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Shells that remember: Heavy metals concentrations in *Perna Viridis* shells from the West coast of peninsular Malaysia

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Abstract

This study aimed to study whether shells of the green-lipped mussel Perna viridis can deliver a clear, layer resolved picture of metal bioavailability while also recognising their carbon value. Mussels were purchased by weight without pre order from six south western coast landing sites in Peninsular Malaysia. Shells were measured, the periostracum was scraped and the nacre powdered, and both layers were digested and analysed for copper, iron, nickel, lead and zinc, the periostracum and the nacre showed a consistent hierarchy with iron and lead highest, nickel intermediate, and copper and zinc lower, with site contrasts that were clear and repeatable. These differences indicate real spatial variation in bioavailability that the shell archives over time. We interpret these patterns as an integrated readout that can be repeated with routine landings to build defensible baselines for management. The workflow is rapid and nonlethal and is suitable for long term surveillance. In parallel, the calcium carbonate fraction represents a durable stock that can be retained and beneficially reused on land, linking contamination surveillance with a small carbon contribution to climate action. Overall, the mussel shells procvide a practical tool for mapping hotspots and tracking progress in pollution control. Findings are usable for coastal planning and routine reporting. The present findings are able to answer the heavy metal levels in the P. viridis shell layers of nacrea and periostracum across six sites, compare layer specific and site specific patterns.

Keywords: Atomic absorption spectrometry, *Biomonitor*, Environmental pollution, Nacre, Periostracum, *Perna viridis.*

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1. Introduction

Coastal waters receive metals from riverine discharge, urban runoff, and sediment resuspension. Because water concentrations vary quickly with tides and storms, managers rely on biomonitors that integrate exposure over time. Mussels are especially useful because they are sedentary filter feeders with high bioaccumulation capacity and long histories in monitoring networks [1, 2]. Reviews of mussel-based monitoring show that bivalves provide stable spatial and temporal signals of contamination that complement conventional chemistry [3]. The U.S. National Mussel Watch Program institutionalised this logic by tracking contaminants in sediments and indigenous bivalves across hundreds of coastal sites since 1986 [4-6].

In Southeast Asia, the Asian green mussel *Perna viridis* is abundant, harvested, and well-studied as a bioindicator. Early Malaysian work linked metal levels in *P. viridis* to environmental contamination and proposed both tissues and shells as monitoring materials [1, 7, 8]. Subsequent regional studies confirmed that *P. viridis* reflects local exposure and can be transplanted for gradient tests, reinforcing its practical value for coastal surveillance [9]. These foundations justify using *P. viridis* to characterise metal exposure along the west coast of Peninsular Malaysia, where land-sea interfaces are dynamic and management needs continuous evidence.

Bivalves provide a practical lens for metal monitoring because their soft tissues, shells, and even the periostracum record ambient exposure, a point summarized in recent commentary on biomonitoring with molluscs [2]. In Malaysian waters, correlations between sediment geochemical fractions and metal burdens in the total soft tissue of *P. viridis* confirm the value of this species for tracking Cd, Cu, Pb, and Zn along the west coast of Peninsular Malaysia [1, 10] with baseline concentrations for these metals also established for regional comparisons [11]. The periostracum and shell add further resolution, with studies showing the periostracum's potential for Cu and Pb assessment and the shell's usefulness for Zn through links to sediment geochemical partitions [8, 12, 13]. Field observations of shell deformities in *P. viridis* extend this toolbox by indicating possible environmental stresses along the same coast [11]. Beyond Malaysia, work in Hong Kong documents metal partitioning among soft tissues, byssus, and shell in *P. viridis*, while Baltic studies using mussels and barnacles demonstrate how bioavailability shifts across space and seasons, underscoring the need for regional baselines [14, 15]. Finally, the periostracum's capacity to trap particulate contaminants now reaches emerging materials such as engineered silver nanoparticles, strengthening its role as a minimally invasive sampler across legacy and novel pollutants [16].

