DISTRIBUTION, BIOCONCENTRATION, AND DEPURATION OF SELECTED HEAVY METALS IN THE RIVER BASIN OF CHANTHABURI COASTAL AREAS

JAKKAPAN POTIPAT

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DOCTOR DEGREE OF
PHILOSOPHY IN ENVIRONMENTAL SCIENCE
FACULTY OF SCIENCE
BURAPHA UNIVERSITY
JANUARY 2015

The dissertation of Jakkapan Potipat has been approved by the examining committee to be partial fulfillment of the requirements for the Doctor Degree of Philosophy in Environmental Science of Burapha University

Advisory Committee

....Principal advisor

(Associate Professor Dr. Nongnud Tangkrock-olan)

....Co-advisor

(Associate Professor Dr. Voravit Cheevaporn)

Examining Committee

....Principal examiner

(Professor Dr. Herbert Helander)

....Member

(Associate Professor Dr. Nongnud Tangkrock-olan)

....Member

(Associate Professor Dr. Voravit Cheevaporn)

....Member

(Assistant Professor Dr. Bhumiphut Pachana)

This dissertation has been approved by the Faculty of Science to be partial fulfillment of the requirements for the Doctor Degree of Philosophy in Environmental Science of Burapha University

....Dean of the Faculty of Science

(Assistant Professor Dr. Ekaruth Srisook)

.....January.....30........., 2015

ACKNOWLEDGEMENT

I wishes to express my deepest gratitude to the principal advisor, Associate Professor Dr. Nongnud Tangkrock-olan, for her assistance, carefulness and friendship to me in many time of this dissertation study. The author is also extremely grateful to the co-advisor, Associate Professor Dr. Voravit Cheevaporn, for his helpful direction, encouragement and invaluable assistance in all time of the research study.

A special thanks to Professor Dr. Herbert F. Helander to look closely at the final version of the dissertation for English style and grammar, correcting both and offering suggestions for improvement which lead to the success of the dissertation. I also thank Dr. Katavut Pachana for the kindly help to me throughout this study.

I would like to express my gratitude to all those who help, support, interest, valuable hints and gave me the possibility to complete this dissertation. Especially, I would like to give my special thanks to my parent whose patient, understanding, encouragement and spiritual support me to complete this research.

I would like to say thank to Mr. Chansak Kammart for the pleasant time since the bachelor degree to Ph.D. student which he was the best teacher, brother and friend for me.

Acknowledgement is also sincerely thanks to Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi Province for support the laboratory facilities of this study. Finally, financial support by the Higher Education Research Promotion (HERB) under Office of the Higher Education Commission, Thailand is acknowledged.

Jakkapan Potipat

54810065: MAJOR: ENVIRONMENTAL SCIENCE; Ph.D. (ENVIRONMENTAL SCIENCE)

KEYWORDS: HEAVY METALS / ENRICHMENT FACTOR / GEOACCUMULATION INDEX / BIOCONCENTRATION FACTOR / DEPURATION

JAKKAPAN POTIPAT: DISTRIBUTION, BIOCONCENTRATION AND DEPURATION OF SELECTED HEAVY METALS IN THE RIVER BASIN OF CHANTHABURI COASTAL AREAS. ADVISORY COMMITTEE: NONGNUD TANGKROCK-OLAN, Ph.D., VORAVIT CHEEVAPORN, Ph.D. 106 P. 2015.

This study investigated the distribution of Pb, Cd, Cr, Fe, Cu and Zn in the bivalves including: cockles (*Anadara granosa*), mussels (*Perna viridis*) and oysters (*Saccostrea cucullata*) as well as the sediment and seawater samples had also been investigated. They were collected from 24 stations of the three river basin namely: the Wang-Ta-Nord, the Chanthaburi and the Welu. The results found that the accumulation of Fe and Zn were highest in the bivalve tissues, whereas the Cu and Zn in the oyster exceeded the permission standard limit of Thailand. All the heavy metals concentration observed in the samples of sediment and seawater were lower than those from the sediment quality guideline (SQG) and the standard of seawater, respectively. The results of the geoaccumulation index and the enrichment factor values of the heavy metals content in the sediments revealed that the study area was unpolluted and not enriched, respectively. The principal component analysis (PCA) and the multiple regression statistics were used to summarize of the distribution of heavy metals in the study area. The results found that the difference between the non-essential elements (NEE) and essential elements (EE) in the bivalves as well as there were the different distribution of heavy metals among the river basin both sediment and seawater samples. The results of multiple regression analysis showed the relationship between the heavy metals concentration and the influential variation of physicochemical environment. The depuration study found that Cu and Zn concentrations in the oysters and mussels were lower than the permission standard limit in food, Thailand when the depuration process was finished at 72 hours.

CONTENTS

CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF TABLES (CONTINUED)

LIST OF TABLES (CONTINUED)

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

LIST OF FIGURES (CONTINUED)

Figure Page

CHAPTER 1
INTRODUCTION

Heavy metals are elements in the periodic system which can be classified as essential and non-essential for organisms. Essential metals (Fe, Cu, Zn, Co, Mn, Cr, Mo, V, Se, Ni and Sn) are vital for living organisms and are strongly bound in metalloproteins or loosely bound in metal-protein complexes (Cheevaporn, 1995). Haemoglobin (contain Fe) and haemocyanin (contain Cu) are metalloproteins carrying oxygen. Examples of other metallo-proteins include carbonic anhydrase, carboxypeptidase A and B and several dehydrogenases (contain Zn), pyruvate carboxylase (contains Mn) vitamin B_{12} (contains Co), xanthine oxidase (contains Fe and Mo), and cytochrome oxidase (contains Fe and Cu) (Vallee & Wacker, 1970).

Some enzymatic reactions require adequate concentrations of appropriate heavy metals. However at higher concentrations of metals, such as Ag, Hg, Cd and Pb, enzyme activities could be inhibited. This may be due to changes in the structure of the enzyme or by decreased response by the active catalyst (Vallee & Wacker, 1970). Non-essential metals are toxic by reducing active sites of organism molecules (Walker, Hopkin, Sibly, & Peakall, 2006). Consequently, most heavy metals, whether essential or not, are potentially toxic to living organism.

In many parts of the world anthropogenic activities including industry, agriculture and transportation create environmental crises due to accumulation of heavy metals in the food chain (Thongra-ar, Musika, Wongsudawan, & Munhapol, 2008). Heavy metals contaminate in many areas, in particular estuarine ecosystems which fate and distribution depend on physical, chemical and biological variability (Bakan & Balkas, 1999).

After the 5th national economic and social development plan of Thailand (1982-1986) to flooding disaster in Thailand (2011) are cause of environmental changing and pollution crisis in Thailand. Chanthaburi coastal areas in the eastern coast of Thailand, which are effected location from these situations. These areas are undergoing a rapid industrial and commercial development, and many areas have been allocated for agriculture, recreation, tourism, fisheries, industrial estates and urban communities. The environmental impact of this development has been considerable.

Estuaries are important sites for the evaluation heavy metals contamination in environment, which generally estuarine regions were chosen for environmental sampling since most heavy metals and other pollutants accumulate here. There are three important river basins in Chanthaburi namely: the Wang-Ta-Nord, the Chanthaburi and the Welu, each of which are affected by various anthropogenic activities, both in upstream and downstream areas.

This study has integrated both environmental indexes and statistical techniques for the investigation of the distribution of selected heavy metals in the estuarine environment, as well as presentation the experimentation and guideline for mitigation the heavy metals impact.

Objectives

1. To investigate the distribution and bioconcentration of selected heavy metals in the river basin of Chanthaburi coastal areas.

2. To study depuration rate of Cu and Zn in the oysters and mussels.

Hypothesis

1. There are significant differences in heavy metals concentrations between each ambient samples and organism tissues, which relate to location and species.

2. There are significant differences of heavy metal concentrations at depuration period, which vary to bivalve species.

Contribution of knowledge

1. Information from this study can be applied for monitoring the heavy metals contamination in the environment, and used as databases for selected each indexes for appropriate assessment and classification of heavy metal pollution.

2. The data from assessment and classification of heavy metal pollution in each index can be used in solving heavy metal problems with efficient management.

Scope of the study

Part 1. The investigation of distribution of selected heavy metals in the river basin of Chanthaburi coastal areas

This research will study many indexes for monitoring heavy metals contamination in the environment including; Geoaccumulation index (I_{geo}), Enrichment factor (EF), and Biococentration factor (BCF). Oyster (*Saccostrea cucullata*), Mussel (*Perna viridis*), and Cockle or Ark shell (*Anadara granosa*) were selected as the bio-indicator species. In addition, statistical techniques including the principle component analysis (PCA) and multiple regressions were chosen for interpretation and summarization to the heavy metals distribution.

Part 2. The experimental depuration of essential elements (Cu and Zn) in the oysters and mussels from several locations

The oysters (*Saccostrea cucullata*) and mussels (*Perna viridis*) used in this part were collected from the three estuaries of Chanthaburi coastal areas. Each of these, areas was contaminated by metals pollution from various anthropogenic activities, such as industrial estates, agriculture, urban communities and conservation areas.

The oysters and mussels were depurated in static system and then tested Cu and Zn concentrations at 0, 1, 3, 6, 12, 24, 48 and 72 hours. Physicochemical factors were controlled by the method of Lee, Lovatelli, and Ababouch (2008).

CHAPTER 2
LITERATURE REVIEWS

In this dissertation "Distribution, Bioconcentration and Depuration of Selected Heavy Metals in the River Basin of Chanthaburi Coastal Areas" the information is divided into 5 parts:

1. Heavy metals
    1.1 Sources of heavy metals in the estuarine
    1.2 Fate and distribution of heavy metal
    1.3 Distribution of heavy metals in estuary
2. The indexes for heavy metals distribution assessment
    2.1 The Geoaccumulation index (I_{geo})
    2.2 Enrichment Factor (EF)
    2.3 Bioconcentration Factor (BCF)
    2.4 Bioindicator
3. Depuration
4. The statistical instrument for analysis the heavy metals distribution
5. Previous research

1. Heavy metals

Heavy metals are elements in periodic table which have many properties such as atomic numbers are 23-34 and 40-52 as well as sequences of Lanthanides and Actinides, the specific gravity greater than 5 and at room temperature are solid except Hg. The physical properties are great electrical and thermal conductivity, glitter, viscosity, reflection and can be bettered to thin piece. The chemical properties have several oxidation numbers and could be transformed to complex compound when interaction with other chemicals particularly inorganic substances. For toxicological discipline heavy metals are poisonous elements in biological and ecological system.

1.1 Sources of heavy metals in the estuarine

There are five major sources of heavy metals in estuarine ecosystem (Cheevaporn, 1995).

1) Geological weathering. This is the source of background levels. It is to be expected that in areas characterized by metal-bearing lithological formation, these metals will also occur in higher levels in the waters of the area. A comparison of natural metal enrichment of sediments by geological weathering with present-day enrichment at the lower than anthropogenic pollution. The greatest increases in concentration levels of these sediments and waters are found for the heavy metals Cu, Zn, Pb, Hg, and Cd where more than 90% of the concentration of these metals originate from man-made sources.

2) Industrial processing of ores and metals. During the processing of ores, metal-bearing dust particles are formed which may only be partially filtered out by air purification systems. Also appreciable quantities of metals go to waste during chemical metal refinement processes (e.g., galvanizing and pickling).

3) The use of metals and metal compounds. Chromium salts are used during processing in tanneries, copper compounds are used as plant protection agents, mercury is used in chlorine-alkali production, zinc is used in the production of water pipes, and tetraethyl lead is used as an anti-knock agent in gasoline.

4) Burning of fossil fuels, production of cement and bricks. Fossil-fuel mobilization is particularly high for arsenic, zinc, cadmium, copper (coal), nickel, and vanadium (oil). Strong emissions of zinc, lead, selenium, and arsenic result from cement production and heavy metal enrichment such as Pb was founded around brickworks.

5) Leaching of metals from garbage and solid waste dumps. The concentration of this source to metal concentration of inland and coastal waters merits close attention. Mine dumps especially can be a serious source of pollution.

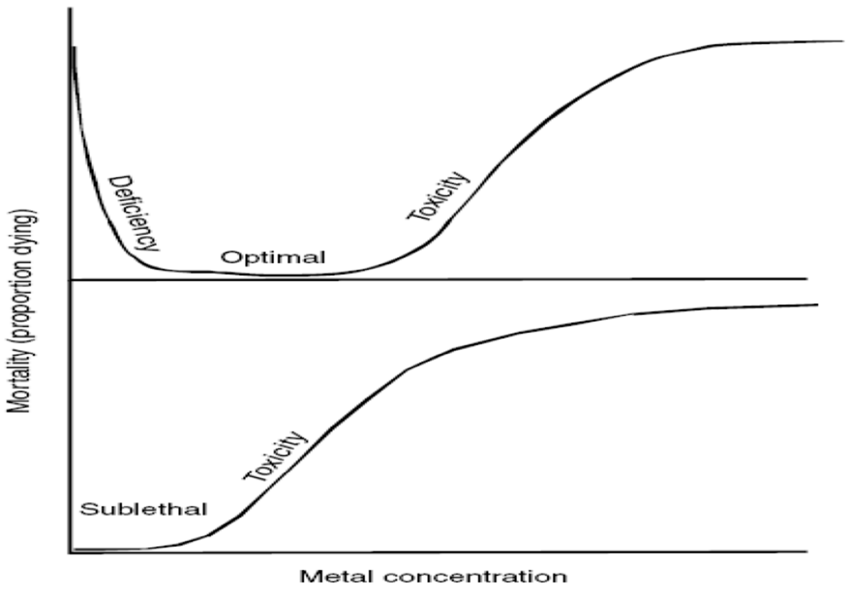


Figure 2-1 Mortality versus metal concentrations for essential (upper panel) and non-essential (lower panel) (Adu, 2010)

1.2 Fate and distribution of heavy metal

In estuarine system, exhibit an extreme level of spatio-temporal variability in their physico-chemical characteristics. As a result, estuaries are regarded as highly complex and dynamic environments that are governed by a variety of natural and anthropogenic stress related gradients.

Processes affecting heavy metals in the environment

When heavy metals are introduced into the environment either naturally or as contaminants, there are processes which can affect the concentrations of these metals in the environment. These include:

1) Adsorption - Heavy metals can be removed from sea water by adsorption at the surfaces of particles such as hydrated ferric oxide, hydrated manganese dioxide and clay minerals. Of the hydrated oxides, ferric oxide is usually more important in coastal regions than manganese dioxide since it is usually more abundant and is much more readily precipitated. The adsorption of trace elements from saline waters on suspended clays, iron and manganese oxides.

One important adsorption mechanism operative in the removal of trace elements from solution during estuarine mixing may be the formation of iron oxide coating on clay particle which have acted as negatively charged nuclei for the adsorption of hydrolysis products of iron. Oxides of iron and manganese are very efficient scavengers of trace elements. The oxide coating formed on detrital particles in estuaries may remove trace elements from solution and may also prevent trace element desorption from ion exchange sites.

2) Precipitation – If the concentration of a metal is higher than the solubility of the least soluble compound that can be formed between the metal and anions in the water which include carbonate, hydroxyl or chloride, then precipitation will occur. In deep basins or in some polluted estuaries and coastal basins there is a great demand for oxygen due to the breakdown of large quantities of organic material. In the absence of oxygen and the presence of H_2S, metals such as Zn, Cu, Cd, Pb, Hg, and Ag have very insoluble sulfides they have a tendency to precipitate. On the other hand, higher concentrations of Fe and Mn are found in this reducing environment because their sulfides are more soluble than the hydrated oxides which are precipitated in oxygenated sea water.

3) Absorption and redistribution by organisms – The removal and deposition of metals from sea water is often promoted by biological processes. For example, in the Monterey Bay, California, the diatom *Phaeodactylum* sp. can store heavy metals over long periods even when the heavy metal content in the water is low. Furthermore, the heavy metal content of diatoms in near-shore waters is about twice as high as the heavy metal contents of diatoms in off-shore waters as a result of anthropogenic influences. A similar observation was also made from other marine phytoplankton. Vertical transport of metals by agents such as the moulted exoskeletons and feces of zooplanktonic animals. The vertical distribution of metals in the sea will be affected by biological action mainly in near-shore waters where nutrients are available from upwelling or from runoff from the land.

In addition to these three processes, there is also remobilization of heavy metals from sediments which may be caused by four types of chemical changes in the waters. They include:

1) Elevated salt concentrations – whereby the alkali and alkali earth cations can compete with the metal ions sorbed onto solid particles.

2) Changes in the redox conditions – whereby under reducing conditions iron and manganese oxides are partly dissolved and so part of the sorbed heavy metal load is released.

3) Lowering of pH – which leads to a dissolution of carbonates and hydroxides, as well as to increase desorption of metal cations due to competition with H^+ ions.

4) Increased use of natural and synthetic complexing agents – which can form stable soluble metal complexes.

