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Distribution of Heavy Metal Concentrations in *Trisidos kiyonoi*: A Biomonitoring Concern

Chee Kong Yap[1*], Franklin Berandah Edward[2], Wan Hee Cheng[3], Krishnan Kumar[3], Hishamuddin Omar[1], Rio Risandiansyah[4], Chee Wah Yap[5] and Meng Chuan Ong[6,7,8]

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2]Natural Resources and Environment Board, Petra Jaya, 93050 Kuching, Sarawak, Malaysia

[3]Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, 71800 Nilai, Negeri Sembilan, Malaysia

[4]Faculty of Medicine, Universitas Islam Malang, Malang, Indonesia

[5]MES SOLUTIONS, 22C-1, Jalan BK 5A/2A, Bandar Kinrara, 47100 Puchong, Selangor, Malaysia

[6]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[7]Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[8]Ocean Pollution and Ecotoxicology (OPEC) Research Group, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu

ABSTRACT

Locally known bivalves "siput pulas", *Trisidos kiyonoi*, were collected from kampong Panchor, Pantai Remis, Perak. The bivalves were then dissected into eight different parts of soft tissue: the remainder, muscle, mantle, byssus, foot, gills, gonads, and shell. The soft tissues were then analyzed for Cu, Cd, Zn, Pb, Ni and Fe. The byssus included the highest quantities of Cd, Cu, Fe, Pb, and Ni among the soft tissues, while the mantle contained the highest concentrations of Zn. This study suggested that byssus is a good biomonitoring organ for Cd, Cu, Fe, Pb and Ni, while shell was suggested as a good biomonitoring tool for Cd, Pb and Ni. This biomonitoring information on the metal distribution in *T. kiyonoi* is vital for future reference.

***Corresponding author**

Chee Kong Yap, Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
E-mail: yapckong@hotmail.com; yapchee@upm.edu.my

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Introduction

Using the whole tissue of mollusks to evaluate heavy metal contamination in coastal areas had a few problems. The use of total tissue to measure heavy metal concentrations may be influenced by a number of factors, such as gonadal circumstances, rainfall, and varied habitat conditions, as opposed to heavy metals caused by human activity [1]. In addition, total tissues may not effectively accumulate heavy metals due to the presence of numerous regulatory mechanisms (easily mobilized compartments) in, which allow certain metals to exist temporarily in the tissues prior to being excreted from the body. In order to evaluate the heavy metal concentrations in coastal areas using a mollusc, the metal must "remain permanently" in the tissue (or a specific organ), making the identification of organs in molluscs that can bind specific metal(s) essential [2].

The phenomena of high concentration variability in soft tissue, such as green-lipped mussel *Perna viridis*, necessitated the proposal of a specific organ in mollusks to improve the precision of heavy metal measurements [3]. According certain metal(s) only attach to specific organ cells that have a high affinity for that metal(s) [3-5]. Therefore, the usage of specific organs in molluscs rather than whole soft tissues is essential (2003). According the binding of hazardous metals to metallothioneins could fix the metals inside various tissues, resulting in a sluggish turnover rate (slow depuration) for these metals [6]. There are also variances in the contact surfaces of various soft tissues. Some tissues (gill) in *P. viridis* may have had the most contact surface with their surrounding seawater, while others (foot and muscle) had the least [3]. In addition, distinct rates of metal buildup and excretion are observed in various soft tissues. The discrepancies in accumulation and depuration rates revealed that they were the product of metal treatment and regulation within the organism [7,8].

In order to monitor certain metals in the marine environment, it is essential to utilize just specific organs. No report on the use of *Trisidos kiyonoi* (Family: Arcidae) to monitor heavy metal contamination has been undertaken in Malaysia prior to this one. In comparison to other species, the metabolic clearance of metals deposited in the soft tissues of molluscs such as *T. kiyonoi* is relatively slow [9]. Therefore, the objective of this study to provide the baseline information on the distribution of six heavy metals in the bivalve T. kiyonoi.

