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Research Article

The Potential of Crystalline Style of Green-Lipped Mussel *Perna viridis* as a Possible Copper Source for Industrial Application

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Abstract

This paper reanalyzed the copper (Cu) data reported by Yap., *et al.* (2006a) on the eight soft tissues of *Perna viridis* (green-lipped mussel) namely, byssus, foot, crystalline style, (CS), gonad, mantle, muscle, gill, and remainder. These Cu data were re-evaluated from the point of the industrial use. The data were re-analysed by using probability analysis and calculated for bioaccumulation factor. In general, it was found that Cu levels were the highest in CS compared to other different organs/soft tissues. Also, the Cu levels in the CS are comparable to habitat surface sediment based on bioaccumulation factor. Based on this accumulation pattern in all the mussel tissues in this study, the main storage and accumulation of Cu in *P. viridis* is CS. Therefore, it is suggested that CS of *P. viridis* is a potential Cu source for industrial application.

Keywords: *Perna viridis; Cu Distribution; Different Soft Tissues*

Introduction

From industrial application point of view, most Cu is used for raw materials for constructions, electronic products, and industrial machineries including heat exchangers and alloys. Due to its high electrical conductivity nature, Cu is an essential material for electrical wiring. Apart from that, commonly used Cu alloys variations in the industry are brass, bronze, and copper-tin-zinc, while cupronickel (combination of Cu and Ni) were preferred for low-denomination coins production [1,2].

Extracellular digestion of bivalves occurs in the crystalline style (CS), a rod-like gelatinous structure, of bivalves consisting of starch-digesting enzymes [3]. These enzymes and stomach content are mixed by rotational action of the CS against the gastric shield. The structure of the CS comprises diverticulum cell lining which produces a firm cellular secretion [4] and it revolves around the stomach, which is a thin-walled sac lined with gastric shield. The rod-like structure CS can be relatively easily well separated from the soft tissues of the other internal soft parts or organs of bivalves, lying within a diverticulum off the stomach. CS of the green-lipped mussel *Perna viridis* has well been reported in various studies in the literature [5-8] but the topic in this paper has not been discussed.

Digestion in bivalves occurs through a CS mediated extracellular digestion of food particles, termed biphasic digestive process, and followed by intracellular digestion which happens in the digestive gland [9]. Olsen., *et al*. [10] found multiple functions of the lysozymes in the CS of *Mytilus edulis*.

To date, numerous studies on the distributions of heavy metals in different parts of *P. viridis* were reported [5-7]. This is due to the accuracy of using a particular organ/tissue of mussel for biomonitoring of heavy metal pollution in the coastal ecosystem [5,6]. Previously, Yap., *et al*. [11] reported the potentials of digestive caecum in mudflat snail *Telescopium telescopium* as a possible cadmium source for industrial application. However, there is no discussion on the industrial application for crystalline style of bivalves.

Objective of the Study

The objective of this paper was to reanalyse the data cited from Yap., *et al*. [5] for probability analysis and bioaccumulation factor.

Materials and Methods

The data of Cu levels in the different soft tissues, namely (byssus, foot, crystalline style, (CS), gonad, mantle, muscle, gill and remainder) of *P. viridis* were cited from Yap., *et al*. [5,7]. However, the sediment data reported in this study is unpublished.

The collected surface sediments were oven-dried and sieved through a 63 µm mesh size. Surface sediment samples were fractioned by sequential extraction technique (the geochemical fractions of easily, freely, exchangeable or leachable (EFLE, F1), acid-reducible (AR, F2), oxidizable-organic (OO, F3) and resistant (Res, F4)) by Badri and Aston [12].

The cited Cu data of the mussels and the Cu levels in the geochemical fractions of the habitat surface sediments, were re-analysed for Probability Analysis (PA) by using Kaleidah Graph (Version 3.08, Synergy Software) and and Bioaccumulation Factor (BAF) (tissue/sediment).

