

Bioconcentration Factor (BCF) and Depuration of Heavy Metals of Oysters (*Saccostrea cucullata*) and Mussels (*Perna viridis*) in the River Basins of Coastal Area of Chanthaburi Province, Gulf of Thailand

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Abstract

This study investigated the distribution of Pb, Cd, Cr, Fe, Cu and Zn in the whole tissues of oysters (*Saccostrea cucullata*) and mussels (*Perna viridis*) collected from the three river basins of the coastal area in Chanthaburi Province, Thailand. The heavy metal concentrations in oysters followed the order: Zn>Fe>Cu>Cd>Cr>Pb, whereas in the mussels it was: Fe>Zn>Cu>Cr>Cd>Pb. The concentrations of Cu and Zn in the oysters were higher than the permission standard, and the bioconcentration factor (BCF) study revealed the ability of the oysters to accumulate the heavy metals. The results of depuration found that Cu and Zn levels in both oysters and mussels were below the permission standard after the end of the process. The time of depuration was related to the levels of Cu and Zn with the higher *r* values and the relationship of the depuration study obtained from the results of multiple regression analysis.

Keywords: bioconcentration factor (BCF); depuration; *Saccostrea cucullata*; *Perna viridis*; Chanthaburi Province

1. Introduction

The heavy metals contaminating in an aquatic environment are a critical issue due to potential threats to marine ecosystem (Jiang *et al.*, 2014). The elements are separated into two categories: essential and non-essential. Essential elements such as Fe, Cu, Zn and Mn have defined biological functions in organisms, whereas non-essential elements such as Pb, Cd, Cr, As and Hg are not involved in any metabolic mechanisms (Ancleto *et al.*, 2015). Heavy metals from natural or anthropogenic origins are transported by rivers, transferred to the boundary of coastal areas and deposited in estuaries (Censi *et al.*, 2006). The heavy metals accumulation in aquatic organisms, especially the filter feeders is of serious concern because of their higher bioaccumulative capacity in the upper trophic levels of the biological magnification process (Zahir *et al.*, 2011).

Several bivalve species live in estuaries that are subjected to anthropogenic pressures, being exposed to high levels of toxic elements (Ancleto *et al.*, 2015). The Nationwide Mussel Watch Programs used the oysters and the mussels as a bioindicator bivalve species that is the Mussel Watch Programs in France, United States and worldwide (Cantillo, 1998; O'Connor and

Lauenstein, 2005). The oyster (*Saccostrea cucullata*) and the mussel (*Perna viridis*) have been used as bioindicator (Boening, 1999). The bivalves of both two species are major organisms of aquaculture, socio-economics and consumption in the Eastern part of Thailand (Amornjaruchit, 1988; Cheevaporn and Menasveta, 2003).

The problems of Cu and Zn accumulated and contaminated in the oysters have also been studied (Phillips and Muttarasin, 1985; Cantillo, 1998; Kwon and Lee, 2001; Chaharlang *et al.*, 2012; Birch *et al.*, 2014). Although, the trace contents of Cu and Zn are essential requirements for the nutrition, the excess Cu and Zn could be harmful to the health (Fosmire, 1990). The depuration studies are an important issue for the health protection demonstrated by the ability of self-purification of contaminated pollutants (Geffard *et al.*, 2002).

The purposes of the present study were to investigate the distribution of heavy metals in the river basins of coastal area of Chanthaburi Province by using the bioconcentration factor (BCF). The depuration study was to display the ability of two bivalve species (*Saccostrea cucullata* and *Perna viridis*) in the elimination process of Cu and Zn.