1.3 Distribution of heavy metals in estuary

An estuary is, by common usage, a place where a river meets an inlet of the sea. It can be defined more precisely in physiographic or geomorphological terms as a river valley that is open to the ocean. Every estuary is unique. Every estuary is subject to differing physical constraints and every estuary is therefore evolving at different rates. The world's estuaries are the ultimate repository for a vast array of substances discharged deliberately or accidentally via human activities

Distribution of heavy metals in seawater of estuarine

The mixing of river water and seawater in an estuary is accompanied by a large number of biogeochemical and physical processes which change the distribution, partitioning and bioavailability of contaminants. Seasonal fluctuation and year to year variation in concentrations of trace metals must be considered in characterizing the importance of biogeochemical processes for the distribution of anthropogenic metals in estuaries.

Distribution of heavy metals in sediment of estuarine

Sediments serve as a potential risk source as well as ultimate sink of heavy metals in aquatic environments and are considered to be a good environmental indicator of metal pollution.

Distribution of heavy metals in organism of estuarine

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. The bioaccumulation of heavy metals by marine organisms may reach many orders of magnitude above background concentrations of certain locality. This phenomenon may demonstrate the potential of some species as a biomonitor of heavy metal pollution. Biomonitoring agents can be assessed by analysing heavy metals in the whole tissues or certain parts or tissues of organisms.

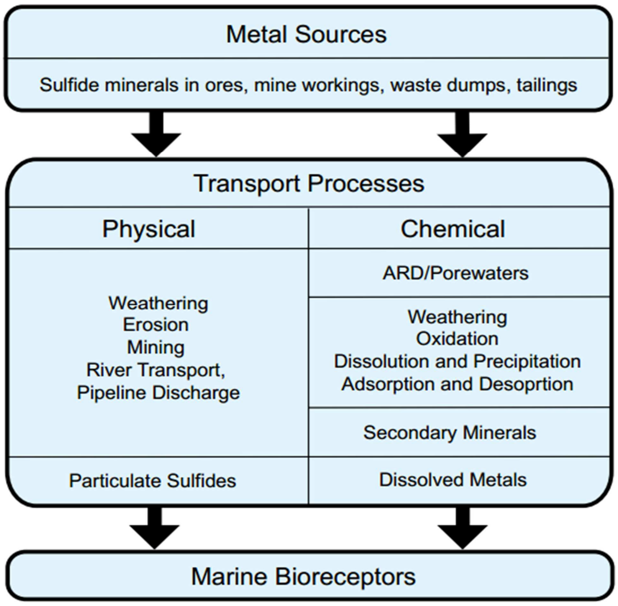


Figure 2-2 A schematic illustration of metal dispersion from source rocks to marine biota (bioreceptors) *ARD-acid rock drainage

2. The indexes of heavy metals distribution assessment

There are many indexes for monitoring heavy metal contaminations in environment, which each indexes are used in different of samples, analysis method, and applied the result to prevent the problems. For this study would like studied three indexes including: geoaccumulation index (I_{geo}), enrichment factor (EF) and biococentration factor (BCF) which were used in determined metal concentrations in bivalve tissues, sediment and seawater. In addition, many reports were used bio-indicator species for monitoring and comparing between different each species in accumulation heavy metals, but this study uses bivalves because its are used to assess environmental quality, and it is assumed that all bivalves in good quality the environment are safety for human living.

2.1 The Geoaccumulation index (I_{geo})

The Geoaccumulation index was used to define the degree of anthropogenic pollution and quantification of metal accumulation in the sediments.

Thongra-ar, Musika, Wongsudawan, and Munhapol (2008) presented the geoaccumulation index (I_{geo}) was used to define the degree of anthropogenic pollution including heavy metal level in the sediment. The index is the enrichment on geological substrates and can be calculated using the following equation:

$$I_{geo} = \log_2 C_n/1.5\times B_n$$

Where C_n is the measured concentration of the examined metal in sediments, B_n is the geochemical background concentration of the metal and 1.5 is the correction factor for variation in background values due to lithogenic effects. However, this index depends on the choice of an appropriate natural background value.

Table 2-1 Geoaccumulation index classification (Förstner et al., 1993)

Sediment Accumulation Index (I_{geo})	I_{geo}Class	Pollution Intensity
>5	6	Very Strong Pollution
>4-5	5	Strong to Very Strong
>3-4	4	Strongly Polluted
>2-3	3	Moderately to Strongly
>1-2	2	Moderately to Polluted
>0-1	1	Unpolluted to Moderate
<0	0	Practically Unpolluted

2.2 Enrichment Factor (EF)

Enrichment Factor (EF) is a good tool to differentiate the metal source between lithogenic and naturally occurring. Enrichment factor is usually distinguished by aluminum because of its high natural concentration, minimal anthropogenic contamination, it is a structural element of clays, and the metals to Al proportions in the crust are relatively constant.

According to Daskalakis and O'Connor (1995) cited in Naji and Ismail (2011) the main advantages of using Fe as a normalizer are:

(1) Fe is associated with fine solid surface.

(2) Its geochemistry is close to that of many trace metals.

(3) Its natural sediment concentration tends to be uniform.

Iron (Fe) has been used successfully by several researchers to normalize metals contamination in river and coastal sediments. The Enrichment Factor (EF) for Fe-normalized data is defined by:

$$EF_{metal} = \frac{(M_x / Fe_x)_{sample}}{(M_c / Fe_c)_{shale}}$$

Where M_x is the concentration of metal in the examined sample, Fe_x is the concentration of Fe in the examined sample, M_c is the concentration of metal in the average shale or undisturbed sediment and Fe_c is the concentration of Fe in the average shale or undisturbed sediment.

In this study Naji and Ismail (2011) used Fe to calculate EF because it is the fourth major element in the earth's crust and most often has no contamination concern. As there were no reported data available, the average shale values used in this study were those by Turekian and Wedepohl (1961) of background levels heavy metals. The undisturbed sediment values utilized were: 20 μg/g for Pb, 0.3 μg/g for Cd, 90 μg/g for Cr, 45 μg/g for Cu, and 95 μg/g for Zn.

Rule (1986) cited in Cheevaporn and San Diego-McGlone (1997) used Fe as a reference metal to normalize for grain-size effect in estuarine and coastal sediments from the inner Virginia shelf as well as Herut and Sandler (2006) recommended to the possible determination of additional normalizer iron (Fe) to better assess basin-wide spatial and temporal trends (Figure 2-3). Din (1992) evaluated normalization ability of Fe by using Al as the reference element found that the covary strongly with Al, with correlation coefficient (r^2) highest than other metals (Table 2-2).

Table 2-2 The correlation coefficient (r^2) between Al and other metals in the sediments from the Straits of Melaka (Din, 1992)

Elements	Correlation (r^2)
Fe	0.930
Cr	0.926
Zn	0.903
Cu	0.893
Pb	0.858
Cd	0.718
Mn	0.194
Ag	0.143
Ni	0.063

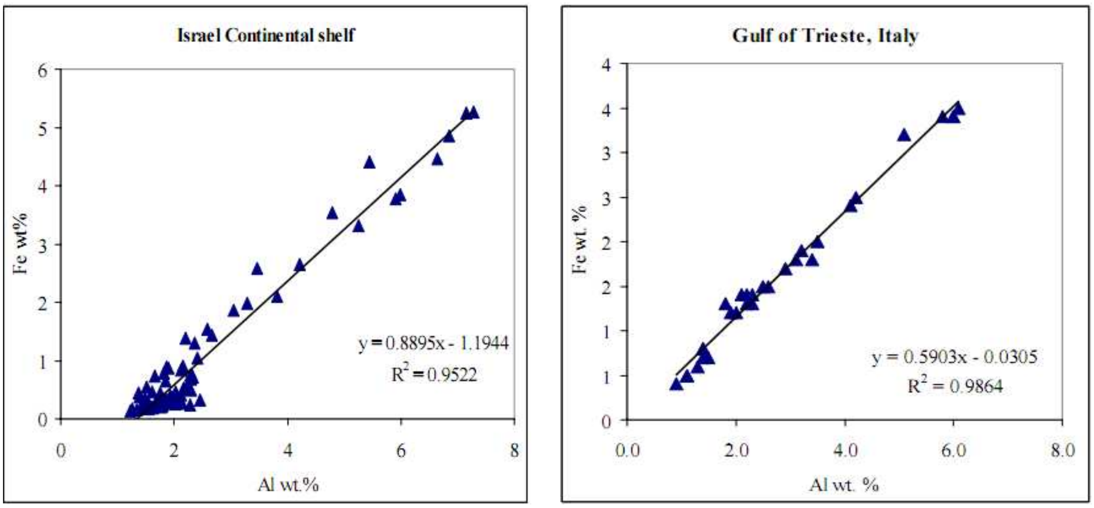


Figure 2-3 Fe versus Al concentrations in sediments at the continental shelf off Israel, gulf of Trieste, Italy (Herut & Sandler, 2006)

Table 2-3 Classification of enrichment factor (Naji & Ismail, 2011)

EF range	Assessment Category	Comment
> 50	VII	extremely severe enrichment
25 – 49.9	VI	very severe enrichment
10 - 24.9	V	severe enrichment
5 - 9.9	IV	moderately severe enrichment
3 - 4.9	III	moderate enrichment
1 - 2.9	II	minor enrichment
< 1	I	no enrichment

2.3 Bioconcentration Factor (BCF)

When organisms are exposed to metals and metalloids in their respective environments, the elements are taken up into or onto the organism, either actively or passively, depending on the element and on a number of environmental conditions. At equilibrium, a living organism usually contains a higher concentration of metal in its tissues than in its immediate environment (water, sediment, soil, air, etc.)

Bioconcentration is a situation in which the level of a heavy metal in an organism exceed the levels of that heavy metals in the ambient environment. Bioconcentration Factor (BCF) representing how much of a heavy metal is in a tissue of organism relative to how much of that heavy metals exists in the environment.

$$\text{Bioconcentration Factor (BCF)} = \frac{\text{Concentration of a heavy metal in an organism}}{\text{Concentration of the same heavy metal in the ambient medium}}$$

Table 2-4 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

2.4 Bioindicator

Adu, O.K. (2010) indicated that the bioindicators are biological indicators of environmental quality that characterize environmental conditions and reflect changes in the condition of an organism resulting from exposure to a toxicant. Their tolerance is usually limited, so their presence or absence, and health state enable the determination some physical and chemical components of the environment without complicated measurements and laboratory analyses and they are indicators of normal status or changes in individuals of a study population.

Bivalves have been shown to be valuable sentinel organisms because they greatly concentrate many chemical elements from seawater and sediment, making analysis easier. At the same time they integrate pollutant levels over time, thereby giving a more realistic indication of the pollution status of the environment, and the knowledge of the concentration factors of metals in bivalves is useful for recognizing the relative ability of the organisms to bioaccumulation selected metals from their environment.

For sediment many parameters can support and explain the changing of heavy metals level, because some of physico-chemical influence the adsorption process in sediments. Thongra-ar, Musika, Wongsudawan, and Munhapol (2008) selected sediment characteristics were determined as follows: pH, calcium carbonate ($CaCO_3$), organic matter (OM), cation exchange capacity (CEC), level of Fe and Mn oxides, and particle size distribution (percent of sand, silt, and clay). Naji and Ismail

(2011) studied support indexes in sediments including three parameters are: total organic carbon (TOC), grain size distribution, and pH.

3. Depuration

Depuration or Self-purification is the removal of xenobiotics from the body of bivalves. The technique applied in many parts of the world for decreases of heavy metal contaminants from consumption of shellfish contaminated with chemical substances is a significant problem.

Depuration consists of placing bivalves in flowing clean seawater that the mollusks resume normal pumping activity and thereby expel contaminants from their gills and intestinal tract over a period of time. The main principles of depuration are:

1. The resumption of filtration activity so that contaminants are expelled
2. The removal of contaminants
3. Avoidance of recontamination
4. Maintenance of viability and quality

Controlled depuration (self-purification) of bivalves is a method that reduces the levels of heavy metals present in bivalve tissues, thus decreasing the potential for accumulations associated with bivalve consumption.

Depuration rate is period which bivalves were excreted the heavy metals from the body. The rate of metal depuration was calculated according to the following formula (Yap, Muhamad Azlan, Cheng, & Tan, 2011) Rate of metal depuration:

$$\frac{\text{Metal level}_{\text{end of metal expose}} - \text{Metal level}_{\text{end of metal depuration}}}{\text{Hour (s) of metal depuration}}$$

The rate of depuration was used applied the concentration factor (CF) was calculated at the end of depuration (Hours) in comparison with the level of metal before exposure, as follow:

$$CF = \frac{\text{Metal level}_{\text{end of metal depuration}}}{\text{Metal level}_{\text{pre-exposure of metal}}}$$

4. The statistical instrument for analysis the heavy metals distribution

There are many techniques of statistical instrument for investigation the heavy metals distribution. For data analysis of this study focused on use multivariate statistic and multiple regression analysis including:

4.1 Principal component analysis (PCA)

PCA is a useful statistical technique that has found application in field such as face recognition and image compression, and is a common technique for finding pattern in data of high dimension. The results of PCA are score plots in which similar samples are located close together and loading plots, which indicate the effects of the original variables on the principal components. PCA does not provide new or additional information, but it helps to draw attention to the relationships in the data which could be found anyway, by a close and time-consuming examination.

The PCA is generally used to reduce a set of data with relatively high number of correlated variables to a smaller set of data of uncorrelated variables (components) which keep most of the information contained in the original data. Each component consists of a number of elements (loads), which represent the correlation of the variables with the component.

PCA could be applied to studied heavy metal contaminations in environment; such as, PCA was carried out to examine the pattern of relationship among all metals in sediments, mussel and oyster tissues obtained from various sites from estuaries (Astudillo Rojas de, Chang Yen, & Bekele, 2005).

Table 2-5 Eigen values (variances) and cumulative variance expressed as percentage of total variance explained by each component score in the PCA for metals in *P. viridis*, oyster (*Crassostrea spp.*) and sediment (Astudillo et al., 2005)

Variable	Principal Components					
	1	2	3	4	5	6
Cu	0.023	-0.727	0.003	-0.573	0.363	-0.106
Zn	0.222	-0.665	0.033	0.610	-0.359	0.084
Cd	0.184	0.050	0.946	-0.178	-0.183	0.051
Cr	-0.570	-0.087	0.090	0.005	-0.345	-0.735
Ni	-0.562	-0.119	-0.052	-0.263	-0.429	0.643
Hg	-0.525	-0.073	0.304	0.446	0.635	0.158
Eigenvalue	2.90	1.733	0.989	0.190	0.152	0.036
% variance	48.3	77.2	93.7	96.9	99.4	100.0

4.2 The multiple linear regression

The regression is one of the most useful methods for processing fuzzy data. An important objective of regression analysis is to the unknown parameters in the regression model. Multiple regression model is regression model that involves more than one regressor variable.

For general model of multiple regression used is indicated in equation of the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + ... + b_kX_k$$

Where: Y is yield or dependent variable

b_0 is the regression constant (intercept)

$b_1 \ldots b_k$ are represent the regression coefficients of dependent variables

$X_1 \ldots X_k$ are independent variables

5. Previous research

Thongra-ar, Musika, Wongsudawan, and Munhapol (2008) calculated geoaccumulation index (*Igeo*) showed that the sediments were moderately polluted with Pb in some locations, particularly at Map Ta Phut Industrial Estate, and were slightly polluted with Cu, Zn and Mn at some sampling stations. All metals (except Cu) were associated with each other in the sediments (Figure 2-4). However, this index depends on the choice of an appropriate natural background value. Since there are no metal background values for this study area, the crustal average values (Taylor, 1964) were used to calculate this index.

