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Zinc Bioaccumulation and Detoxification Mechanisms in *Pomacea insularum*: Implications for Biomonitoring in Metal-Contaminated Ecosystems

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Abstract

This study investigates the bioaccumulation and detoxification mechanisms of zinc (Zn) in *Pomacea insularum* collected from 13 sites in Peninsular Malaysia. By examining the distribution of Zn across various tissues, including the digestive caecum (DC), cephalic tentacle (CT), remaining soft tissues (REM), and shell, we identify tissue-specific roles in Zn absorption, redistribution, and long-term storage. The results reveal that the DC serves as the primary absorption site for ingested Zn, while the CT and foot absorb Zn from the surrounding environment. Zn is then redistributed to metabolically active tissues such as the mantle and REM, where it is temporarily stored or utilized before being sequestered in the shell for detoxification. The shell, which exhibits the lowest Zn concentrations, functions as a long-term storage site, minimizing the metal's bioavailability and preventing toxicity. These findings underscore the importance of *P. insularum* as a biomonitor for assessing both short-term and long-term Zn contamination in aquatic ecosystems, particularly in environments with fluctuating metal concentrations.

Introduction

Heavy metal contamination in aquatic ecosystems is a critical environmental issue, particularly in regions affected by industrial, agricultural, and urban runoff (Rasyid & Dody, 2018; Marimoutou et al., 2023). While zinc (Zn) is an essential metal for biological functions, excessive levels can disrupt metabolic processes and pose serious risks to aquatic organisms (Maher et al., 2016; Taylor & Maher, 2006). Molluscs, including snails and mussels, are widely used as biomonitors due to their ability to bioaccumulate metals through sediment ingestion and direct exposure to contaminated water (Bu-Olayan & Thomas, 2001; Praveen Kumar & Uma Devi, 1998). Their capacity to absorb, redistribute, and store metals makes them valuable indicators of pollution levels (Krupnova et al., 2018; Flessas et al., 2000; Athalye & Gokhale, 1994; Liu et al., 2022).

Pomacea insularum, a freshwater snail found in Southeast Asia, is known for its resilience in polluted environments and its efficiency in metal bioaccumulation (Yap et al., 2009; Fitria et al., 2023). As a sediment-feeder and water-filterer, it integrates contaminants from its surroundings, making it a relevant species for monitoring Zn pollution (Nigariga et al., 2023; Reolid et al., 2024). Previous studies have highlighted the role of molluscs in metal detoxification through specific tissues, such as the digestive caecum (DC) for absorption, soft tissues for redistribution, and shells for long-term storage (Chukaeva & Petrov, 2023; Edward et al., 2010; Simonyi-Poirier et al., 2003; Conti et al., 2012).

This study investigates Zn accumulation in *P. insularum*, focusing on the distribution of the metal across different tissues, including the DC, cephalic tentacle (CT), foot, mantle, remaining soft tissues (REM),

and shell. By examining these patterns, we aim to enhance understanding of the species' physiological adaptations to metal exposure and its effectiveness as a biomonitor in Zn-contaminated aquatic ecosystems (Fang et al., 2001; Emami et al., 2024; Sabri et al., 2014; Vidyalakshmi et al., 2024).

Materials and Methods

Sample Collection

Pomacea insularum specimens were collected from 13 sites in Peninsular Malaysia, specifically in areas known for elevated levels of Zn contamination due to industrial and agricultural runoff (Yap et al., 2009; Yap et al., 2010). Sampling was conducted between January and March 2024, during which at least 20 individuals of snails were collected from each sampling site using a convenient sampling approach. This approach ensures the representativeness of varying levels of Zn contamination across sites, aligning with established collection protocols for assessing heavy metal exposure in molluscs (Yap et al., 2015; Yap et al., 2023). The collected specimens were immediately rinsed with deionized water to remove any external debris and sediments, following methods outlined by Yap et al. (2009). The snails were transported to the laboratory in a cool box (below 10°C) for further processing.

Tissue Dissection and Preparation

In the laboratory, eight different parts (CT, mantle, pineal sac (PS), REM, foot, DC, operculum, and shell) were separated and they were pooled from 20 individuals. Later, three replicates from each pooled part were analysed for metals. The dissection was carried out using stainless steel scalpels and tweezers to avoid metal contamination, in line with the practices recommended by Yap et al. (2010). Each tissue was carefully weighed and documented for subsequent analysis. The tissues were then dried in an oven at 60°C for 48 hours to obtain a constant dry weight, consistent with procedures for minimizing variability in dry weight analysis (Yap et al., 2003).

Sample Digestion

Dried tissue samples were digested using nitric acid (HNO_3) to analyse Zn concentrations. Approximately 0.5 g of each dried tissue was placed into a 50 mL Teflon digestion vessel. The digestion process began by adding 10 mL of concentrated nitric acid (HNO_3, 65%) to each sample. The vessels were placed on a hot plate, and the digestion proceeded in two stages: the samples were first heated at 40°C for one hour to initiate the digestion, followed by an increase in temperature to 140°C for three hours to ensure complete dissolution of the tissues (Yap et al., 2009; Yap et al., 2015). The digested samples were then allowed to cool, and each sample was filtered through Whatman No. 42 filter paper to remove any undissolved particles. The final digested solutions were diluted with deionized water to a final volume of 50 mL and stored in polyethene bottles for subsequent analysis.

Zinc Analysis

Zn concentrations in the digested samples were determined using atomic absorption spectrophotometry (AAS) with a PerkinElmer AAnalyst 800 model. Calibration curves were prepared using standard Zn solutions with concentrations ranging from 0 to 10 mg/L, and the instrument was calibrated before each batch of sample analysis. Quality control measures, including the use of blanks and standard reference materials (SRM 2976, mussel tissue), were employed to ensure accuracy and precision, following the established protocol by Yap et al. (2004). All measurements were conducted in triplicate to account for any variability, and the mean concentration for each tissue was calculated.