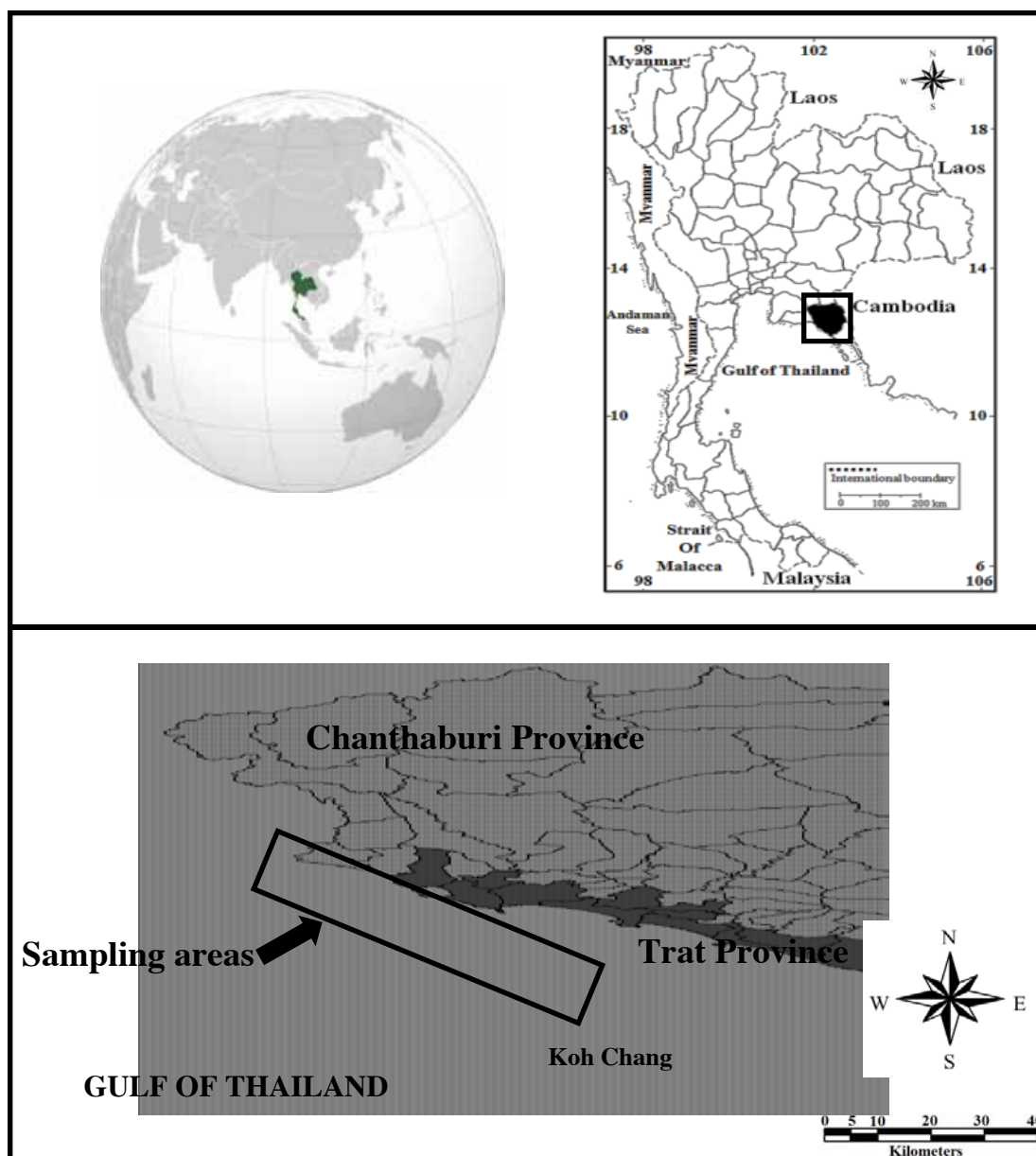


Figure 1. The map of coastal area of Chanthaburi Province

2. Materials and Methods

2.1. Study area and collection of bivalves

The coastal area of Chanthaburi Province is the tropical aquatic ecosystem in the eastern part of Thailand. The bivalve samples were collected between latitudes $12^{\circ}14'N$ and $12^{\circ}38'N$ and longitudes $101^{\circ}50'E$ and $102^{\circ}20'E$. The sampling site receives the large amounts of runoff, erosion and sewage from the three river basins namely: the Wang-Ta-Nord, the Chanthaburi and the Welu, which have a catchment area of $1,722 \text{ km}^2$.

The two bivalve species (*Saccostrea cucullata* and *Perna viridis*) were collected in March 2012 at the sampling sites (Fig.1). The bivalves were cleaned and

washed with seawater and transported in the isothermal insulated boxes to laboratory within two hours.

2.2. Depuration experiment

After being rinsed, the bivalves were transferred to the spherical depuration tanks (diameter, 40 cm; height, 50 cm). Each tank contained around 2-5 kg of adult bivalves and 20 L of natural seawater which was continuously aerated (dissolved oxygen > 60%), temperature ($25-30^{\circ}C$), salinity (25-29 g/L) and pH (7.4-8.3) (Lee *et al.* 2008). Subsamples of oysters and mussels were randomly removed at: 0, 1, 3, 6, 12, 24, 48 and 72 hour for analysis. The samples of bivalve tissues were stored at $-20^{\circ}C$ in a freezer until further analysis. All measurements were performed in 5 replicates.