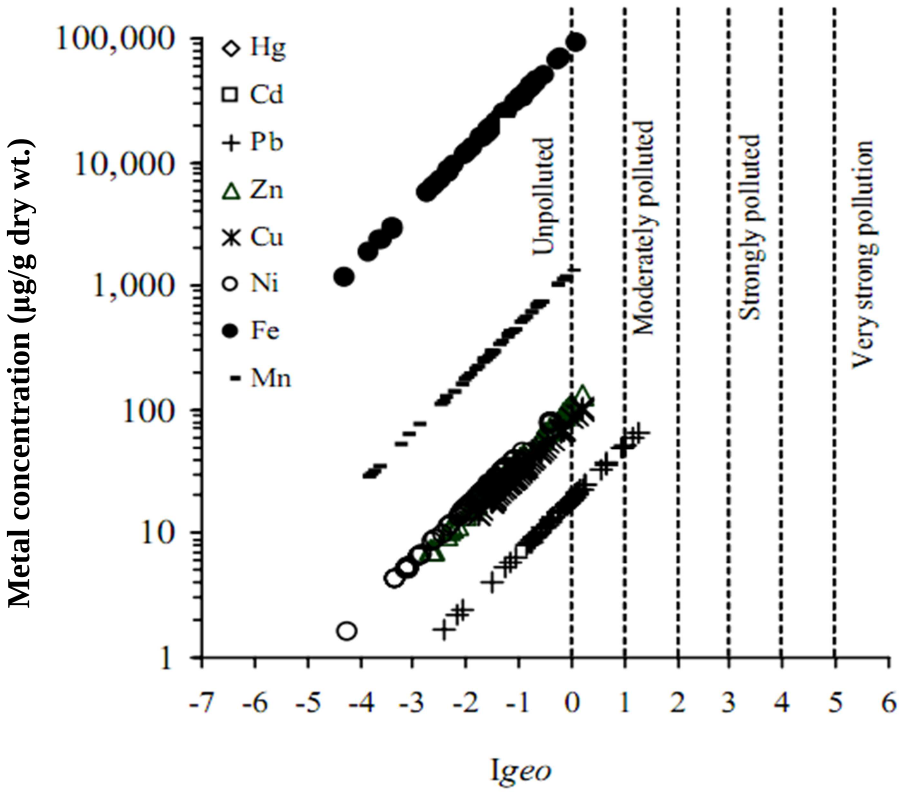


Figure 2-4 Plots of heavy metal concentrations and I_{geo} index (Thongra-ar, Musika, Wongsudawan, & Munhapol, 2008)

Table 2-6 Geoaccumulation index (*Igeo*) for different metals at different sampling stations (Suthar, Nema, Chabukdhara, & Gupta, 2009)

Sampling station	*Igeo* (Cd)	*Igeo* (Cu)	*Igeo* (Cr)	*Igeo* (Fe)	*Igeo* (Mn)	*Igeo* (Zn)	*Igeo* (Pb)
S-1	2.64	0.28	0.35	1.01	0.05	0.18	0.56
S-2	4.14	1.56	1.43	1.03	0.07	0.24	0.45
S-3	5.56	0.35	0.65	1.03	0.06	0.18	0.37
S-4	5.51	0.29	0.39	1.03	0.06	0.20	0.59
S-5	3.42	0.29	0.43	1.02	0.05	0.18	0.45
S-6	6.70	0.08	0.25	0.96	0.02	0.01	0.05
Minimum	2.64	0.08	0.25	0.96	0.02	0.01	0.05
Maximum	6.70	1.56	1.43	1.03	0.07	0.24	0.59
Mean	4.67	0.47	0.58	1.01	0.05	0.16	0.41
SD[a]	1.47	0.07	0.41	0.02	0.015	0.07	0.18

[a] Standard deviation

Suthar, Nema, Chabukdhara, and Gupta (2009) was to assess the level of heavy metals (Cd, Cr, Cu, Fe, Mn, Zn and Pb) in water and sediments of Hindon River in industrialized city Ghaziabad, India. The geoaccumulation index (*Igeo*) suggested “unpolluted to moderate pollution” of Mn, Pb and Zn (*Igeo* < 1), “moderate pollution” of Cu, Cr, Fe (*Igeo* < 2), and “very strong pollution” of Cd (*Igeo* > 5) in River Hindon at Ghaziabad city. The industrial and urban discharges in river catchment areas were the major sources of heavy metals in river. (Table 2-6)

The result from the present investigation showed that EF of Cd ranged from 5.38 to 19.18, EF from 0.99 to 7.15 for Zn, from 0.26 to 0.97 for Ni, from 0.69 to 3.78 for Cu and from 3.23 to 9.08 for Pb. The EF values of Ni in all the stations were found to be less than 1 (EF<1) which indicated that this metal had no enrichment. For the geoaccumulation index (*Igeo*) of heavy metals in this study revealed that 83.33% of the elements belonged to *Igeo* classes 0 and 1 (unpolluted and unpolluted to moderately polluted), 9.52% belonged to *Igeo* class 2 (moderately polluted) and only 7.14% is classified at *Igeo* class 3 (moderately to strongly polluted). (Table 2-7)

Table 2-7 Metal enrichment factor (EF) and geoaccumulation index (*Igeo*) values in surface sediments of Klang river (Naji & Ismail, 2011)

Station	Cd		Zn		Ni		Cu		Pb		Fe	
	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*
1	5.58	0.34	0.99	-2.16	0.26	-4.11	0.69	-2.68	3.90	-0.18	-	-2.16
2	9.24	1.18	3.72	-0.13	0.45	-3.18	1.14	-1.84	5.73	0.49	-	-2.03
3	8.67	1.40	3.38	0.04	0.46	-2.84	1.83	-0.84	6.22	0.92	-	-1.72
4	8.91	1.32	2.19	-0.71	0.39	-3.20	1.63	-1.13	5.98	0.74	-	-1.84
5	12.36	1.54	5.26	0.31	0.72	-2.56	3.26	-0.38	9.08	1.10	-	-2.09
6	6.59	1.12	2.21	-0.46	0.33	-3.19	1.13	-1.42	3.71	0.29	-	-1.66
7	7.00	1.33	2.71	-0.04	0.43	-2.69	1.26	-1.15	3.81	0.45	-	-1.48
8	5.38	1.21	2.84	0.29	0.35	-2.75	1.51	-0.62	4.46	0.94	-	-1.22
9	16.50	2.28	4.27	0.33	0.60	-2.51	1.70	-1.00	6.76	1.00	-	-1.76
10	17.88	1.98	3.08	-0.56	0.67	-2.75	1.32	-1.78	5.43	0.26	-	-2.18
11	19.18	2.24	7.15	0.82	0.97	-2.06	3.78	-0.10	6.92	0.77	-	-2.02
12	17.83	2.19	7.05	0.86	0.73	-2.42	2.03	-0.94	6.39	0.71	-	-1.96
13	13.32	1.97	6.50	0.93	0.64	-2.42	2.01	-0.76	5.91	0.79	-	-1.77
14	14.25	1.84	7.07	0.82	0.81	-2.30	3.12	-0.36	6.06	0.60	-	-1.99
15	10.95	1.37	4.54	0.09	0.68	-2.65	2.01	-1.08	5.90	0.47	-	-2.09
16	15.03	2.23	5.87	0.87	0.71	-2.18	2.53	-0.34	6.37	0.99	-	-1.68
17	15.92	2.09	5.77	0.61	0.74	-2.35	2.18	-0.79	6.98	0.90	-	-1.91
18	14.07	2.24	3.38	0.17	0.64	-2.24	2.34	-0.36	5.75	0.94	-	-1.59
19	13.32	2.26	2.95	0.09	0.52	-2.42	2.03	-0.45	0.94	0.83	-	-1.47
20	17.50	2.09	3.48	-0.24	0.60	-2.78	2.64	-0.64	7.12	0.79	-	-2.05
21	12.19	1.64	2.38	-0.72	0.43	-3.17	1.01	-1.97	3.23	-0.28	-	-1.97
Max	19.18	2.28	7.15	0.93	0.97	-2.06	3.78	-0.34	9.08	1.10	-	-1.22
Min	5.38	0.34	0.99	-2.16	0.26	-4.11	0.69	-2.68	3.23	-0.27	-	-2.18
Mean	13.08	1.71	4.13	0.06	0.58	-2.70	1.98	-0.98	5.78	0.64	-	-1.84
SD	5.24	0.52	1.83	0.72	0.18	0.47	0.79	0.64	1.38	0.37	-	0.26

Kwon and Lee (2001) found the BCF of Zinc and Copper in oyster was particularly higher than these of other metals in biota. This report recommended careful examination of Zn fate and transport in the compartments will give a good indicator for ecological risk assessment due to its abundance and increasing bioaccumulation trend in sediment and biological sample. (Table 2-8)

Table 2-8 Bioconcentration factors (BCF) of heavy metals in bivalve (Kwon & Lee, 2001)

		Zn	Pb	Cd	Ni	Cu	Cr	Sr
Seawater (μg/L)		2.17	0.64	0.08	0.72	0.91	0.19	-
Sediment (mg/kg)		206.81	38.76	2.07	27.46	42.87	45.38	5.69
Bivalves	Mussel (mg/kg)	26.81	0.14	0.28	0.16	1.06	0.08	0.86
	Oyster (mg/kg)	393.32	0.14	0.47	0.08	24.88	0.04	0.44
BCF	Mussel/Seawater	12,355	220	3,475	228	1,165	434	-
	Oyster /Seawater	181,256	216	5,818	111	27,339	185	-
	Mussel/Sediment	0.130	0.004	0.134	0.006	0.025	0.002	0.160
	Oyster /Sediment	1.902	0.004	0.225	0.003	0.580	0.001	0.081

Abdullah, Sidi, and Aris (2007) investigated of heavy metals in bivalves, seawater and sediment samples collected from two estuaries of different environmentally background. The study areas are located at Likas estuary and Kota Belud estuary on the west coast of Sabah, Malaysia. This study found that the mollusk has a potential to be used as bioindicator for the contamination of Cd and Zn in water and sediment of an estuarine environment, as indicated by its high bioconcentration factors (BCFs) values. (Table 2-9 and Table 2-10)

Table 2-9 Comparison of BCF of heavy metals from seawater between mollusks in the west coast of Sabah, Malaysia (Abdullah et al., 2007)

Heavy metals	Cumulative factor		
	M. meretrix R.	*Anadara granosa*	*Crassostrea iredalei*
Cd	5.5×10^{2}	1.0×10^{2}	1.1×10^{2}
Cu	1.6×10^{2}	1.6×10^{2}	3.1×10^{2}
Cr	2.8×10^{2}	nd	nd
Pb	1.7×10	4.7×10	4.6×10
Zn	2.5×10^{3}	2.2×10^{3}	9.2×10^{3}

*nd=not detected

Table 2-10 Comparison of BCF of heavy metals from sediments between mollusks in the west coast of Sabah, Malaysia (Abdullah et al., 2007)

Heavy metals	Cumulative factor		
	M. meretrix R.	*Anadara granosa*	*Crassostrea iredalei*
Cd	0.8	0.2	0.2
Cu	0.1	0.1	0.2
Cr	0.1	nd	nd
Pb	0.1	0.2	0.2
Zn	0.3	0.3	1.1

*nd=not detected

Yap et al. (2011) studied the accumulation and depuration of Cu and Zn in the soft tissues of cockles. For both metals, the metal levels were found to increase during the accumulation period but they decreased during the depuration period. Basically, there were only slightly differences in the accumulation and depuration patterns of these two metals. Both metals were found in significantly ($p< 0.05$) higher levels in the soft tissues at the end of accumulation (Day 6) and at the end of depuration (Day 10) as compared to those in the control treatments. (Table 2-11 and Table 2-12)

Table 2-11 Concentrations (mean µg/g dry weight ± standard error) of Cu and Zn during the accumulation and depuration in the soft and hard tissues (shell) of *Anadara granosa* (*n*=3) (Yap et al., 2011)

Metal/Tissue		During accumulation peroid				During depuration period	
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Cu	Soft tissue	6.92	10.99±0.20	14.27±0.42	18.20±0.16	14.59±0.05	11.83±0.15
	Shell	6.56	6.69±0.09	6.85±0.06	6.94±0.06	6.70±0.03	6.63±0.03
Control	Soft tissue	6.92	5.17±0.09	4.60±0.03	5.30±0.01	5.75±0.10	5.93±0.10
	Shell	6.56	6.59±0.04	6.52±0.11	6.51±0.07	6.45±0.14	6.39±0.05
Zn	Soft tissue	106.9	182.04±2.32	258.3±0.68	264.6±1.25	201.8±1.72	187.0±0.87
	Shell	4.17	5.05±0.08	5.22±0.06	5.57±0.05	5.40±0.05	5.30±0.06
Control	Soft tissue	106.9	106.08±0.39	107.9±0.46	106.1±0.37	109.2±0.50	107.5±0.51
	Shell	4.17	4.16±0.03	4.22±0.04	4.43±0.07	4.20±0.09	4.12±0.04

Table 2-12 T-test result of the metals between the end of accumulation and the control treatment, and between end of depuration and control treatment (Yap et al., 2011)

		End of accumulation (Day 6)	End of depuration (Day 10)
Cu	Soft tissues	$p<0.05$	$p<0.05$
	Shells	$p>0.05$	$p>0.05$
Zn	Soft tissues	$p<0.05$	$p<0.05$
	Shells	$p>0.05$	$p>0.05$

Amaral, Rebelo, Torres, and Pfeiffer (2005) study Zn and Cd accumulation and depuration, a set of oysters, *Crassostrea rhizophorae*, were transplanted to a metal contaminated coastal lagoon and another one was harvested there and transplanted to a non-polluted site. In the present study, researchers observed that *C. rhizophorae* is capable of better metal accumulation than depuration. In the long term, oysters can provide information about uptake rates, bioavailability and relative concentrations rather than levels of environmental contamination. (Figure 2-5 and Figure 2-6)

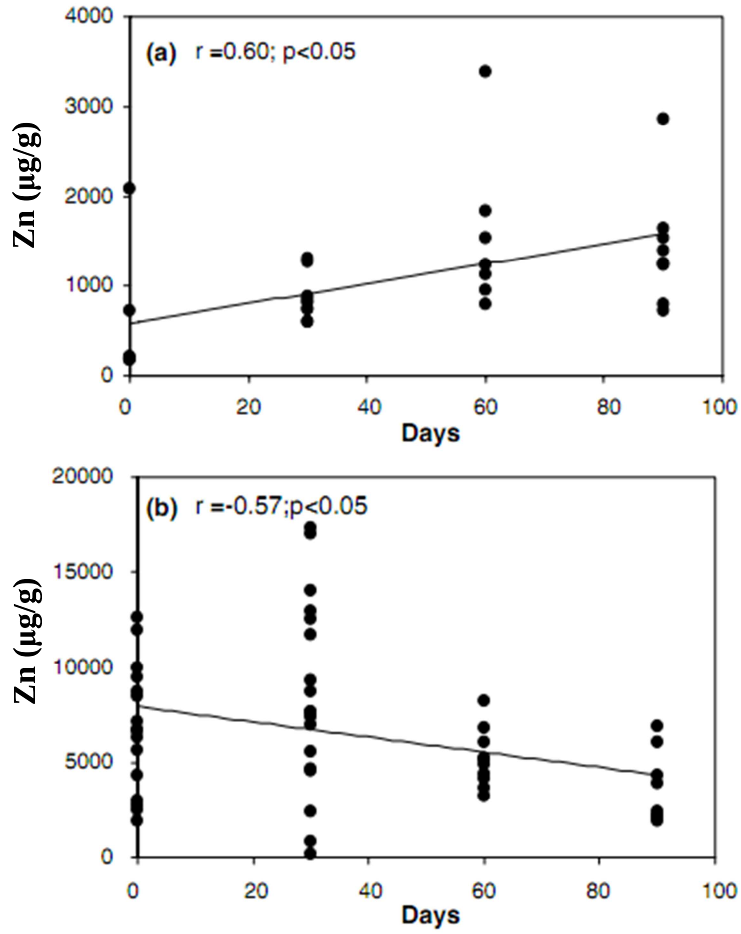


Figure 2-5 Temporal variation of Zn concentrations (µg/g dry weight) in oysters transplanted to Sepetiba Bay ((a); contaminated) and Cabo Frio Island ((b); clean) Spearman correlation significant when $p < 0.05$ (Amaral et al., 2005)

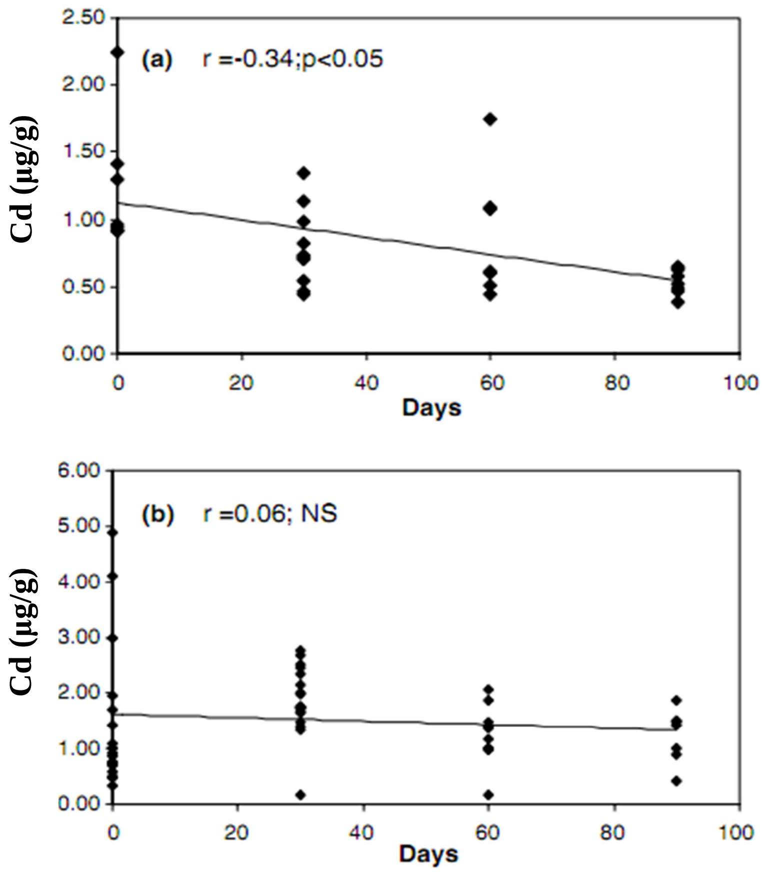


Figure 2-6 Temporal variation of Cd concentrations (µg/g dry weight) in oysters transplanted to Sepetiba Bay ((a); contaminated) and Cabo Frio Island ((b); clean) Spearman correlation significant when $p < 0.05$ (Amaral et al., 2005)

