human health through seafood consumption (Foster and Chacko, 1995; Bellotto and Miekeley, 2007; Montojo et al., 2021; Budiawan et al., 2020). Effective biomonitoring is essential for ecosystem health and pollution management, by offering insights into contamination trends (Goldberg, 1975; Yap et al., 2003; Zhong et al., 2024; Zhou et al., 2023).

The green mussel, *Perna viridis*, is an effective biomonitor for heavy metal pollution due to its capacity to bioaccumulate metals (Phillips and Rainbow, 1993; Krishnan et al., 2023; Neethu et al., 2024; Haeruddin et al., 2021). While soft tissues are frequently analyzed, their metal levels can fluctuate due to metabolic processes, complicating long-term monitoring (Soto et al., 2000; Mayk et al., 2022; Meng et al., 2023; Liu et al., 2024). Mussel shells provide a more stable indicator, reflecting cumulative contamination over time and requiring minimal preservation (Bourgoin, 1990; Juncharoenwongsa et al., 2011; Lin et al., 2021).

Analyzing *P. viridis* shells for Cd monitoring has distinct advantages. The periostracum, the shell's outer layer, interacts directly with water and captures Cd from bioavailable fractions in sediments. Shell components correlate with sediment-bound Cd, particularly bioavailable forms, making them effective for environmental assessments (Yap et al., 2002; Pérez-Mayol et al., 2014; Baudrimont et al., 2020; Canli and Canli, 2024). This study analyzed the periostracum, shell components, and sediments, along with shell morphometrics, to evaluate Cd bioaccumulation dynamics and relationships across these components, providing detailed insights into coastal pollution (Carriker et al., 1980; Stewart et al., 2021; Falfushynska et al., 2024; Cai et al., 2025).

The objective of this study was to evaluate the suitability of *P. viridis* shells as biomonitoring tools for Cd contamination in coastal ecosystems of Peninsular Malaysia. By comparing Cd concentrations in the periostracum and total shell components with those in soft tissues and surface sediments across 16 coastal sites, we aimed to assess the shells' potential for providing a stable, long-term record of environmental Cd exposure. The findings were intended to support the development of sustainable, cost-effective biomonitoring methods that align with environmental management goals and the United Nations Sustainable Development Goals (UNSDGs) related to ecosystem conservation and pollution control.

Materials and methods

This study was conducted across 16 coastal sites in Peninsular Malaysia, encompassing a variety of environments, including industrial areas, aquaculture zones, recreational sites, and urbanized coastal areas (*Fig. 1*; *Table 1*).

Table 1. *Site description and sampling dates*

No.	Sites	Lat (N)	Long (E)	Sampling dates	Site description
1	Penang	05° 353863'	100° 352554'	2005	Industrial area
2	**Bagan Tiang**	05° 08.517'	100° 22.459'	2002	Offshore and fish aquaculture a
3	**Kukup**	01° 19.551'	105° 26.500'	2004	Fish aquaculture and jetty and ferry activities in the vicinity
4	**Minyak Beku**	01° 47.732'	102° 53.37'	2005	Recreational site
5	**Kg.P.Puteh-1**	01° 26'	103° 41'	2002	Ports, industrial and urban areas
6	**P.Lido-1**	01° 28'	103° 44'	2002	A small jetty and a floating restaurant
7	Senggarang	01° 77'	103° 01'	10 Aug2004	Bought from roadside stall
8	**Gelang Patah**	01° 28'	103° 40'	11 Aug 2004	Aquaculture area
9	**Kg.S.Melayu-2**	01° 27'	103° 42'	11 Aug 2004	Mussel aquaculture area
10	**Kg.S.Melayu-1**	01° 28'	103° 43'	11 Aug 2004	Mussel aquaculture area
11	**P.Lido-2**	01° 28'	103° 44'	11 Aug 2004	A small jetty and a floating restaurant
12	**Senibong**	01° 29'	103° 49'	11 Aug 2004	Mussel aquaculture area
13	**Teluk Jawa**	01° 29'	103° 50'	11 Aug 2004	Ports, industrial and urban areas
14	**Kg.Masai**	01° 28'	103° 52'	11 Aug 2004	Ports, industrial and urban areas
15	**Kg.P.Puteh-2**	01° 26'	103° 41'	11 Aug 2004	Ports, industrial and urban areas
16	Sg.Belungkor	01° 29'	104° 11'	12 Aug 2004	Fish aquaculture areas

Sampling sites in bold are the mussel populations where different soft tissues of *P. viridis* are reported together with their habitat surface sediments

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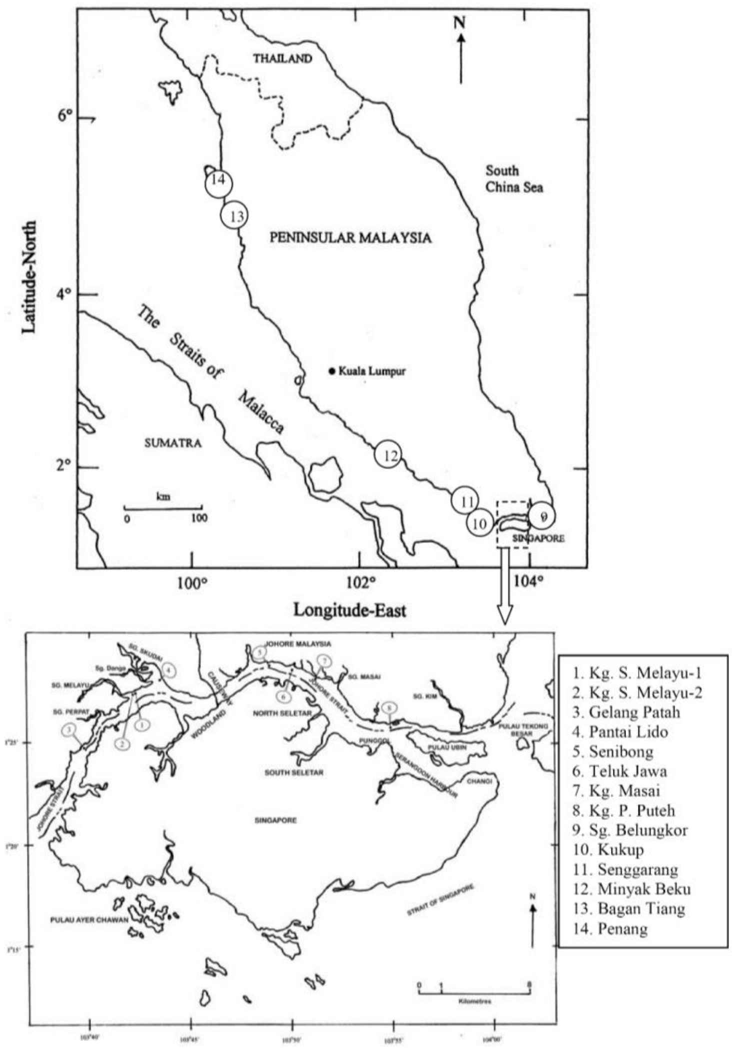

Figure 1. *Sampling sites of Perna viridis in the coastal waters of Peninsular Malaysia*

These sites were selected to capture a range of pollution sources, from industrial and urban runoff to aquaculture and recreational activities, providing a comprehensive assessment of Cd contamination in different coastal contexts. Latitude and longitude coordinates were recorded for each site, except for Penang, to ensure precise geographic documentation. The sampling period spanned from 2002 to 2005, offering data across multiple years to evaluate temporal variations in Cd contamination.

Noteworthy sites include Bagan Tiang (offshore aquaculture area), and Kukup (aquaculture with jetty and ferry activities), Minyak Beku (recreational site), Pantai Lido (recreational site), Kg. Sungai Melayu (fish aquacultural site), Senibong (shipping and mussel aquacultural site), Telok Jawa (shipping and mussel aquacultural site), and Kg. Pasir Puteh (ports, industrial, and urban areas) (*Fig. 2*), further contributed to the dataset, representing areas with varying contamination levels. The mussels were collected directly from these environments, either from aquaculture areas or roadside stalls where they were freshly harvested. This ensured that the samples reflected local environmental conditions, thereby enhancing the relevance of the data for biomonitoring Cd pollution in coastal waters.