Table 1. The results of the recovered percentage analysis of the certified reference material (Marine Dogfish Reference Materials: DORM-3; Marine Sediment Reference Materials: MESS-3) from the National Research Council, Canada

Metal	Sample	Certified value	Measured value	Percentages of recovery
Pb	DORM-3 (Marine dogfish)	0.395 ± 0.050	0.351 ± 0.034	88.8
	MESS-3 (Marine sediment)	21.1 ± 0.7	18.8 ± 0.7	89.0
Cd	DORM-3 (Marine dogfish)	0.290 ± 0.020	0.238 ± 0.018	82.1
	MESS-3 (Marine sediment)	0.24 ± 0.01	0.20 ± 0.02	84.2
Cr	DORM-3 (Marine dogfish)	1.890 ± 0.170	1.721 ± 0.159	91.1
	MESS-3 (Marine sediment)	105 ± 4	93 ± 5	88.8
Fe	DORM-3 (Marine dogfish)	347 ± 20	312 ± 15	89.9
	MESS-3 (Marine sediment)	43,400 ± 1,100	38,870 ± 850	89.6
Cu	DORM-3 (Marine dogfish)	15.50 ± 0.63	16.73 ± 0.32	107.9
	MESS-3 (Marine sediment)	33.9 ± 1.6	37.5 ± 1.6	110.6
Zn	DORM-3 (Marine dogfish)	51.3 ± 3.1	53.7 ± 2.0	104.8
	MESS-3 (Marine sediment)	159 ± 8	168 ± 7	105.7

2.3. Heavy metals analysis

The samples of bivalve tissues were digested with concentrated nitric acid (ULTRAPUR[®] Grade; HNO₃, 65%) as described by Yap and Edward (2010). We determined Pb, Cd and Cr by using a graphite furnace atomic absorption spectrophotometer (Model SpectrAA-640, Varian), whereas Fe, Cu and Zn were analyzed with an air-acetylene flame atomic absorption spectrophotometer (Model Spectrometer 3110, Perkin-Elmer).

The precision and accuracy of the analytical techniques were assessed by comparing with those of the Marine Dogfish Reference Materials (DORM-3) and the Marine Sediment Reference Material (MESS-3) from the National Research Council of Canada. The results were close to the certified values (Table 1).

2.4. Statistical analysis

The statistical analyses were performed with the Statistical Package for Social Science (SPSS) Version 18 for Windows (Serial No.5083337). The correlation coefficient and the multiple regression analysis were used to explain the relationship between the depuration rates and the depuration factors (time of depuration and weight of bivalves). The results of the concentrations of Cu and Zn in the oysters (*Saccostrea cucullata*) were transformed into the log values before being calculated by using the multiple regression equation.

3. Results and Discussion

3.1. The distribution of heavy metals

The results of the distribution of heavy metals and the permission standard guidelines are shown in

Table 2. The average heavy metal concentrations in the oysters (*Saccostrea cucullata*) were 0.011 ± 0.003 µg/g for Pb, 0.752 ± 0.193 µg/g for Cd, 0.201 ± 0.051 µg/g for Cr, 126 ± 13 µg/g for Fe, 32.577 ± 5.860 µg/g for Cu and 186.180 ± 10.688 µg/g for Zn (means ± SD, *n* = 24). While, the average heavy metals concentrations in the mussels (*Perna viridis*) were 0.010 ± 0.002 µg/g for Pb, 0.093 ± 0.023 µg/g for Cd, 0.161 ± 0.033 µg/g for Cr, 436 ± 68 µg/g for Fe, 10.039 ± 4.224 µg/g for Cu and 31.472 ± 4.084 µg/g for Zn (means ± SD, *n* = 24). The results of the investigation showed that Cu and Zn concentrations in the oysters were higher than the permission standard limit. O'Connor and Lauenstein (2005) indicated that higher concentrations of Cu and Zn in oysters were due to the metals within the membrane-enclosed granular hemocytes. In addition, both Cu and Zn act as structural ions with high affinity to interact with metallothioneins proteins which are also important in transporting both essential and toxic metals in oysters (Burger and Gochfeld, 2006).

The average heavy metal concentrations in the sediment from the three river basins of Chanthaburi coastal areas were 1.818 ± 0.525 µg/g for Pb, 0.018 ± 0.005 µg/g for Cd, 8.644 ± 1.648 µg/g for Cr, 17,860 ± 3,385 µg/g for Fe, 7.414 ± 1.952 µg/g for Cu and 18.122 ± 3.367 µg/g for Zn (means ± SD, *n* = 24). Whereas, the average concentration of the heavy metals in the seawater samples collected from the three river basins of Chanthaburi coastal areas were 1.365 ± 0.291 µg/L for Pb, 0.009 ± 0.004 µg/L for Cd, 0.075 ± 0.020 µg/L for Cr, 108.413 ± 18.097 µg/L for Fe, 2.857 ± 0.261 µg/L for Cu and 17.841 ± 2.164 µg/L for Zn (means ± SD, *n* = 24). The distribution of heavy metals in the sediment and seawater were comparable to those in natural levels as indicated by comparing with the worldwide average and the standard levels of Thailand (Potipat, 2015). Furthermore, Potipat *et al.* (2015) used

Table 2. The concentration of heavy metals in oysters (*Saccostrea cucullata*), mussels (*Perna viridis*), sediment and seawater from the three river basins of Chanthaburi coastal areas