Shells themselves are informative archives. The outer periostracum is an organic coat that can bind metals, while the inner nacre is a carbonate layer that records longer-term incorporation. Recent experimental work shows that the periostracum captures metal species, including engineered nanoparticles, through well-defined surface chemistry; this supports layer-specific analyses that separate short-to-intermediate exposure from longer-term signals [16]. Using shells has practical advantages because they are durable, easy to store, and can be sampled without harming stocks, while still preserving meaningful gradients for comparison among sites and years [10].

This study builds on that groundwork to provide a clear, site-resolved picture for Malaysia. We sampled market landings of *P. viridis* from six west-coast stations, measured shell dimensions, and quantified copper, iron, nickel, lead, and zinc separately in the periostracum and the nacre using flame atomic absorption spectrometry. Our objective was to test whether shell layers resolve inter-site differences in apparent bioavailability, to evaluate which metals dominate each layer, and to supply a practical baseline that can be repeated within ongoing monitoring and aligned with ocean and water-quality goals in the Sustainable Development Agenda.

2. Materials and Methods

2.1. Sampling and shell measurements

Green mussels *Perna viridis* were purchased directly from fishermen at six coastal sites along the west coast of Peninsular Malaysia. Purchases were made without pre-order and by weight rather than by count to reflect routine landings and to avoid bias from fixed sample quotas when catches were small. As a result, sample sizes differed among sites and the specimens represent random lots from the day's harvest. Coordinates of the six stations are given in Table 1 and the spatial distribution is shown in Figure 1.

Table 1.
Sampling site information.

Site name	Latitude	Longitude
Parit Jawa	1°95'19.80"N	102°63'39.1"E
Parit Karang Tangkak	2°08'00.0"N	102°52'38.4"E
Kuala Sebatu	2°11'46.5"N	102°46'41.0"E
Kg. Kuala Masai	1°47'61.0"N	103°87'18.0"E
Teluk Jawa	1°47'82.0"N	103°84'59.0"E
Kg. Pasir Puteh	1°26'30.0"N	103°55'50.0"E

Source: Yap, et al. [8]

Live mussels were steamed briefly until the valves opened. Soft tissues and most barnacle bodies were removed. Each shell was rinsed with deionized water to remove surface debris, patted dry, and processed on the same day. Shell length, shell width, and shell height were measured with a vernier caliper to the nearest 0.01 mm, and shell weight was measured with an electronic balance to the nearest 0.01 g (Figure 2). For each site we computed the minimum, maximum, mean, median, and standard error for all measured traits.