Panutrakul and Khamdee (2008) developed small close circulate depuration system for live oyster to reduce heavy metal contents in live oyster to safety level for human consumption. Oysters were placed in close circulation depuration system with a density of 14 oysters per 100 liters of seawater at 25 ppt. Oysters were collected at 0, 6, 12, 24, and 48 hours for heavy metal determination. All of the heavy metals concentrations except Pb in oysters showed reducing patterns with increasing depuration period. Heavy metal concentrations in oyster after the depuration process were within safety standard for human consumption except for Zn which is still slightly higher than the standard. (Table 2-13)

Table 2-13 Mean ± S.D. of heavy metal concentrations in the oyster at different hour after passed depuration process (µg/g wet weight) (Panutrakul & Khamdee, 2008)

Hour	Zn (µg/g)	Cu (µg/g)	Cd (µg/g)	Hg (µg/g)	Pb (µg/g)
0	195.73±73.43	26.84±6.08	0.080±0.020	4.12±0.74	0.174±0.022
6	176.10±37.19	21.94±10.22	0.071±0.017	3.64±1.06	0.220±0.078
12	170.51±67.76	18.77±8.95	0.071±0.031	3.22±1.32	0.288±0.174
24	125.63±63.88	21.52±2.36	0.069±0.034	2.82±0.76	0.260±0.193
48	107.69±42.93	15.87±4.03	0.030±0.013	2.65±0.33	0.150±0.038

Geffard, Amiard, and Amiard-Triquet (2002) studied half-lives of metals (days) of oysters which transferred from the metal-rich areas to a clean site by used half-lives equation calculated ($T1/2 = \log 2/b$). The sequence of increasing half-lives in the whole soft tissues of oyster is Cd < Zn < Cu. In this study the half-lives or effective period is the time needed to eliminate 50% of the metal quantity initially present. For elimination kinetics were modeled the following equation: $\log y = a + bx$ where x is the length of depuration; y is the metal quantity; a is the intercept; and b is the slope. (Table 2-14)

Table 2-14 Half-lives of metals (days) determined from elimination curves (log y = a + bx) in oysters (*Crassostrea gigas*) translocated from the metal-rich Gironde estuary (Fr.) to a clean site (Bay of Bourgneuf; Fr) (Geffard et al., 2002)

		Whole soft tissues	Gills	Digestive gland
Cadmium	Slope	-0.0022	-0.00235	-0.0034
	Intercept	1.0006	0.286	0.3697
	Half-lives	137	128	89
Copper	Slope	-0.0007	-0.00080	-0.00225
	Intercept	2.5822	2.0333	1.9273
	Half-lives	430[a]	376[b]	134[c]
Zinc	Slope	-0.000899	-0.0008	-0.0018
	Intercept	3.2388	2.629	2.539
	Half-lives	335[a]	376[bA]	167[cA]

In the whole soft tissues and different organs, half lives with the same small letter were not significantly different (at a level of 95%) between metals. Half-lives with the same capital letter were not significantly different (at a level of 95%) between gills and digestive gland.

Berandah, Kong, and Ismail (2010) found that the essential metals, Cu, Zn and Fe distributed differently in the different soft tissues of the molluscs. The differences to the affinities of the metals to the binding sites of metallothioneins (MT) in the different soft tissues 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. This 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.

Astudillo et al. (2005) used PCA (Principal component analysis) carried out to examine the pattern of relationship among all metals in sediments, mussel and oyster tissues obtained from various sites from the Gulf of Paria (Figure 2-7). The first two PCA axes were selected because they explain the majority of variance of the heavy metals in sediments, in the green mussel (*Perna viridis*) and oyster (*Crassostrea spp.*)

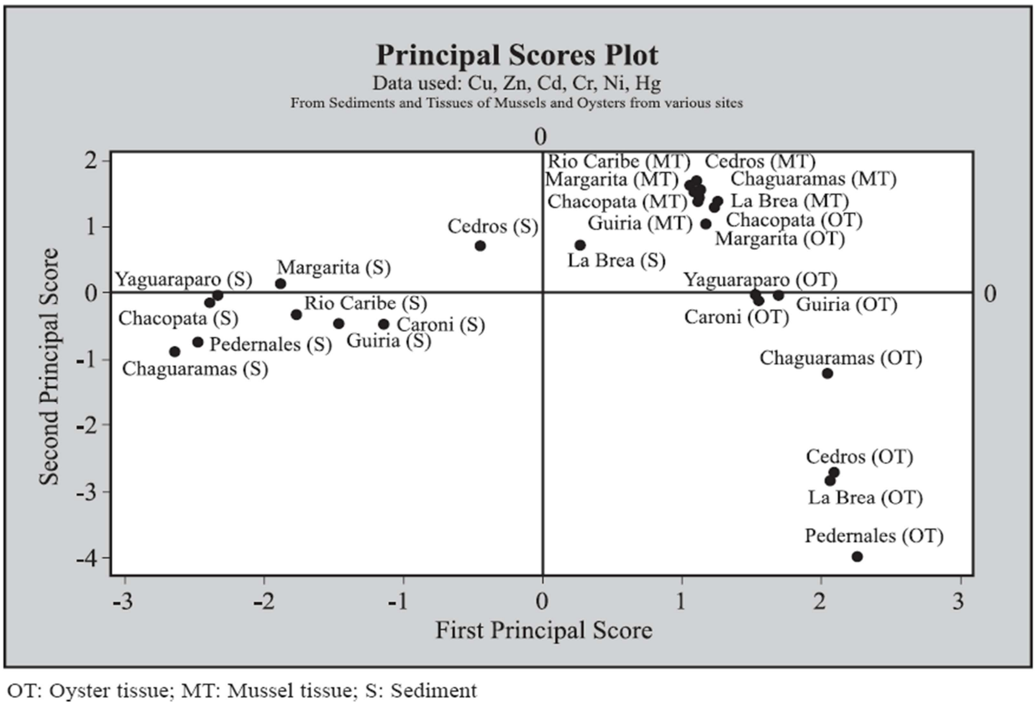


Figure 2-7 Principal scores plot based on heavy metal levels of sediments and tissues of oysters and mussels sampled from various sites in Trinidad and Venezuela (Astudillo et al., 2005)

Spooner, Maher, and Otway (2003) showed the PCA of all sediment data illustrates the grouping of sites within the bay. The principal component biplot (PC 1 explaining 93.01% of variation, PC 2 explaining 2.66% of variation) illustrates a close grouping of site 2, 3, and 4, which are all located along the southern shoreline. Sediment at these sites have significantly lower zinc, copper, and lead concentrations than other sites. Site 5 and 6 are separated from these stations in southern area because of the high zinc and copper concentrations found in sediments, which had a relatively positive variance from the standardized zero point on PC 1. (Figure 2-8)

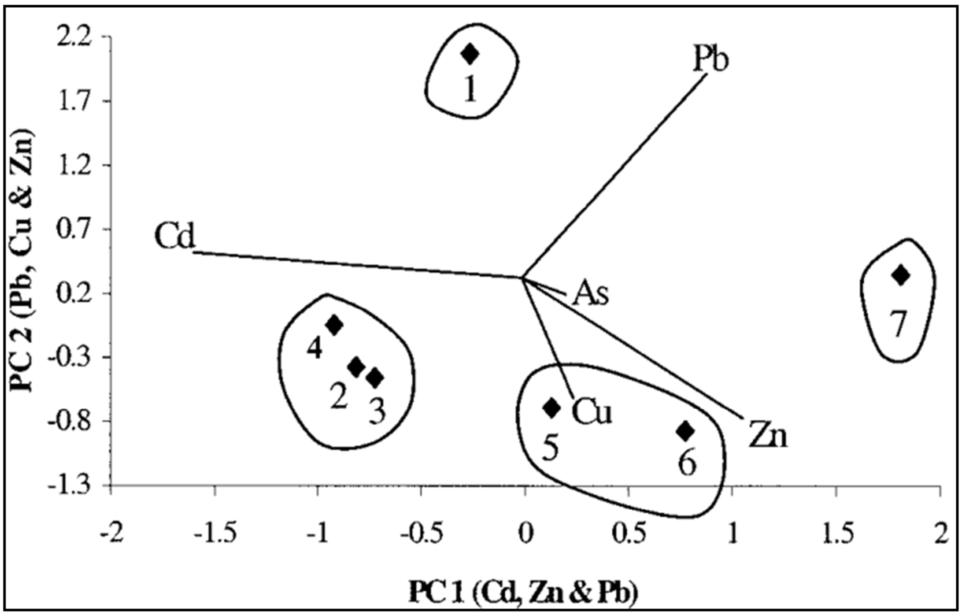


Figure 2-8 PCA biplot for Botany Bay surficial sediment trace metal concentration data. Vector direction is proportional to the strength of the factor loading on each axis. (Spoone et al., 2003)

Lares, Flores-Muñoz, and Lara-Lara (2002) studied temporal variability of bioavailable Cd, Hg, Zn, Mn and Al in an upwelling regime. The assumption of this study is the investigation relationship between climatic and hydrographic conditions to the physiological state of the mussels (condition index) by PCA. The PCA showed the correlation of metals (Hg, Zn, Mn and Al) to pluvial precipitation and to the condition index. By axes of all these variables high loads (positive) in Component 1, which it reveal the relation of metal concentrations with rainy influence. (Figure 2-9)

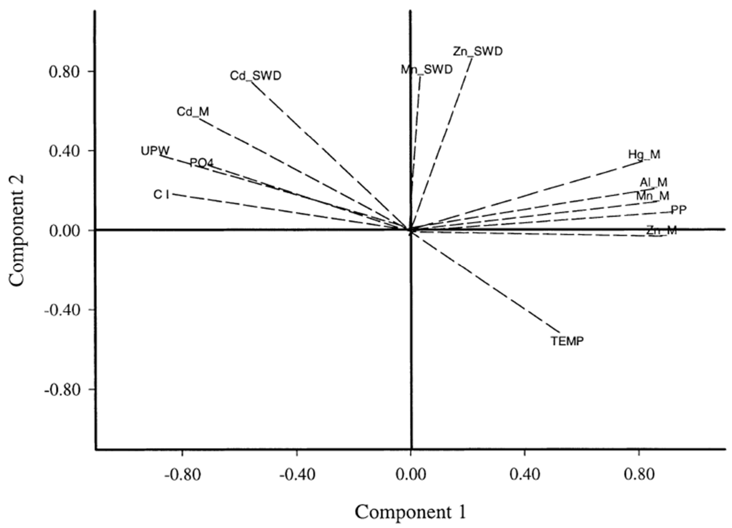


Figure 2-9 Principal component analysis with all variables studies of bioavailable Cd, Hg, Zn, Mn and Al in an upwelling regime (Lares et al., 2002)

Yap, Edward, and Tan (2010) used multivariate analysis including correlation and multiple stepwise linear regression for investigation the heavy metal concentrations (Cd, Cu, Fe, Ni, Pb and Zn) in the different parts of bivalves. The multiple stepwise linear regression show that heavy metals in the total soft tissues were influenced by the accumulation in the different types of soft tissues. (Table 2-15)

Table2-15 Multiple stepwise linear regression between the soft tissues and the different of soft tissues of the selected bivalves based on the concentrations of Cd, Cu, Fe, Ni, Pb and Zn (Yap et al., 2010)

Species (Bivalves)	Multiple stepwise linear regression equation	*R*	R^2
Donax faba	Total tissue=0.353-0.260 (remainder) -5.610 (mantle) +2.738 (gill) +6.680 (siphon) -0.172 (foot) -3.433 (muscle)	0.994	0.988
Polymesoda erosa	Total tissue=0.261+0.758 (remainder) +0.010 (muscle) +0.546 (foot) +0.770 (mantle) -0.977 (gill)	0.990	0.981

CHAPTER 3
RESEARCH METHODOLOGY

Sampling stations

The samples were collected from along the river basin of Chanthaburi coastal areas. The samples were Bivalves (Oyster, Mussel and Cockle), surface sediments and seawater. The sampling stations were divided into 3 zones symbolically: A, B and C which focusing on industrial, urban community and conservation areas, respectively (Figure 3-1). All sampling at each station was carried out during March 2012 to March 2013.

Table 3-1 GPS location and description of sampling areas in the river basin of Chanthaburi coastal areas

Sampling Areas	GPS Location	Description
A (Wang-Ta-Nord Basin)	Lat. 12.538166° Long. 101.947492°	Upstream site in agriculture areas and downstream site near Map Ta Phut industrial estate.
B (Chanthaburi Basin)	Lat. 12.484102° Long. 102.060256°	Several anthropogenic activities (urban, oxidation pond treatment, aqua culture in river mouth areas)
C (Welu Basin)	Lat. 12.386656° Long. 102.370192°	Upstream site in agriculture areas and river mouth near conservation areas for eco-tourism.

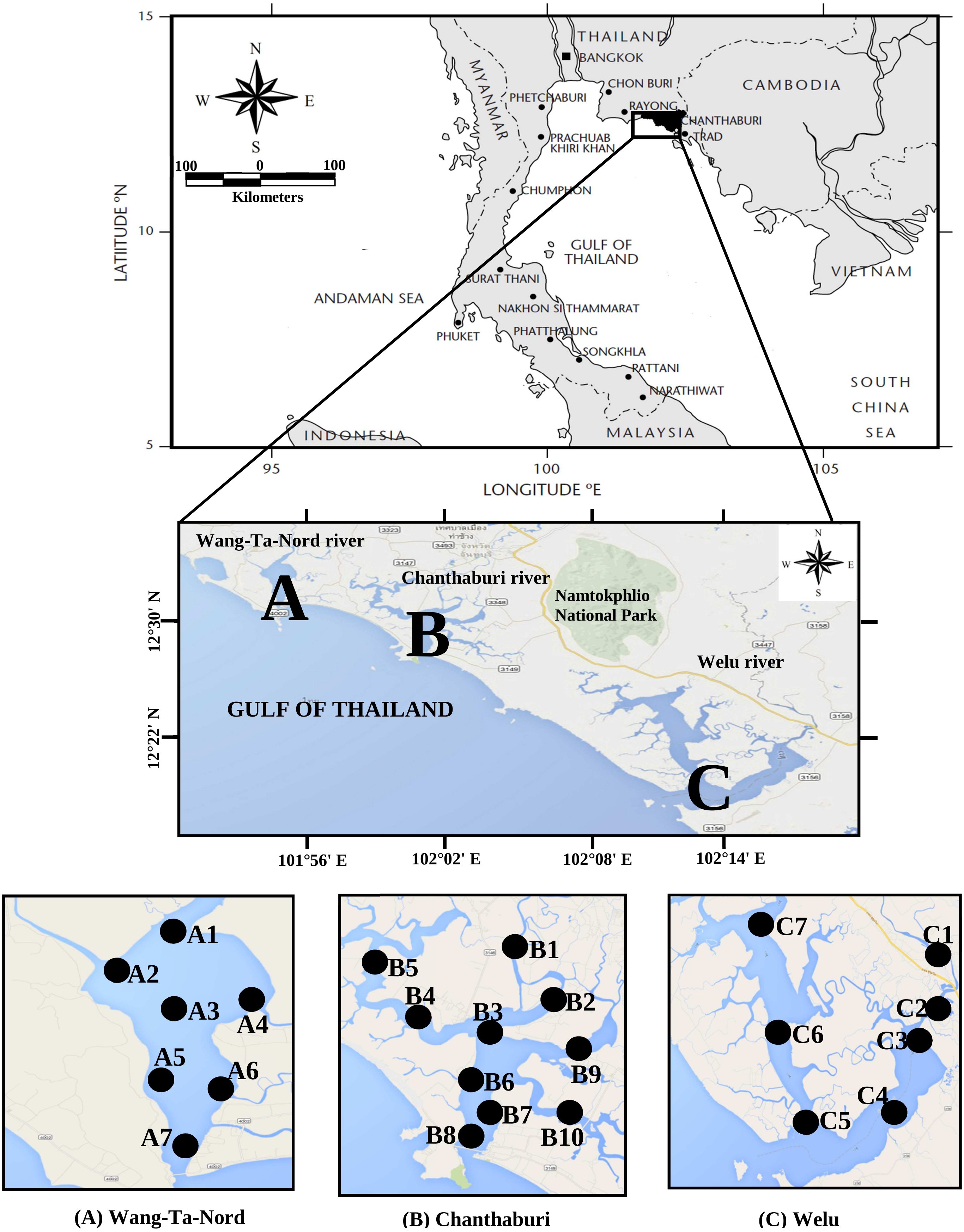


Figure 3-1 Map of river basin of Chanthaburi coastal area and sampling locations

Sample preparation

1) Bivalve samples were prepared by digestion in concentrated nitric acid (ULTRAPUR® Grade, Merck Chemicals, HNO_3 65%, Germany), which this procedure followed (Yap & Edward, 2010).