Samples		Concentration of heavy metals (µg/g wet weight)						Reference
		Pb	Cd	Cr	Fe	Cu	Zn	
Oysters (<i>Saccostrea cucullata</i>)	Mean	0.011	0.752	0.201	126	32.577	186.180	
	SD	0.003	0.193	0.051	13	5.860	10.688	
Mussels (<i>Perna viridis</i>)	Mean	0.010	0.093	0.161	436	10.039	31.472	
	SD	0.002	0.023	0.033	68	4.224	4.084	
Sediments	Mean	1.818	0.018	8.644	17,860	7.414	18.122	
	SD	0.525	0.005	1.648	3,385	1.952	3.367	
Seawater (µg/L)	Mean	1.356	0.009	0.075	108.413	2.857	17.841	
	SD	0.291	0.004	0.020	18.097	0.261	2.164	
Permission standard limit in food and sea food (Ministry of Public Health, Thailand)		0.5	2.0	-	-	20	100	PCD (1999)
Permission standard limit in food (WHO)		2.0	1.0	50	-	30	100	WHO (1982)
Maximum permissible levels (Brazilian Ministry of Health)		10	5	-	-	150	250	Heidari <i>et al.</i> (2013)
Maximum permissible limit (MPL) of heavy metals in sea food products (µg/g wet weight) according to international standards		0.5	0.005	-	-	30	30	FAO (1983)
		1.5	0.5	-	-	30	150	FAO/WHO (1984)
		2.0	0.2	-	-	20	50	England (MAFF, 2000)
		2.0	-	-	-	30	100	Malaysia (MFR, 1985)

the geoaccumulation index and the enrichment factor to demonstrate that the sediment from the study area was unpolluted and not enriched by the heavy metal contents.

3.2. Bioconcentration factor (BCF)

Mountouris *et al.* (2002) defined bioconcentration factor (BCF) that is used to calculate the distribution of heavy metals between biota and ambient medium (sediment and seawater) as:

$$BCF = \frac{C_{\text{biota}}}{C_{\text{ambient medium}}}$$

Where C_{biota} is the heavy metals concentration in the biota and $C_{\text{ambient medium}}$ is the heavy metals concentration in the ambient.

In this study, the $BCF_{\text{bivalves/water}}$ ($BCF_{\text{b/w}}$) refers to the ratio of heavy metal concentrations in bivalve tissues and seawater, whereas the $BCF_{\text{bivalves/sediment}}$ ($BCF_{\text{b/s}}$) is the ratio of the heavy metals concentration in the bivalve tissues and sediment. The oysters

(*Saccostrea cucullata*) and the mussels (*Perna viridis*) were chosen and represented for calculation of the BCF. The BCF results from the present investigation are shown in Table 3 and the classification of BCF values shown in Table 4.

The $BCF_{\text{b/w}}$ values of all heavy metals were found to be very high ($BCF > 1,000$), except for Pb which indicated that a high ability to dissolve in seawater. In contrast, the $BCF_{\text{b/s}}$ values were low ($BCF < 30$) indicating a limited ability to accumulate heavy metals from the sediment. Kwon and Lee (2001) suggested the use of BCF to interpret the relationship of partition and bioavailability in the marine environment, while the results from this study found that the accumulation heavy metals were decreasing as follows: bivalves > sediment > seawater, except the $BCF_{\text{b/s}}$ values for Pb, Cr and Fe.

The results of $BCF_{\text{b/w}}$ and $BCF_{\text{b/s}}$ showed the ability of the oysters to accumulate heavy metals. This capacity has been described in several previous reports (Otchere, 2003; Chaharlang *et al.*, 2012; Gawade *et al.*, 2013; Birch *et al.*, 2014).

Table 3. The BCF values of heavy metals of the oysters (*Saccostrea cucullata*) and the mussels (*Perna viridis*) in the river basin of coastal area, Chanthaburi Province

Heavy metals	BCF _{b/w}		BCF _{b/s}	
	Oysters	Mussels	Oysters	Mussels
Pb	7.80	7.00	0.006	0.005
Cd	85,231	10,690	43	5.346
Cr	2,578	2,110	0.023	0.019
Fe	1,183	4,080	0.007	0.024
Cu	11,446	3,300	4.380	1.263
Zn	11,331	1,720	10.378	1.724

Table 4. Classification of bioconcentration factor (Bernd, 2000)

BCF range	Assessment Category	Comment
> 1,000	IV	Very High BCF
100-1,000	III	High BCF
30-100	II	Moderate BCF
< 30	I	Low BCF

3.3. Depuration

The contamination of heavy metals in seafood is a major health concern worldwide. The contaminations of Cu and Zn in the oysters in this study and several other reports (e.g. Phillips and Yim, 1981) exceeded the permission standard limit from both WHO (1989) and PCD (1999); in contrast to low accumulations in mussels (Table 5). Han *et al.* (1993) found that Cu played different roles in the oyster metabolism than that in the mussel (e.g. the essential structure of haemocyanin). Blackmore (2001) also demonstrated that oysters accumulate metals from the marine

environment by adsorbing onto inorganic elements while absorbing onto organic elements.

