

Bioaccumulation and Distribution of Heavy Metals (Cd, Cu, Fe, Ni, Pb and Zn) in the Different Tissues of *Chicoreus capucinus* Lamarck (Mollusca: Muricidae) Collected from Sungai Janggut, Kuala Langat, Malaysia

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Abstract

Knowledge on accumulation and distribution of metals in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals in the different parts of molluscs such as *Chicoreus capucinus*. *Chicoreus capucinus* was collected from intertidal areas of Sungai Janggut mudflat, Kuala Langat, Selangor and analysed for heavy metals content in the tissues. The capability of the different parts to accumulate heavy metal from the environment was measured by calculating their Biota-Sediment Accumulation Factor (BSAF) values. From this preliminary investigation, it was found that the highest concentrations of Cu were found in the caecum ($194 \pm 24.4 \mu\text{g/g dw}$), Cd in digestive gland ($32.9 \pm 0.000 \mu\text{g/g dw}$) and Fe in operculum ($971 \pm 2.50 \mu\text{g/g dw}$). For Ni and Pb, high concentrations in shell were observed and Zinc high levels in most of the tissues studied except shell and operculum. On the other hand, highest BSAF values were obtained in caecum for Cu (101.2), Zn (27.4) and Cd (53.1), while highest BSAF values were obtained in shell for Pb (32.6) and Ni (8.88). However, in general, most of the different parts of the gastropod could be suggested as macro concentrator organs, since the BSAF values were greater than 2. More studies should be conducted in the future to determine the potential of *C. capucinus* as biomonitor.

Keywords: heavy metals; sequential extraction; sediment; mollusc; biomonitor

1. Introduction

Numerous factors may affect the bio-concentrations of heavy metal in mollusks tissues. The concentrations of heavy metal accumulated by marine organisms are not only depending on the water quality but also seasonal factor, temperature, salinity, diet or food intake, spawning and individual variation (Hamed and Emara, 2006). The bioaccumulation of heavy metals by marine mollusc and other marine organisms may reach many orders of magnitude above background concentrations of certain locality. This phenomenon may demonstrate the potential of these species as a biomonitor of heavy metal pollution (Chan, 1989; Hamed and Emara, 2006). Biomonitoring agents can be assessed by analysing heavy metals in the whole tissues or certain parts or tissues of organisms. Many studies have shown that intertidal molluscs can be good biomonitoring organisms (Ismail, 2006). Due to heavy metals contamination are very localised and closed to discharge point sources (Ismail *et al.*, 1993) and molluscs inhabit in different microhabitat of intertidal areas, a detail studies on different species and tissues are important.

In this study, different parts of *Chicoreus capucinus* were analysed for heavy metals in order to propose this species as biomonitor. The use of different tissues is believed could overcome the inaccuracies incurred when determining heavy metal levels in the total soft tissues since it may not be accurately reflective for certain contaminant concentrations in individual target tissues of the organism. This was based on the fact that different tissues accumulate metals at different rates and that the biological half-lives of metals at each type of soft tissues also differ from one another (Lakshmanan and Nambisan, 1989; Yap *et al.*, 2007). Each different parts of the gastropod may play a different function whether in its metabolic or physiological roles. This may influence the distribution of metals in the different soft tissues of molluscs. Therefore, knowledge on metal distribution in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals in the different parts of molluscs, thus, helping in proposing the potential biomonitor to monitor heavy metal contamination in Sungai Janggut or similar types of environment.

Sungai Janggut areas are well-known for its

aquaculture activities for prawn. Beside industries, urbanisations, transportation and shipping activities, aquaculture also may contribute to the elevation of heavy metals in the coastal environment. Many studies have shown that shrimp farming contributed to the elevation of heavy metals in the environment (Ismail, 2008).