2) Sediment samples were digested by method for the acid digestion of sediment (Dean, 2003).

Additional, Sediment samples were analyzed organic carbon with Weight Loss on Ignition technique (Combs & Nathan, 1998) and grain size was determined by sieve analysis (American Society for Testing and Materials, 2007)

3) Seawater samples were cleared with concentrated nitric acid which modification from (The Perkin Elmer Corporation, 1994)

Determination of heavy metals

The instrumental analysis was carried out by using flame atomic absorption spectrophotometer model Spectrometer 3110 Perkin Elmer for analysis essential elements (Fe, Cu, and Zn) and graphite furnace atomic absorption spectrophotometer model SpectrAA-640 Varian for analysis non-essential elements (Pb, Cd, and Cr)**.**

Quality control

The accuracy and precision of the analyses were determined by measurements of standard reference materials from National Research Council Canada. Recoveries were done by using prepared standard solutions for each metal. In addition, the analytical procedures for the bivalves and sediment were checked with the Certified Reference Material (CRM) for dogfish muscle (DORM-3, National Research Council Canada) and marine sediment (MESS-3, National Research Council Canada). The recoveries of all the metal were satisfactory (80-110%). Moreover, to avoid possible contamination, all glassware and equipment used were acid-washed.

Procedure of indexes for assessment of heavy metals distribution

Procedure of investigation of heavy metals distribution in each indexes, which including: geoaccumulation index (I_{geo}), enrichment factor and bioconcentration factor could be followed for each process in Figure 3-2, Figure 3-3, and Figure 3-4.

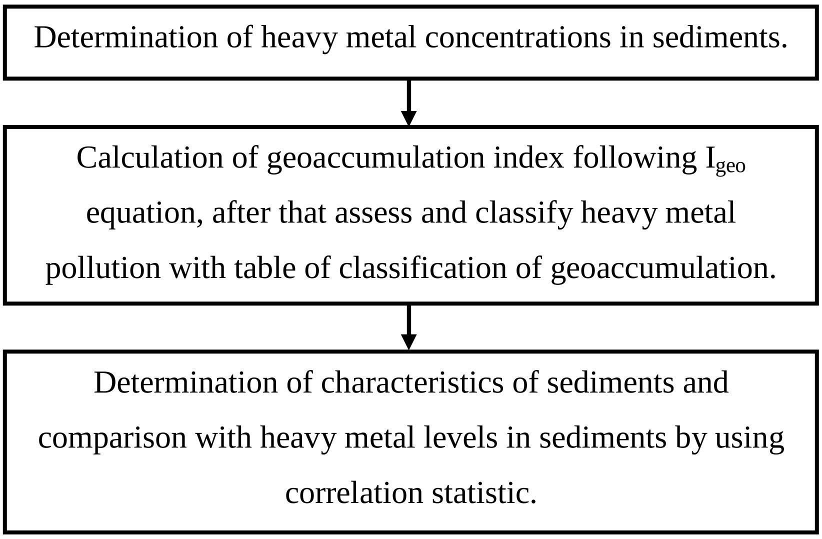


Figure 3-2 Procedure of geoaccumulation index (I_{geo}) for heavy metals monitoring

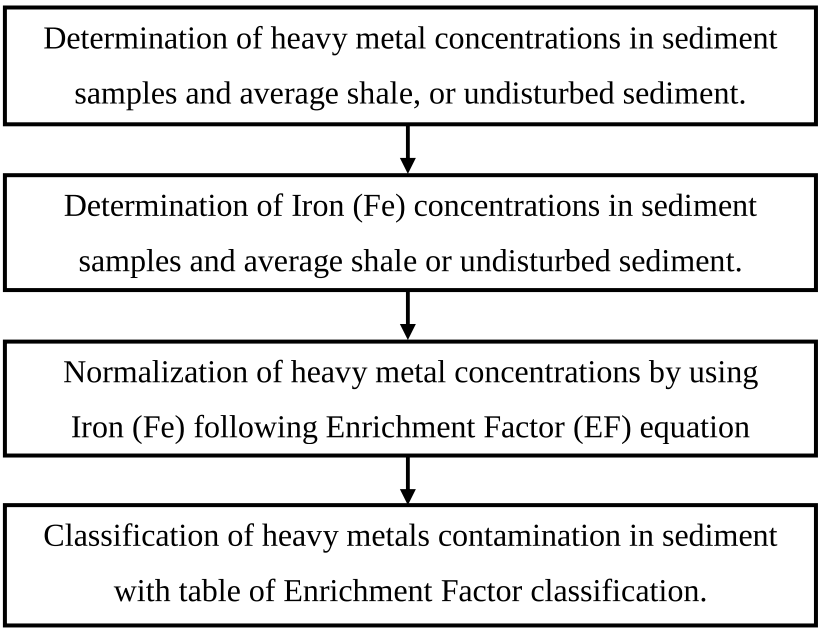


Figure 3-3 Procedure of enrichment factor (EF) for heavy metals monitoring

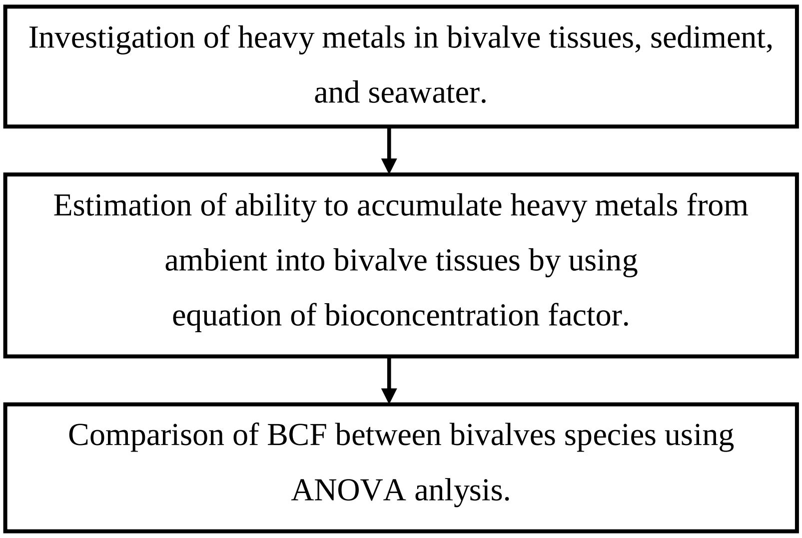


Figure 3-4 Procedure of bioconcentration factor (BCF) for heavy metals monitoring

Experiment of depuration

Prior to the depuration experiment, oysters and mussels were placed in a tank containing 50 L of seawater with 20 sampling groups per tank. Depuration assays were undertaken during an 8-hours period. Oysters and mussels were removed at 0, 1, 3, 6, 12, 24, 48 and 72 hour for analysis. Oyster and mussel activity as well as water temperature were routinely monitored throughout depuration, as were DO, salinity, and pH. After finishing the collection, each samples were put in a plastic bag and stored at 4°C. At the end of the depuration assay, the samples were transported to the laboratory for the analysis.

Once placed in the system, the physicochemical conditions were kept as described in Table 3-2, in order to obtain an optimum activity of the bivalves. The oysters and mussels were experimented at different hours of depuration as shown in Table 3-3.

Table 3-2 Physicochemical parameters under which the depuration experiments were performed (Lee et al., 2008)

	Temp range (°C)	Salinity range (g/L)	pH	DO (% saturation)
Depuration	25-30	25-29	7.4-8.3	> 60

Table 3-3 The experimental depuration of Cu and Zn in oysters and mussels

Metals	Species	Station	Hours of depuration	Types of systems
Cu	Oysters	A	0, 1, 3, 6, 12, 24, 48 and 72	Static
	Mussels	B		
		C		
Zn	Oysters	A	0, 1, 3, 6, 12, 24, 48 and 72	Static
	Mussels	B		
		C		

Statistical and data analysis

All data were tested for normality and homogeneity. When required, the data sets were transformed to a normal distribution by using either a $\log_{10}$ or a square root transform function. All the statistical analyses were either undertaken on data with a normal distribution and statistical tests including:

1. Descriptive Statistics including: means, standard deviation and percentages were used for presentation and conclusion the metal levels in different parts and other heavy metal indexes.

2. The differences of concentration between the different metal levels, locations, and species were checked using analysis of variance (ANOVA).

3. The correlation coefficient analysis was used to analyze the relationships between interdependencies of bivalves (oysters, mussel and cockle), sediments, seawater and other indexes.

4. The principal component analysis (PCA) was used to reduce the highly dimensional of heavy metals distribution in many local areas into minimal components to find the original variables with minimal variance. Two-dimensional biplot was produced by PCA technique to illustrate the located grouping and interaction between the non essential elements (NEE) and the essential elements.

5. The multiple regression analysis was selected to investigation to what extent metal concentrations in many partitions of environment such as bivalves, sediment and seawater. Moreover, these equations were used for prediction of heavy metals distribution in the study areas. In addition, the independent variables in equations were selected from only influential variables which were significant from the correlation coefficient analysis.

The 0.05 significance level was adopted for all statistical tests.

Table 3-4 Indexes for heavy metals contamination assessment in the river basin of Chanthaburi coastal areas

Indexes	Samples	Heavy metals
Statistical Model (Multiple Linear Regression) and PCA (Principal component analysis)	Bivalves, Sediments and Seawater	Zn, Cu, Fe, Cd, Pb and Cr
Geoaccumulation index (*Igeo*) and Enrichment Factor (EF)	Sediments	Zn, Cu, Fe, Cd, Pb and Cr
Bioconcentration factors (BCF)	Bivalves, Sediments and Seawater	Zn, Cu, Fe, Cd, Pb and Cr
Depuration rate	Oysters Mussels	Zn and Cu

(Bivalves: Cockle, Mussel and Oyster)

CHAPTER 4
RESULTS

Part 1. The investigation of distribution of selected heavy metals in the river basin of Chanthaburi coastal areas

The distributed study of selected heavy metals (Pb, Cd, Cr, Fe, Cu and Zn) were integrated and combined with scientific process, environmental index and statistical techniques including: the geoaccumulation index (I_{geo}), the enrichment factor (EF), the bioconcentration factor (BCF), the principal component analysis (PCA) and the multiple regression analysis.

The distribution of heavy metals

The distributed study of selected heavy metals (Pb, Cd, Cr, Fe, Cu and Zn) in the three river basin of Chanthaburi coastal areas showed that the average heavy metals concentration of three bivalves including: Cockles (*Anadara granosa*), Mussels (*Perna viridis*) and Oysters (*Saccostrea cucullata*). The average of heavy metals concentration of the cockles (*Anadara granosa*) are 0.022 ± 0.006 µg/g for Pb, 0.426 ± 0.214 µg/g for Cd, 0.132 ± 0.015 µg/g for Cr, 493 ± 79 µg/g for Fe, 9.663 ± 6.003 µg/g for Cu and 33.870 ± 13.271 µg/g for Zn (means ± *SD*, *n*=24). The average of heavy metals concentration of the mussels (*Perna viridis*) are 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 average of heavy metals concentration of the oysters (*Saccostrea cucullata*) are 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). The ANOVA analysis showed that Pb, Cd, Cu and Zn concentration of the different bivalve species and the river basin were significantly different ($p<0.05$), whereas Cr and Fe concentration only differ significantly of the bivalve species.

The averages of heavy metals concentration in sediment of the river basin of Chanthaburi coastal areas from various stations are 1.818 ± 0.525µg/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). The ANOVA testing found that there was no significant difference of the station (p>0.05), except Pb. The Pb concentration was highest level in the Chanthaburi river basin.

The averages of heavy metals concentration in seawater of the river basin of Chanthaburi coastal areas from various stations are 1.365 ± 0.291µg/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 concentration of Cd, Fe, Cu and Zn in the river basin were significantly difference of the station (p<0.05), whereas Pb and Cr were not found. The Cd, Fe and Cu, Zn were highest level in the Chanthaburi river basin and the Welu river basin, respectively.

The geoaccumulation index (I_{geo})

The result from this study showed that I_{geo} values of Pb ranged from -4.16 to (-2.63), I_{geo} from -8.67 to -3.43 for Cd, from -4.92 to -3.74 for Cr, from -3.07 to -1.90 for Fe, from -4.47 to -2.93 for Cu and from -8.67 to -3.43 for Zn. Table 4-4 show the negative values (I_{geo}<0) indicated that there was no pollution from the heavy metals in all the river basin of Chanthaburi coastline.

The ANOVA testing found that there was no significant difference of the station (p>0.05), except Pb. The Turkey analysis revealed that the I_{geo} value of Pb was the highest value in the Chanthaburi river basin. Fe and Zn were highest I_{geo} values and concentration in sediment as shown in Figure 4-3.

The enrichment factor (EF)

As there were no reported data available, the average shale values used in this study were those by Turekian and Wedepohl (1961) of background levels heavy metals. The undisturbed sediment values utilized were: 20 µg/g for Pb, 0.3 µg/g for Cd, 90 µg/g for Cr, 45 µg/g for Cu, and 95 µg/g for Zn. The result of EF study showed that EF of Pb ranged from 0.15 to 0.38, EF from 0.08 to 0.26 for Cd, from 0.16 to 0.36 for Cr, from 0.18 to 0.68 for Cu and from 0.34 to 0.69 for Zn. The EF results from the present investigation were shown in Table 4-4.

The EF values of all heavy metals at all stations were found to be less than 1 (EF<1) which indicated that there was no heavy metals enrichment detected in the study area as shown in Figure4-4. The ANOVA testing showed that there was no significant difference of the station (p>0.05), except Cu. Turkey analysis revealed that the EF of Cu was different between the Chanthaburi river basin and the Welu river basin.

Table 4-1 Mean of heavy metals concentration (μg/g) in bivalve species in the river basin of Chanthaburi coastal areas

Bivalves	Location	Concentration of heavy metals (μg/g)					
		Pb	Cd	Cr	Fe	Cu	Zn
Cockles (*Anadara granosa*)	Wang-Ta-Nord	0.028	0.659	0.131	499	5.657	28.535
	Chathaburi	0.020	0.377	0.138	481	15.776	47.369
	Welu	0.017	0.263	0.126	502	4.934	19.921
	Mean	0.022	0.426	0.132	493	9.663	33.870
	SD	0.006	0.214	0.015	79	6.003	13.271
Mussels (*Perna viridis*)	Wang-Ta-Nord	0.009	0.097	0.181	394	7.873	28.454
	Chathaburi	0.010	0.100	0.154	472	13.826	34.883
	Welu	0.010	0.081	0.150	428	6.796	29.615
	Mean	0.010	0.093	0.161	436	10.039	31.472
	SD	0.002	0.023	0.033	68	4.224	4.084
Oysters (*Saccostrea cucullata*)	Wang-Ta-Nord	0.009	0.704	0.183	122	39.337	192.489
	Chathaburi	0.013	0.848	0.224	131	29.974	184.219
	Welu	0.010	0.664	0.186	122	29.536	182.671
	Mean	0.011	0.752	0.201	126	32.577	186.180
	SD	0.003	0.193	0.051	13	5.860	10.688
Permission standard limit in food and sea food (Ministry of Public Health)		0.5	2.0			20	100
Permission standard limit in food WHO (1989)				50	1,000		

Table 4-2 Concentration of heavy metals (μg/g) in sediments in the river basin Chanthaburi coastal areas

Location		Concentration of heavy metals (μg/g)						pH	OM %	Particle Size Distribution		
		Pb	Cd	Cr	Fe	Cu	Zn			Sand %	Silt %	Clay %
Wang-Ta-Nord (7 stations)	Mean	1.536	0.016	8.192	16,689	7.542	17.930	7.66	2.4	41.5	41.7	16.8
	Max	2.511	0.024	10.731	20,492	9.800	21.392	8.11	3.3	45.3	45.2	20.6
	Min	1.045	0.010	6.327	10,733	5.831	13.322	7.26	1.9	37.5	39.3	13.9
Chathaburi (10 stations)	Mean	2.209	0.020	9.322	19,615	6.653	19.199	7.77	2.7	38.9	41.6	19.5
	Max	3.039	0.028	11.308	22,671	8.998	21.941	8.23	3.6	42.3	45.6	24.3
	Min	1.577	0.017	7.740	16,981	3.692	14.272	7.23	1.9	33.8	37.0	16.3
Welu (7 stations)	Mean	1.542	0.016	8.127	16,526	8.372	16.774	7.49	3.1	39.1	40.8	20.1
	Max	2.075	0.027	10.763	20,767	10.832	22.675	8.06	4.2	42.8	43.7	25.2
	Min	1.084	0.09	4.948	10,059	5.938	11.644	7.04	2.5	35.6	39.2	16.9
Sediments in river basin Chanthaburi coastal areas (24 stations)	Mean	1.818	0.018	8.644	17,860	7.414	18.122	7.66	2.7	39.7	41.4	18.9
	SD	0.525	0.005	1.648	3,385	1.952	3.367	0.37	0.6	2.8	2.3	2.9
World average sediment Sparks (2003)		19	0.17	72	41,000	33	95					
Proposed sediment quality guidelines (SQG) for Thailand by PCD (2006)*		35.8	0.99	81	-	31.6	121					

* Equilibrium Partitioning Approach (EqP) Method

Table 4-3 Concentration of heavy metals (µg/L) in seawater in the river basin Chanthaburi coastal areas

Location		Concentration of heavy metals (µg/L)					
		Pb	Cd	Cr	Fe	Cu	Zn
Wang-Ta-Nord (7 stations)	Mean	1.396	0.006	0.087	101.081	2.815	18.735
	Max	1.744	0.009	0.112	105.735	3.047	20.321
	Min	1.140	0.004	0.053	92.845	2.522	15.627
Chathaburi (10 stations)	Mean	1.307	0.013	0.066	127.104	2.709	16.389
	Max	1.736	0.017	0.092	138.802	2.985	18.757
	Min	0.930	0.010	0.044	110.724	2.363	12.795
Welu (7 stations)	Mean	1.387	0.007	0.077	89.042	3.112	19.023
	Max	1.901	0.011	0.104	98.699	3.337	20.800
	Min	1.009	0.004	0.058	79.825	2.733	16.744
Seawater in river basin Chanthaburi coastal areas (24 stations)	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
Criterion*		8.5	5.0	100	300	8.0	50

*The announcement of the National Environment Board No.27 (B.E. 2549) regarding the specification of the standard of sea water.