Results and Discussion

The distributions of Cu levels in the soft tissues of mussels from the 21 sampling sites in Peninsular Malaysia are presented in table 1. In general, CS shows the highest Cu levels among all the eight soft tissues studied. CS from 14 out of 21 populations recorded the highest Cu concentrations. The overall statistics of Cu levels among the eight parts of mussels (Table 2) show that CS recorded the highest mean Cu levels, followed by byssus, remainder, gill, mantle, gonad, foot and muscle.

Sites	Sampling date	Bys	CS	Gill	Mantle	Foot	Gonad	Muscle	REM	F1	F2	F3	F4	SUM	NR
K. Pontian	10 May 2007	11.6	**90.7**	3.64	2.37	0.24	3.16	0.09	4.35	0.08	0.38	1.50	5.50	7.46	1.96
Kg. Pasir Puteh	10 May 2007	**51.4**	38.5	11.3	12.3	13.1	9.75	7.83	17.2	0.29	0.53	13.7	28.2	42.7	14.5
Senibong	10 May 2007	**50.9**	24.6	13.9	20.3	31.9	11.4	11.5	20.7	0.99	0.55	69.5	51.8	123	71.1
Pantai Lido	10 May 2007	13.9	**28.2**	4.33	11.0	0.10	6.75	0.04	6.63	1.30	0.63	69.6	52.4	124	71.6
Tg. Kupang	10 May 2007	10.4	**46.9**	2.36	6.08	3.37	6.50	3.25	8.47	0.15	0.40	3.88	13.8	18.2	4.43
Kukup	9 May 2007	12.9	**31.8**	6.23	5.85	3.29	5.86	2.89	9.32	0.17	0.51	1.01	12.2	13.9	1.69
G. Patah	11 Aug 2004	37.1	**58.2**	10.7	7.96	8.19	8.55	6.15	10.8	0.39	0.46	3.66	13.2	17.8	4.51
Senibong	11 Aug 2004	**43.4**	30.2	14.7	10.4	9.32	8.47	8.97	12.9	1.78	0.44	132	53.3	187	134

T. Jawa	11 Aug 2004	**71.6**	34.0	13.1	12.5	8.64	9.54	8.20	14.2	0.88	0.26	94.8	48.4	144	95.9
Kg. Masai	11 Aug 2004	50.2	**56.0**	13.4	11.3	10.0	9.49	9.20	12.2	1.80	0.43	118	64.1	184	120
P.Lido	11 Aug 2004	**61.7**	9.69	12.1	8.87	8.53	9.11	8.89	15.7	0.75	0.44	30.8	25.9	57.9	31.9
Nenasi	8 Apr 2004	18.3	**33.4**	4.54	1.44	4.24	5.92	3.09	5.00	0.08	0.40	2.85	11.8	15.1	3.34
K. Pon-tian	4 Aug 2004	22.2	**68.5**	12.3	8.66	7.88	9.59	5.23	13.5	0.12	0.33	2.15	9.40	12.0	2.60
Sebatu	17 Apr 2002	18.9	**33.5**	19.7	10.9	9.05	7.63	7.90	11.2	0.15	0.36	0.84	10.2	11.5	1.35
Kg.Pasir Puteh	19 Jan 2005	**23.7**	6.35	12.4	11.4	6.95	9.40	7.68	12.5	1.14	0.80	119	66.1	187	121
Minyak Beku	18 Jan 2005	23.7	**40.0**	7.92	6.38	6.46	7.68	6.19	10.2	0.19	0.05	22.8	22.8	45.8	23.1
Pantai Lido	19 Jan 2005	**61.7**	9.69	12.1	8.87	8.53	9.11	8.89	15.7	0.39	0.25	29.4	17.6	47.7	30.1
B. Tiang	19 Apr 2005	22.2	**39.9**	7.44	7.17	6.93	7.03	8.46	17.8	0.26	0.19	5.86	14.3	20.6	6.30
Bayan Lepas	2008	21.6	**33.8**	14.2	9.34	11.3	8.62	4.78	11.2	10.5	0.15	90.2	59.2	160	101
K.Sg. Ayam	13 May 2008	41.7	**54.9**	12.6	9.33	2.75	5.05	12.3	11.5	0.16	0.10	6.87	28.2	35.3	7.13
Pantai Lido	13 May 2008	9.21	**53.1**	10.4	7.76	4.97	9.60	7.05	10.5	0.61	0.35	11.1	10.7	22.7	12.1