Material and Procedure

On 25 February 2006, samples of *T. kiyonoi* were purchased from a stall located at Kampung Panchor, Pantai Remis, Perak (N 04°31' 39.5" E 100°39' 16.5") (Figure 1). The bivalves were placed in a clean plastic bag, frozen, and transported to the laboratory. When the samples arrived at the laboratory, they were held at -10° until analysis. The bivalves were thawed at room temperature on clean tissue paper before to examination. The tissue was then completely removed from its shell. The tissue was subsequently divided into eight distinct components, including the remnant, muscle, mantle, byssus, foot, gill, and shell. The silt that adheres to the shell was also gathered. The samples were dried to a constant weight for 72 hours at 60° [10].

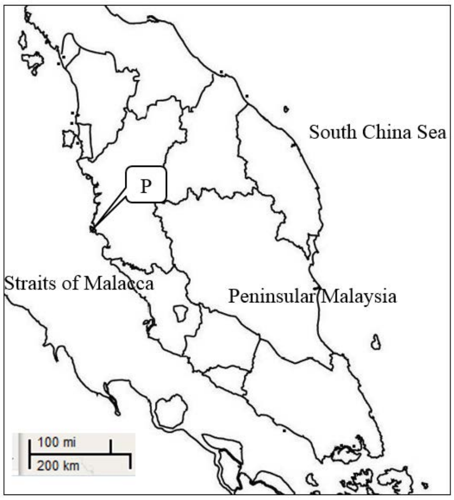

Figure 1: Map showing the sampling sites for *Trisidos kiyonoi* in Pantai Remis (P), in the west coast of Peninsular Malaysia

The soft tissues of T. kiyonoi and sediments were digested in a 69% nitric acid solution. They were exposed to a low temperature of 40°C for 1 hour before being placed in a hot block digester for at least 3 hours at a temperature of 140°C to ensure thorough digestion. The digested samples were then diluted with double-distilled water to a specific volume. The sample was subsequently filtered via Whatman No. 1 filter paper, and the filtering was stored until metal analysis. The information was reported in micrograms per gramme of dry weight. The concentrations of the samples were calculated using calibration curves that were produced from the analysis of multi-level calibration standards. Each metal's 1,000 mg/l stock solution was used to prepare standard solutions (Merck Titrisol). The analytical methods for gastropods were confirmed using a standard reference sample, dogfish liver (DOLT-3, National Research Council of Canada) and all metal recoveries were satisfactory (85-115%).

Results and Discussion

Table 1 displays the mean concentrations (mg/kg dry weight) of Cd, Cu, Cd, Zn, Pb, and Ni, and Fe in the various soft tissues of *T. kiyonoi* and its habitat sediments, as well as a comparison to the food-safe limits. For muscle, the Cu concentrations ranged from 3.04-3.67; Cd 1.78-2.10; Zn 55.4-60.8; Pb 2.27-3.66; Ni 1.33-1.97; and Fe 328-368. For mantle, Cu concentrations ranged from 3.24 to 4.74, Cd from 2.05-2.56, Zn from 67.8 to 82.1, Pb from 3.63 to 4.55, Ni from 2.32 to 3.53, and Fe from 500 to 636.

For foot, the Cu concentrations ranged from 6.61-7.43; Cd 1.51-1.86; Zn 56.7-59.4; Pb 2.71-3.19; Ni 0.717-1.083; and Fe 555-556. For gill, the Cu ranged from 5.36-6.16; Cd is 3.50-3.97; Zn 56.66-61.1; Pb 3.54-4.62; Ni 2.99-4.12; and Fe 627-873. In contrast, for the gonad, the Cu concentrations ranged from 2.95-3.81, Cd 1.66-1.78, Zn 41.6-49.8, Pb 3.85-4.65, Ni 0.892-1.39, and Fe 264-362. For shells, the Cu concentrations ranged from 2.64-3.80, Cd 3.44-3.84, Zn 5.77-7.17, Pb 30.6-47.5, Ni 27.2-28.9, and Fe 66.4-365.