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Figure 2. *Some sampling observational photos during the sampling of mussels and their sediments in the present study*

At each site, *P. viridis* individuals were measured for shell morphometric parameters, including shell length, width, height, and total shell weight. Between 6 to 12 mussels were collected per site (*Table 2*), providing a representative sample size for statistical analysis. There were 13 sampling sites where surface sediments (0-10 cm) were collected from the mussel habitat using an ekman grab or clean stainless steel spatula (Yap et al., 2002a, b).

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Shell measurements, including length (cm), width (cm), height (cm), and total weight (g), were obtained using a digital caliper and a precision balance. Values were reported as means with standard errors to reflect within-site variability accurately.

Table 2. *Values [mean ± standard error] of shell lengths (cm), widths (cm) and heights (cm) and total shell weights (g) of Perna viridis used in the present study*

No.	Sites	N	Shell length	Shell width	Shell height	Total shell weight
1	Penang	7	7.47 ± 0.44	2.28 ± 0.14	3.66 ± 0.17	13.52 ± 1.66
2	**Bagan Tiang**	12	5.68 ± 0.11	1.83 ± 0.04	2.91 ± 0.06	6.36 ± 0.40
3	**Kukup**	6	8.26 ± 0.19	2.50 ± 0.05	3.57 ± 0.06	12.38 ± 0.65
4	**Minyak Beku**	7	7.64 ± 0.26	2.37 ± 0.07	3.58 ± 0.12	12.21 ± 1.35
5	**Kg.P.Puteh-1**	12	7.02 ± 0.19	2.26 ± 0.05	3.19 ± 0.1	9.43 ± 0.46
6	**P.Lido-1**	11	6.47 ± 0.13	2.39 ± 0.06	3.02 ± 0.09	8.54 ± 0.52
7	Senggarang	12	6.26 ± 0.18	2.02 ± 0.03	2.54 ± 0.04	5.84 ± 0.39
8	**Gelang Patah**	12	4.38 ± 0.2	1.54 ± 0.18	2.45 ± 0.05	4.08 ± 0.37
9	**Kg.S.Melayu-2**	12	5.20 ± 0.15	1.79 ± 0.01	2.40 ± 0.01	4.79 ± 0.33
10	**Kg.S.Melayu-1**	12	5.35 ± 0.08	1.98 ± 0.03	2.70 ± 0.01	4.37 ± 0.4
11	**P.Lido-2**	12	3.05 ± 0.03	1.17 ± 0.04	1.64 ± 0.05	2.90 ± 0.05
12	**Senibong**	12	6.14 ± 0.06	1.99 ± 0.09	2.96 ± 0.08	5.98 ± 0.29
13	**Teluk Jawa**	12	10.64 ± 0.11	3.22 ± 0.03	3.88 ± 0.04	23.04 ± 0.38
14	**Kg.Masai**	12	4.92 ± 0.31	1.76 ± 0.06	2.57 ± 0.14	6.66 ± 0.24
15	**Kg.P.Puteh-2**	12	7.02 ± 0.43	2.09 ± 0.08	3.24 ± 0.08	9.13 ± 1.07
16	Sg.Belungkor	12	6.05 ± 0.09	1.79 ± 0.05	2.99 ± 0.04	7.89 ± 0.84

Sampling sites in bold are the mussel populations where different soft tissues of P. viridis are reported together with their habitat surface sediments

Morphometric data revealed significant differences across sites, with shell lengths ranging from 3.05 ± 0.03 cm at P. Lido-2 to 10.64 ± 0.11 cm at Teluk Jawa. Shell widths varied from 1.17 ± 0.04 cm (P. Lido-2) to 2.50 ± 0.05 cm (Kukup), while shell heights ranged from 1.64 ± 0.05 cm (P. Lido-2) to 3.22 ± 0.03 cm (Teluk Jawa). Total shell weights showed substantial variation, with the heaviest shells recorded at Teluk Jawa (23.04 ± 0.38 g) and the lightest at P. Lido-2 (2.90 ± 0.05 g). These morphometric variations allowed for the assessment of shell size and weight as factors potentially influencing Cd bioaccumulation.

Upon collection, *P. viridis* samples were carefully cleaned to remove any surface debris and contaminants. Each mussel was then separated into seven distinct parts: gill, mantle, foot, gonad, muscle, crystalline style (CS), and remining soft tissues (REM), in addition to the total soft tissues (TST) for comprehensive analysis of Cd distribution across different body components.

The washed shells of *P. viridis* were dried at 60°C for 72 h in an oven. Later, these dried shells were ignited heated to 400°C for 18 h in a furnace in order to separate of the calcite (outer) and nacreous layers in *P. viridis* shells as described by Bourgoin (1988). After the ignition, the outer layer (the periostracum) was separated from the inner part of the shells for metal analysis (Yap et al., 2003).

All the periostracum, total shells and different soft tissues were subjected to acid digestion for Cd analysis, following a standardized protocol to ensure consistency and

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accuracy. The different soft tissues, periostracum and total shell samples were weighed (approximately 0.50 g each) and digested with concentrated nitric acid (HNO_3) in a closed digestion vessel. The digestion process began with heating at 40°C for 1 h, followed by an increase in temperature to 140°C for an additional 3 h to ensure complete dissolution of the shell material. This staged heating approach minimized potential Cd loss by volatilization at lower temperatures, while the prolonged high-temperature digestion ensured complete breakdown of the mineral matrix (Yap et al., 2004).

In addition to the mussel samples, surface sediment samples were collected from selected sites as indicated in *Table 1*. These sediment samples underwent a separate acid digestion protocol, where each sample was treated with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 4:1 ratio (Yap et al., 2002a, b). The geochemical fractions analyzed include F1 (easily, leachable or exchangeable), F2 (acid-reducible), F3 (organic-oxidizable), and F4 (resistant), with a total summation (SUM) of all fractions, followed the methodology as described by Badri and Aston (1983).

All digested solutions, including those from shell, soft tissue, and sediment samples, were analyzed for Cd concentration using a PerkinElmer AAnalyst 800 Atomic Absorption Spectrometer (AAS). This model provides high sensitivity and precision in trace metal detection, essential for accurately quantifying low levels of Cd in environmental samples. The Cd data was verified using certified reference materials (CRMs) for Dogfish and MESS sediments, to validate the method's accuracy and reproducibility, ensuring the reliability of the Cd measurements obtained in this study. The recoveries were acceptable between 80-110% (Yap et al., 2003, 2004).

Descriptive statistics were calculated to determine the means and standard errors for shell morphometric parameters and Cd concentrations across the different tissues and shell components. Pearson's correlation analysis was performed to investigate the relationships between shell morphometrics and Cd levels, as well as between Cd concentrations in shells and sediment-bound geochemical fractions. Multiple linear regression analysis (MLRA) and multiple linear stepwise regression analysis (MLSRA) were used to identify significant predictors of Cd accumulation in different total and periostracum of mussel shells and drying procedures (60 and 400) (as dependent variable (DV)), with sediment fractions and morphometric parameters, (or different tissues) considered as independent covariates (IC). To identify which specific group means are significantly different from each other, post-hoc analysis using Tukey test was conducted.

All statistical analyses were conducted using JASP (JASP Team, 2024). JASP (Version 0.19.0 Computer software) statistical software, an open-source platform known for its user-friendly interface and robust statistical testing capabilities. A significance level of $P < 0.05$ was maintained across all analyses to ensure that only statistically meaningful relationships were interpreted. For the visual representation of data, including graphs presented in this article, the latest version of KaleidaGraph software (Version 5.0; Synergy Software) was used. KaleidaGraph is well-regarded for its flexibility in creating high-quality, customizable scientific graphs, allowing for clear and accurate visualization of results.