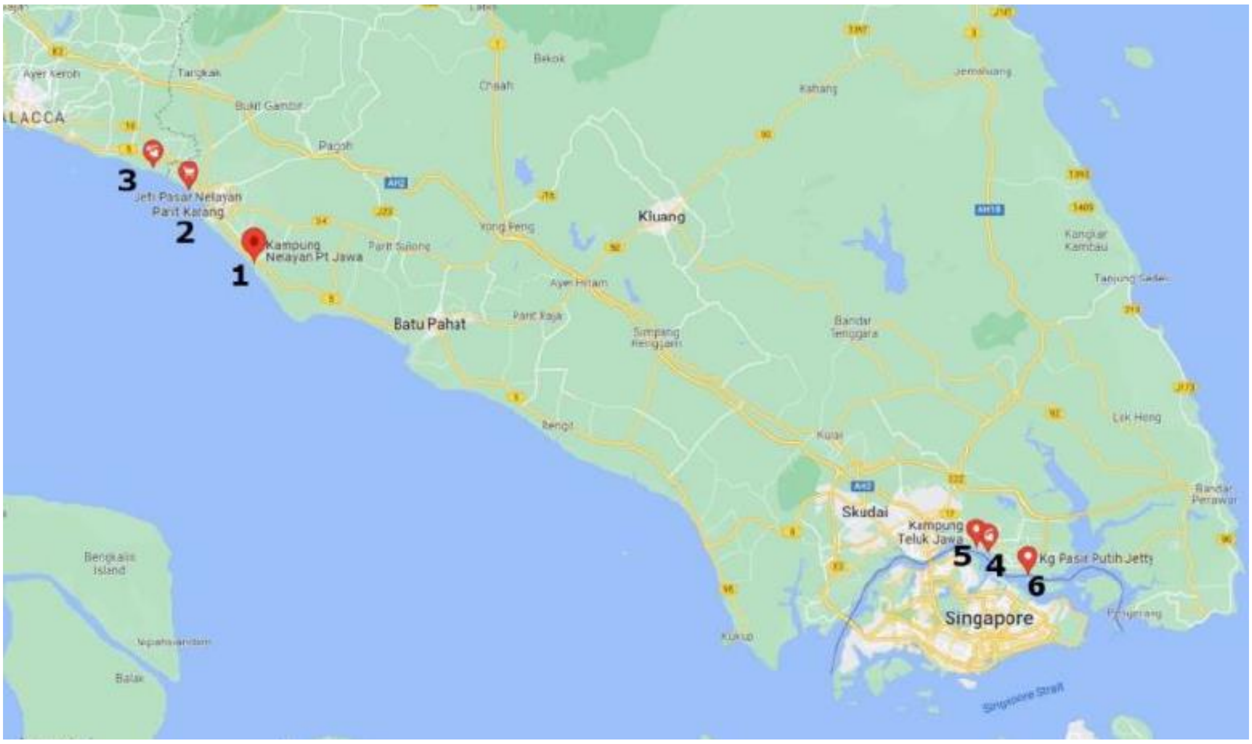

Figure 1.
Google map showing sampling stations of *P. viridis* from the west coast of Peninsular Malaysia Yap, et al. [11].