Table 4-4 Heavy metals enrichment factor (EF) and geoaccumulation index (*Igeo*) values in the river basin Chanthaburi coastal areas

Station	Pb		Cd		Cr		Fe		Cu		Zn	
	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*	EF	*Igeo*
A1	0.28	-3.88	0.15	-4.92	0.36	-4.35	-	-2.98	0.68	-3.57	0.69	-2.83
A2	0.35	-2.90	0.22	-3.64	0.22	-4.41	-	-2.31	0.61	-3.07	0.51	-2.59
A3	0.21	-3.56	0.15	-4.13	0.26	-4.08	-	-2.24	0.34	-3.81	0.60	-2.29
A4	0.17	-4.16	0.18	-4.13	0.29	-4.18	-	-2.51	0.44	-3.74	0.64	-2.46
A5	0.20	-3.41	0.15	-3.99	0.27	-3.80	-	-2.04	0.46	-3.20	0.48	-2.42
A6	0.20	-3.86	0.13	-4.54	0.30	-4.08	-	-2.44	0.48	-3.54	0.43	-2.98
A7	0.15	-3.88	0.12	-4.32	0.16	-4.57	-	-2.05	0.41	-3.37	0.48	-2.41
B1	0.38	-2.63	0.18	-3.84	0.32	-3.74	-	-2.17	0.34	-3.78	0.46	-2.60
B2	0.31	-3.06	0.26	-3.43	0.28	-4.04	-	-2.31	0.44	-3.54	0.61	-2.34
B3	0.19	-3.57	0.13	-4.13	0.27	-3.90	-	-2.08	0.47	-3.20	0.43	-2.59
B4	0.26	-3.01	0.13	-4.13	0.22	-4.08	-	-2.02	0.29	-3.88	0.48	-2.40
B5	0.30	-2.90	0.17	-3.78	0.26	-3.94	-	-2.08	0.42	-3.37	0.51	-2.37
B6	0.27	-3.02	0.14	-4.06	0.23	-4.08	-	-2.06	0.19	-4.47	0.35	-2.88
B7	0.18	-3.41	0.14	-3.90	0.21	-4.06	-	-1.90	0.18	-4.38	0.48	-2.27
B8	0.26	-3.04	0.18	-3.64	0.25	-3.92	-	-2.02	0.29	-3.84	0.50	-2.33
B9	0.25	-3.22	0.17	-3.84	0.21	-4.27	-	-2.15	0.50	-3.20	0.46	-2.58
B10	0.28	-3.20	0.17	-4.06	0.27	-4.13	-	-2.31	0.51	-3.31	0.64	-2.26
C1	0.38	-3.56	0.17	-4.76	0.26	-4.92	-	-3.07	0.62	-3.80	0.58	-3.17
C2	0.24	-4.11	0.13	-5.06	0.28	-4.72	-	-2.98	0.59	-3.78	0.54	-3.17
C3	0.15	-4.01	0.08	-5.06	0.19	-4.47	-	-2.20	0.37	-3.66	0.34	-3.04
C4	0.18	-3.76	0.16	-4.06	0.25	-4.08	-	-2.22	0.51	-3.24	0.49	-2.56
C5	0.19	-3.56	0.14	-4.06	0.26	-3.90	-	-2.06	0.56	-2.93	0.56	-2.21
C6	0.28	-3.17	0.22	-3.64	0.30	-3.92	-	-2.28	0.59	-3.08	0.57	-2.40
C7	0.22	-3.31	0.20	-3.47	0.27	-3.80	-	-2.02	0.54	-2.93	0.50	-2.33
Max	0.38	-2.63	0.26	-3.43	0.36	-3.74	-	-1.90	0.68	-2.93	0.69	-2.21
Min	0.15	-4.16	0.08	-8.67	0.16	-4.92	-	-3.07	0.18	-4.47	0.34	-3.17
Mean	0.24	-3.42	0.16	-4.29	0.26	-4.14	-	-2.27	0.45	-3.53	0.51	-2.56
SD	0.07	0.42	0.04	1.04	0.04	0.30	-	0.32	0.13	0.41	0.09	0.29
Average crust (μg/g) [a]	12.5		0.2		100		56,300		55		70	
Average shale (μg/g) [b]	20		0.3		90		47,200		45		95	

(A-Wang-Ta-Nord, B-Chanthaburi and C-Welu)

[a] Taylor (1964)

[b] Turekian and Wedenphol (1961)

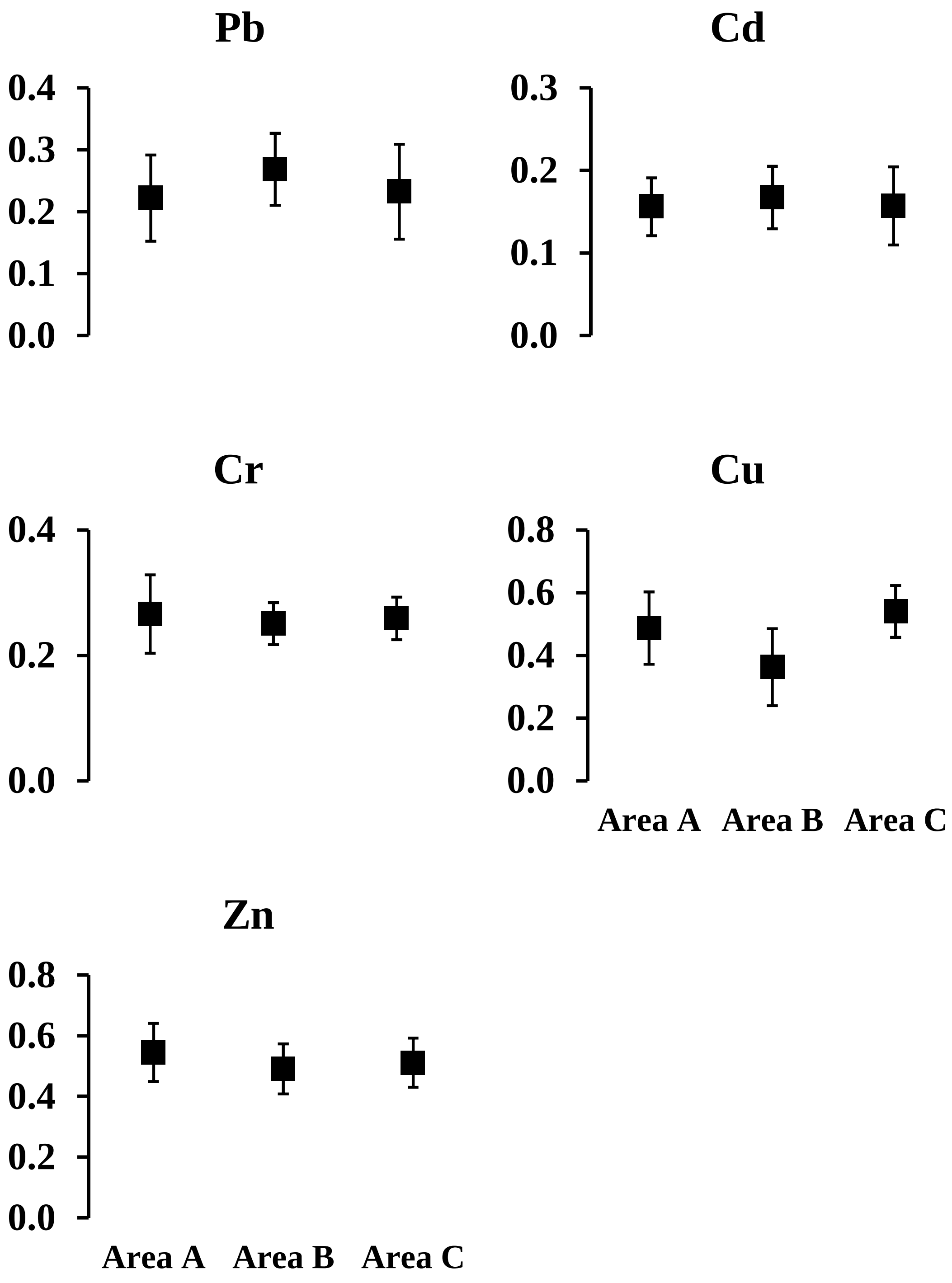


Figure 4-1 Enrichment of heavy metals in the river basin Chanthaburi coastal areas

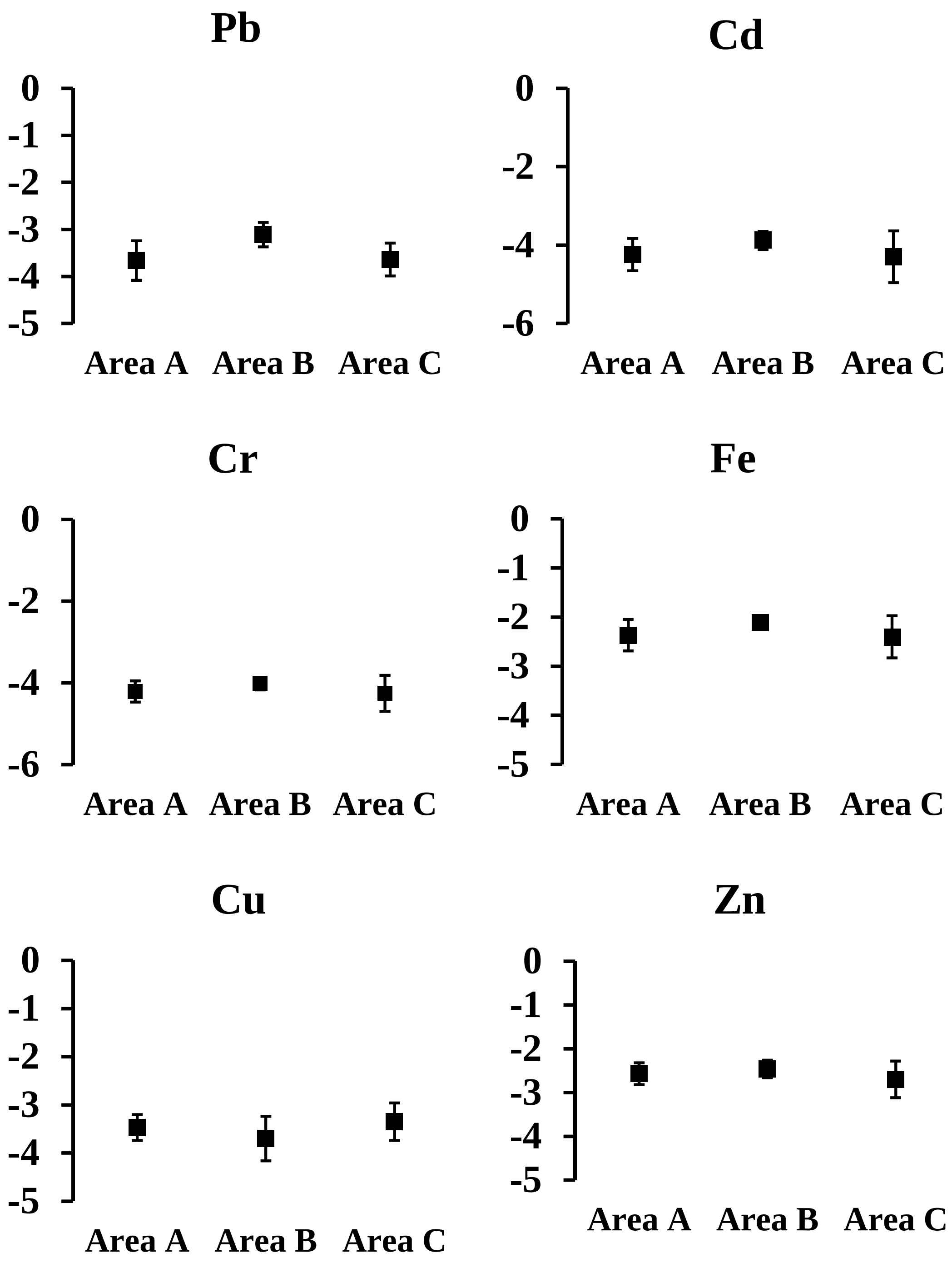
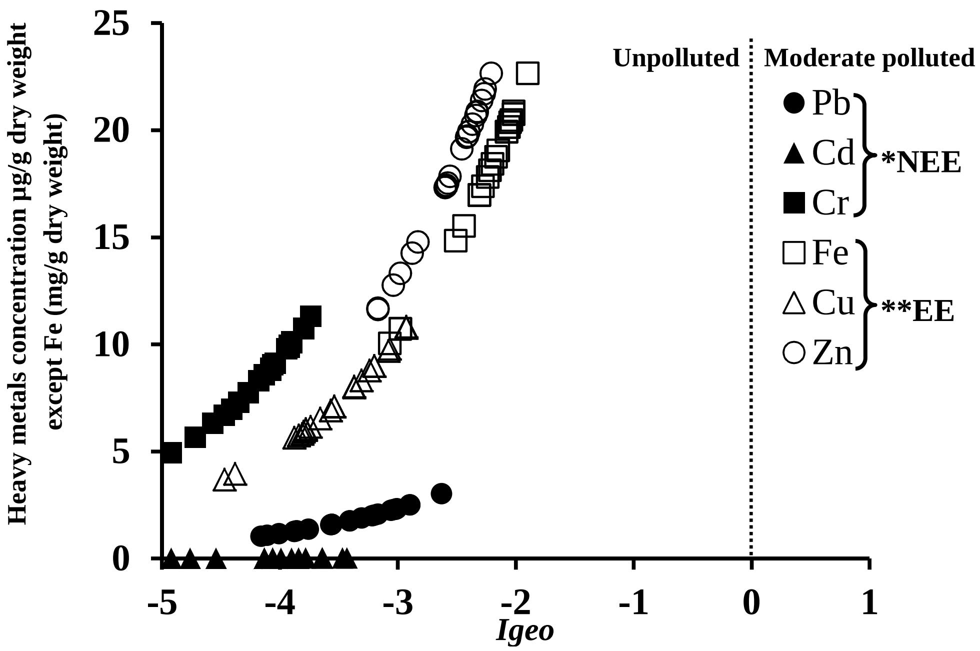
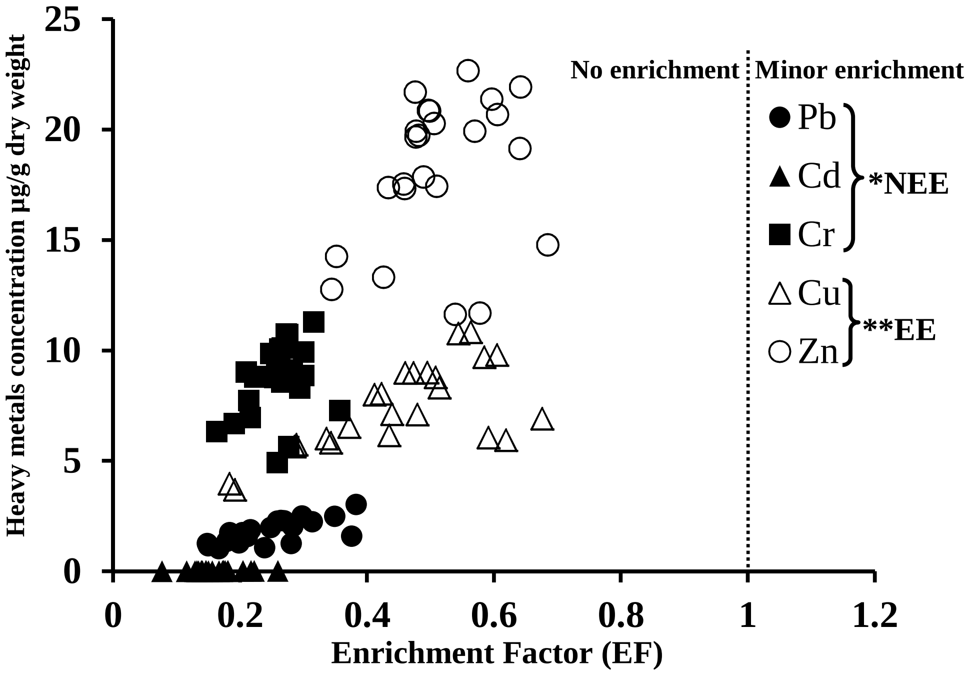


Figure 4-2 Geoaccumulation (*Igeo*) values of heavy metals in the river basin Chanthaburi coastal areas



*NEE-Non Essential Elements, **EE- Essential Elements

Figure 4-3 Plot of heavy metals concentration and *Igeo* index



*NEE-Non Essential Elements, **EE- Essential Elements

Figure 4-4 Plot of heavy metals concentration and enrichment factor (EF)

The bioconcentration factor (BCF)

The result of $BCF_{Bivalves/Seawater}$ in the cockles (*Anadara granosa*) showed that BCF values of Pb ranged from 12.3 to 20.1, from 29,000 to 109,833 for Cd, from 1,505 to 2,090 for Cr, from 3,787 to 5,640 for Fe, from 1,585 to 5,823 for Cu and from 1,047 to 2,890 for Zn, the mussels (*Perna viridis*) showed that BCF values of Pb ranged from 6.4 to 7.2, from 7,690 to 16,170 for Cd, from 1,950 to 2,330 for Cr, from 3,720 to 4,810 for Fe, from 2,180 to 5,100 for Cu and from 1,520 to 2,130 for Zn and the oyster (*Saccostrea cucullata*) showed that BCF values of Pb ranged from 6.4 to 9.9, from 65,231 to 117,333 for Cd, from 2,103 to 3,394 for Cr, from 1,031 to 1,370 for Fe, from 9,491 to 13,974 for Cu and from 9,603 to 11,240 for Zn (Table 4-5, Table 4-6, and Table 4-7). The ANOVA analysis showed that $BCF_{Bivalves/Seawater}$ of all heavy metals the different bivalve species were significantly different ($p<0.05$), except Cr. The different station did not found all heavy metals.