***Table 1:** Distributions of copper levels in the different parts of green-lipped mussel Perna viridis.*

Note: REM: Remaining Soft Tissues. Values in bold show the highest Cu levels among the eight parts of mussels.

	Minimum	Maximum	Mean	Std Deviation	Std Error	Skewness	Kurtosis
BYS	9.21	71.64	32.30	19.40	4.23	0.55	-1.03
CS	6.35	90.73	39.15	20.09	4.38	0.56	0.50
Gill	2.36	19.65	10.45	4.36	0.95	-0.23	-0.46
Mantle	1.44	20.30	9.05	3.90	0.85	0.61	1.98
Foot	0.10	31.97	7.89	6.48	1.41	2.41	7.29
Gonad	3.16	11.36	8.01	1.94	0.42	-0.71	0.12
Muscle	0.04	12.30	6.60	3.30	0.72	-0.46	-0.39
Remainder	4.35	20.68	11.99	4.10	0.90	0.08	-0.24
F1	0.08	10.52	1.06	2.24	0.49	3.86	13.89
F2	0.05	0.80	0.38	0.18	0.04	0.13	0.05
F3	0.84	132.00	39.50	45.98	10.03	0.87	-0.82
F4	5.50	66.14	29.47	20.77	4.53	0.57	-1.27
SUM	7.46	187.00	70.35	66.99	14.62	0.76	-1.09

***Table 2:** Overall statistics of Copper levels in the different parts of green-lipped mussel Perna viridis. N= 21.*

Note: SUM: Summation of F1, F2, F3 and F4 of the geochemical fractions in the surface sediments.

Table 3 and 4 shows the BAF values of Cu in the different soft tissues of *P. viridis* from 21 sampling sites. When the sediment is represented by the non-resistant fraction, it is found that byssus (13/21) and CS (12/21) most of the of sampling sites with BAF values higher than 1. The current data showed that byssus and CS has higher Cu levels as compared to those in the habitat surface sediment.

Sites	Bys	CS	Gill	Mantle	Foot	Gonad	Muscle	Remainder
K. Pontian	5.93	46.29	1.86	1.21	0.12	1.61	0.05	2.22
Kg. Pasir Puteh	3.54	2.65	0.78	0.85	0.90	0.67	0.54	1.18
Senibong	0.72	0.35	0.20	0.29	0.45	0.16	0.16	0.29
Pantai Lido	0.19	0.39	0.06	0.15	0.00	0.09	0.00	0.09
Tg. Kupang	2.36	10.62	0.53	1.37	0.76	1.47	0.74	1.91
Kukup	7.67	18.81	3.68	3.46	1.94	3.46	1.71	5.50
G. Patah	8.22	12.90	2.37	1.77	1.82	1.90	1.36	2.40
Senibong	0.32	0.22	0.11	0.08	0.07	0.06	0.07	0.10
T. Jawa	0.75	0.35	0.14	0.13	0.09	0.10	0.09	0.15
Kg. Masai	0.42	0.47	0.11	0.09	0.08	0.08	0.08	0.10
P.Lido	1.93	0.30	0.38	0.28	0.27	0.28	0.28	0.49
Nenasi	5.47	10.00	1.36	0.43	1.27	1.77	0.92	1.50
K. Pontian	8.53	26.31	4.72	3.33	3.03	3.69	2.01	5.17
Sebatu	14.02	24.83	14.56	8.04	6.71	5.65	5.85	8.31
Kg.Pasir Puteh	0.20	0.05	0.10	0.09	0.06	0.08	0.06	0.10
Minyak Beku	1.03	1.74	0.34	0.28	0.28	0.33	0.27	0.44
Pantai Lido	2.05	0.32	0.40	0.30	0.28	0.30	0.30	0.52
B. Tiang	3.53	6.33	1.18	1.14	1.10	1.12	1.34	2.83
Bayan Lepas	0.21	0.33	0.14	0.09	0.11	0.09	0.05	0.11
K.Sg. Ayam	5.85	7.71	1.77	1.31	0.39	0.71	1.73	1.62
Pantai Lido	0.76	4.40	0.86	0.64	0.41	0.79	0.58	0.87
No. of site > 1.0.	13	12	8	8	6	8	6	10