This combined use of JASP for statistical analysis and KaleidaGraph for data visualization facilitated a comprehensive and accessible presentation of the findings, enabling a robust assessment of the factors influencing Cd bioaccumulation in *P. viridis* and the suitability of shells as biomonitoring materials for coastal Cd contamination.

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Results

Comparative analysis of Cd concentrations in the periostracum and total shells of Perna viridis

The results illustrated in *Figure 3* provide a comparative analysis of Cd concentrations in the periostracum (P) and total shells (T) of *P. viridis*, collected from various sites in Peninsular Malaysia. Cd concentrations were measured in samples dried at 60°C (P60Cd, T60Cd) and ignited at 400°C (P400Cd, T400Cd), highlighting the influence of temperature on Cd retention across different sample types.

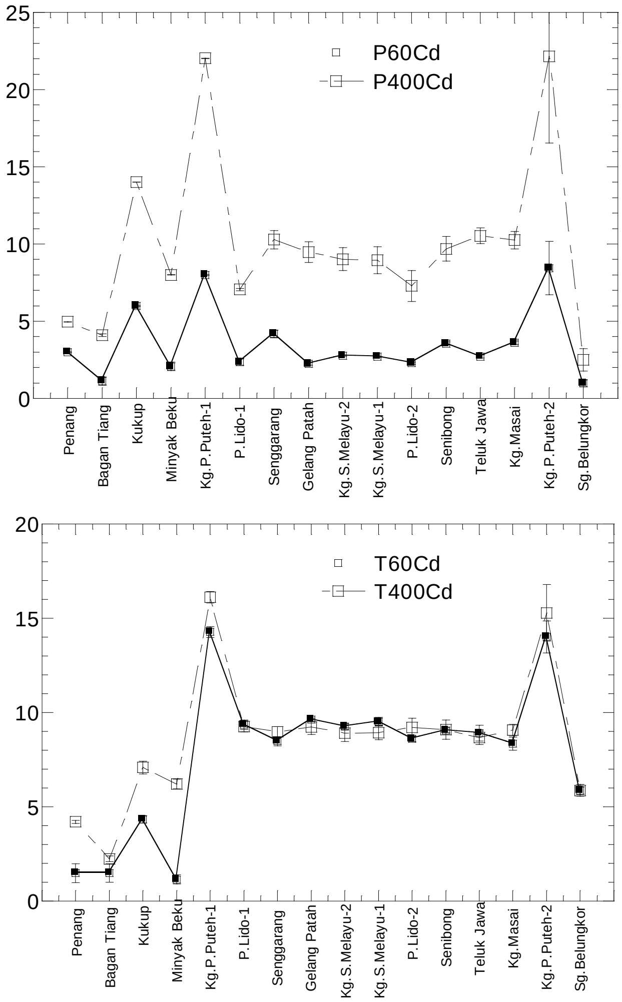

Figure 3. *Comparison of Cd concentrations (µg/g dry weight ± standard error) between 60 °C dried and 400 °C ignited periostracum (P) and total shells (T) of Perna viridis*

For the periostracum samples, the Cd concentrations show a noticeable variation across sites, with P60Cd values ranging from approximately 2 µg/g to 25 µg/g dry weight. P60Cd concentrations were consistently higher than P400Cd across most sampling sites, indicating that the periostracum retains higher Cd levels when dried at 60°C compared to the ignited samples. This trend suggests that igniting samples at 400°C potentially volatilizes some of the Cd content or causes changes in the periostracum structure that reduce Cd retention.

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The periostracum samples from Kg. P. Puteh-1 and Kg. P. Puteh-2 stand out with the highest P60Cd concentrations, reaching around 25 µg/g, significantly higher than the concentrations observed at other sites. This marked increase in Cd at these specific locations indicates a localized contamination source, possibly linked to anthropogenic activities such as industrial discharge or agricultural runoff. Sites like Teluk Jawa and Sg. Belungkor also showed elevated Cd concentrations, but these were comparatively lower than the peaks at the Kg. P. Puteh sites.

P400Cd concentrations, on the other hand, remained relatively stable across sites, with most values ranging between 5 µg/g and 15 µg/g. The reduced variability in P400Cd compared to P60Cd suggests that igniting the samples may diminish the Cd concentration variability, likely due to a loss of loosely bound Cd compounds or matrix modifications in the periostracum layer.

T60Cd values varied between 1.15 µg/g and 14.27 µg/g across sampling sites, with the highest concentration detected at Kg. P. Puteh-1. This site again emerged as a notable hotspot for Cd accumulation, reinforcing the hypothesis of site-specific contamination. T400Cd concentrations ranged from 2.24 to 16.12 µg/g across the sampling sites (*Table 3*). Like the periostracum samples, the T400Cd values were consistently lower than T60Cd, which indicates that higher temperatures might reduce Cd retention in the shell matrix. The overall similarity in Cd concentrations across various sites in total shell samples suggests a relatively uniform level of Cd exposure for *P. viridis* in the majority of the locations. However, the observed peaks at Kg. P. Puteh-1 and Kg. P. Puteh-2 for T60Cd highlight that localized Cd contamination affects the bioaccumulation in both the periostracum and total shell components of the organism.

Cd concentrations in the shells and soft tissues of Perna viridis

The results presented in *Figure 3* highlight the Cd concentrations in the shells and soft tissues of *P. viridis* collected from various locations across Peninsular Malaysia.

Table 3. *Overall concentrations of Cd between 60℃ dried and 400℃ ignited periostracum (P) and total shells (T) of Perna viridis (N = 16 populations)*

		Mean	Minimum	Maximum
Periostracum	P60Cd	3.50 ± 0.55^{a}	0.98	8.44
	P400Cd	10.0 ± 1.37^{c}	2.51	22.16
Total shells	T60Cd	7.74 ± 0.99^{b}	1.15	14.27
	T400Cd	8.65 ± 0.86^{b}	2.24	16.12
Allometric data	Shell length	6.35 ± 0.44	3.05	10.64
	Shell height	2.06 ± 0.12	1.17	3.22
	Shell width	2.96 ± 0.14	1.64	3.88
	Total shell weight	8.57 ± 1.24	2.9	23.04

Post-hoc analysis using Tukey test in which the different alphabets are significant at $P < 0.05$

In the periostracum samples, P60 values ranged from 0.98 µg/g (Sg. Belungkor) to 8.44 µg/g (Kg. P. Puteh-2). When ignited at 400°C, periostracum Cd concentrations (P400) ranged from 2.51 µg/g (Bagan Tiang) to 22.16 µg/g (Kg. P. Puteh-2), indicating that some sites retained significant Cd even after ignition, likely due to stronger binding within the matrix or differences in environmental exposure.

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In the total shell samples, the T60 values varied from 1.15 µg/g (Penang) to 14.27 µg/g (Kg. P. Puteh-1). When the samples were ignited at 400°C, T400 values ranged from 2.24 µg/g (Bagan Tiang) to 16.12 µg/g (Kg. P. Puteh-1), with a slight reduction in variability compared to T60. This reduction suggests that drying at 60°C retains more loosely bound Cd compared to 400°C, where some Cd may be lost due to volatilization or structural changes in the shell matrix.

Cd concentrations of Cd in the total soft tissues of Perna viridis and the four geochemical fractions of their habitat sediment

The soft tissues (ST) of *P. viridis* generally showed lower Cd concentrations across the sites, ranging from 0.66 µg/g (Bagan Tiang) to 5.55 µg/g (Kg. P. Puteh-2) (*Table 4*). From *Table 4*, the Cd concentrations in *P. viridis* and its geochemical fractions varied across 13 sites, with the highest TST at Kg. P. Puteh-2 (5.55 µg/g) and the lowest at Bagan Tiang (0.66 µg/g). The acid-reducible fraction (F2) was highest at P.Lido-2 (2.24 µg/g), indicating high Cd bioavailability under reducing conditions, while the resistant fraction (F4) peaked in Senibong (2.31 µg/g), suggesting lower bioavailability. The exchangeable fraction (F1) remained relatively low, with the highest at Kg.P.Puteh-2 (0.68 µg/g). The total Cd in sediments (SUM) was highest at P.Lido-2 (4.19 µg/g), followed by Gelang Patah (3.33 µg/g) and Senibong (3.23 µg/g), indicating potential Cd contamination hotspots. These variations suggest site-specific differences influenced by pollution sources, sediment composition, and hydrodynamic factors, with a predominance of F4 and F2 fractions, highlighting the need for further investigation into Cd bioavailability and environmental risks in these areas.