Statistical Analysis

The collected data were analyzed using NCSS 2024 statistical software. Descriptive statistics (mean, standard deviation, minimum, and maximum values) were calculated for Zn concentrations in each tissue. A one-way analysis of variance (ANOVA) was conducted to assess the differences in Zn concentrations between tissues, followed by Tukey's Honestly Significant Difference (HSD) test to identify statistically significant differences ($P<0.05$) between tissue means. Pearson correlation coefficients were calculated to examine the relationships between Zn concentrations in different tissues. Factor analysis with varimax rotation was performed to explore underlying patterns in tissue-specific Zn accumulation, and multiple regression analysis was conducted to identify predictors of Zn levels in the shell, using the Zn concentrations in other tissues as independent variables (Yap et al., 2009; Yap et al., 2015).

Quality Assurance and Control

To ensure the reliability of the data, all glassware and equipment used in the experiment were acid-washed with 10% HNO_3 and rinsed with deionized water prior to use, in accordance with Yap et al. (2003). Blank samples were included in every batch of tissue digestion to check for contamination during sample preparation and analysis. Additionally, the standard reference material was included in each batch of analyses to verify the accuracy of the AAS measurements (Yap et al., 2010). The limits of detection (LOD) for Zn were determined for each analysis, and any results below the LOD were reported as non-detectable.

Results

Overall Zn Distribution

Table 1 provides a comprehensive overview of the Zn concentrations (mg/kg dry weight) across eight distinct tissues of *P. insularum*, which were collected from 13 sites in Peninsular Malaysia. The DC exhibited the highest Zn concentrations, with a mean value of 279 mg/kg and a maximum recorded concentration of 471 mg/kg, emphasizing its primary role in Zn absorption from ingested sediment. This suggests that the DC is the major organ responsible for Zn assimilation, likely due to its exposure to ingested particulates from sediment, which are a primary source of heavy metals in this aquatic environment.

The REM also showed elevated Zn levels, with a mean of 75.4 mg/kg and a maximum concentration of 117 mg/kg, indicating their involvement in Zn storage and redistribution within the organism. The mantle and CT showed moderate Zn concentrations, with mean values of 74.9 mg/kg and 64.8 mg/kg, respectively. These tissues likely participate in Zn absorption and redistribution, particularly for maintaining metabolic functions, as molluscs often use their mantle for shell secretion and tissue repair processes. The foot and operculum, which also had moderate Zn levels (66.8 mg/kg and 38.9 mg/kg, respectively), are likely involved in locomotion and environmental interaction, making them sites for transferring Zn from the surrounding water and sediment.

Interestingly, the shell exhibited the lowest Zn concentration, with a mean of 20.6 mg/kg and a maximum of 38.9 mg/kg. This finding aligns with the shell's known role as a long-term detoxification site for Zn, where excess Zn is stored in an inert form to prevent its accumulation in metabolically active tissues. The low Zn concentration in the shell suggests that *P. insularum* uses its shell primarily for metal sequestration, which protects the soft tissues from potential Zn toxicity, thus preventing Zn overload in organs critical for physiological processes. This distribution pattern demonstrates a sophisticated metal management strategy in which certain tissues, such as the DC and soft tissues, handle the initial metal absorption while the shell functions as the final storage and detoxification site.

Correlation of Zn Levels

Figure 1 and Table 2 present the correlation coefficients of Zn concentrations between the eight tissues of *P. insularum*, revealing the extent of Zn redistribution between various organs. A high correlation between the CT and foot (R=0.95) suggests that these two tissues are closely linked in Zn uptake and redistribution. The CT, which acts as the primary interface with the environment, likely absorbs Zn from waterborne sources, while the foot, which is in contact with both water and sediment, complements the Zn redistribution process. The strong correlation between these two tissues highlights their cooperative role in managing Zn bioavailability and ensuring its proper distribution across the organism.

Similarly, the mantle exhibited strong correlations with the CT (R=0.80) and REM (R=0.82), emphasizing its involvement in the redistribution of Zn absorbed by other tissues. The mantle, being a metabolically active tissue involved in shell formation and overall tissue growth, likely processes Zn for essential metabolic functions before redistributing excess Zn to other storage tissues. The moderate correlation between the DC and the foot (R=-0.17) suggests that Zn absorbed by the digestive system follows an independent processing pathway before being redistributed to other tissues, such as the foot and shell.

The low correlations observed between the shell and most soft tissues support the hypothesis that the shell serves as a final detoxification and storage site. Once Zn is transferred to the shell, it is no longer actively involved in redistribution, preventing the accumulation of potentially toxic levels of Zn in other tissues. This pattern of low correlation with the shell emphasizes its role as a detoxification sink, where Zn is permanently sequestered to prevent its bioavailability in metabolically active tissues. The relatively weak correlation between the DC and other tissues further indicates the distinct role of the DC in handling ingested Zn separately from waterborne Zn absorbed by other tissues like the gill or foot.

Table 1. Overall statistics of Zn concentrations (mg/kg dry weight) in the eight parts of *Pomacea insularum* collected from 13 sites in Peninsular Malaysia. N= 39.

Variable	Mean± SD	Minimum	Maximum
CT	64.8±11.7^{c}	39.38	84.2
Mantle	74.9±23.8^{c}	23.5	115
PS	44.5±13.7^{b}	21.9	77.8
REM	75.4±19.2^{c}	42.7	117
Foot	66.8±12.4^{c}	42.6	92.2
DC	279±60.9^{d}	191	471
Oper	38.9±37.6^{b}	7.87	160
Shell	20.6±9.14^{a}	8.66	38.9

Note: Oper= operculum; CT= cephalic tentacle; PS= pineal sac; DC= digestive caecum; REM= remaining soft tissues. Superscript letters (e.g., a, b, c) were assigned to mean values in Table 1 to represent statistically distinct groups based on the Tukey HSD test results. Note**:** Different superscripts indicate statistically significant differences between tissue means at P<0.05.

Factor Structure Summary for Zn

Table 3 shows the results of a factor analysis, which helps to elucidate the patterns of Zn accumulation and the tissue-specific roles in Zn management across *P. insularum*. The mantle exhibited a strong negative loading on Factor 1 (-0.70), which suggests that it plays a key role in Zn absorption and redistribution, particularly for metabolic use. As the mantle is involved in key physiological functions such as tissue repair and shell formation, its high loading indicates that it actively processes Zn before redistributing it to other tissues.