The average amounts of heavy metals (in mg/kg dry weight) in *T. kiyonoi* samples of different sizes are shown in Figure 3. It is clearly shown that smaller sized (59.5 to 62.6mm) group of *T. kiyonoi* accumulated higher amounts of Cu, Zn, and Fe, which recorded concentrations of 8.14, 64.7, and 557, respectively, compared to the larger size group. The dilution effect due to unequal growth relative to metal accumulation is believed to be responsible for the lower concentration of metals in larger individuals compared to smaller individuals [11, 12]. Gilek et al found that the size of the mussel *M. edulis* had an impact on its bioaccumulation because tissue concentrations decreased as body size increased and size-dependent variations in the absorption rate were mostly responsible for the observed changes in bioaccumulation. In contrast, Cd, Pb, and Ni concentrations were higher in the bigger bivalves than in the smaller ones, accumulating 2.64, 8.00, and 4.93, respectively. In fact, it has been discovered that the clam *Macoma bathica* body size and the amount of Ag present in its soft tissues correlate positively [13-15]. This is because some bivalves can accumulate large amounts of Ag by encapsulating it in AgS2, a stable, insoluble, and non-toxic compound [16-19]. Therefore, in *T. kiyonoi*, the presence of a Cd, Pb, and Ni detoxification/sequestration mechanism that is somewhat more efficient in larger individuals could account for the observed increase in bioconcentration efficiency with size. Accumulation of high amounts of metals (either large or little) may also be associated with feeding habits, morphological or physiological characteristics of the bivalve [20].

Figure 2: The *Trisidos kiyonoi* bought from Kampung Panchor, Pantai Remis, Perak