Table 4. *Concentrations of Cd in the total soft tissues of Perna viridis and the four geochemical fractions of their habitat sediment*

No.	Sites	TST	F1	F2	F3	F4	SUM
1	Bagan Tiang	0.66^{a}	0.16^{ab}	0.21^{a}	0.20^{ab}	1.13^{b}	1.69^{a}
2	Kukup	2.29^{c}	0.14^{ab}	0.09^{a}	0.07^{a}	0.93^{b}	1.23^{a}
3	Minyak Beku	0.96^{a}	0.20^{b}	0.12^{a}	0.15^{ab}	1.86^{bc}	2.33^{b}
4	Kg.P.Puteh-1	2.87^{c}	0.29^{b}	0.80^{b}	0.70^{b}	1.37^{b}	3.15^{c}
5	P.Lido-1	1.13^{a}	0.30^{b}	0.47^{ab}	0.28^{ab}	1.16^{b}	2.21^{b}
6	Gelang Patah	2.47^{c}	0.16^{a}	1.65^{c}	0.23^{ab}	1.28^{b}	3.33^{c}
7	Kg.S.Melayu-2	0.85^{a}	0.16^{a}	0.70^{ab}	0.38^{ab}	1.21^{b}	2.46^{b}
8	Kg.S.Melayu-1	1.37^{b}	0.08^{a}	1.63^{c}	0.41^{b}	1.20^{b}	3.32
9	P.Lido-2	0.97^{a}	0.10^{a}	2.24	0.65^{b}	1.20^{b}	4.19
10	Senibong	1.15^{a}	0.22^{b}	0.25^{a}	0.46^{b}	2.31^{c}	3.23^{c}
11	Teluk Jawa	2.12^{c}	0.22^{b}	0.46^{ab}	0.31^{ab}	1.92^{c}	2.90^{bc}
12	Kg.Masai	1.58^{b}	0.23^{b}	0.59^{ab}	0.23^{ab}	1.79^{bc}	2.84^{bc}
13	Kg.P.Puteh-2	5.55^{d}	0.68^{c}	0.72^{b}	0.84^{c}	0.16^{a}	2.40^{bc}

F1 = easily, leachable, freely or exchangeable fraction; F2 = acid-reducible fraction; F3 = organic-oxidizable fraction; F4 = resistant fraction; SUM = summation of F1, F2, F3 and F4. Post-hoc analysis using Tukey test in which the different alphabets are significant at $P < 0.05$. N = 13

Probability plot of Cd concentrations in the periostracum (P) and total shells (T)

In addition to the concentration data presented in *Table 3, Figure 4* provides a probability plot of Cd concentrations in the periostracum (P) and total shells (T) of *P.*

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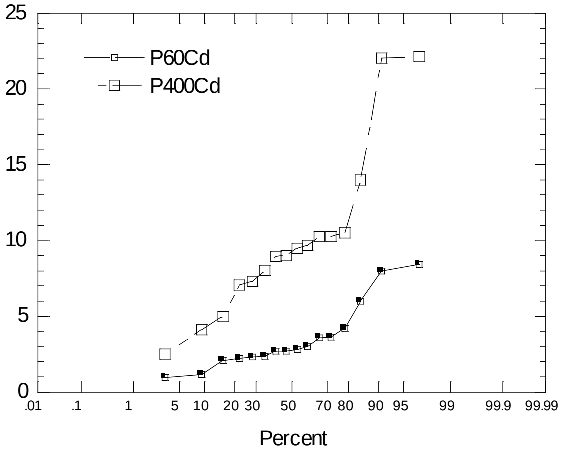
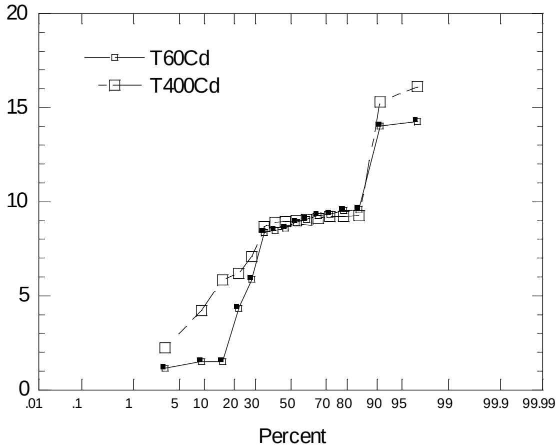
viridis dried at 60°C (P60Cd, T60Cd) and ignited at 400°C (P400Cd, T400Cd) across 16 populations. This plot highlights the distribution and variability of Cd concentrations across samples, providing insight into the cumulative probability of Cd levels in both sample types under different thermal treatments.



(a)



(b)

Figure 4. *Probabilities of Cd concentrations (µg/g dry weight ± standard error) between 60 ℃ dried and 400 ℃ ignited periostracum (P) and total shells (T) of Perna viridis. Based on 16 populations*

Figure 4a shows the cumulative distribution of Cd concentrations for periostracum samples. The P60Cd data reveals a distinct upward trend, with most values clustering between 2 µg/g and 10 µg/g for the lower percentiles (10% to 70%). However, a sharp increase is observed at the higher percentiles, with P60Cd concentrations reaching up to approximately 25 µg/g at the 99.9th percentile. This sharp rise reflects the highly localized contamination observed in certain sites, particularly Kg. P. Puteh-1 and Kg. P. Puteh-2, as noted in the individual site data.

In contrast, the P400Cd data exhibits a more gradual increase across the percentiles, with a narrower distribution and lower peak values, reaching up to around 15 µg/g at the 99.9th percentile. The reduced variability in P400Cd concentrations compared to P60Cd suggests that igniting samples at 400°C reduces the retention of loosely bound Cd, resulting in a more stable distribution across the populations.

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Figure 4b shows the probability plot for total shell Cd concentrations (T60Cd and T400Cd). The T60Cd distribution is similar to the periostracum, with a gradual increase up to approximately 10 µg/g for most of the sample population, followed by a significant rise in the upper percentiles. This trend is consistent with the high Cd concentrations detected at sites like Kg. P. Puteh-1, where localized contamination is evident.

The T400Cd concentrations demonstrate a generally stable increase across the percentiles, peaking at about 15 µg/g at the uppermost percentiles, with less dramatic variation compared to T60Cd. This stability in T400Cd distribution, similar to the pattern seen in P400Cd, further supports the hypothesis that the 400°C treatment minimizes variability by reducing the presence of loosely bound Cd.

Pearson's correlation coefficients of Cd levels between the shell and different soft tissues of Perna viridis

In addition to the concentration and distribution data, *Table 5* provides a heatmap based on Pearson's correlation coefficients of Cd levels between the shell (P60, P400, T60, and T400) and different soft tissues (eight parts) of *P. viridis*, using a sample size of N = 13. This table reveals significant correlations, suggesting interdependence in Cd accumulation patterns between the shell and various soft tissues.