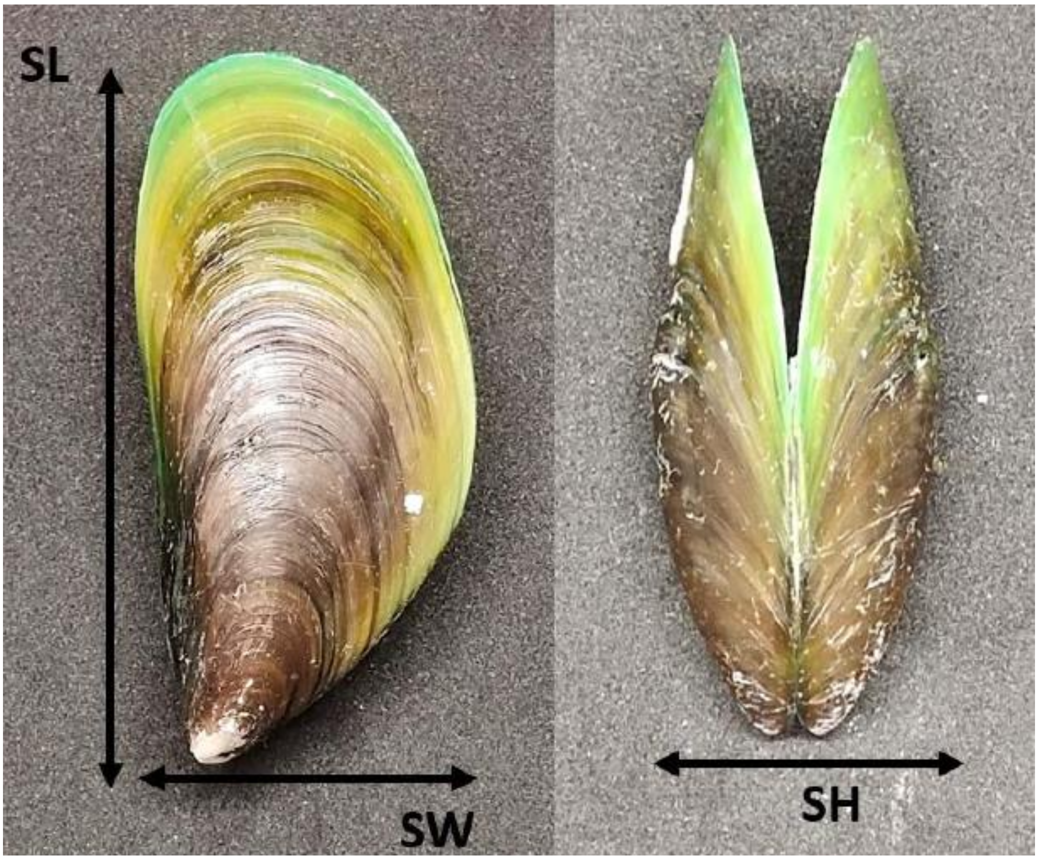

Figure 2.
Measurement of morphological traits for *P. viridis*. (SL: shell length; SW: shell width; SH: shell height) Yap, et al. [11].

2.2. Shell Processing and Layer Separation

To analyze the outer organic coat and the mineral layer separately, we followed a simple physical separation. The periostracum was gently scraped from the external shell surface using clean plastic blades and collected into pre-weighed acid-washed polypropylene vials. The remaining carbonate shell was rinsed with deionized water, air-dried, and the inner nacre was lightly powdered with an agate mortar and pestle. All tools and containers were soaked in ten percent nitric acid overnight and rinsed thoroughly with deionized water before use to minimize trace-metal contamination. Layer-specific sampling improves interpretability because the periostracum can bind dissolved metals through catechol chemistry, while nacre records longer-term carbonate-bound incorporation [16, 17].

2.3. Acid Digestion for Metal Determination

Subsamples of periostracum and nacre were digested in acid-cleaned vessels. For each digest, approximately 0.50 g of shell powder or the entire periostracal scrapings from an individual were wetted with ultrapure water, followed by the slow addition of trace-metal grade concentrated nitric acid. Vessels were covered and heated gently on a hotblock digestor for 1 hour at 40°C and increased to 140°C until the reaction subsided and the solution cleared. After cooling, digests were brought to 40 mL with double distilled water. Reagent blanks were prepared with each batch.

Copper, iron, nickel, lead, and zinc were quantified by flame atomic absorption spectrometry using a PerkinElmer AAnalyst 800 operated with an air–acetylene flame. To control contamination and verify accuracy, we analyzed procedural blanks, duplicate digests, and fortified matrix spikes with every batch. Accuracy was further checked with a certified reference material for trace metals in biological matrices, analyzed alongside samples; acceptable recoveries (80-120%) for all metals were recorded.

2.4. Data Treatment and Statistics

All measurements were entered into a single database and screened for transcription errors. For each site and shell layer we summarized metals and morphometrics by minimum, maximum, mean, median, and standard error. Where appropriate, metals were compared among sites descriptively because the sampling design was opportunistic at landing points and sample sizes were unbalanced by site. These summaries are the basis for the Results section and the site-wise contrasts reported there.

3. Results

3.1. Shell Size and Mass

Table 2 shows the shell dimensions of *P. viridis* varied among the six sites. At Parit Jawa, shells ranged from 53.59 to 109.46 mm in length with a mean of 76.04 mm, 18.13 to 34.13 mm in width with a mean of 25.47 mm, and 26.32 to 41.55