The CT displayed a significant negative loading on Factor 2 (-0.90), highlighting its role in Zn absorption from environmental sources but its limited function in long-term storage. This finding aligns with the CT's role as a sensory and environmental interface, where it absorbs Zn from both water and sediment but quickly redistributes it to other tissues, such as the mantle or foot. The DC showed a notable loading on Factor 4 (0.48), suggesting that while it is crucial for Zn absorption, it does not serve as a long-term storage organ. This reflects its role in processing and redistributing ingested Zn rather than detoxifying or sequestering it.

The shell showed a strong positive loading on Factor 2 (0.61), confirming its primary role as the final repository for Zn. The shell's ability to store Zn in a

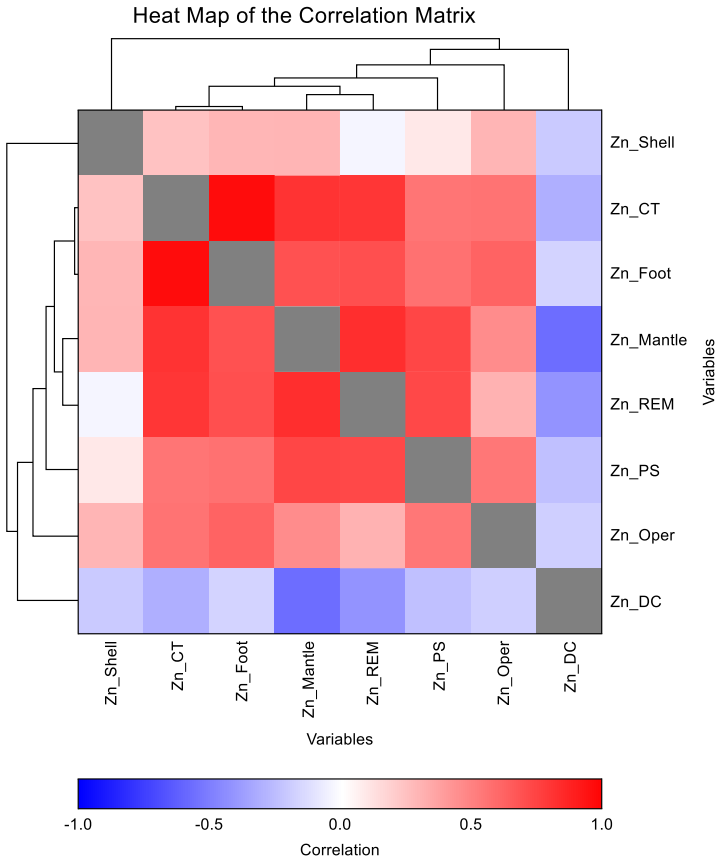

Figure 1. A heat map based on correlation coefficients of Zn levels the seven parts of tissues in *Pomacea insularum* based on 13 sampling sites collected from west part of Peninsular Malaysia (N=39). Note: Oper= operculum; CT= cephalic tentacle; PS= pineal sac; DC= digestive caecum; REM= remaining soft tissues.

Table 2. Correlation coefficients of Zn levels between in the eight parts of *Pomacea insularum* collected from 13 sites in Peninsular Malaysia. N=39

	CT	Mantle	PS	REM	Foot	DC	Oper
CT	1.00	**0.80**	0.54	**0.79**	**0.95**	-0.31	0.55
Mantle		1.00	**0.72**	**0.82**	**0.68**	-0.57	0.45
PS			1.00	**0.72**	0.56	-0.25	0.53
REM				1.00	**0.69**	-0.42	0.30
Foot					1.00	-0.17	0.61
DC						1.00	-0.19
Oper							1.00
Shell							

Note: Oper= operculum; CT= cephalic tentacle; PS= pineal sac; DC= digestive caecum; REM= remaining soft tissues. Values in bold are significant at P<0.05.

detoxified form prevents Zn from accumulating toxic levels in metabolically active tissues. The REM, which exhibited a high loading on Factor 3 (-0.57), is likely involved in intermediate storage, acting as a temporary holding site for Zn before transferring it to the shell or other tissues. The operculum, with a significant loading on Factor 4 (0.48), appears to play a role in Zn storage, particularly in protecting soft tissues from excess Zn, although it is less involved than the shell in long-term detoxification.

Multiple Regression Analysis of Zn

Table 4 provides the results of the multiple regression analysis, indicating the predictors of Zn concentration across the various tissues of *P. insularum*. The CT emerges as a significant predictor of Zn levels in several tissues, including the shell and the mantle. This suggests that Zn absorbed by the CT is redistributed to the mantle for immediate metabolic use and then to the shell for long-term detoxification. The mantle also showed significant regression coefficients with the DC, indicating that Zn absorbed through the digestive system is redistributed to the mantle before being stored in the shell.

The soft tissues, particularly the REM, play a crucial role in Zn redistribution, as evidenced by their significant regression coefficients with other tissues. For example, the REM had strong coefficients with the foot, suggesting that Zn absorbed from ingested material is processed through these tissues before being redistributed or stored. The foot, which also showed strong coefficients with the shell and other tissues, plays a central role in redistributing Zn absorbed through environmental interactions.

Interestingly, the shell had a significant regression relationship with the DC, further emphasizing its role in detoxifying and storing Zn absorbed from ingested material. The results confirm that multiple tissues in *P. insularum* are involved in a sophisticated Zn absorption, redistribution, and long-term storage system. This system allows the organism to balance Zn availability for metabolic functions while protecting itself from potential toxicity through efficient detoxification mechanisms. The shell serves as the final detoxification site, sequestering Zn in an inert form and preventing its accumulation in more sensitive tissues.