The concentrations of Cu and Zn determined in the oysters and the mussels during various times of depuration are given in Fig. 2 and the illustrated data as shown in Table 6. After 24 hours of depuration, Cu and Zn levels in two species had decreased to levels below the permission standard, except the Zn level in oysters. The study of depuration found that the difference of Cu and Zn levels after the end of the process which Han *et al.* (1993) explained that the Zn concentration stays longer in the bivalves than Cu due to the different ability in regulating Cu and Zn.

Table 5. The mean concentrations of Cu and Zn in tissues of oysters and mussels (µg/g) in different countries

Oyster species	Location	Dry/Wet wt	Cu	Zn	Reference
<i>Crassostrea virginica</i>	World average	Dry wt	160	1,600	Cantillo (1998)
<i>Saccostrea glomerata</i>	Sydney estuary, Australia	Wet wt	193	949	Birch <i>et al.</i> (2014)
<i>Saccostrea cucullata</i>	Persian Gulf, Iran	Dry wt	324	747	Chaharlang <i>et al.</i> (2012)
<i>Saccostrea cucullata</i>	Masan Bay, Korea	Wet wt	25	393	Kwon and Lee (2001)
<i>Saccostrea cucullata</i>	Eastern coast, Thailand	Wet wt	44	280	Panutrakul <i>et al.</i> (2007)
<i>Saccostrea cucullata</i>	Coastal area of Chanthaburi Province	Wet wt	33	186	This study
Mussel species	Location	Dry/Wet wt	Cu	Zn	Reference
<i>Mytilus edulis</i>	World average	Dry wt	8	30	Cantillo (1998)
<i>Perna viridis</i>	Peninsular, Malaysia	Wet wt	3.4	22	Yap <i>et al.</i> (2004)
<i>Perna viridis</i>	Gulf of Thailand	Dry wt	10	94	Ruangwises and Ruangwises (1998)
<i>Perna viridis</i>	Inner Gulf of Thailand	Dry wt	8	51	Cheevaporn and Menasveta (2003)
<i>Perna viridis</i>	Coastal area of Chanthaburi Province	Wet wt	10	31	This study

Table 6. Mean \pm SD of Cu and Zn concentrations ($\mu\text{g/g}$) in soft tissues of oysters and mussels at different times after passed depuration process ($\mu\text{g/g}$ wet weight)

Hours	Oysters (<i>Saccostrea cucullata</i>)		Mussels (<i>Perna viridis</i>)	
	Cu	Zn	Cu	Zn
0	39.748 \pm 6.453	178.554 \pm 3.924	9.898 \pm 0.881	32.234 \pm 2.337
1	38.528 \pm 3.241	172.787 \pm 3.127	9.761 \pm 0.646	30.728 \pm 1.677
3	37.636 \pm 2.468	168.261 \pm 4.083	9.484 \pm 0.366	30.076 \pm 1.126
6	33.736 \pm 2.920	162.318 \pm 3.386	9.317 \pm 0.508	29.513 \pm 1.218
12	30.572 \pm 2.080	153.436 \pm 4.289	9.129 \pm 0.709	28.970 \pm 1.069
24	22.832 \pm 2.374	127.306 \pm 4.223	8.403 \pm 0.469	25.786 \pm 2.036
48	18.650 \pm 1.731	106.861 \pm 2.699	8.275 \pm 0.435	22.114 \pm 1.681
72	17.645 \pm 1.649	98.687 \pm 3.929	8.137 \pm 0.577	19.572 \pm 1.113

The results of Zn depuration in oysters demonstrated that Zn concentrations were still close to the permission standard limit after the depuration was finished (72 hours). Amaral *et al.* (2005) reported incomplete metal elimination in longer period because the metals in the dissolved form are quickly depurated, while metals in the amorphous granules are kept for longer period in the tissues. The depuration rates of Cu and Zn in the oysters obtained by Han *et al.* (1993) decreased until a steady state when there was no apparent change of the Cu and Zn concentrations between incubation in seawater and the oyster tissues. Langston and Bebianno (1998) also found that the most of bivalve species modulated the essential elements in whole tissues with the homeostasis and transformed Cu and Zn to the metalloenzyme.

During depuration the Cu and Zn concentrations in oysters exhibited a significant linear decrease: $r=-0.873$, $p<0.001$ for Cu and $r=-0.954$, $p<0.001$ for Zn (Fig. 3). Similarly, the Cu and Zn concentrations in mussels showed a significant linear decrease: $r=-0.694$, $p<0.001$ for Cu and $r=-0.931$, $p<0.001$ for Zn (Fig. 4). Pearson correlation analyses revealed that the decrease in Cu and

Zn concentrations in oyster and mussel tissues showed negative relation toward the time of depuration. Several researchers have suggested that longtime of depuration was the most probable reason for the decrease of the contaminating elements in the bivalves. Chan *et al.* (1999) found that the rates of metal reduction were controlled with the time of transplanted depuration and the metals half-life.