Chicoreus capucinus is the commonest predators of barnacles and bivalves available in Sungai Janggut area. This predator snail prey their victims by drilling a hole through the shell and sucking out the contents using their narrow proboscis (Lim et al., 2001). This group of snails is known to have boring organ under their foot, which can produce carbonic acid to soften the shell and later make a hole for the proboscis to suck the prey. This species, which shells are usually measured at 4-5 cm in height, is thick with sculptured ridges down the length. They are sometimes seen abundant in the mangrove trees (Tan and Chou, 2000). Its position in the tropics levels of intertidal food webs and very limited movement in its habitat, this study could propose its possibility to be a good biomonitor for heavy metals for its kind of habitat. Thus, the objective of this study is to provide important information of the metal distribution in the different parts of *C. capucinus*, investigate its capability to accumulate heavy metal from the environment by

determining its Biota-Sediment Accumulation Factor (BSAF) and suggesting its possibility as biomonitor for heavy metals.

2. Materials and Methods

2.1. Sampling, storage and sample preparation

A sampling was conducted in Sungai Janggut, Kuala Langat Selangor (N 03° 9' 36" E 101° 19' 12.0") in April 2006. Sungai Janggut (Fig. 1) is a mangrove area where fishing and prawn aquaculture activities are conducted. To avoid differences in metal concentrations because of size or reproductive stage, only the commercial-sized individuals were collected and analysed (Saavedra et al., 2004). About 25-30 of *C. capucinus* (sizes 40-55 mm) were hand picked from the study site for the analysis of heavy metals in their different tissues. Collected samples were brought back to laboratory in iced box and kept frozen prior to dissection and analysis. The gastropods were then dissected and pooled into respective tissues of digestive caecum, foot, mantle, muscle, remainder and tentacle. All the different group of tissues were pooled together accordingly and dried at 60°C to constant dry weights. Besides these soft tissues, all the shells and operculum were also pooled and analysed.

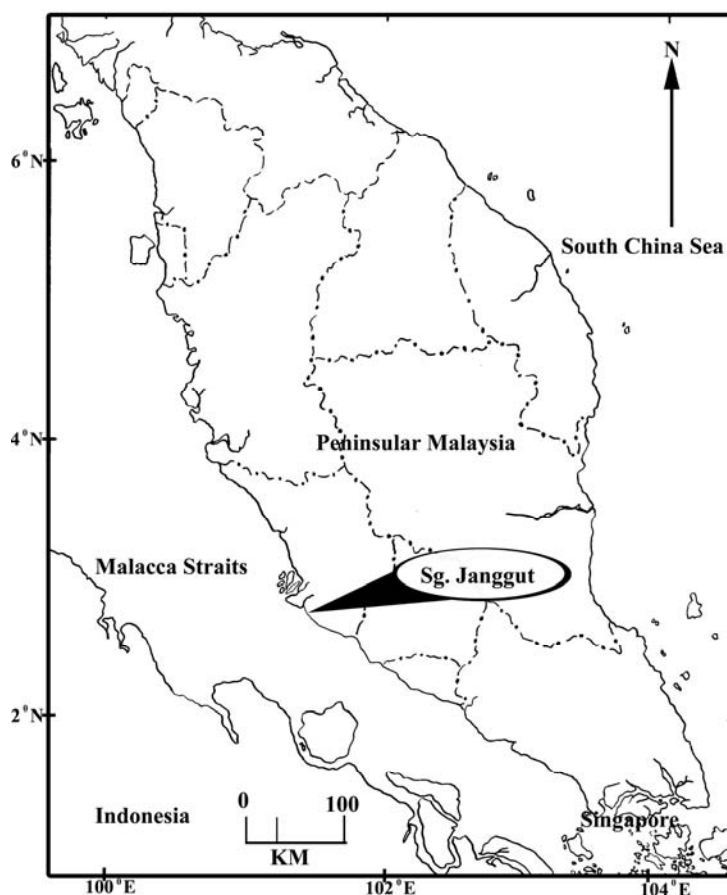


Figure 1. The sampling location of *Chicoreus capucinus* in Sungai Janggut, Kuala Langat, Selangor.