Table 5. *Pearsons' correlation coefficients of Cd levels between shells and different soft tissues (8 parts) of Perna viridis*

Variable	P60	P400	T60	T400	TST	CS	Gill	Mantle	Foot	Gonad	Muscle	REM
P60	—											
P400	**0.98**	—										
T60	**0.60**	**0.67**	—									
T400	**0.80**	**0.86**	**0.92**	—								
TST	**0.82**	**0.86**	**0.61**	**0.72**	—							
CS	-0.13	-0.07	-0.40	-0.15	0.00	—						
Gill	0.38	0.31	-0.07	0.05	0.40	-0.03	—					
Mantle	**0.83**	**0.84**	0.36	0.53	**0.71**	-0.22	0.48	—				
Foot	0.48	0.51	0.44	0.44	**0.76**	-0.09	**0.59**	0.51	—			
Gonad	**0.56**	0.48	-0.01	0.12	**0.64**	0.06	0.44	**0.58**	0.50	—		
Muscle	**0.76**	**0.82**	0.51	**0.61**	**0.87**	-0.11	**0.56**	**0.84**	**0.70**	0.49	—	
REM	**0.59**	**0.64**	0.32	0.38	**0.67**	-0.34	0.48	**0.88**	0.52	0.48	**0.87**	—

Values in bold are significantly correlated at P < 0.05. REM = remaining soft tissues; TST = total soft tissues; CS = crystalline style. N = 13

Strong correlations were observed between the shell components, particularly between P60 and P400 (r = 0.98), indicating a consistent Cd retention pattern in the periostracum regardless of the temperature treatment. Similarly, a high correlation exists between T60 and T400 (r = 0.92), suggesting stability in Cd levels within the total shell across drying temperatures. These strong correlations imply that temperature effects on Cd retention are consistent across the shell types and that either drying condition can reflect similar Cd levels for comparative purposes.

The total soft tissues (TST) exhibit notable correlations with the shell components, showing significant positive correlations with P60 (r = 0.82), P400 (r = 0.86), T60 (r = 0.61), and T400 (r = 0.72). These correlations suggest that total soft tissues accumulate Cd levels in relation to those in the shell, indicating a potential relationship between shell-bound Cd and bioaccumulation in the soft tissues. This strong association

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reinforces the shell's role as a biomonitor for environmental Cd exposure, with soft tissue Cd levels likely reflecting ambient Cd availability.

Individual soft tissues also show varying degrees of correlation with the shell. For example:

The mantle shows significant correlations with P60 (r = 0.83) and P400 (r = 0.84), implying that mantle tissue accumulates Cd in a manner closely associated with periostracum Cd levels. Muscle tissue also has a significant positive correlation with T400 (r = 0.61), suggesting that Cd in the muscle may be influenced by total shell Cd levels under high-temperature conditions. Other soft tissues, such as the gill and foot, display lower or non-significant correlations with the shell, indicating variability in Cd accumulation among tissue types. The CS shows weak and non-significant correlations with all shell variables (ranging from -0.40 to -0.07), suggesting that this tissue does not reflect Cd levels found in the shell and may be less suitable as a biomonitor for Cd exposure.

Pearson's correlation coefficients between Cd levels in the shells and the geochemical fractions of Cd in the habitat surface sediments

To further examine the environmental relevance of Cd accumulation in *P. viridis* shells, *Table 6* presents a heatmap based on Pearson's correlation coefficients between Cd levels in the shells (P60, P400, T60, T400) and the geochemical fractions of Cd in the habitat surface sediments.

Table 6. *Pearsons' correlation coefficients of Cd levels between shells of Perna viridis and their geochemical fractions of the habitat surface sediments*

Variable	P60	P400	T60	T400	F1	F2	F3	F4	SUM
P60	—								
P400	**0.98**	—							
T60	**0.60**	**0.67**	—						
T400	**0.80**	**0.86**	**0.92**	—					
F1	**0.67**	**0.69**	0.51	**0.63**	—				
F2	-0.14	-0.06	0.40	0.26	-0.24	—			
F3	**0.58**	**0.62**	**0.78**	**0.79**	**0.57**	0.42	—		
F4	-0.45	-0.41	-0.26	-0.29	-0.48	-0.22	-0.36	—	
SUM	-0.13	-0.02	0.49	0.38	-0.18	**0.79**	0.52	0.29	—

Values in bold are significantly correlated at $P < 0.05$. F1 = easily, leachable, freely or exchangeable fraction; F2 = acid-reducible fraction; F3 = organic-oxidizable fraction; F4 = resistant fraction; SUM = summation of F1, F2, F3 and F4. N = 13

The results reveal several significant correlations between the Cd levels in the shell components and specific geochemical fractions of the habitat sediments, suggesting an interaction between sediment-bound Cd and its bioaccumulation in *P. viridis*.

The F1 fraction, representing the most bioavailable Cd, shows positive correlations with shell components P60 (r = 0.67), P400 (r = 0.69), and T400 (r = 0.63). This indicates that Cd in the periostracum and total shell, particularly when dried or ignited, is influenced by the easily leachable fraction of Cd in the sediment. These positive correlations suggest that *P. viridis* shells, especially in the periostracum, may reflect

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ambient bioavailable Cd levels in their habitat, highlighting the potential of using *P. viridis* as a biomonitoring tool for bioavailable Cd in sediments.

No significant correlations were found between the F2 fraction and the shell components, with correlation coefficients ranging from -0.14 to 0.40. This indicates that the acid-reducible Cd fraction in sediments may not significantly contribute to the Cd levels accumulated in the shells of *P. viridis*. The lack of correlation suggests that Cd in this fraction is less bioavailable or less accessible for uptake by *P. viridis* under natural conditions.

Significant correlations were observed between the F3 fraction and several shell components, including T60 ($r = 0.78$) and T400 ($r = 0.79$). These strong correlations suggest that Cd associated with organic matter and oxidizable compounds in sediments is accessible to *P. viridis* and accumulates in the shell, particularly in total shell samples subjected to both drying and ignition treatments. This relationship underscores the role of organic matter in enhancing Cd bioavailability in sediment and influencing bioaccumulation in *P. viridis*.

The F4, representing Cd strongly bound in mineral structures, does not show significant correlations with any shell component, with coefficients ranging from -0.45 to -0.26. This lack of correlation implies that the resistant fraction is biologically inaccessible to *P. viridis*, as expected due to its strong binding within sediment mineral matrices, rendering it unavailable for uptake.

The SUM does not significantly correlate with any shell components, with correlation values close to zero, indicating no clear relationship between total sediment Cd and shell Cd levels. This suggests that specific geochemical fractions, rather than the total sediment Cd, are more critical in influencing Cd bioavailability and uptake in *P. viridis*.

Predictor outputs from multiple linear regression analysis (MLRA) and multiple linear stepwise regression analysis (MLSRA)

Table 7 provides the predictor outputs from MLRA and MLSRA, examining the relationships between Cd concentrations in different parts of the shell of *P. viridis* and various geochemical fractions in the sediment. In these analyses, each shell component (P60, P400, T60, and T400) is treated as the DV, while the geochemical fractions F1, F2, F3, F4, and their SUM serve as ICs.

The MLRA results provide regression coefficients (β) for each predictor variable relative to the different shell components. For P60 as the DV, the intercept (Int) is 0.73, and among the geochemical fractions, F1, F2, F3, and F4 have notable positive β values (4.11, 5.73, 7.31, and 5.72, respectively). This indicates that all geochemical fractions contribute positively to Cd levels in P60, with F3 showing the highest influence. The negative coefficient for SUM ($\beta = -6.39$) suggests an overall inverse relationship with the total geochemical content, possibly reflecting complex interactions within sediment-bound Cd.

For P400 as DV, the intercept (Int) is -0.05, and F1 to F4 show positive aβ values, indicating these fractions contribute positively to Cd levels in P400. Similar to P60, F3 has the highest impact, suggesting the organic-oxidizable fraction plays a major role in Cd retention within the periostracum.

For T60 as the DV, the intercept (Int) is 0.01, and T400 has a strong positive effect ($\beta = 1.60$), highlighting the relationship between Cd in T60 and T400. Among the geochemical fractions, F1 through F4 all show significant positive impacts, with F1 having the strongest influence. This underscores the importance of bioavailable Cd in influencing total shell Cd levels.