Table 7 showed the relative depuration of Cu and Zn determined by multiple linear regressions. The results revealed that the depuration rates of Cu and Zn during the experimental depuration of both oysters and mussels have a significantly positive relationship with the length of depuration (hour) and the weight of bivalves (gram). The effectiveness of depuration process depends on various factors, e.g., the physiology of bivalves, the duration of depuration and the environmental conditions (Anacleto *et al.*, 2015).

4. Conclusion

The results of this study found that the distribution of the essential elements (Fe, Cu and Zn) were higher

Table 7. Multiple regression equations of depuration Cu and Zn in the oysters (*Saccostrea cucullata*) and the mussels (*Perna viridis*)

Bivalves	Depuration rate ($\mu\text{g/g}$)	Multiple regression equation	R ²	Significance of regression
Oysters (<i>Saccostrea cucullata</i>)	Cu	$\text{LogY}_{\text{Cu}} = -4.0905 + 0.001 (\text{Time; hour}) + 0.100 (\text{Weight; g})$	0.961	0.000
	Zn	$\text{LogY}_{\text{Zn}} = -1.020 + 0.002 (\text{Time; hour}) + 0.050 (\text{Weight; g})$	0.983	0.000
Mussels (<i>Perna viridis</i>)	Cu	$\text{Y}_{\text{Cu}} = -15.849 + 0.020 (\text{Time; hour}) + 1.684 (\text{Weight; g})$	0.904	0.003
	Zn	$\text{Y}_{\text{Zn}} = -14.853 + 0.087 (\text{Time; hour}) + 3.036 (\text{Weight; g})$	0.977	0.000