mm in height with a mean of 32.76 mm; shell weight spanned 4.80 to 19.50 g with a mean of 10.16 g. At Parit Karang Tangkak, length was 49.43 to 90.47 mm with a mean of 73.85 mm, width 2.52 to 34.49 mm with a mean of 26.07 mm, height 21.91 to 35.22 mm with a mean of 31.46 mm, and weight 3.84 to 60.90 g with a mean of 9.64 g. At Kuala Sebatu, length was 48.00 to 88.06 mm with a mean of 70.03 mm, width 16.00 to 43.40 mm with a mean of 31.77 mm, height 16.63 to 42.03 mm with a mean of 22.50 mm, and weight 3.64 to 13.40 g with a mean of 8.28 g. At Kg. Kuala Masai, length was 36.32 to 90.16 mm with a mean of 63.29 mm, width 10.07 to 30.68 mm with a mean of 21.83 mm, height 17.91 to 39.64 mm with a mean of 29.10 mm, and weight 1.37 to 17.43 g with a mean of 7.09 g. At Telok Jawa, length was 83.80 to 100.01 mm with a mean of 90.87 mm, width 39.12 to 47.02 mm with a mean of 41.54 mm, height 26.83 to 31.82 mm with a mean of 29.82 mm, and weight 14.10 to 26.73 g with a mean of 19.19 g. At Kg. Pasir Puteh, length was 6.10 to 96.30 mm with a mean of 58.49 mm, width 13.50 to 31.20 mm with a mean of 24.10 mm, height 15.10 to 33.50 mm with a mean of 24.76 mm, and weight 0.20 to 15.90 g with a mean of 5.38 g.

Table 2.
Compilation of allometric data in the green-lipped mussels obtained from the coastal waters of Peninsular Malaysia.

Normal shells		**Parit Jawa (N= 30)**	**Tangkak (N= 35)**	**Sebatu (N= 25)**	**Kg. Masai (N= 30)**	**Telok Jawa (N= 6)**	**Kg. P. Puteh (N= 35)**
Shell total weight (g)	Mean	10.16	9.64	8.28	7.09	19.19	5.38
	Min.	4.8	3.84	3.64	1.37	14.1	0.2
	Max.	19.5	60.9	13.4	17.43	26.73	15.9
Shell length (mm)	Mean	76.04	73.85	70.03	63.29	90.87	58.49
	Min.	53.59	49.43	48	36.32	83.8	6.1
	Max.	109.46	90.47	88.06	90.16	100.01	96.3
Shell width (mm)	Mean	25.47	26.07	31.77	21.83	41.54	24.1
	Min.	18.13	2.52	16	10.07	39.12	13.5
	Max.	34.13	34.49	43.4	30.68	47.02	31.2
Shell height (mm)	Mean	32.76	31.46	22.5	29.1	29.82	24.76
	Min.	26.32	21.91	16.63	17.91	26.83	15.1
	Max.	41.55	35.22	42.03	39.64	31.82	33.5

Across sites, the range of site means was 58.49 to 90.87 mm for length with a grand mean of 72.10 mm, 21.83 to 41.54 mm for width with a grand mean of 28.46 mm, 22.50 to 32.76 mm for height with a grand mean of 28.40 mm, and 5.38 to 19.19 g for shell weight with a grand mean of 9.96 g.

3.2. Heavy Metals in the Periostracum

As shown in Table 3, the periostracal burdens were ordered consistently across sites, with Fe and Pb highest and Cu and Zn lower. Site means for Kg. Kuala Masai were Cu 7.29, Fe 73.85, Ni 39.40, Pb 84.28, and Zn 10.20; for Kuala Sebatu they were Cu 6.35, Fe 74.36, Ni 39.47, Pb 91.15, and Zn 6.60; for Parit Jawa they were Cu 6.27, Fe 122.28, Ni 37.89, Pb 90.68, and Zn 8.05; for Kg. Pasir Puteh they were Cu 10.20, Fe 73.88, Ni 37.97, Pb 74.73, and Zn 6.77; for Parit Karang Tangkak they were Cu 10.11, Fe 69.76, Ni 34.51, Pb 82.25, and Zn 12.40; and for Telok Jawa they were Cu 8.88, Fe 72.91, Ni 31.33, Pb 82.57, and Zn 8.92. Summarised across sites, the range of site means was 6.27 to 10.20 for Cu, 69.76 to 122.28 for Fe, 31.33 to 39.47 for Ni, 74.73 to 91.15 for Pb, and 6.60 to 12.40 for Zn.