Table 3. Factor Structure Summary after Varimax Rotation based on Zn levels in the eight parts *Pomacea insularum* collected from 13 sites in Peninsular Malaysia. N= 39. Values in bold are the tissues selected using the factor analysis

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Shell	-0.10	**0.61**	-0.15	0.01
CT	**-0.90**	0.18	-0.30	0.23
Mantle	**-0.49**	0.18	**-0.70**	**0.42**
PS	-0.30	0.00	-0.27	**0.87**
REM	**-0.64**	-0.31	**-0.57**	**0.41**
Foot	**-0.91**	0.27	-0.08	0.31
DC	0.07	-0.18	**0.68**	-0.07
Oper	-0.38	**0.44**	-0.02	**0.48**

Note: Oper= operculum; CT= cephalic tentacle; PS= pineal sac; DC= digestive caecum; REM= remaining soft tissues. Values in bold are significant at P<0.05.

Table 4. Multiple regression analysis output on the selected part with their predictors of Zn levels in the other seven parts of *Pomacea insularum* collected from 13 sites in Peninsular Malaysia. N= 39. Values in bold are significant at P<0.05.

Shell	b(i)	CT	b(i)	PS	b(i)	Mantle	b(i)
Intercept	24.12	Intercept	7.04	Intercept	13.75	Intercept	-12.56
CT	-1.00	Mantle	**0.18**	Shell	-0.11	PS	**0.85**
Mantle	**0.44**	PS	**-0.23**	CT	**-2.23**	REM	-0.05
PS	-0.19	REM	**0.11**	Mantle	**0.53**	Foot	**-1.75**
REM	**-0.37**	Foot	**0.67**	REM	**0.47**	DC	**-0.07**
Foot	**1.05**	DC	0.01	Foot	**1.28**	Oper	-0.05
DC	-0.02	Oper	0.02	DC	**0.05**	Shell	**0.42**
Oper	-0.01	Shell	-0.06	Oper	**0.12**	CT	**2.81**
R^2	0.449	R^2	0.980	R^2	0.855	R^2	0.922
Oper	b(i)	DC	b(i)	Foot	b(i)	REM	b(i)
Intercept	-53.01	Intercept	239.92	Intercept	-8.16	Intercept	-1.60
Shell	-0.07	Shell	-0.65	Shell	**0.12**	Shell	**-0.34**
CT	3.43	CT	3.93	CT	**1.27**	CT	**1.75**
Mantle	-0.63	Mantle	**-2.72**	Mantle	**-0.21**	Mantle	-0.04
PS	**2.31**	Oper	-0.55	DC	0.00	Foot	-0.60
REM	**-1.81**	PS	**2.97**	Oper	0.00	DC	-0.05
Foot	-0.03	REM	-1.90	PS	**0.25**	Oper	**-0.14**
DC	-0.17	Foot	0.51	REM	-0.07	PS	**0.73**
R^2	0.618	R^2	0.526	R^2	0.966	R^2	0.884

Note: Oper= operculum; CT= cephalic tentacle; PS= pineal sac; DC= digestive caecum; REM= remaining soft tissues. Values in bold are significant predictors at P<0.05.

Discussion

Zinc Absorption

The findings of this study reveal a complex, well-regulated system of Zn absorption and redistribution in *P. insularum*, with each tissue playing a distinct role. The DC, exhibiting the highest Zn concentrations, is the primary organ for Zn absorption. The high mean Zn concentration in the DC suggests that *P. insularum* ingests Zn-rich sediment as part of its diet, which the DC processes for nutrient extraction (Rasyid & Dody, 2018; Krupnova et al., 2018). This aligns with the ecological role of *P. insularum* as a grazer and sediment feeder, where sediment provides both a food source and a pathway for metal uptake (Athalye & Gokhale, 1994; Praveen Kumar & Uma Devi, 1998; Kutluyer Kocabaş et al., 2023). The substantial Zn concentration in the DC highlights its role as a major pathway for Zn exposure, making it a focal tissue for understanding Zn bioaccumulation patterns in contaminated environments (Bu-Olayan & Thomas, 2001; Conti et al., 2012; Ibrahim et al., 2023).

The REM also showed elevated Zn levels, indicating that they act as intermediate storage sites for Zn after absorption in the DC (Maher et al., 2016; Liu et al., 2022). The mantle and foot, with moderate Zn concentrations, are vital for redistributing absorbed Zn to other tissues and supporting metabolic processes. The mantle is particularly important for shell formation and tissue repair, which require Zn for enzymatic activity and structural maintenance (Fang et al., 2001; Edward et al., 2010; Benhamdoun et al., 2024). The CT, which interfaces directly with the environment, likely absorbs Zn from water and transfers it to the mantle, foot, and REM for further processing or storage. A strong correlation between the CT and foot (R=0.95) underscores their cooperative roles in managing Zn bioavailability, as the foot, in contact with sediment, also absorbs Zn, complementing the CT's function (Yap et al., 2010; Marimoutou et al., 2023; Ayanda et al., 2024).

The elevated Zn levels in these soft tissues suggest that *P. insularum* can maintain bioavailable Zn for metabolic needs. However, the variation in Zn concentrations across tissues, as indicated by significant standard deviations, suggests a dynamic distribution, with tissues like the mantle and REM serving as temporary storage before Zn is utilized or transferred to the shell for detoxification (Boulajfene et al., 2017; Emami et al., 2024; Messina et al., 2025). The transfer of Zn from soft tissues to the shell represents a critical mechanism by which *P. insularum* manages Zn exposure and prevents toxicity, ensuring that Zn levels in metabolically active tissues remain below harmful thresholds (Greville & Morgan, 1989; Taylor & Maher, 2006; Dallinger, 2024).

Fast Zn Regulation in Pineal Sacs (PS)

The PS in *P. insularum* play a pivotal role in the fast regulation of Zn, particularly in comparison to other soft tissues within the organism. The findings of this study suggest that the PS exhibits the most rapid response to fluctuating Zn levels, absorbing and redistributing Zn at a much faster rate than other tissues (Simonyi-Poirier et al., 2003; Vidyalakshmi et al., 2024). This rapid regulation likely reflects the physiological function of the PS as a site of Zn detoxification, ensuring that excess Zn does not accumulate in metabolically critical areas such as the muscle or DC (Conti et al., 2012; Nigariga et al., 2023).