Table 3: *Bioaccumulation factor (BAF; tissues/non-resistant fractions in sediment) of Cu in the different soft tissues of Perna virdis from 21 sampling sites in Peninsular Malaysia.*

Note: Non-resistant fractions are summation of F1, F2 and F3 of the geochemical fractions in the surface sediments.

Sites	Bys	CS	Gill	Mantle	Foot	Gonad	Muscle	Remainder
K. Pontian	1.56	12.16	0.49	0.32	0.03	0.42	0.01	0.58
Kg. Pasir Puteh	1.20	0.90	0.26	0.29	0.31	0.23	0.18	0.40
Senibong	0.41	0.20	0.11	0.17	0.26	0.09	0.09	0.17
Pantai Lido	0.11	0.23	0.03	0.09	0.00	0.05	0.00	0.05
Tg. Kupang	0.57	2.58	0.13	0.33	0.19	0.36	0.18	0.46
Kukup	0.94	2.30	0.45	0.42	0.24	0.42	0.21	0.67
G. Patah	2.08	3.27	0.60	0.45	0.46	0.48	0.35	0.61
Senibong	0.23	0.16	0.08	0.06	0.05	0.05	0.05	0.07
T. Jawa	0.50	0.24	0.09	0.09	0.06	0.07	0.06	0.10
Kg. Masai	0.27	0.30	0.07	0.06	0.05	0.05	0.05	0.07
P.Lido	1.06	0.17	0.21	0.15	0.15	0.16	0.15	0.27
Nenasi	1.21	2.21	0.30	0.10	0.28	0.39	0.20	0.33
K. Pontian	1.85	5.71	1.02	0.72	0.66	0.80	0.44	1.12
Sebatu	1.64	2.91	1.71	0.94	0.79	0.66	0.69	0.97
Kg.Pasir Puteh	0.13	0.03	0.07	0.06	0.04	0.05	0.04	0.07
Minyak Beku	0.52	0.87	0.17	0.14	0.14	0.17	0.14	0.22
Pantai Lido	1.29	0.20	0.25	0.19	0.18	0.19	0.19	0.33
B. Tiang	1.08	1.94	0.36	0.35	0.34	0.34	0.41	0.87
Bayan Lepas	0.14	0.21	0.09	0.06	0.07	0.05	0.03	0.07
K.Sg. Ayam	1.18	1.56	0.36	0.26	0.08	0.14	0.35	0.33
Pantai Lido	0.41	2.34	0.46	0.34	0.22	0.42	0.31	0.46
No. of site > 1.0.	10	10	2	0	0	0	0	0

Table 4: *Bioaccumulation factor (BAF; tissues/total SUM in sediment) of Cu in the different soft tissues of Perna virdis from 21 sampling sites in Peninsular Malaysia.*

Note: SUM= Summation of F1, F2, F3 and F4 of the geochemical fractions in the surface sediments.