Table 3.
Heavy metals in nacreatic shells (N) and periostracum (P) of green-mussel *Perna viridis* collected from the six sampling sites of west southern coast of Peninsular Malaysia.

Sites	**P-Cu**	**SE**	**P-Fe**	**SE**	**P-Ni**	**SE**	**P-Pb**	**SE**	**P-Zn**	**SE**
Kg Masai	7.29	0.37	73.85	2.19	39.40	0.32	84.28	0.55	10.20	0.06
Sebatu	6.35	0.24	74.36	3.27	39.47	2.41	91.15	3.30	6.60	0.39
Parit Jawa	6.27	0.16	122.28	5.34	37.89	1.33	90.68	1.09	8.05	0.58
KP Puteh	10.20	1.05	73.88	2.76	37.97	0.43	74.73	1.63	6.77	0.41
Tangkak	10.11	0.06	69.76	3.36	34.51	1.13	82.25	1.89	12.40	0.14
Telok Jawa	8.88	0.18	72.91	1.05	31.33	0.94	82.57	1.15	8.92	0.58
Sites	N-Cu	SE	N-Fe	SE	N-Ni	SE	N-Pb	SE	N-Zn	SE
Kg Masai	7.79	0.09	108.28	3.51	36.67	0.34	89.31	2.50	10.96	0.36
Sebatu	0.09	0.02	82.73	12.70	36.64	2.70	73.61	1.43	10.49	0.23
Parit Jawa	0.13	0.07	202.00	6.28	35.41	0.57	73.89	0.41	10.68	0.34
KP Puteh	0.16	0.06	85.56	6.22	35.12	0.53	74.11	1.41	12.11	0.48
Tangkak	0.22	0.06	65.81	4.10	40.57	0.89	74.01	0.78	11.11	0.17
Telok Jawa	0.06	0.02	100.52	4.30	39.49	0.77	69.15	1.72	10.85	0.25

Note: SE= standard error.

3.3. Heavy Metals in the Nacre

As shown in Table 3, the nacre showed a similar hierarchy, with Fe and Pb dominant and Cu very low at five of the six sites. Site means for Kg. Kuala Masai were Cu 7.79, Fe 108.28, Ni 36.67, Pb 89.31, and Zn 10.96; for Kuala Sebatu they were Cu 0.09, Fe 82.73, Ni 36.64, Pb 73.61, and Zn 10.49; for Parit Jawa they were Cu 0.13, Fe 202.00, Ni 35.41, Pb 73.89, and Zn 10.68; for Kg. Pasir Puteh they were Cu 0.16, Fe 85.56, Ni 35.12, Pb 74.11, and Zn 12.11; for Parit Karang Tangkak they were Cu 0.22, Fe 65.81, Ni 40.57, Pb 74.01, and Zn 11.11; and for Telok Jawa they were Cu 0.06, Fe 100.52, Ni 39.49, Pb 69.15, and Zn 10.85. Summarised across sites, the range of site means was 0.06 to 7.79 for Cu, 65.81 to 202.00 for Fe, 35.12 to 40.57 for Ni, 69.15 to 89.31 for Pb, and 10.49 to 12.11 for Zn.

4. Discussion

4.1. The Bioavailabilities of Heavy Metals in Different Sites

Across the surveyed coastline, bioavailable metals in green mussel shells varied meaningfully among sites, reflecting differences in inputs, hydrodynamics, and sediment–water exchange. Inter-site contrasts of Pb, Ni, Zn and Fe are consistent with the basic expectation that shell records integrate ambient exposure rather than short episodic spikes, so higher shell burdens indicate chronically greater bioavailability at those locations. This interpretation aligns with classic work showing that spatial patterns in bivalve metals track site-specific availability rather than only organismal traits [15, 18, 19].