Table 1. Analytical results for the reference material and the certified value for each metal ($\mu\text{g/g}$ dry weight)

Metal	Sample	Certified reference material (CRM)	Measured value	Percentage of recovery, %
Cd	DOLT-3 Dogfish-liver	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
	NCS DC73319-Soil	4.3	4.91 ± 0.069	114 ± 1.60
Cu	DOLT-3 Dogfish-liver	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
	NCS DC73319-Soil	21.0	19.2 ± 0.171	91.4 ± 1.15
Fe	DOLT-3 Dogfish-liver	1484 ± 57.0	1070	72.1
	NCS DC73319-Soil	NA	NA	NA
Pb	DOLT-3 Dogfish-liver	NA	NA	NA
	NCS DC73319-Soil	98.0	94.7 ± 1.40	96.7 ± 2.02
Ni	DOLT-3 Dogfish-liver	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
	NCS DC73319-Soil	20.4	21.3 ± 0.176	105 ± 0.861
Zn	DOLT-3 Dogfish-liver	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24
	NCS DC73319-Soil	680	394 ± 2.80	57.9 ± 41.2

Note: NA=Not Available

2.2. Sample digestion

About 0.5 - 0.7 g of dried tissues from each category were weighed and placed in acid-washed digestion tubes. 10 ml of concentrated nitric acid (AnalaR grade, BDH 69%) was added to the digestion tube to digest the tissues. The tubes were placed in a digestion block at 40°C for 1 hour and the tissues were then fully digested at 140°C for 3 hours as described by Ismail and Ramli (1997). After being cooled, the content of each tube was diluted to 40 ml with double de-ionised water. The digested samples were then filtered through Whatman No.1 (filter speed: medium) filter papers in funnels into acid-washed pill boxes.

For sediment samples, about one gram of each dried sample was digested in a combination of concentrated nitric acid (69%) and perchloric acid (60%) in the ratio of 4:1, first at low temperature (40°C) for one hour and then the temperature was increased to 140°C for three hours (Ismail and Roberts, 1992) and

followed by similar steps as above.

2.3. Speciation of Cd, Cu, Ni, Pb and Zn in sediments

Geochemical fractions of Cd, Cu, Ni, Pb and Zn in the sediments were obtained by using the modified SET as described by Badri and Aston (1983) and Tessier and Campbell (1987).

2.4. Determination of Cd, Cu, Fe, Ni, Pb and Zn

The digests were then analyzed for heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) by using an air-acetylene Perkin-Elmer™ flame atomic absorption spectrophotometer model AAnalyst 800. Blank determination was carried out for calibration of the instrument. Standard solutions were prepared from 1000 ppm stock solutions provided by MERCK Titrisol for Cd, Cu, Ni, Pb and Zn and the data obtained from the AAS were presented in $\mu\text{g/g}$ dry weight basis.

Table 2. Heavy metal concentrations ($\mu\text{g/g}$ dry weight) in geochemical fractions of sediment collected from Sungai Janggut, Kuala Langat, Selangor.

Metal	Geochemical fraction of heavy metals				Total Digestion
	EFLE	Acid-reducible	Oxidisable-organic	resistant	
Cu	0.270-0.350	0.280-0.320	1.18-1.42	1.52-1.76	2.94-3.55
Zn	1.41-1.73	3.13-3.49	6.73-7.46	11.39-11.88	21.13-22.55
Pb	0.490-0.630	0.070-0.520	1.13-4.07	17.5-22.4	22.7-24.3
Ni	0.160-0.350	0.390-0.470	1.61-3.62	4.39-6.99	7.49-8.44
Cd	0.210-0.230	0.010-0.030	0.170-0.280	1.63-2.12	1.95-2.31

2.5. Quality control

Recoveries were done by using prepared standard solutions for each metal. In addition, the analytical procedures for the molluscs and sediment were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada) and soil (Soil-5, International Atomic Energy Agency, Vienna, Austria). The recoveries of all the metals were satisfactory (80-110%) as is shown in Table 1. On the other hand, to avoid possible contamination, all glassware and equipment used were acid-washed.

2.6. Statistical analyses

The mean and standard error of the data were analyzed using the KaleidaGraph 3.08. Softwares, Microsoft EXCEL and STATISTICA 99 edition were then applied for graphic assignment. In order to estimate the proportion in which metal occurs in the organism and in associated sediment, BSAFs were calculated for selected metals in the molluscs studied according to formula (Szefer *et al.*, 1999).

$$BSAF = C_x/C_s$$

Where C_x and C_s are the mean concentrations of metal in the organism and in associated sediment, respectively. In the present study, the summation of non-resistant geochemical fractions namely the EFLE, acid-reducible and oxidisable-organic were applied to the BSAF formula due to its bioavailabilities characteristic to the living organisms.