The high variability in Zn concentrations within the PS, as seen in Table 1 (mean=44.5 mg/kg, SD=13.7 mg/kg), indicates that this tissue can quickly adapt to changes in environmental Zn availability. The correlation data in Table 2 further supports the capacity for fast regulation, which show moderate to high correlations between the PS and other tissues, such as the mantle and REM. These correlations suggest that the PS works in conjunction with other soft tissues to redistribute absorbed Zn, ensuring that it is either utilized in metabolic processes or transferred to detoxified storage sites like the shell or byssus (Maher et al., 2016; Priawandiputra et al., 2020).

Referring to Figure 2, the fast regulation of Zn in the PS can be explained through the available metabolically [A] component of Zn uptake (Rainbow, 2002). The PS appears to regulate Zn by ensuring that metabolically required Zn [AR] is quickly absorbed and made available for essential functions, while excess Zn [AE] is rapidly processed and either excreted [E] or transferred to detoxified stores (Ubrihien et al., 2017; Reolid et al., 2024). The PS's role as a Zn regulator is crucial in maintaining the organism's Zn homeostasis, preventing the accumulation of toxic levels in other soft tissues.

The unique physiological properties of the PS enable it to act as an initial Zn regulator, absorbing Zn quickly from the environment, particularly from water and sediment. The rapid turnover of Zn within the PS ensures that Zn is efficiently redistributed, preventing buildup in critical tissues like the muscle, which could impair metabolic processes (Yap et al., 2009; Bouzahouane et al., 2024). The ability of the PS to act as a "buffer" for Zn regulation highlights its importance in managing metal exposure in *P. insularum*, particularly in environments with fluctuating Zn concentrations (Athalye & Gokhale, 1994; Daka et al., 2006).

Hence, the PS in *P. insularum* functions as the primary site for fast Zn regulation, allowing the organism to respond quickly to changes in environmental Zn availability. The efficient absorption, redistribution, and detoxification of Zn in the PS contribute to the overall metal management strategy of *P. insularum*, ensuring that essential metabolic functions are preserved while minimizing the risk of Zn toxicity (Flessas et al., 2000;

Boulajfene et al., 2017). This rapid regulatory mechanism underscores the ecological resilience of *P. insularum* in Zn-contaminated environments and enhances its potential as a biomonitor for metal pollution in aquatic ecosystems (Conti et al., 2012; Vidyalakshmi et al., 2024).

Low Zn Accumulation in the Shells Due to Its Not Being a Storage Site for Zn

The model shown in Figure 2 illustrates the regulation of Zn in soft tissues, with key processes such as uptake [U], metabolically available forms [A], and excretion [E]. This model is applicable for understanding Zn dynamics in soft tissues of snails like *P. insularum* (Praveen Kumar & Uma Devi, 1998; Rasyid & Dody, 2018; Ibrahim et al., 2023). However, it is insufficient to explain the role of hard tissues, such as shells, in Zn storage and detoxification. In *P. insularum*, the shells have been shown to accumulate the lowest levels of Zn, unlike their role in storing other metals like Cd or Ni (Athalye & Gokhale, 1994; Liu et al., 2022; Benhamdoun et al., 2024). This suggests that the shell is not a primary storage site for excreted Zn, and the regulation of Zn occurs primarily in soft tissues (Maher et al., 2016; Krupnova et al., 2018; Messina et al., 2025).

The comparison of Zn with Ni and Cd in this study is particularly relevant due to the contrasting roles of hard and soft tissues in metal storage and detoxification. Unlike Ni and Cd, which have been reported to accumulate in the shells of *P. insularum* (Athalye & Gokhale, 1994; Liu et al., 2022; Wu et al., 2023), Zn shows a different regulatory mechanism, with preferential accumulation in soft tissues rather than shells (Maher et al., 2016; Krupnova et al., 2018; Kutluyer Kocabaş et al., 2023).

Snails employ different metal sequestration strategies, with shells often acting as passive sinks for non-essential metals like Ni and Cd, enabling long-term detoxification. In contrast, *P. insularum* regulates Zn primarily through physiological and biochemical pathways in soft tissues rather than direct deposition into shells. Unlike Cd and Ni, which are typically incorporated into shells or excreted as part of detoxification mechanisms, Zn is an essential metal regulated through homeostatic control in metabolic processes. The lower Zn accumulation in shells suggests that snails prioritize active excretion or redistribution within soft tissues rather than structural deposition (Bici et al., 2023; Ibrahim et al., 2023).

This distinction has important implications for biomonitoring. Previous studies (Athalye & Gokhale, 1994; Liu et al., 2022; Benhamdoun et al., 2024) highlight the effectiveness of shells in storing Cd and Ni, making them reliable indicators of long-term exposure. However, the low Zn accumulation in shells indicates that Zn monitoring should focus on soft tissues, where active regulation occurs. Relying solely on shell-based assessments may underestimate Zn contamination in aquatic environments. Therefore, soft tissue analysis is crucial for accurately assessing Zn bioaccumulation and detoxification mechanisms in *P. insularum*, ensuring a comprehensive understanding of metal exposure and ecological risk. Certain metals, such as Pb, Cr, and Co, follow different sequestration pathways in snails and fall beyond the scope of this study. Similarly, Hg and As exhibit distinct toxicokinetics and were not included due to their unique mechanisms of uptake and detoxification (Bici et al., 2024; Messina et al., 2025).

The model in Figure 2 proposes that Zn absorbed from the environment enters the metabolically available pool [A], where it is either utilized for essential