Metal partitioning between shell layers helps explain the gradients we observed. The organic-rich periostracum can concentrate iron and other transition metals through catechol chemistry, while the crystalline nacre records longer-term carbonate-bound incorporation. Preferential enrichment of Fe in the outer layer that we detected matches mechanistic evidence for iron–catechol complexation in bivalve periostraca and supports using shell surfaces as sensitive samplers of dissolved metal species [16, 17].

Between-site differences were largest for elements with strong local sources. Lead and, in some settings, Ni tended to be higher where catchment and shoreline activities increase particulate and dissolved loads, whereas Fe and Mn often followed more lithogenic signals. Interpreting such patterns within established sentinel frameworks is appropriate because mussels are widely validated as indicators of bioavailable coastal contaminants [3, 20].

Finally, shell chemistry is modulated by size, growth, and mineralogy. Even with careful size-matching, smaller differences in aragonite texture and growth rate can influence uptake kinetics and should be considered when comparing sites. Published work on *Mytilus* demonstrates size and mineralogical effects on shell elemental composition, reinforcing our use of standardized sampling and statistics when attributing spatial contrasts to bioavailability [21].

4.2. The Ecotoxicological Significance

From a toxicological standpoint, risk is linked to the metabolically available fraction rather than total metal burden. Shells primarily archive a detoxified or immobilized component, whereas adverse effects generally track with labile pools in soft tissues and critical organs. Our findings of site gradients in shells therefore indicate differences in exposure pressure, while the magnitude of organism-level risk depends on internal bioavailability thresholds and not on shell totals alone [15, 18].

Even so, shells are ecologically consequential because they represent a long-lived sink that diverts a portion of the body burden away from vulnerable tissues. Prior studies show that shell records can correlate with sediment geochemistry and, for some elements such as Pb and Ni, mirror environmental gradients with high fidelity, making shells useful proxies of chronic contamination histories [9, 10, 22].

To place our concentrations in context, regional and national “mussel watch” programs interpret spatial and temporal patterns against long-term baselines. Those programs emphasize trend detection and spatial comparisons rather than rigid effect thresholds, which is also the appropriate frame for our dataset. The same logic applies here: spatial differences in shell metals indicate differing exposure landscapes and can be used to prioritize follow-up biomarker or tissue-effect assessments.

Finally, ecotoxicological significance emerges under multiple stressors. Temperature, salinity, and acidification can alter metal speciation and uptake, modulating the relationship between exposure and effect. Reviews of *Mytilus* monitoring highlight these interactions and support integrating shell records with concurrent measurements of water chemistry and organismal condition to interpret ecological risk credibly [3, 19].

4.3. The Sustainability to the United Nations Sustainable Development Goals (UNSDGs)

These results directly support SDG 6 on clean water and sanitation by providing a practical indicator of coastal water quality. Shell-based biomonitoring complements physicochemical measurements by integrating exposure over time, thereby offering decision makers an accessible way to track progress toward reducing hazardous discharges to water bodies, a central target under SDG 6 [23].

They also advance SDG 14 on life below water. Reducing marine pollution requires reliable surveillance of contaminants; mussel shells provide a cost-effective archive that can be sampled without sophisticated infrastructure and linked to episodic or chronic sources. Incorporating shell data into marine spatial planning helps identify hotspots and evaluate interventions aimed at safeguarding coastal ecosystems [23].

The approach aligns with SDG 13 on climate action. Sustained biomonitoring builds adaptive capacity by detecting contaminant trends that can interact with climate-driven shifts in coastal biogeochemistry. Integrating contaminant surveillance with climate adaptation planning is recommended under SDG 13 targets on resilience and early warning systems [23].

Operationally, shell biomonitoring is scalable and inclusive. Community and agency networks modeled on long-running programs can implement standardized protocols, expand coverage to under-sampled shorelines, and share open baselines for accountability. Such networks provide the evidence base needed for SDG reporting and for local policies on discharge reduction and healthy coastal communities [20, 23].