Based on PA, figure 1 shows the probability graph for the Cu levels in CS of *P. viridis* in comparison to other different soft tissues and non-resistant of the surface sediments. This obviously show that Cu levels in CS are higher than those in the foot, mantle, muscle, gill, gonad and remainder. This is evidently shown that the Cu levels in the CS are comparable to non-resistant fractions (Table 3) and total summations of all geochemical fractions (Table 4) in the sediments based on BAF values. This strongly indicated that CS is the main target organ for Cu storage and accumulation based on accumulation pattern of Cu in the eight soft tissues (Table 1), overall highest Cu levels (Table 2), PA (Figure 1), and most BAF values with > 1.0 (Table 3 and 4).

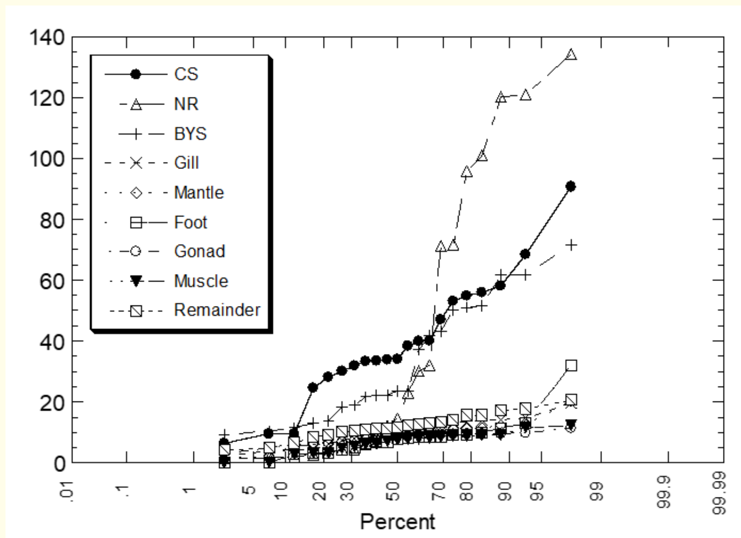

Figure 1: *Probability graph for the copper levels in crystalline styles (CS) of Perna viridis in comparison to other different soft tissues and habitat surface sediment (NR). Note: BYS: Byssus; NR= Summation of F1, F2 and F3 of the geochemical fractions in the surface sediments.*

The higher levels of Cu in the CS of *P. viridis* are in accordance with those reported by Yap., *et al*. [5,6] in which the high level of Cu in the CS could be explained through the biochemical nature of mussels. The main oxygen carrier pigment in mollusks is the Cu-containing hemocyanin, instead of the Fe-containing haemoglobin found in vertebrates [13]. The high Cu level in CS could be due to Cu in hemocyanin, which serves as the main enzyme cofactor for metabolism [13]. Cheung and Wong [14] also reported the detoxification and translocation of toxic metals by oyster, *Crassostrea gigas* involves accumulation of high levels of Cu by leukocytes in the tissues of the oyster.

The present high Cu levels found in the CS of *P. viridis*, which formed part of the digestive glands indicated that CS is a storage site for this essential Cu, plausibly in the form of metallothionein or granules [15]. The separated CS can be utilized as a potential material for industrial uses, needing further research studies.

Conclusion

This paper reanalyzed the Cu data that was previously reported by Yap., *et al*. [5,6] on the different soft tissues of *Perna viridis*. These Cu data were re-evaluated from the point of the industrial use. The data were re-analyzed by using probability analysis and calculated for bioaccumulation factor. In general, it was found that Cu levels were the highest in CS compared to other different organs/soft tissues. Also, the Cu levels in the CS are comparable to habitat surface sediment based on bioaccumulation factor. This strongly indicated that CS is the main storage and accumulation organ for CS as shown from the accumulation pattern of CS in the eight soft tissues. Therefore, it is suggested that CS of *P. viridis* is a potential Cu source for industrial application.

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