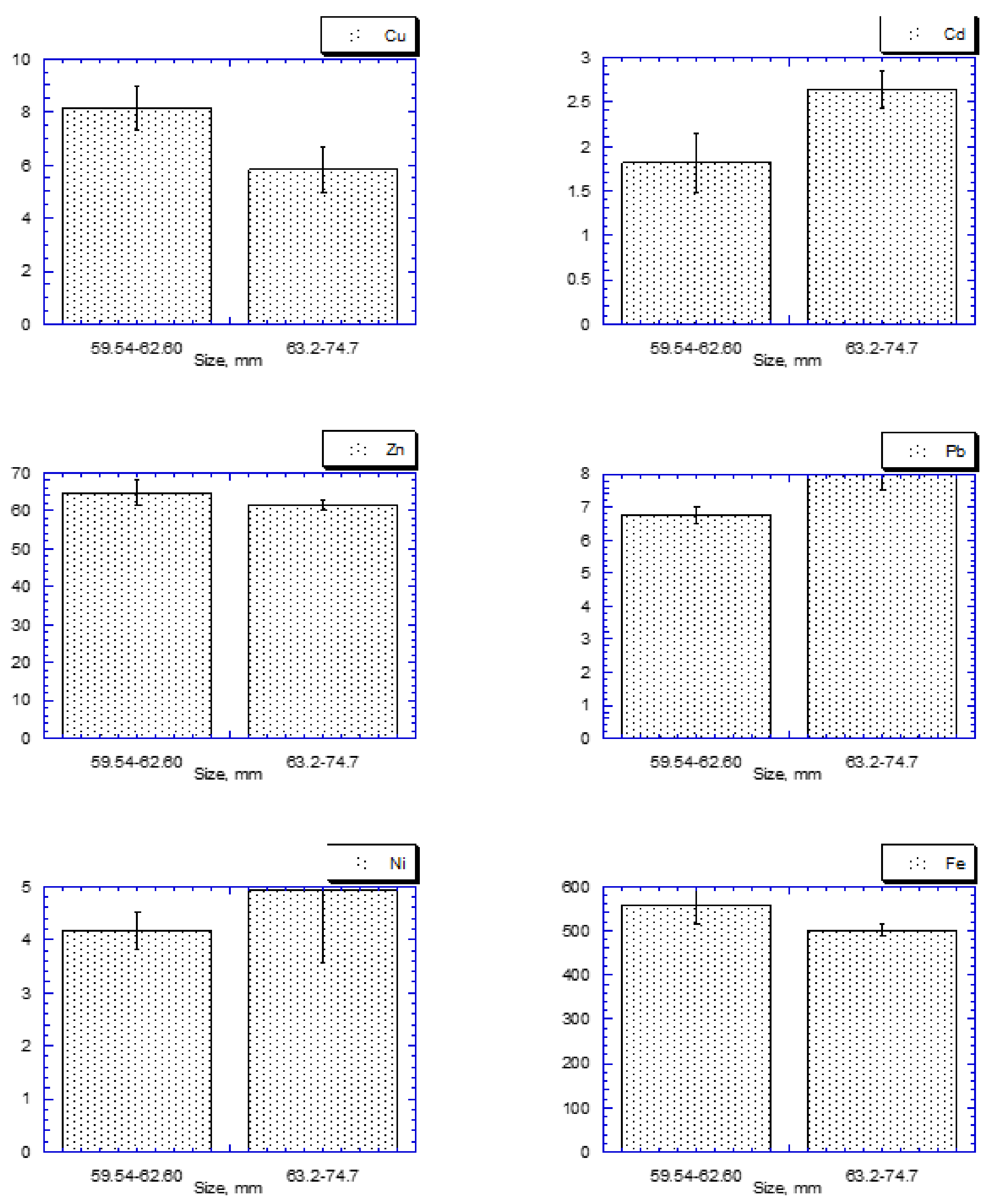

Figure 3: Concentrations (mean ± standard error, mg/kg dry weight) of heavy metals (Cu, Cd, Zn, Pb, Ni and Fe) in the different sizes of *Trisidos kiyonoi*.

According to Table 1, byssus accumulated the highest Cu concentrations (66.1), while shell accumulated the lowest (3.22). Sediment recorded the greatest levels of Cd (4.97), while the remainder recorded the lowest Cd level (1.35). The mantle reported the highest concentration of Zn (73.4), whereas the shell recorded the lowest Zn concentration (6.30). Shell accumulated the greatest Pb level (40.2), whilst the remainder accumulated the lowest Pb level (2.84). The shell accumulated the highest level of Ni (28.3), whereas foot accumulated the lowest Ni concentration (0.90). The byssus had the highest Fe concentrations (2256), whilst shell had the lowest Fe concentrations (186).

Table 1: Concentrations of (mean ± standard error, mg/kg dry) of heavy metals (Cu, Cd, Zn, Pb, Ni and Fe) in the different part of soft tissues of Trisidos kiyonoi and its comparison with the maximum permissible limits

Tissues	Cu	Cd	Zn	Pb	Ni	Fe
Remainder	6.45 ± 0.51	1.35 ± 0.15	50.7 ± 3.47	2.84 ± 0.16	2.20 ± 0.22	376 ± 32.1
Muscle	3.31 ± 0.19	1.93 ± 0.09	57.5 ± 1.71	2.91 ± 0.41	1.74 ± 0.20	349 ± 11.6
Mantle	3.92 ± 0.44	2.34 ± 0.15	73.4 ± 4.46	4.22 ± 0.29	2.76 ± 0.39	587 ± 43.8
Byssus	66.1 ± 0.01	4.69 ± 0.01	53.1 ± 0.001	30.3 ± 0.01	16.4 ± 0.01	2256 ± 0.01
Foot	7.02 ± 0.41	1.69 ± 0.17	58.1 - 1.34	2.95 ± 0.24	0.90 ± 0.18	555 ± 0.50
Gill	5.73 ± 0.43	3.74 ± 0.23	58.8 ± 2.24	4.08 ± 0.54	3.55 ± 0.57	750 ± 123
Gonad	3.38 ± 0.43	1.72 ± 0.06	45.7 ± 4.09	4.25 ± 0.40	1.14 ± 0.25	313 ± 49.1
Shell	3.22 ± 0.33	3.60 ± 0.12	6.30 ± 0.437	40.2 ± 5.01	28.3 ± 0.54	186 ± 91.2
ABIA (1991) [34]	150	5.00	250	10.0	NA	NA
MPHT (1986) [35]	133	NA	667	6.67	NA	NA
USFDA (1990) [37]	NA	25.0	NA	11.5	NA	NA
NHMRC (1987) [37]	350	10.0	750	NA	NA	NA