The result of $BCF_{Bivalves/Sediment}$ in the cockles (*Anadara granosa*) showed that BCF values of Pb ranged from 0.009 to 0.018, from 16.850 to 41.188 for Cd, from 0.015 to 0.016 for Cr, from 0.025 to 0.030 for Fe, from 0.589 to 2.371 for Cu and from 1.188 to 2.467 for Zn, the mussels (*Perna viridis*) showed that BCF values of Pb ranged from 0.005 to 0.006, from 5.000 to 6.063 for Cd, from 0.017 to 0.022 for Cr, from 0.024 to 0.026 for Fe, from 0.812 to 2.078 for Cu and from 1.587 to 1.817 for Zn and the oyster (*Saccostrea cucullata*) showed that BCF values of Pb ranged from 0.006 to 0.006, from 42 to 44 for Cd, from 0.022 to 0.024 for Cr, from 0.007 to 0.007 for Fe, from 3.528 to 5.216 for Cu and from 9.595 to 10.890 for Zn (Table 4-5, Table 4-6, and Table 4-7). The ANOVA analysis showed that $BCF_{Bivalves/Sediment}$ of all heavy metals the different bivalve species were significantly different ($p<0.05$), whereas did not found the different station.

Table 4-5 Accumulation of heavy metals in cockles (*Anadara granosa*) from the three river basin Chanthaburi coastal areas

Heavy metals	Bioconcentration factor					
	Seawater ($BCF_{Bivalves/Seawater}$)			Sediment ($BCF_{Bivalves/Sediment}$)		
	Wang-Ta-Nord	Chathaburi	Welu	Wang-Ta-Nord	Chathaburi	Welu
Pb	20.1	15.3	12.3	0.018	0.009	0.011
Cd	109,833	29,000	37,571	41.188	18.850	16.438
Cr	1,505	2,090	1,636	0.016	0.015	0.016
Fe	4,940	3,787	5,640	0.030	0.025	0.030
Cu	2,009	5,823	1,585	0.750	2.371	0.589
Zn	1,523	2,890	1,047	1.591	2.467	1.188

Table 4-6 Accumulation of heavy metals in mussels (*Perna viridis*) from the three river basin Chanthaburi coastal areas

Heavy metals	Bioconcentration factor					
	Seawater ($BCF_{Bivalves/Seawater}$)			Sediment ($BCF_{Bivalves/Sediment}$)		
	Wang-Ta-Nord	Chathaburi	Welu	Wang-Ta-Nord	Chathaburi	Welu
Pb	6.4	7.7	7.2	0.006	0.005	0.006
Cd	16,170	7,690	11,570	6.063	5.000	5.063
Cr	2,080	2,330	1,950	0.022	0.017	0.018
Fe	3,900	3,720	4,810	0.024	0.024	0.026
Cu	2,800	5,100	2,180	1.044	2.078	0.812
Zn	1,520	2,130	1,560	1.587	1.817	1.766

Table 4-7 Accumulation of heavy metals in oysters (*Saccostrea cucullata*) from the three river basin Chanthaburi coastal areas

Heavy metals	Bioconcentration factor					
	Seawater ($BCF_{Bivalves/Seawater}$)			Sediment ($BCF_{Bivalves/Sediment}$)		
	Wang-Ta-Nord	Chathaburi	Welu	Wang-Ta-Nord	Chathaburi	Welu
Pb	6.4	9.9	7.2	0.006	0.006	0.006
Cd	117,333	65,231	94,857	44	42	42
Cr	2,103	3,394	2,416	0.022	0.024	0.023
Fe	1,208	1,031	1,370	0.007	0.007	0.007
Cu	13,974	11,065	9,491	5.216	4.505	3.528
Zn	10,274	11,240	9,603	10.736	9.595	10.890

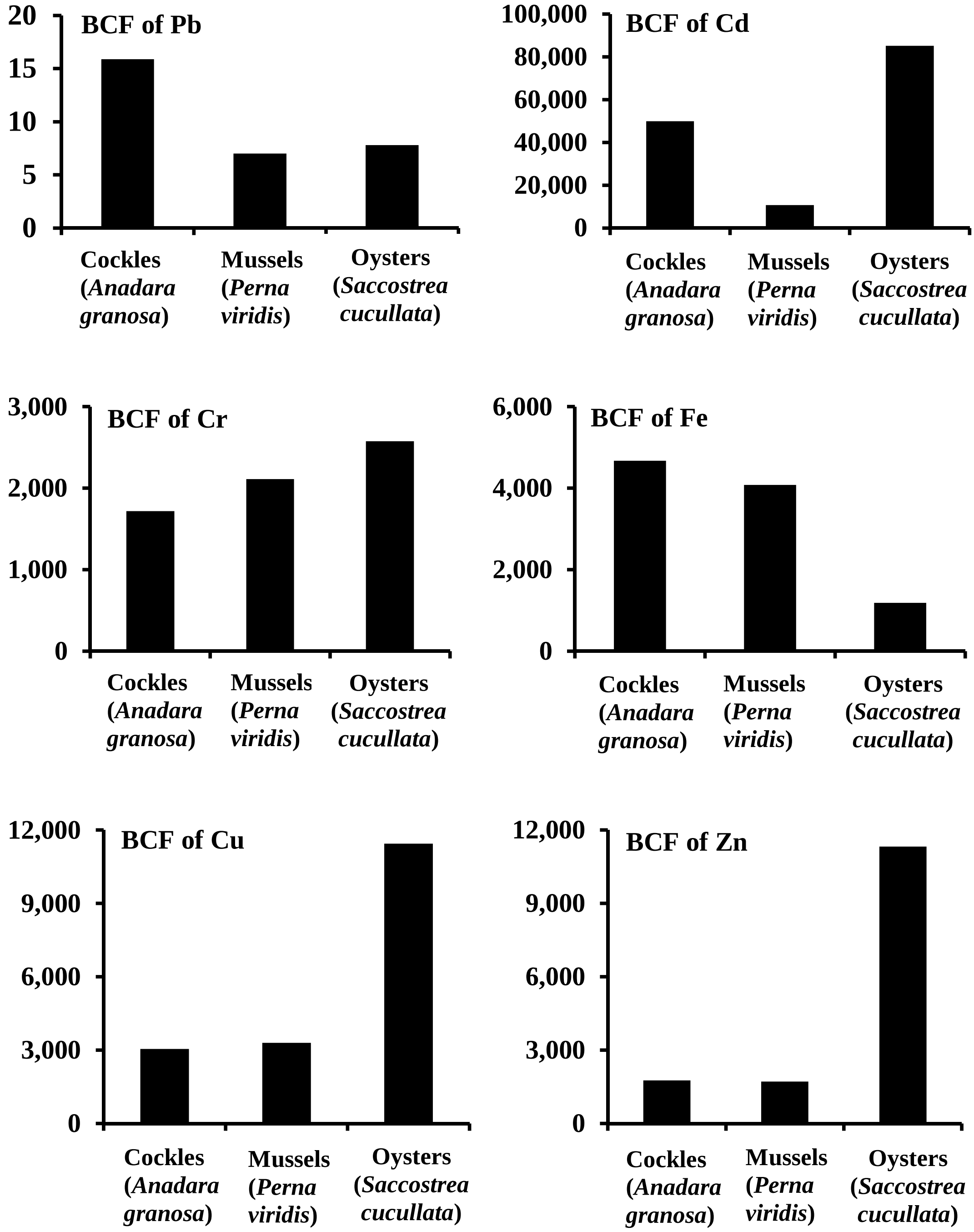


Figure 4-5 Comparison of $BCF_{Bivalves/Seawater}$ between bivalve species in the river basin Chanthaburi coastal areas

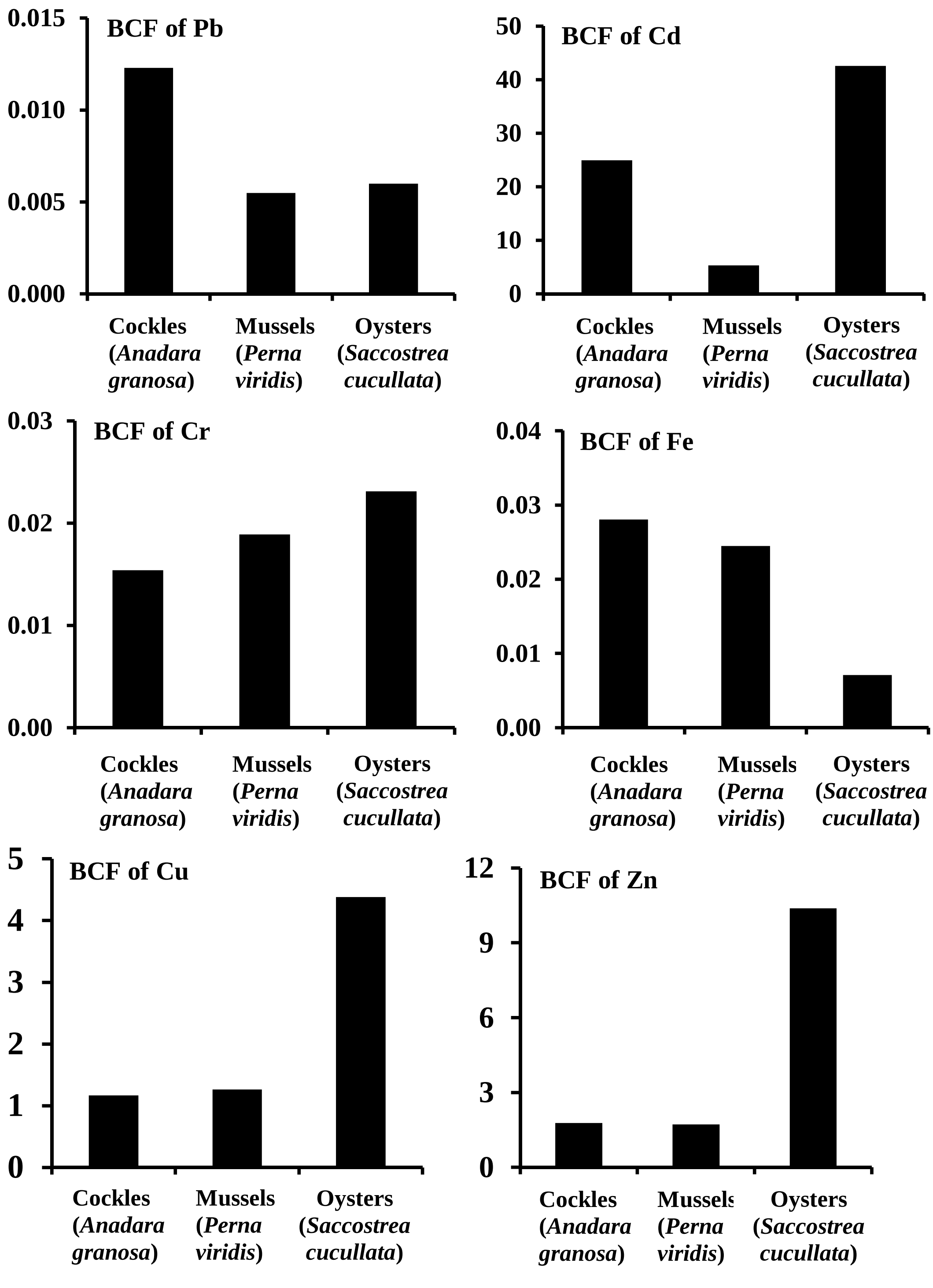
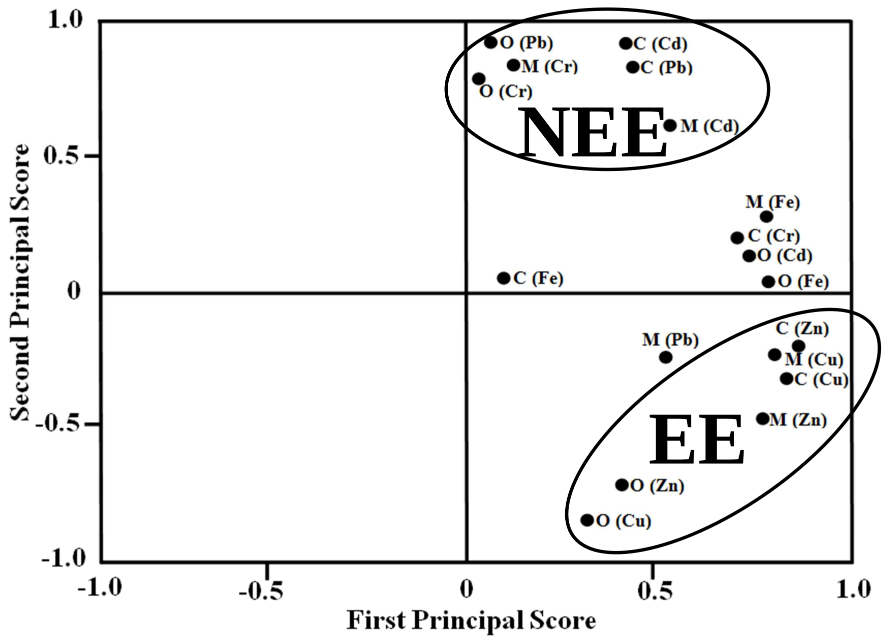


Figure 4-6 Comparison of $BCF_{Bivalves/Sediment}$ between bivalve species in the river basin Chanthaburi coastal areas

The principal component analysis (PCA)

The PCA analysis revealed that the distribution of heavy metals in bivalves depended on different between the Non Essential Elements (NEE) and the Essential Elements (EE) (Figure 4-7). The distribution of heavy metals in sediment and seawater were classified and obviously separated to the different river basin (Figure 4-8 and Figure 4-9).

The first principal score axis contained the all data of the distribution heavy metals in the bivalves in the right of the plot as shown in Figure 4-7. Whereas, the second principal score axis revealed that the difference between the Non Essential Elements (NEE) and the Essential Elements (EE). The first group containing the EE (Cu and Zn) in the bivalves could be distinguished in the lower right of the plot, whereas Fe in all bivalves are located in the middle of the second principal score axis. The second group are mostly the NEE in the bivalves was found in the upper right of the plot.