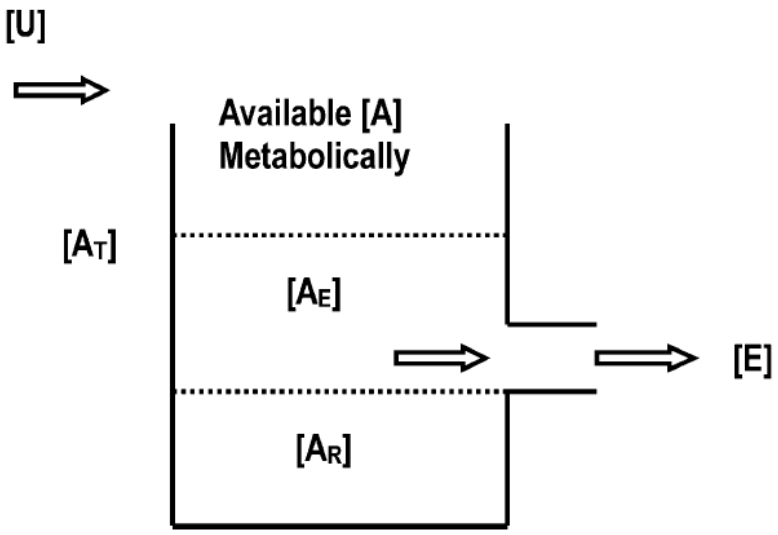

Figure 2. The trace metal accumulation pattern of an aquatic invertebrate which regulates the total body metal concentration of an essential metal by balancing uptake [U] with excretion [E]. All metal is accumulated in the metabolically available component [A], itself subdivided into the essential metal required for metabolic purposes [A_R], and excess metal [A_E] over and above this metabolic requirement (Rainbow, 2002).

metabolic functions [AR] or excreted [E] to prevent toxicity. In the case of *P. insularum*, this model suggests that soft tissues like the DC, foot, mantle, and CT actively regulate Zn levels through absorption and redistribution, ensuring that Zn concentrations remain within a safe range (Simonyi-Poirier et al., 2003; Ubrihien et al., 2017; Ibrahim et al., 2023). On the other hand, the shells do not participate significantly in Zn storage, as evidenced by the relatively low levels of Zn found in these tissues (Fang et al., 2001; Bouzahouane et al., 2024; Dallinger, 2024).

This finding aligns with the biological functions of the shell and its composition. The shell primarily comprises calcium carbonate and serves as a structural and protective barrier, rather than a detoxification site for metals like Zn (Reolid et al., 2024; Emami et al., 2024; Messina et al., 2025). Unlike Cd or Ni, which can be sequestered in the shell to prevent their accumulation in soft tissues, Zn is more actively regulated within the metabolic processes of the soft tissues (Taylor & Maher, 2006; Daka et al., 2006; Wu et al., 2023). The shell's low Zn levels indicate that it is not a repository for detoxified Zn, as is the case with other metals, but rather that Zn regulation is handled primarily through metabolic pathways in the soft tissues (Conti et al., 2012; Marimoutou et al., 2023; Ayanda et al., 2024).

Zn is essential for soft tissue enzymatic functions, cellular repair, and metabolic processes. The DC, in particular, plays a critical role in absorbing Zn from ingested material, while the mantle and foot contribute to the redistribution and utilization of Zn in physiological functions (Chukaeva & Petrov, 2023; Nigariga et al., 2023; Messina et al., 2025). The excretion mechanisms in these tissues ensure that excess Zn does not accumulate to toxic levels. For instance, Zn absorbed by the CT from environmental water is rapidly redistributed or excreted, preventing buildup (Greville & Morgan, 1989; Yap et al., 2009; Wu et al., 2023). This active regulation explains why soft tissues exhibit moderate Zn levels while the shell remains a minimal storage site (Brough & White, 1990; Menon et al., 2023; Benhamdoun et al., 2024).

The shell's minimal role in Zn storage can be further understood by considering the detoxification processes for other metals. Metals like Cd and Ni, which are more toxic at lower concentrations than Zn, require long-term storage sites to prevent their interference with metabolic processes. In these cases, the shell serves as a detoxification sink, where metals are stored inactive (Bu-Olayan & Thomas, 2001; Yap et al., 2010; Paul & Aditya, 2024). On the other hand, Zn is less toxic and more actively utilized in metabolic processes, meaning that the organism relies on metabolic regulation in soft tissues rather than passive storage in the shell (Flessas et al., 2000; Fang et al., 2001; Bici et al., 2024).

Therefore, the low Zn accumulation in the shells of *P. insularum* indicates that the shell is not a storage site for excreted Zn, in contrast to its role in storing other metals like Cd and Ni. The regulation of Zn occurs primarily in soft tissues, where metabolic processes control Zn absorption, redistribution, and excretion (Edward et al., 2010; Boulajfene et al., 2017; Ibrahim et al., 2023). This model emphasizes the active role of soft tissues in maintaining Zn homeostasis while the shell remains uninvolved in Zn detoxification. Therefore, while the model effectively explains Zn dynamics in soft tissues, it does not apply to hard tissues like the shell, which do not serve as storage sites for Zn (Yap et al., 2009; Maher et al., 2016; Dallinger, 2024).

Accumulation Without Excretion in the Digestive Caecum (DC)

The regulation of Zn within *P. insularum* demonstrates a sophisticated mechanism where the DC plays a pivotal role in Zn accumulation without subsequent excretion (Figure 3). The findings of this study reveal that the DC exhibits the highest Zn concentrations among the examined tissues, with a mean of 279 mg/kg, underscoring its primary function in Zn absorption (Priawandiputra et al., 2020; Marimoutou et al., 2023). This elevated Zn level in the DC indicates that *P. insularum* ingests Zn-laden sediment as a fundamental component of its diet. As a grazer and sediment feeder, *P. insularum* relies heavily on sediment particles as a food source and a medium for metal uptake (Simonyi-Poirier et al., 2003; Conti et al., 2012). The DC processes these ingested sediments, extracting nutrients while concurrently accumulating Zn from the particulate matter. This dual role of the DC as both a digestive and metal-absorbing organ highlights its significance in the overall bioaccumulation strategy of *P. insularum*, making it a critical focus for understanding Zn dynamics in contaminated environments (Maher et al., 2016; Nigariga et al., 2023).

The accumulation of Zn in the DC without active excretion mechanisms suggests that *P. insularum* employs a strategy of internal sequestration to manage excess Zn. Unlike other tissues that participate in Zn redistribution and detoxification, the DC acts as a primary storage site where Zn is retained rather than expelled (Priawandiputra et al., 2020; Nigariga et al., 2023). This accumulation without excretion can be interpreted as a regulatory adaptation that allows *P. insularum* to maintain essential Zn levels for metabolic functions while preventing the toxicity associated with excessive Zn exposure (Conti et al., 2012; Liu et al., 2022). The high Zn concentration in the DC indicates that Zn is primarily retained within this tissue, minimizing its bioavailability in other metabolically active tissues and thereby reducing the risk of Zn-induced toxicity (Maher et al., 2016; Emami et al., 2024).