Note: NA=Not available
Maximum permissible levels established by Brazilian Ministry of Health (ABIA)
Permissible limit set by Ministry of Public Health, Thailand (MPHT)
Food and Drug Administration of the United States (USFDA)
Australia Legal Requirements (NHMRC)

Several points were uncovered through this analysis. The presence of higher heavy metal (Cu, Cd, and Fe) concentrations in the byssus paralleled the high concentrations of these metals in the sediment. For high amounts of Pb and Ni in sediment, higher concentrations of that metal were also detected in the shell. This event revealed that byssus is an effective biomonitoring tool for Cu, Cd, and Fe, while shell was proposed for Pb and Ni. The high Cu, Cd, and Fe concentrations in byssus may be a result of its role as a metal excretion pathway. In *P. viridis* green-lipped mussels, excessive metal concentrations, which become poisonous at high levels, are expelled through the byssus before they reach the threshold level. Yap et al reported the significance of byssus as a pathway for the elimination of high metal concentrations [21-24]. The buildup of elevated levels of Cd and Zn in the byssus tissue, which were higher than in the majority of other soft tissues of the mussel, provided evidence that the byssus serves as the short-term compartment for Cd and Zn removal [23]. According to Yap et al., the highest rates of accumulation and depurations and the lowest percentage of decrease in byssus indicated that byssus was a sensitive organ for Cu and that it operated as a possible exist route for Cu. Byssus is composed of collagen fibres and grows continuously; heavy metals may be discharged through the byssus and exhibit varying quantities of heavy metals in the byssus [21,25]. The presence of high concentrations of Pb and Ni in the shell of *T. kiyonoi* may be a result of the metals being incorporated into the crystalline lattices of the shell, similar to what occurred during the depuration period with elevated concentrations of Cd, Pb, and Zn in the shell of P. viridis. This can be explained by the fact that some trace metals are either linked to the organic matrix of the shell or are incorporated into the molluscs and barnacle shells by replacing the calcium ion in the crystalline phase of the shell [26-32]. Yap et al. (2003f) showed that Cd and Pb concentrations in the shells of *P. viridis* are less variable than in the entire soft tissues of this species. Bourgoin also discovered that Pb concentrations in the shells of *M. edulis* are less variable than in the soft tissues. The aforementioned evidence supported the appropriateness of *T. kiyonoi* shell as a biomonitoring tool for Pb and Ni contamination in their natural environment. Elevated Zn contents in the mantle of *T. kiyonoi* suggested that it is a useful biomonitoring organ for Zn contamination. The mantle may have high binding affinities for Zn, and metallothionein may play a key role in Zn detoxification; hence, the mantle has large quantities of Zn [33].

Meanwhile, the foot, gill, gonad, and muscle accumulate low levels of heavy metals. This may be because softer tissues have less surface interactions with the surrounding water. The low level of metal concentrations in soft tissues may also be influenced by gonadal state. In addition, it is possible that the low binding affinities of metals in soft tissues contributed to the inability of metal ions to bind to soft tissue cells, hence reducing the accumulated metal concentrations. The comparison of the maximum permissible limits of metals in the edible soft tissues of *T. kiyonoi* in Table 1 revealed that the quantities of heavy metals in all edible tissues were below the food-permissible limit established by authorities in Brazil, Thailand, the United States, and Australia. This lead to a conclusion that the *T. kiyonoi* bought from Kampung Panchor, Pantai Remis, Perak are safe for human consumption.

Conclusion

The *T. kiyonoi*'s byssus (for Cu, Cd, and Fe), mantle (for Zn), and shell (for Pb and Ni) have the potential to accumulate heavy metal concentrations, as demonstrated by this study. The capability is determined by the heavy metal concentrations recorded, which mirrored the concentrations recorded in the bivalve shell sediment. To establish its potential as a biomonitor for heavy metal pollution in Malaysian coastal waters, additional research is required. Consequently, the current baseline information is essential for future reference.

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