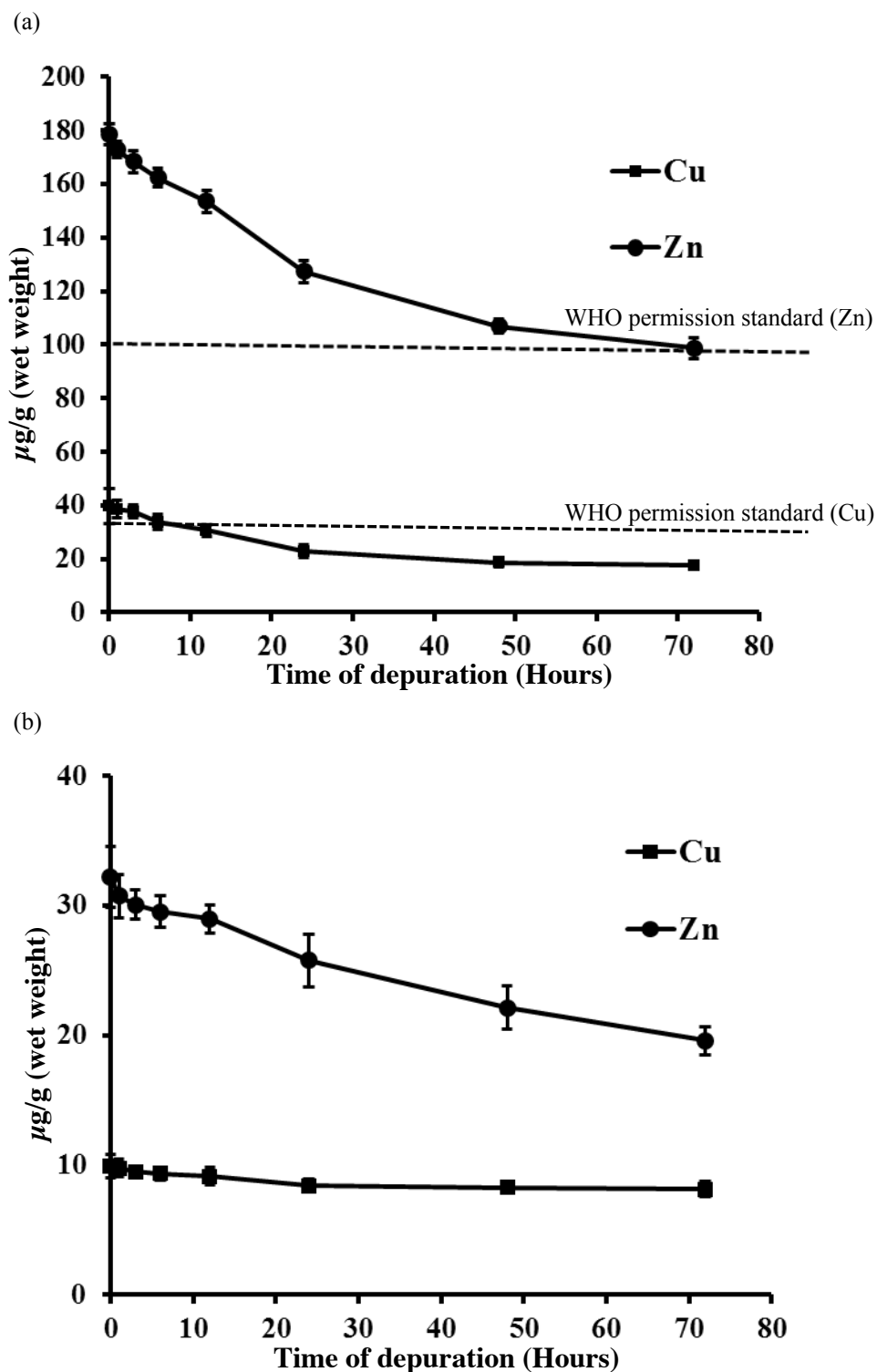


Figure 2. The concentrations of Cu and Zn in soft tissues of oysters (a) and mussels (b) at different times of depuration process ($\mu\text{g/g}$ wet weight)

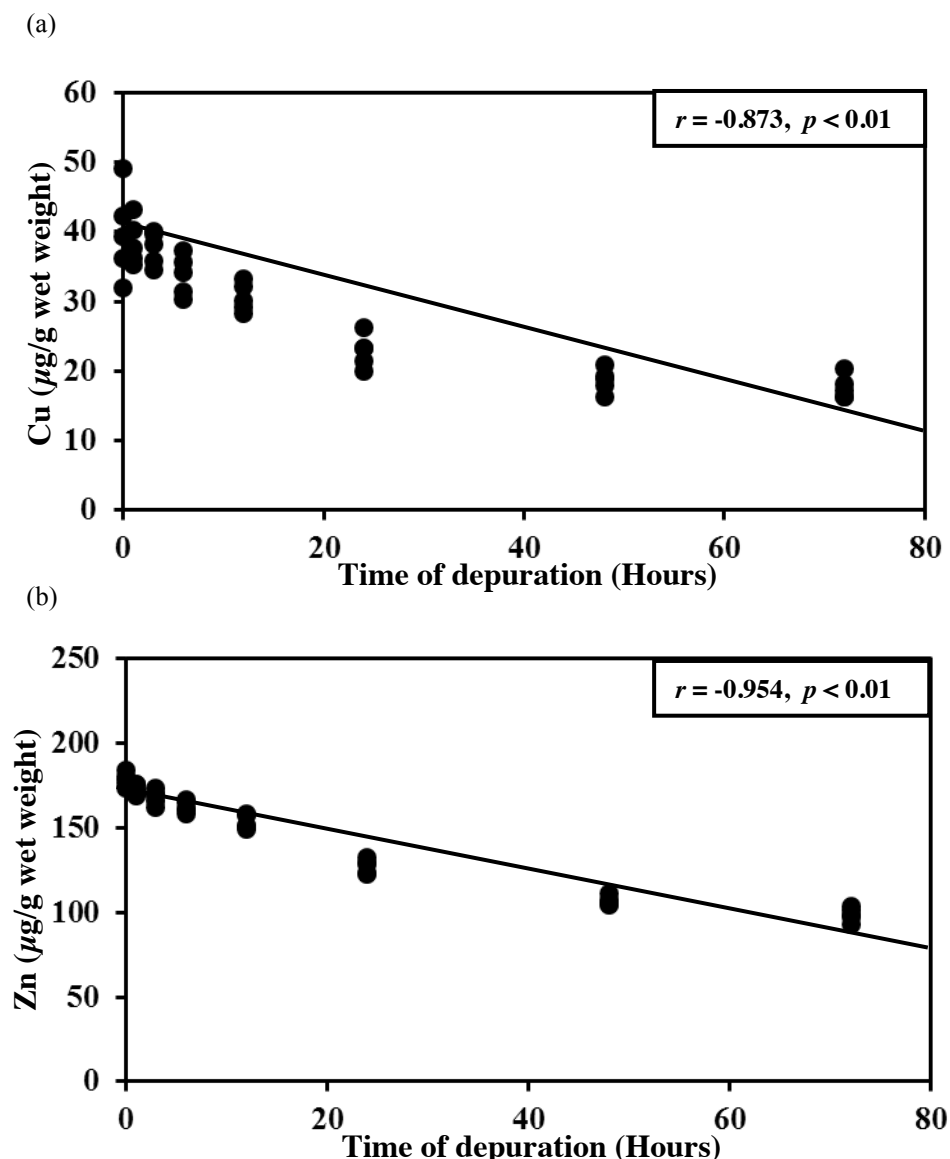


Figure 3. Variation of Cu (a) and Zn (b) concentrations (µg/g wet weight) in the oysters (*Saccostrea cucullata*) during the depuration periods. Pearson correlation significant at $p < 0.01$.