Furthermore, the elevated Zn levels in the DC reflect the organism's reliance on sediment ingestion as a significant route of metal exposure. The dynamic distribution of Zn within *P. insularum*'s tissues is evident from the substantial Zn concentrations observed in the

REM, which serve as intermediate storage sites (Simonyi-Poirier et al., 2003; Edward et al., 2010). The mantle and foot, exhibiting moderate Zn concentrations, facilitate the redistribution of Zn absorbed by the DC to other tissues involved in metabolic processes and eventual detoxification (Krupnova et al., 2018; Rasyid & Dody, 2018). The CT, with its high correlation with the foot (R=0.95), plays a crucial role in absorbing Zn directly from water, complementing the sediment-based absorption in the DC (Athalye & Gokhale, 1994; Vidyalakshmi et al., 2024). This interconnected network of tissues ensures that Zn is efficiently managed within the organism, with the DC acting as a central hub for Zn accumulation (Bu-Olayan & Thomas, 2001; Yap et al., 2010).

The absence of significant Zn excretion from the DC highlights a unique aspect of *P. insularum*'s metal regulation system. Instead of actively removing excess Zn, *P. insularum* relies on internal sequestration within the DC and subsequent redistribution to detoxification sites such as the shell and byssus (Menon et al., 2023; Reolid et al., 2024). This strategy minimizes the physiological burden associated with metal excretion and leverages the DC's role as a primary storage organ to maintain Zn homeostasis. By accumulating Zn within the DC, *P. insularum* effectively buffers against fluctuating environmental Zn levels, ensuring that essential metabolic processes remain unaffected by external Zn variability (Taylor & Maher, 2006; Chukaeva & Petrov, 2023). This mechanism of accumulation without excretion underscores the organism's resilience and adaptability to Zn-contaminated environments, making it a robust biomonitor for assessing long-term Zn exposure in coastal ecosystems (Greville & Morgan, 1989; Marimoutou et al., 2023).

Therefore, the DC's role in *P. insularum* exemplifies an effective strategy for Zn regulation through accumulation without excretion. This mechanism allows the organism to sustain necessary Zn levels for metabolic functions while preventing toxic Zn accumulation in critical tissues (Yap et al., 2009; Conti et al., 2012). Understanding this tissue-specific accumulation pattern provides valuable insights into the metal-handling capabilities of *P. insularum* and reinforces its utility as a biomonitor for Zn contamination in marine environments.

Zinc Detoxification and Storage in the Shell

The shell plays a vital role in detoxifying and storing excess Zn, effectively preventing the accumulation of toxic levels in metabolically active tissues (Maher et al., 2016; Boulajfene et al., 2017). While the shell exhibited the lowest Zn concentrations compared to soft tissues, its role as a long-term storage site is critical (Bu-Olayan & Thomas, 2001; Liu et al., 2022). The shell's ability to sequester Zn in an inert form allows *P. insularum* to tolerate environments with high metal contamination without suffering from Zn-induced toxicity (Krupnova et al., 2018; Menon et al., 2023). This finding is consistent with studies on other molluscs, where calcified structures like shells or exoskeletons serve as repositories for heavy metals, protecting soft tissues from metal overload (Simonyi-Poirier et al., 2003; Edward et al., 2010). The significant regression relationship between the shell and other tissues, particularly the CT and DC, further emphasizes the shell's role as the final destination for Zn that has been processed and redistributed by soft tissues (Boulajfene et al., 2017; Marimoutou et al., 2023).

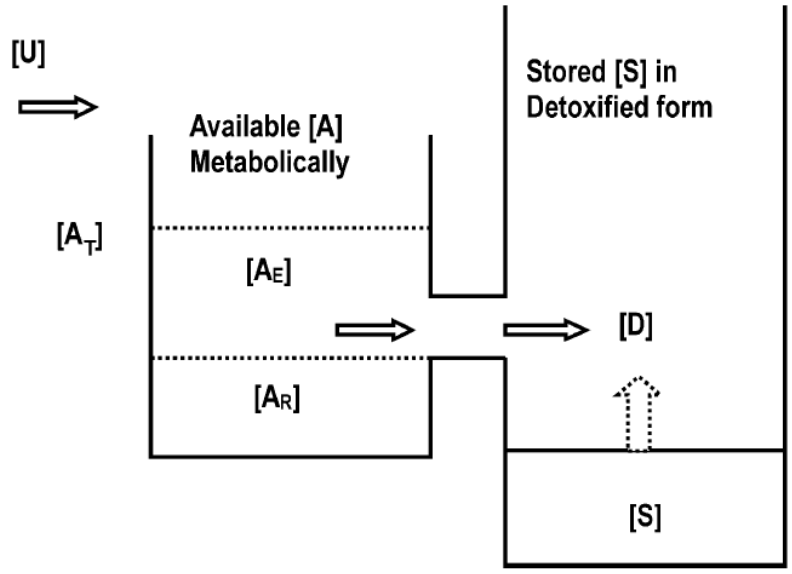

Figure 3. The trace metal accumulation pattern of an aquatic invertebrate that is a net accumulator of an essential metal without excretion of metal taken up. Metabolically available metal in excess of requirements is detoxified [D] to be stored [S] as the detoxified component of accumulated metal with no upper concentration limit. Other details as in Figure 1. This accumulation pattern is exemplified by zinc in barnacles (Rainbow, 2002).

The low correlation between the shell and other soft tissues supports the hypothesis that once Zn is transferred to the shell, it is no longer actively involved in redistribution. This finding aligns with the understanding that the shell acts as a detoxification sink, where Zn is permanently stored, minimizing its bioavailability and preventing potential toxic effects (Greville & Morgan, 1989; Conti et al., 2012). The low variability in Zn concentrations within the shell across the 13 sampling sites suggests that the shell provides a reliable indicator of long-term Zn exposure, making it a valuable tissue for biomonitoring efforts in environments with fluctuating Zn contamination (Praveen Kumar & Uma Devi, 1998; Chukaeva & Petrov, 2023). Additionally, the shell's structural stability allows it to accumulate Zn over the organism's lifetime, providing a cumulative record of metal exposure that reflects both past and present environmental conditions (Brough & White, 1990; Krupnova et al., 2018).