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Table 7. *Predictor outputs based on multiple linear regression analysis (MLRA) and multiple linear stepwise regression analysis (MLSRA) with the selected mussel shell part as dependent variable (DV), and the independent covariates (ICs) considered included F1, F2, F3, F4, SUM. N = 13*

MLRA	**DV = P60**	**B**	**DV = P400**	**β**	**DV = T60**	**β**	**DV = T400**	**β**
	Int	0.73	Int	-0.05	Int	0.01	Int	-0.89
	T60	-0.02	T60	-0.16	P400	-0.34	P400	0.29
	T400	0.05	T400	0.61	T400	1.60	P60	0.11
	P400	0.38	P60	1.79	P60	-0.16	F1	-63.7
	F1	4.11	F1	40.9	F1	67.8	F2	-65.7
	F2	5.73	F2	38.9	F2	69.1	F3	-64.1
	F3	7.31	F3	35.5	F3	67.9	F4	-64.9
	F4	5.72	F4	38.5	F4	67.8	SUM	66.2
	SUM	-6.39	SUM	-38.4	SUM	-69.0	T60	0.37
MLSRA	**DV = P60**	**B**	**DV = P400**	**β**	**DV = T60**	**β**	**DV = T400**	**β**
	Int	-0.88	Int	0.89	Int	-1.19	Int	1.493
	P400	0.42	P60	1.88	T400	1.03	T60	0.548
			T400	0.345			P400	0.286
ICs considered but not included	T60, T400, F1, F2, F3, F4, SUM.		T60, F1, F2, F3, F4, SUM.		P400, P60, F1, F2, F3, F4, SUM.		P60, F1, F2, F3, F4, SUM.	

Int = Intercept; F1 = easily, leachable, freely or exchangeable fraction; F2 = acid-reducible fraction; F3 = organic-oxidizable fraction; F4 = resistant fraction; SUM = summation of F1, F2, F3 and F4

For T400 as the DV, the intercept (Int) is -0.89, with significant positive coefficients for F1, F2, F3, and F4 (with negative signs ranging from -63.7 to -64.9), indicating inverse relationships between the resistant fraction and T400. This pattern suggests that higher levels of resistant fraction Cd correlate with lower Cd retention in ignited shell samples.

The MLSRA results reveal the stepwise regression findings, listing only the most significant predictors that best explain the variation in each shell component. For P60: The intercept (Int) is -0.88, with P400 as the primary predictor (β = 0.42), suggesting a close relationship between Cd levels in P60 and P400, while other geochemical fractions were not included in the final model.

For P400, the intercept (Int) is 0.89, with T400 as the significant predictor (β = 0.345), indicating that the Cd levels in T400 are the main contributor to the variability in P400, likely due to a shared exposure source or retention mechanism.

For T60, the intercept (Int) is -1.19, with T400 as a strong predictor (β = 1.03), demonstrating that Cd in ignited total shell samples (T400) explains a large portion of the variability in T60 Cd levels.

For T400, the intercept (Int) is 1.493, with T60 (β = 0.548) and P400 (β = 0.286) as significant predictors, highlighting a close association between Cd in different shell treatments, likely reflecting consistent environmental exposure.

Predictor outputs from multiple linear regression analysis (MLRA) and multiple linear stepwise regression analysis (MLSRA)

Table 8 presents the predictor outputs from MLRA and MLSRA, focusing on Cd levels in the shell of *P. viridis* as the DV and the Cd concentrations in eight different soft tissues (gill, mantle, foot, gonad, muscle, CS, TST, and REM), as IC.

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Table 8. *Predictor outputs based on multiple linear regression analysis (MLRA) and multiple linear stepwise regression analysis (MLSRA) with the selected mussel shell part as the dependent variable (DV), and the independent covariates (ICs) considered are the eight different soft tissues of Perna viridis. N = 13*

MLRA	**DV = P60**	**β**	**DV = P400**	**β**	**DV = T400**	**β**	**DV = T60**	**β**
	Int	-0.42	Int	0.78	Int	-0.16	Int	-0.39
	T60	-0.16	T60	0.16	T60	0.80	P400	0.59
	T400	0.05	T400	0.05	P400	0.14	TST	-0.71
	P400	0.61	TST	0.40	TST	0.35	CS	-0.17
	TST	-0.24	CS	0.06	CS	0.11	Gill	-0.65
	CS	-0.05	Gill	-0.56	Gill	0.93	Mantle	-1.57
	Gill	0.29	Mantle	1.38	Mantle	0.40	Foot	0.61
	Mantle	-0.78	Foot	-0.34	Foot	-0.27	Gonad	0.74
	Foot	0.17	Gonad	-0.35	Gonad	-0.46	Muscle	3.45
	Gonad	0.26	Muscle	1.41	Muscle	-4.14	REM	-1.76
	Muscle	-0.51	REM	-0.22	REM	1.85	P60	-1.45
	REM	-0.12	P60	1.51	P60	0.36	T400	1.04
MLSRA	**DV = P60**	**β**	**DV = P400**	**β**	**DV = T400**	**β**	**DV = T60**	**β**
	Int	-0.88	Int	0.21	Int	0.66	Int	-0.59
	P400	0.42	P60	1.41	T60	0.64	T400	1.30
			T400	0.66	P400	0.25	CS	-0.10
			Muscle	2.95	CS	0.06	P60	-0.59
			Gill	-1.06				
			T60	-0.34				
ICs considered but not included	T60, T400, TST, CS, Gill, Mantle, Foot, Gonad, Muscle, REM		TST, CS, Mantle, Foot, Gonad, REM		TST, Gill, Mantle, Foot, Gonad, Muscle, REM, P60		P400, TST, Gill, Mantle, Foot, Gonad, Muscle, REM	

Values in bold are significantly correlated at P < 0.05. REM = remaining soft tissues; TST = total soft tissues; CS = crystalline style

The MLRA results show the regression coefficients (β) for each tissue predictor relative to the different shell components:

For P60 as DV, the intercept (Int) for P60 is -0.42, with P400 (β = 0.61) and TST (β = 0.24) emerging as positive predictors, suggesting that Cd levels in the periostracum at 60°C are influenced by both the periostracum dried at 400°C and total soft tissues. The muscle and mantle tissues show negative correlations (β = −0.51 and β = −0.78, respectively), indicating an inverse relationship with Cd levels in P60.

For P400 as DV, the intercept (Int) for P400 is 0.78, and the gill and gonad tissues show significant positive effects (β = 1.38 and β = 0.60, respectively), implying that Cd in the periostracum dried at 400°C is influenced by Cd levels in these tissues. The negative coefficient for CS (β = −0.56) indicates a weaker relationship with the CS.

For T400 as DV, the intercept (Int) is -0.16, with the gill (β = 0.93) and REM (β = 1.85) showing significant positive contributions, indicating these tissues are associated with Cd levels in the total shell ignited at 400°C. Muscle (β = −4.14) demonstrates a strong inverse relationship, suggesting that higher muscle Cd is associated with lower total shell Cd under the 400°C treatment.

For T60 as DV, the intercept (Int) for T60 is -0.39, and T400 shows a significant positive relationship (β = 1.04), indicating that the Cd levels in the total shell dried at 60°C are closely linked to those in T400. The gonad tissue (β = 3.45) also contributes positively, while the mantle and REM tissues show negative correlations (β = −1.57 and β = −1.76, respectively), suggesting these tissues inversely relate to Cd in T60.

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The MLSRA identifies the most significant predictors for each shell component. For P60, the intercept (Int) is -0.88, with P400 as the primary predictor ($\beta = 0.42$), emphasizing the close association between Cd levels in P60 and P400.

For P400, the intercept (Int) is 0.21, with P60 ($\beta = 1.41$), T400 ($\beta = 0.66$), and muscle ($\beta = 2.95$) as significant predictors, indicating that Cd levels in P400 are influenced by these shell and tissue components, with muscle playing a strong role.

For T400, the intercept (Int) is 0.66, with T60 ($\beta = 0.64$) and P400 ($\beta = 0.25$) as significant predictors, suggesting that Cd levels in T400 are influenced by the periostracum and total shell across treatments.

For T60, the intercept (Int) is -0.59, with T400 ($\beta = 1.30$) as the strongest predictor, underscoring the influence of Cd levels in the ignited total shell on those in the dried total shell.