than those of the non-essential elements (Pb, Cd and Cr); Fe and Zn concentrations reaching the highest accumulation. The accumulation of heavy metals was decreasing as follows: bivalves>sediment>seawater as demonstrated in the BCF study. The bivalves in this study were used as biomonitoring indicator whereas oysters (*Saccostrea cucullata*) expressed as a strong bioaccumulator with the high levels of Cu and Zn. The contamination of Cu and Zn in oysters and the mussels was not of serious problem after their depuration process. The depuration has been mandatory to decrease Cu and Zn in the bivalves to the acceptable levels for human consumption. We emphasized that the

monitoring of heavy metals contamination is necessary to prevent the toxicity of metals.

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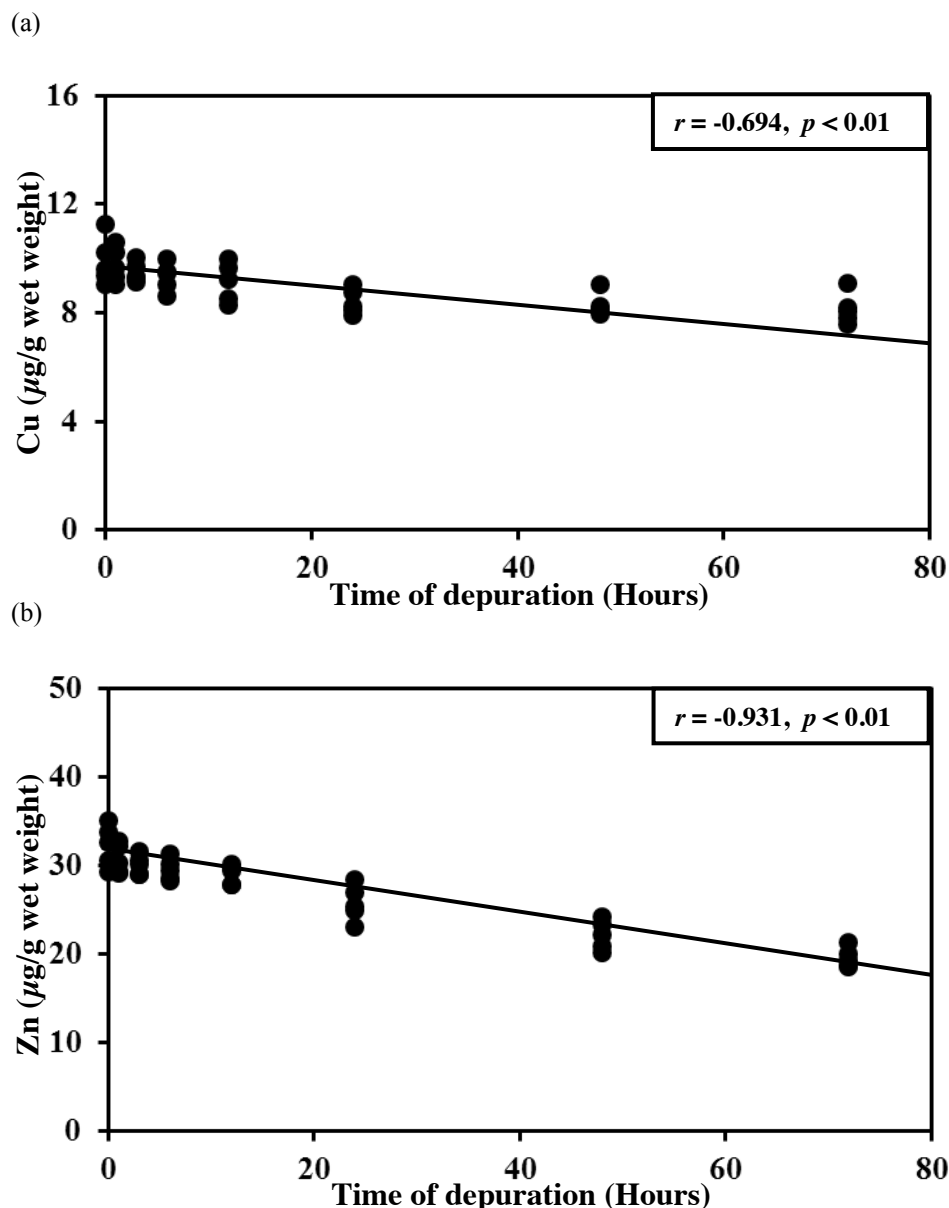


Figure 4. Variation of Cu (a) and Zn (b) concentrations (µg/g wet weight) in the mussels (*Perna viridis*) during the depuration periods. Pearson correlation significant at $p < 0.01$.

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