Interestingly, the shell's relatively low Zn concentrations, despite its role in long-term storage, may indicate that *P. insularum* regulates the total amount of Zn it stores in calcified structures. This regulation likely prevents excessive Zn accumulation, which could otherwise interfere with the shell's structural integrity or contribute to shell fragility (Flessas et al., 2000; Emami et al., 2024). Sequestering Zn in the shell while maintaining its mechanical properties is crucial for *P. insularum*, as it provides physical protection against predators and environmental stressors (Athalye & Gokhale, 1994; Rasyid & Dody, 2018). Therefore, the organism's ability to balance Zn detoxification with maintaining shell strength highlights a sophisticated biological strategy for coping with metal exposure in contaminated habitats (Fang et al., 2001; Reolid et al., 2024).

Tissue-Specific Roles in Zinc Redistribution

The correlation and factor analyses further elucidate the tissue-specific roles in Zn redistribution, which highlight the cooperative nature of Zn management across different organs (Simonyi-Poirier et al., 2003; Edward et al., 2010). The mantle's strong negative loading on Factor 1 (-0.70) and its significant correlations with other tissues, particularly the CT and REM, suggest that the mantle plays a key role in processing Zn absorbed from various sources (Praveen Kumar & Uma Devi, 1998; Krupnova et al., 2018). As the mantle is involved in shell formation and other metabolic processes, it likely processes Zn for immediate use, particularly for enzymatic reactions and structural development. Once the Zn is no longer needed for these metabolic functions, the mantle redistributes it to other tissues, such as the REM and shell, for storage or detoxification (Flessas et al., 2000; Maher et al., 2016).

The CT, with its strong correlation with the foot and significant regression relationship with the shell, plays an essential role in Zn absorption from the environment (Rasyid & Dody, 2018; Nigariga et al., 2023). The CT's involvement in Zn redistribution is crucial for managing the bioavailability of Zn in tissues involved in environmental interaction, such as the foot (Conti et al., 2012; Boulajfene et al., 2017). The high loading of the REM on Factor 3 suggests that these tissues serve as intermediate storage sites, holding Zn temporarily before transferring it to the shell for long-term detoxification. This process ensures that Zn is kept in bioavailable form for metabolic use but is eventually sequestered in the shell to prevent toxicity (Liu et al., 2022; Marimoutou et al., 2023).

The DC, primarily responsible for absorbing Zn from ingested sediment, exhibited a moderate loading on Factor 4 (0.48). This loading indicates that the DC plays a significant role in Zn absorption but does not participate in long-term storage (Ubrihien et al., 2017; Reolid et al., 2024). The correlation between the DC and other tissues, such as the foot and mantle, highlights the tissue's role in distributing Zn to other organs after processing it from ingested material. The DC's function as the primary organ for handling ingested Zn reinforces the idea that *P. insularum* manages Zn through multiple pathways, with different tissues specializing in either absorption, redistribution, or storage (Maher et al., 2016; Chukaeva & Petrov, 2023).

Implications for Biomonitoring and Environmental Health

The ability of *P. insularum* to regulate Zn through tissue-specific absorption, redistribution, and detoxification highlights its effectiveness as a biomonitor for aquatic ecosystems (Bu-Olayan & Thomas, 2001; Fang et al., 2001). The shell, serving as a long-term repository, provides a stable record of Zn exposure, making it particularly useful for assessing chronic contamination across different environments (Flessas et al., 2000; Taylor & Maher, 2006). Its consistent Zn concentrations across sampling sites further support its reliability in long-term monitoring, especially in areas impacted by industrial and agricultural runoff (Athalye & Gokhale, 1994; Emami et al., 2024).

In contrast, the CT, foot, and mantle tissues respond more dynamically to environmental changes, making them valuable indicators of short-term fluctuations in Zn levels (Greville & Morgan, 1989; Priawandiputra et al., 2020). These tissues rapidly absorb and redistribute Zn, enabling early detection of pollution events before they cause significant ecological damage (Menon et al., 2023; Vidyalakshmi et al., 2024).

By efficiently sequestering Zn in its shell without impairing metabolic functions, *P. insularum* demonstrates resilience in metal-contaminated environments (Marimoutou et al., 2023; Nigariga et al., 2023). Understanding Zn bioaccumulation patterns across different tissues enhances environmental

monitoring strategies and informs mitigation efforts. This study provides valuable insights into how *P. insularum* can be utilized in biomonitoring programs, contributing to improved assessment and management of Zn pollution in aquatic ecosystems (Yap et al., 2009; Conti et al., 2012).

Conclusion

Pomacea insularum employs a tissue-specific strategy to regulate Zn exposure, with the DC absorbing Zn from sediment, while the CT and foot facilitate uptake from water and sediment. Redistribution across soft tissues, including the mantle, REM, and foot, ensures Zn availability for metabolic functions, while the shell acts as a long-term detoxification site. The relatively stable but low Zn concentrations in the shell provide a historical archive of exposure, reinforcing its role as a biomonitor for metal contamination. Strong correlations and regression relationships between Zn levels in soft tissues and the shell indicate an active, dynamic transfer over time, supporting a multi-tissue regulatory mechanism. These findings highlight the significance of *P. insularum* as a biomonitor, integrating short-term Zn dynamics in soft tissues with long-term accumulation in the shell, making it a valuable model for assessing Zn bioaccumulation and environmental metal pollution in aquatic ecosystems.

Ethical Statement

Not applicable since all the snails were collected from the wild in abundance.

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Author Contribution

Conceptualisation, C.K.Y. and K.A.A.-M.; methodology and validation, C.K.Y. and K.A.A.-M.; formal ana lysis, C.K.Y.; investigation, C.K.Y.; resources, K.A.A.-M.; data curation, C.K.Y.; writing—original draft preparation, C.K. Y.; writing—review and editing, C.K.Y. and K.A.A.-M. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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