Discussion

Potential of Perna viridis Shells as biomonitoring tools for Cd contamination

The increasing levels of Cd and other heavy metals in coastal ecosystems present serious risks to marine biodiversity, public health, and overall environmental sustainability (Langston and Bebianno, 1998; Phillips and Rainbow, 1993; Puente et al., 1996; Zhong et al., 2024; Zhou et al., 2023; Falfushynska et al., 2024). Cd, a toxic and persistent pollutant, bioaccumulates in marine organisms and magnifies through food chains, leading to detrimental ecological and human health effects (Forstner and Wittmann, 1981; Luoma, 1983; Bellotto and Miekeley, 2007; Montojo et al., 2021; Budiawan et al., 2020; Neethu et al., 2024). The bioaccumulation of Cd in bivalves has been well-documented, with recent studies emphasizing its toxic effects on physiological functions, reproductive health, and survival in various molluscan species (Meng et al., 2023; Liu et al., 2024; Haeruddin et al., 2021). Effective biomonitoring is therefore essential for assessing and managing Cd contamination in marine environments (Yap et al., 2003; Phillips, 1980; Zver'kova, 2009; Baudrimont et al., 2020; Canli and Canli, 2024; Amachree et al., 2024).

Traditionally, soft tissues of marine organisms have been used for monitoring heavy metals, but these tissues are limited by their susceptibility to rapid metabolic turnover, which can result in fluctuating metal concentrations that fail to accurately represent long-term environmental exposure (Bourgoin, 1990; Cravo et al., 2004; Wang et al., 2008; Cai et al., 2025; Blanc et al., 2023). This study highlights the potential of using *P. viridis* shells, particularly the periostracum and total shell components, as more stable and reliable biomonitors for Cd in coastal waters (Yap et al., 2004; Fischer, 1984; Mayk et al., 2022; Lin et al., 2021). The use of shells as biomonitors has been explored in recent research, showing strong correlations between shell Cd content and environmental Cd levels, confirming their potential as long-term indicators of pollution (Gomez-Delgado et al., 2023; Liu et al., 2024; Zhong et al., 2024).

The utility of *P. viridis* shells as biomonitors lies in their ability to retain Cd consistently over time, reflecting cumulative environmental exposure with minimal influence from short-term physiological changes (Bourgoin and Risk, 1987; Sturesson, 1978; Krishnan et al., 2023; Richardson et al., 2001; Dauphin et al., 2003; Reolid et al., 2024). Unlike soft tissues, which are affected by metabolic processes such as growth, excretion, and tissue turnover, shells provide a more durable and static record of metal accumulation (Falfushynska et al., 2024; Amachree et al., 2024; Liu et al., 2024). Our

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findings demonstrate significant positive correlations between Cd levels in *P. viridis* shells and sediment-bound Cd, particularly with the bioavailable (F1) and organic-oxidizable (F3) fractions, which are the most ecologically relevant forms of Cd (Yap et al., 2002; Pourang et al., 2019; Baudrimont et al., 2020; Canli and Canli, 2024). These fractions represent Cd that is readily available or can be mobilized in the environment, making their presence in shell components a strong indicator of ambient contamination (Markich et al., 2002; Richardson, 1993; Lin et al., 2021; Zhou et al., 2023). The periostracum’s high correlation with the F1 fraction ($r = 0.67$) suggests that it is particularly effective at capturing Cd that is readily leachable and exchangeable, making it an ideal material for detecting Cd hotspots in polluted areas (Bourgoin, 1988; Yap et al., 2004; Zhong et al., 2024; Meng et al., 2023).

Additionally, the use of shells as biomonitors aligns with global efforts toward sustainable environmental monitoring. Shells are easy to collect, durable over time, and retain metal contaminants without requiring complex preservation methods, making them suitable for large-scale, long-term studies (Brand et al., 1987; Richardson, 1993; Juncharoenwongsa et al., 2011; Cai et al., 2025; Budiawan et al., 2020). This durability allows shells to archive historical pollution data, enabling trend analysis and facilitating the identification of chronic contamination sources (Ravera et al., 2005; Sturesson, 1978; Ben-Eliahu et al., 2020; Blanc et al., 2023; Falfushynska et al., 2024). The significant relationships observed between shell Cd levels and sediment-bound fractions support the use of *P. viridis* shells as a scientifically robust, practical solution for biomonitoring in coastal regions (Goldberg, 1980; Carriker et al., 1982; Pérez-Mayol et al., 2014; Gomez-Delgado et al., 2023; Haeruddin et al., 2021).

This approach is particularly valuable in light of UNSDGs, as it supports sustainable marine management practices that protect ecosystem health (Goal 14: Life Below Water), ensure clean water (Goal 6: Clean Water and Sanitation), and promote responsible production and consumption (Goal 12: Responsible Consumption and Production) through non-invasive monitoring methods (Langston and Bebianno, 1998; Yap et al., 2003; Pearce and Mann, 2006; Zhong et al., 2024; Neethu et al., 2024; Zhou et al., 2023). The integration of *P. viridis* shells into routine biomonitoring programs offers a sustainable, cost-effective, and scientifically reliable method to track heavy metal pollution over time, contributing to global efforts in environmental conservation and pollution management (Montojo et al., 2021; Cai et al., 2025; Baudrimont et al., 2020).

Enhanced stability of Cd retention in shells versus soft tissues

The results of this study reveal that *P. viridis* shells retain Cd in a stable manner that is not significantly influenced by short-term environmental or physiological fluctuations, unlike soft tissues, which tend to exhibit inconsistent metal levels (Cravo et al., 2004; Lares et al., 2005; Stewart et al., 2021). This stability is evident from the comparative data across sites, showing that Cd levels in periostracum (P60Cd) and total shell components (T60Cd) correlate strongly with sediment-bound Cd fractions, particularly the bioavailable (F1) and organic-oxidizable (F3) forms (Yap et al., 2002; Sturesson, 1984; Bellotto and Miekeley, 2007). Soft tissues, on the other hand, show variable and less predictable Cd levels due to the influence of physiological processes such as growth, excretion, and tissue regeneration (Soto et al., 2000; Carriker et al., 1991; Wang et al., 2008). These processes may cause Cd concentrations in soft tissues to fluctuate, complicating the interpretation of data for long-term contamination assessment (Phillips, 1985; Langston and Bebianno, 1998).

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The consistent retention of Cd in shells is especially important for monitoring cumulative exposure, as shells integrate metal levels over time, providing a historical record of environmental conditions (Dauphin, 2003; Markich et al., 2002). This characteristic allows shells to capture chronic exposure trends, making them ideal for evaluating sites with prolonged contamination (Yasoshima and Takano, 2001; Carriker et al., 1982; Stewart et al., 2021). The shell-based approach also minimizes the need for continuous sample collection and processing, reducing the logistical burden and cost of monitoring programs (Watson et al., 1995; Richardson et al., 2001; Krishnan et al., 2023). The inherent durability and reliability of shells ensure that they provide a continuous record of metal contamination, even under conditions that may destabilize other biomonitoring materials, making them a robust and adaptable option for long-term environmental monitoring, aligned with UNSDGs focused on sustainable development (Geist et al., 2005; Phillips, 1980; Puente et al., 1996).

Statistical justification of shell-based biomonitoring through correlations and regression models

The strong, statistically significant correlations between Cd levels in shells and sediment-bound Cd, particularly in the F1 and F3 fractions, underline the scientific validity of using *P. viridis* shells as biomonitors (Yap et al., 2002; Cravo et al., 2004). These fractions, representing bioavailable and organically-bound Cd, respectively, are ecologically relevant forms that are more likely to affect marine organisms, making their association with shell Cd levels both meaningful and practical (Forstner and Wittmann, 1981; Luoma, 1983). Regression analysis further supports this finding, with MLRA and MLSRA models indicating that shell Cd levels are strongly influenced by these sediment fractions (Bourgoin, 1990; Phillips and Rainbow, 1993). These statistical models provide insight into the predictability of Cd accumulation in shells, demonstrating that shell Cd levels can be systematically related to specific geochemical fractions in sediments, unlike soft tissues which lack consistent predictive power (Richardson et al., 2001; Soto et al., 2000).