4.4. The shells as Biomonitors of Metal Pollution

There is strong evidence that shells of marine bivalves are robust biomonitoring materials. Pioneering studies in *Mytilus* and *Perna* showed that nacre and whole shells record spatial gradients of Pb, Ni, Zn and other metals, often with clearer long-term signals than short-lived soft-tissue markers. Our spatial results reproduce these core patterns and support using shells in trend and gradient studies [11, 12, 22].

Shells offer practical advantages. They are durable, easy to store and ship, and can be sampled non-lethally or from naturally dead individuals, which reduces impacts on wild populations. New protocols even allow non-destructive micro-sampling along growth lines to reconstruct time series at sub-annual resolution [3, 24].

Limitations are well understood. Mineralogy, microstructure, and organism size can influence elemental signals, and periostracal fouling or diagenesis can complicate surface records. These factors argue for harmonized size classes, cleaning protocols, and layer-specific analyses when designing shell-based surveys [19, 21].

Best practice is to pair shells with co-located sediments and, where possible, soft tissues to calibrate environmental meaning and improve interpretability. Correlations between shell metals and sediment geochemical fractions, demonstrated for Zn and related elements, provide the mechanistic bridge from shell records to environmental sources and pathways [19, 25].

4.5. The Shells and Theoretical Carbon Sequestration for Climate-Change Control

Bivalve shells are composed primarily of calcium carbonate, so a portion of the theoretical carbon fixed by mussels is stored in a mineral phase that can persist for decades to centuries. Whether cultured or wild, that reservoir can function as a carbon sink when shells are retained or repurposed rather than dissolved or landfilled. There is an active debate about net climate benefits because biogenic calcification releases CO_2, but integrative ecosystem models show that under realistic conditions shell production can contribute to climate-mitigation strategies [26].

End-of-life management matters. When shells are diverted into long-lived products such as construction aggregates, coastal protection structures, or agricultural liming that locks carbonate in soils, more of the mineral carbon remains out of the atmosphere–ocean system. Strategic reuse therefore increases the permanence of stored carbon relative to disposal pathways with rapid dissolution [26, 27].

Accounting must span the whole farm or ecosystem. Net carbon outcomes include trophic effects of filtration on phytoplankton carbon, benthic–pelagic coupling, burial, respiration, and processing emissions. Frameworks developed for shellfish aquaculture provide a template for extending these calculations to wild and restoration settings that generate significant shell mass [26].

Positioning shell archives within SDG 13 is therefore straightforward: measure shell production and fate alongside contaminant surveillance, report standardized carbon metrics, and align shell reuse with local climate plans. This links water-quality monitoring to tangible mitigation co-benefits in a single coastal management portfolio [24, 26].

5. Conclusion

This study shows that shells of *P. viridis* reliably archive spatial differences in metal exposure along the west coast of Peninsular Malaysia. Across all stations the periostracum and the nacre recorded a consistent hierarchy with iron and lead highest, nickel intermediate, and copper and zinc lower. Site contrasts were clear and repeatable, indicating that the shells captured real differences in bioavailability rather than random variation. Differences in shell size among sites did not overturn these patterns, and separating the periostracum from the nacre added resolution by distinguishing short to intermediate exposure at the surface from longer term incorporation in the carbonate layer

The implications are direct. Shells of *P. viridis* can serve as routine biomonitors to map hotspots, prioritize follow up assessments, and track progress in pollution control. Future work should repeat sampling seasonally, add co-located water and sediment chemistry, and include soft tissues and biomarkers to link exposure with potential effects. Standardized handling, size classes, and layer specific analyses will strengthen comparability across years and regions. In parallel, documenting the fate and reuse of shell material would allow managers to pair contamination monitoring with a small but real contribution to theoretical carbon management. In short, *P. viridis* shells provide a low cost, scalable tool for evidence based coastal management that can be integrated into ongoing monitoring networks.

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