3. Results

Distributions of heavy metal in the different parts of *C. capucinus* are shown in Fig. 2. It was found that the mantle and caecum of gastropod were accumulative of Cu. The digestive gland and caecum were found to accumulate higher concentrations of Cd. As for the accumulation of Zn, most of the different soft tissues were accumulative of the metal. Meanwhile, the shell of *C. capucinus* accumulated highest concentrations of Pb and Ni. The operculum of the gastropod was found to accumulate higher concentrations of Fe compared to other different parts.

On the other hand, the heavy metal concentrations in geochemical fractions of sediment were shown in

Table 2. In general, among the non-resistant fractions, it was observed that the ranges oxidisable-organic fractions were higher than other non-resistant fractions namely the EFLE and acid-reducible. Higher concentrations ranges of oxidisable-organic fractions are due to the high organic compound characterized the mangrove area.

4. Discussion

The essential metals, Cu, Zn and Fe distributed differently in the different soft tissues of the molluscs studied. The differences to the affinities of the metals to the binding sites of metallothioneins (MT) in the different soft tissues (Roesijadi, 1980; Viarengo *et al.*, 1985) could affect the different metal levels found in the molluscs. The digestive gland of the gastropods plays an important role in heavy metal metabolism and contributes to their metal detoxification (Viarengo, 1989; Saha *et al.*, 2006). This could explain the high metal concentrations in these organs. The present study revealed that the shell of the molluscs had high level of non-essential metals like Ni and Pb. The metals found in the shell could be explained on the basis that some trace metals are incorporated into the shells of the molluscs through substitution of the calcium ions in the crystalline phase of the shell or are associated with the organic matrix of the shell (Foster and Chacko, 1995).

The purpose to obtain the BSAF values as shown in Table 3 is to estimate the proportion in which metal occurs in the organism and in associated sediment. Based on the values calculated, the different parts of the gastropod could be classified into a few groups such as macro concentrator (BSAF >2), micro concentrator (1 < BSAF < 2) or deconcentrator (BSAF < 1) as proposed by Dallinger (1993). In general, based on that proposal, most of the different parts could be classified as macro concentrator. The caecum was found to record the highest BSAF values for the accumulation of Cu, Zn and Cd. Meanwhile the shell was recorded the highest BSAF values for Pb and Ni. According to Bohac (1999), macro concentrators can be particularly suggested as suitable biomonitor (or biomonitoring organ/material). Therefore, it is useful for recognising of molluscs as a potential biomonitor to determine a relationship between concentrations of a given metal in the organisms and its bioavailable form in the associated sediment.