The ability to predict Cd levels in shells based on sediment composition is invaluable for coastal pollution monitoring programs, as it allows for accurate assessment of contamination levels using shell samples alone (Phillips, 1985; Sturesson, 1978). This predictive relationship means that environmental managers can rely on shell-based data to make informed decisions about contamination hotspots, pollution trends, and potential mitigation strategies (Nicholson and Szefer, 2003; Markich et al., 2002). The statistical robustness of these models highlights the suitability of shells not only as passive indicators but also as active tools for quantitative environmental assessment, enhancing the quality and reliability of pollution monitoring data (Gardiner, 1982; Goldberg, 1980). Such statistical justification is essential for biomonitoring programs operating under the UNSDGs, as it supports the establishment of science-based, scalable solutions for managing coastal and marine pollution (Langston and Bebianno, 1998; Yap et al., 2003).

Advantages of using shells for long-term and climate-resilient biomonitoring

The resilience of *P. viridis* shells to environmental degradation offers an advantage for monitoring in regions where climate change may intensify pollution impacts (Dauphin, 2003). Coastal areas are increasingly subjected to climate-driven phenomena,

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such as rising temperatures, ocean acidification, and shifts in precipitation patterns, which can alter the release and transport of contaminants like Cd (Geist et al., 2005; Lécuyer et al., 2004). Soft tissues, due to their physiological responsiveness, may reflect these changes rapidly, but such fluctuations may obscure long-term contamination trends. Shells, however, due to their mineralized structure, are less affected by short-term environmental variations, making them resilient, climate-stable indicators for monitoring cumulative metal exposure (Bertine and Goldberg, 1972; Sturesson, 1984). This resilience positions shells as an ideal biomonitoring material, capable of providing reliable data even under climate-induced stress (Richardson, 1993; Cravo et al., 2002).

As coastal ecosystems face complex challenges due to climate variability, the ability of shells to retain pollutants consistently and predictably supports adaptive management strategies aimed at protecting marine resources (Carell et al., 1987; Goldberg, 1980). Monitoring Cd levels in shells over time allows environmental agencies to identify emerging pollution trends and assess the effectiveness of pollution control measures (Langston and Bebianno, 1998; Richardson et al., 2001). This aligns with Goal 13 (Climate Action) by enabling proactive responses to pollution linked to climate impacts, such as intensified runoff or increased sedimentation due to extreme weather events (Szefer et al., 2002). Additionally, the long-term stability of shell Cd concentrations means that shells can serve as archival biomonitoring materials, providing historical contamination records critical for tracking shifts in ecosystem health over extended periods, thus supporting the conservation goals outlined in the UNSDGs (Puente et al., 1996; Yap et al., 2003).

Alignment with UNSDGs for sustainable and resilient coastal monitoring

By employing *Perna viridis* shells as biomonitoring materials, this approach addresses multiple UNSDGs related to environmental health, ecosystem protection, and sustainable resource management (Phillips and Rainbow, 1993; Langston and Bebianno, 1998). Goal 14 (Life Below Water) emphasizes the conservation of marine ecosystems, which are increasingly threatened by heavy metal pollution from anthropogenic sources (Kennedy, 1986; Yap et al., 2004). The shell-based approach allows for accurate assessment of pollution without harming the organism, promoting non-invasive, ecologically sensitive monitoring practices (Cravo et al., 2004; Fischer, 1984). Furthermore, shell-based biomonitoring aligns with Goal 6 (Clean Water and Sanitation) by enabling the detection of harmful pollutants in coastal waters, supporting water quality assessment and restoration initiatives critical for marine and coastal ecosystem sustainability (Richardson et al., 2001; Carriker et al., 1980).

Moreover, this sustainable approach minimizes the environmental footprint of monitoring programs, which is especially relevant for Goal 12 (Responsible Consumption and Production). Using shells as biomonitors reduces the need for frequent, resource-intensive sampling and preserves natural populations by avoiding destructive tissue sampling (Bourgoin, 1990; Soto et al., 2000). This practice aligns with the principles of sustainable development by promoting resource-efficient, low-impact monitoring solutions that provide high-quality data for coastal management (Sturesson, 1976; Luoma, 1983). Additionally, given that Cd contamination poses risks to both biodiversity and human health, monitoring Cd levels in shells supports Goal 3 (Good Health and Well-Being) by contributing to the safeguarding of seafood safety, thus protecting communities that rely on marine resources for their livelihoods (Goldberg, 1975; Yasoshima and Takano, 2001).

Future implications and policy recommendations for shell-based biomonitoring

The evidence from this study suggests that *Perna viridis* shells can be integrated into regulatory frameworks for monitoring coastal contamination, offering a scientifically justified, cost-effective, and sustainable solution for environmental assessment (Gardiner, 1982; Phillips, 1980; Abderrahmani et al., 2020). Policymakers could incorporate shell-based biomonitoring into existing marine health indicators, using shells to monitor chronic contamination in both industrialized and rural coastal areas (Geist et al., 2005; Yap et al., 2002; von Hellfeld et al., 2022). Such integration would facilitate early detection of pollution hotspots, allow for the tracking of contamination trends over time, and enable proactive interventions to prevent ecosystem degradation (Richardson, 1993; Szefer and Szefer, 1985; Omeragic et al., 2020). Shell-based monitoring could also complement traditional water and sediment analyses, providing an additional layer of data for comprehensive environmental assessments (Dauphin et al., 2003; Fuge et al., 1993; Wachholz et al., 2023).

Furthermore, shell-based biomonitoring could be especially valuable in regions with limited resources for extensive laboratory testing or frequent sample collection (Langston and Bebianno, 1998; Ravera et al., 2005; Abderrahmani et al., 2020). The durability of shells makes them suitable for long-term storage and analysis, enabling retrospective studies that can provide insights into historical contamination levels and trends (Puente et al., 1996; Markich et al., 2002; Omeragic et al., 2020). This approach supports the UNSDGs by promoting scalable, adaptable monitoring practices that can be implemented in diverse geographic and socio-economic contexts (Phillips, 1985; Cravo et al., 2002; von Hellfeld et al., 2022). By establishing shell-based monitoring as a standardized practice, environmental agencies and governments can make data-driven decisions to protect marine ecosystems, support climate adaptation efforts, and fulfil global commitments to sustainable development (Watson et al., 1995; Yap et al., 2003; Wachholz et al., 2023).

Conclusion

The findings of this study underscore the scientific validity and practical advantages of using *Perna viridis* shells, particularly the periostracum and total shell components, as biomonitors for Cd pollution in coastal waters. Strong positive correlations between shell Cd levels and bioavailable and organic-bound sediment fractions indicate that shells reliably capture ambient Cd, reflecting long-term environmental exposure. This stability, in contrast to the metabolic fluctuations seen in soft tissues, makes shells superior indicators of chronic contamination, providing a stable, cumulative record less affected by short-term physiological changes. These qualities fulfil key criteria for effective biomonitoring, establishing shells as valuable tools for assessing persistent pollution (Huanxin et al., 2000). Shell-based monitoring allows for the accurate assessment of contamination levels and trends over time, supporting informed, data-driven decisions to protect marine biodiversity, ensure food safety, and promote resilient coastal communities in a context of increasing environmental change.

Moreover, the use of *P. viridis* shells aligns closely with global sustainability goals, including the UNSDGs. Shell-based monitoring offers an efficient, low-cost, and environmentally sustainable approach that enhances ecosystem conservation (Goal 14), supports clean water initiatives (Goal 6), and promotes responsible production and consumption practices (Goal 12). By enabling non-invasive, durable, and scalable

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monitoring, shell biomonitoring is well-suited to adaptive management and climate resilience, offering a robust framework for assessing and mitigating the impacts of Cd contamination on coastal ecosystems. As a policy-relevant and ecologically responsible solution, shell biomonitoring enhances our ability to track marine pollution and contributes to broader efforts to protect human health and biodiversity, meeting critical sustainability targets amid the growing pressures of environmental change.

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