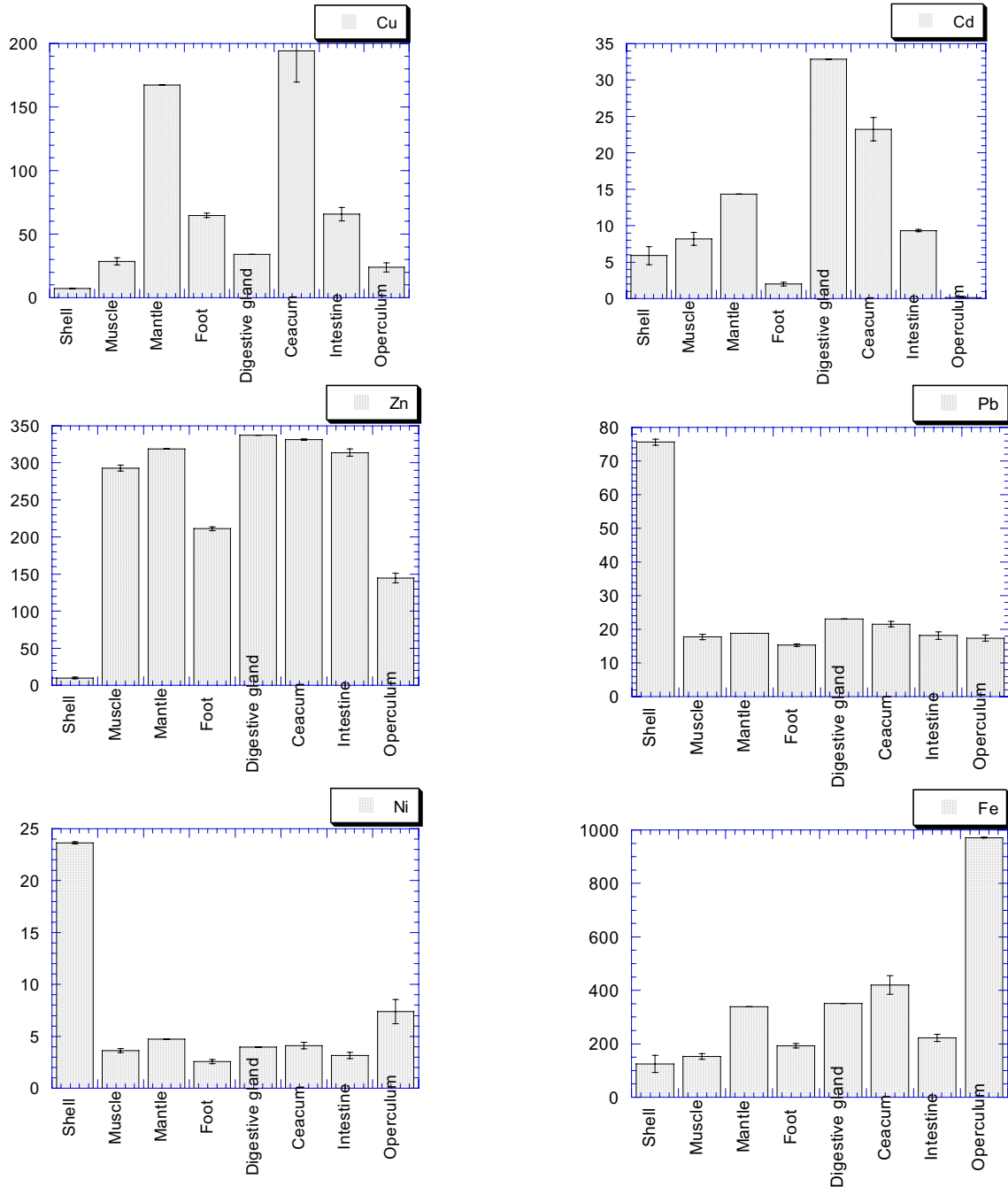


Figure 2. Heavy metal (Cu, Cd, Zn, Pb, Ni and Fe) concentrations ($\mu\text{g/g}$ dry weight) in the different tissues of *Chicoreus capucinus* collected from Sungai Janggut, Kuala Langat, Selangor.

From the discussion above, it is suggested that further studies should focus on the gastropods as potential biomonitor. Their feeding habit and location should be taken into account when proposing a biomonitor. Misunderstanding on the gastropods' "profiles" could result in wrong interpretation of data in a biomonitor. On the other hand, certain soft tissues such as caecum, digestive gland and mantle should

be focused upon as biomonitoring organs/materials for essential metals, while the shells are for non-essential metals. The affinities of the metals to the binding sites of metallothioneins (MT) in certain soft tissues, location, function and BSAF value of a tissue should be considered before proposing the different parts of the *C. capucinus* as biomonitoring organs/materials.

Table 3. Biota-sediment (non-resistant fractions of SET) accumulation factors (BSAF) based on the selected different parts of *Chicoreus capucinus*.

Different part	Cu	Zn	Pb	Ni	Cd
Shell	3.80	0.83	32.59	8.88	13.57
Caecum	101.18	27.39	9.31	1.57	53.13
Operculum	12.25	11.94	7.58	2.85	0.75
Foot	33.53	17.45	6.50	0.98	4.54

5. Conclusion

As for the conclusions, most of the different parts of *C. capucinus* were macro concentrator organs due to their capability to accumulate heavy metals from the sediment. However, among the different soft tissues, it could be suggested that the caecum was good accumulator of Cu, Zn and Cd and shell was for Pb based on the highest BSAF values recorded. Future studies should focus on the caecum and shell of *C. capucinus* as potential biomonitor of intertidal area.

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Received 2 Mayl 2010

Accepted 30 June 2010

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