C-Cockles, M-Mussels and O-Oysters

NEE-Non Essential Elements and EE- Essential Elements

Figure 4-7 Principal component analysis of heavy metal concentrations in the cockles, mussels and oysters from the three river basin of Chanthaburi coastal areas

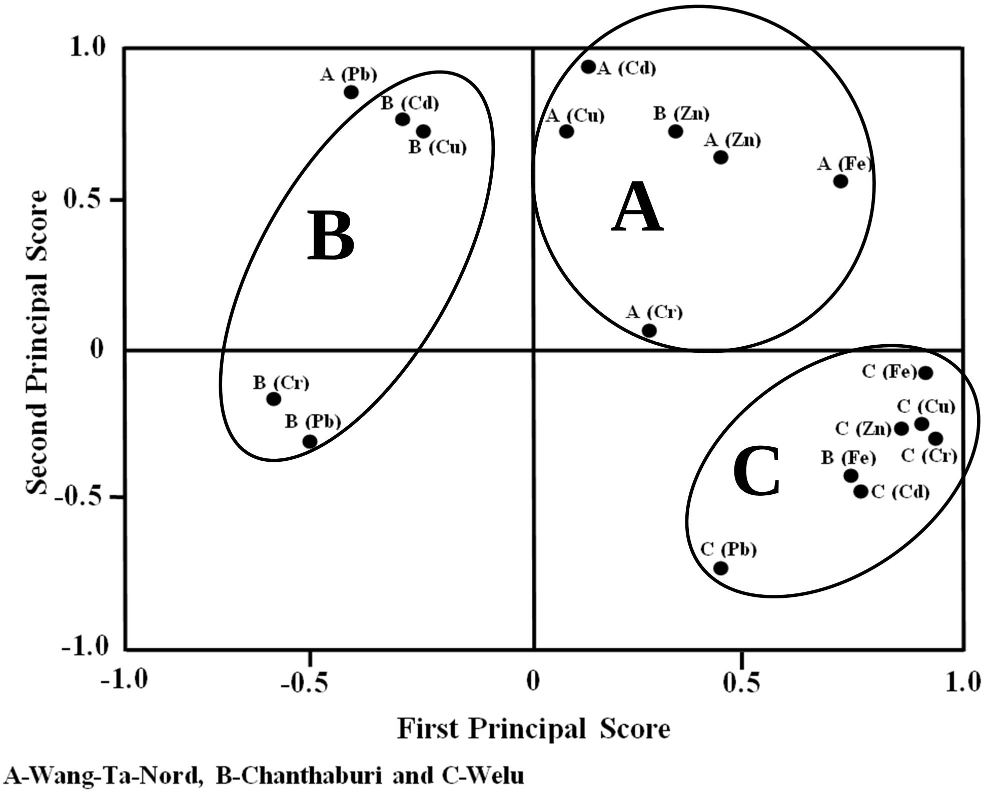


Figure 4-8 Principal component analysis of heavy metal concentrations in sediments from the three river basin of Chanthaburi coastal areas

The results of the PCA study on the sediment of the three sampling sites point out that the tendency of the heavy metals distribution was distinctly located which revealed an obvious separation in the first principal score axis as shown in Figure 4-8. The first group containing the sample from the Welu river basin (C) could be distinguished in the lower right of the plot. The second group are mostly the sample from the Wang-Ta-Nord river basin (A) is located in the upper right of the plot. The third group of samples from the Chanthaburi river basin (B) was found in the left of the first principal score axis.

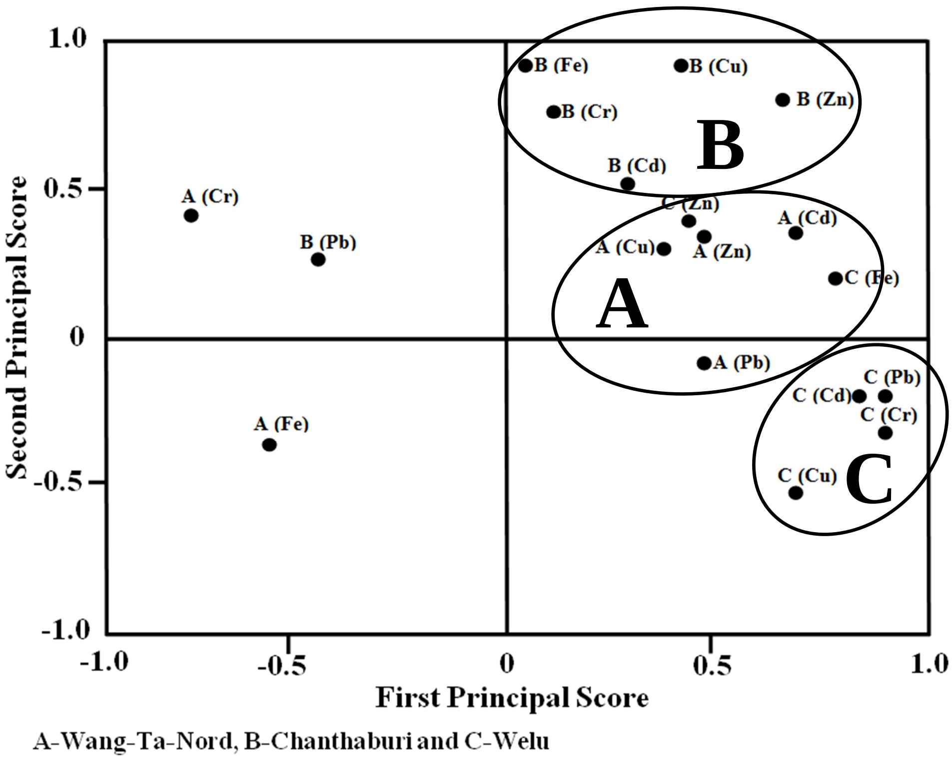


Figure 4-9 Principal component analysis of heavy metal concentrations in seawater from the three river basin of Chanthaburi coastal areas

The results of the PCA study on the seawater of the three sampling sites point out that the tendency of the heavy metals distribution was distinctly located which revealed an obvious separation in the second principal score axis as shown in Figure 4-9. The first group containing the sample from the Welu river basin (C) could be distinguished in the lower right of the plot. The second group are mostly the sample from the Wang-Ta-Nord river basin (A) is located in middle of the second principal score axis. The third group of samples from the Chanthaburi river basin (B) was found in the upper right of the plot

The multiple regression analysis

The multiple regression analysis is used for study the correlation between heavy metal contents in environmental samples and the influence variable. These multiple regression equations of bivalve have as dependent variable: the heavy metals concentration in bivalves and as independent variable: Physicochemical of sediment and seawater including: 1) percent of sand, silt and clay particles, percent of organic carbon and pH in sediments, 2) pH, EC and salinity in seawater as well as the weight of bivalves.

For sediments the multiple regression equations assumes that heavy metals concentration in sediments depend on physicochemical sediments including: percent of sand, silt and clay particles, percent of organic carbon, pH of sediments. Seawaters multiple regression equations were focused the three independent variables including: pH, EC and salinity which affect to heavy metals concentration in seawaters.

Table 4-8 Simple and multiple regression equation of heavy metals concentration in cockles (*Anadara granosa*) from the three river basin Chanthaburi coastal areas

Heavy metals (µg/g)	Simple and multiple regression equation	R^2	Significance of regression
Pb	Y_{Pb} = 0.018 - 0.002(OM; %) - 1.019(Clay; %) +0.001(EC; µS/cm) + 0.001(Salinity: %)	0.512	0.006
Cd	Y_{Cd} = 0.536 - 0.084(OM; %) - 0.012(Clay; %) +0.025(EC; µS/cm)	0.328	0.043
Cr	Y_{Cr} = 0.085 - 0.006(OM; %) + 0.002(pH) + 0.015(Weight; g)	0.308	0.056
Fe	LogY_{Fe} = 2.384 + 0.040(pH)	0.645	0.004
Cu	Y_{Cu} = -18.587 + 2.018(pH) + 3.831(Weight; g)	0.365	0.059
Zn	Y_{Zn} = -23.186 - 2.516(OM; %) + 8.347(pH)	0.419	0.079

Table 4-9 Simple and multiple regression equation of heavy metals concentration in mussels (*Perna viridis*) from the three river basin Chanthaburi coastal areas

Heavy metals (µg/g)	Simple and multiple regression equation	R^2	Significance of regression
Pb	Y_{Pb} = 0.013 - 0.001(OM; %)	0.256	0.264
Cd	Y_{Cd} = 0.138 - 0.010(OM; %) - 0.001(Clay; %)	0.221	0.259
Cr	Y_{Cr} = 0.230 - 0.011(OM; %) + 0.003(Salinity; %)	0.365	0.089
Fe	Y_{Fe} = 97.942 + 33.262(pH) + 6.510(Salinity; %)	0.348	0.087
Cu	Y_{Cu} = -27.022 - 0.501(OM; %) + 5.019(pH)	0.225	0.069
Zn	Y_{Zn} = 19.329 - 0.280(Clay; %) + 0.535(Salinity; %)	0.233	0.062

Table 4-10 Simple and multiple regression equation of heavy metals concentration in oysters (*Saccostrea cucullata*) from the three river basin Chanthaburi coastal areas

Heavy metals (µg/g)	Simple and multiple regression equation	R^2	Significance of regression
Pb	$LogY_{Pb}$ = -2.317 - 0.063(OM; %) + 0.041(pH) + 0.015(Salinity; %)	0.264	0.098
Cd	Y_{Cd} = -0.932 + 0.187(pH) + 0.019(Salinity; %)	0.279	0.032
Cr	Y_{Cr} = -0.174 - 0.013(OM; %) - 0.003(Clay; %) + 0.007(Weight; g)	0.153	0.335
Fe	Y_{Fe} = -84.068 + 10.113(pH) + 1.131(Salinity; %) + 1.861(Weight; g)	0.319	0.049
Cu	Y_{Cu} = 32.431 - 0.490(Clay; %) + 0.691(EC; µS/cm)	0.267	0.038
Zn	Y_{Zn} = 98.724 + 1.162(EC; µS/cm) + 1.132(Weight; g)	0.162	0.156

Table 4-8 showed that the cockles had relationship to the organic matter and the clay particle with the significantly negative relationship for Pb and Cd, whereas the pH had significantly positive relationship to Fe and Cu. Cr and Zn had also significantly positive and negative relationship to the pH and the organic matter, respectively. The distribution of heavy metals in mussels had relationship to the organic matter, the clay particle, the pH and the salinity. The organic matter is the major major independent variable of Pb, Cd, Cr and Cu with the significantly negative relationship (Table 4-9). The distribution of heavy metals in oyster had relationship to many independent variable including: the organic matter, the pH, the salinity, the clay particle, the elctroconductivity and the weight. The pH and the weight had significantly positive relationship to all heavy metals except Cu (Table 4-10).

Table 4-11 Multiple regression equation of heavy metals concentration in sediments from the three river basin Chanthaburi coastal areas

Heavy metals (µg/g)	Multiple regression equation	R^2	Significance of regression
Pb	Y_{Pb} = 1.630 + 0.634(OM; %) + 0.101(Clay; %)	0.657	0.000
Cd	Y_{Cd} = 0.022 + 0.005(OM; %) + 0.001(Clay; %)	0.699	0.000
Cr	Y_{Cr} = 11.584 + 0.047(OM; %) + 0.149(Clay; %)	0.618	0.002
Fe	LogY_{Fe} = 3.397 + 0.038(OM; %) + 0.012(Clay; %) – 0.095(pH)	0.412	0.030
Cu	Y_{Cu} = 13.038 + 1.415(OM; %) + 0.094(Clay; %)	0.541	0.023
Zn	Y_{Zn} = 9.817 + 1.729(OM; %) + 0.002(Clay; %) – 1.696(pH)	0.433	0.032

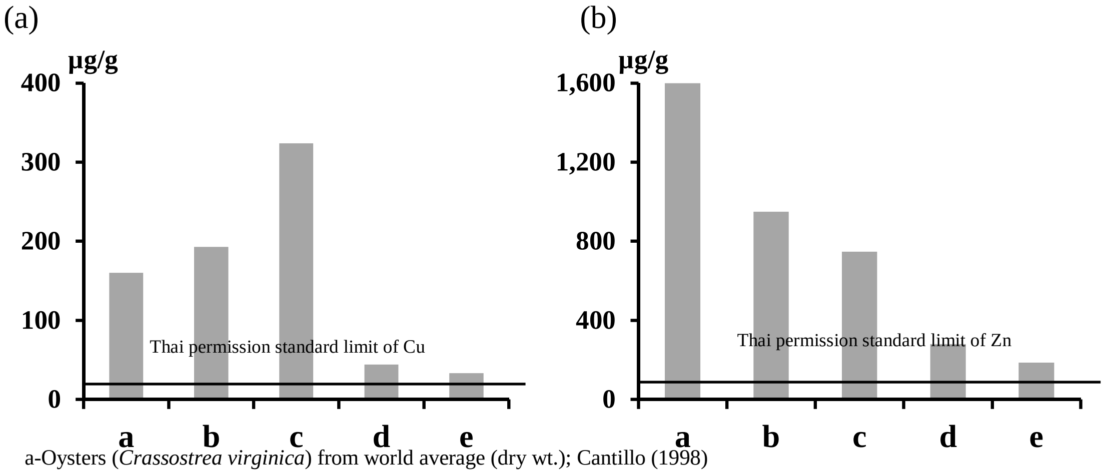
Table 4-12 Simple and multiple regression equation of heavy metals concentration in seawater from the three river basin Chanthaburi coastal areas

Heavy metals (μg/L)	Simple and multiple regression equation	R^2	Significance of regression
Pb	Y_{Pb} = 1.246 + 0.009(Salinity; %)	0.528	0.002
Cd	Y_{Cd} = -0.041 - 0.005(pH) + 1.288(Salinity; %)	0.412	0.004
Cr	Y_{Cr} = 0.198 - 0.016(pH)	0.491	0.052
Fe	Y_{Fe} = 128.826 + 1.498(EC; μS/cm)	0.376	0.091
Cu	Y_{Cu} = 2.4230 + 0.014(EC; μS/cm) + 0.020(Salinity; %)	0.210	0.296
Zn	Y_{Zn} = 13.380 + 0.328(EC; μS/cm)	0.255	0.012

The relationship between the heavy metals concentration and the sediment characteristics could be explained by the multiple regression equations as displayed in Table 4-11. The results of statistical analysis revealed that all heavy metals had significantly positive relationship with the organic matter (OM) and the clay particle size whereas Fe and Zn had also significantly negative relationship with the pH.

The Table 4-12 showed the simple regression equation for Pb, Cr, Fe, and Zn whereas Cd and Cu are shown the multiple regression equation. These equations revealed that the heavy metals had significantly positive relationship with the salinity and electroconductivity (EC), whereas had significantly negative relationship with the pH.

Part 2. The experimental depuration of essential elements (Cu and Zn) in the oysters (*Saccostrea cucullata*) and mussels (*Perna viridis*) in the river basin of Chanthaburi coastal areas



a-Oysters (*Crassostrea virginica*) from world average (dry wt.); Cantillo (1998)

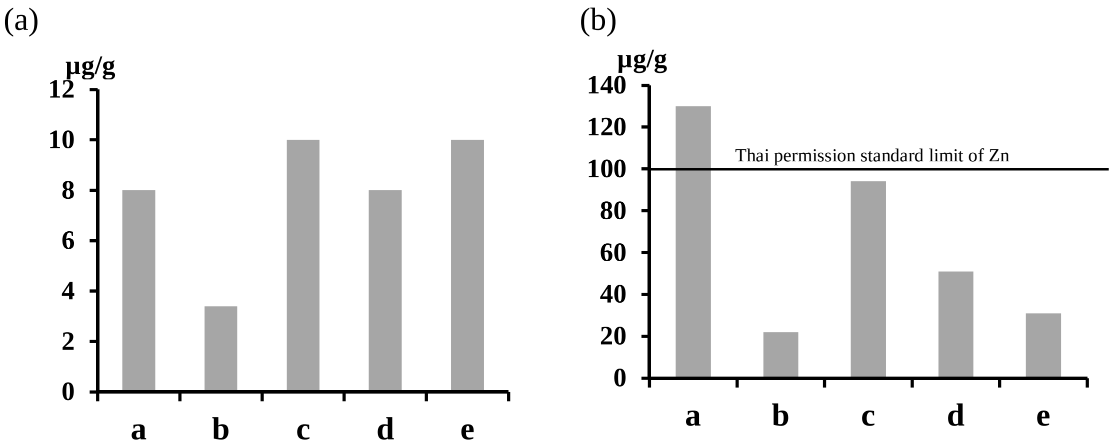
b-Oysters (*Saccostrea glomerata*) from Sydney estuary, Australia (wet wt.); Birch, Melwani, Lee, and Apostolatos (2014)

c-Oysters (*Saccostrea cucullata*) from Persian Gulf, Iran (dry wt.); Chaharlang, Bakhtiari, and Yavari (2012)

d-Oysters (*Saccostrea cucullata*) from East coast of Thailand (wet wt.); Panutrakul, Khaesejan, and Thungkao (2007)

e-This study

Figure 4-10 Mean concentrations (µg/g) of Cu (a) and Zn (b) in soft tissues of oysters in many part of the world



a-Mussels (*Mytilus edulis*) from world average (dry wt.); Cantillo (1998)

b-Mussels (*Perna viridis*) from West coast of Peninsular, Malaysia (wet wt.); Yap, Ismail, and Tan (2004)

c-Mussels (*Perna viridis*) from Gulf of Thailand (dry wt.); Ruangwises and Ruangwises (1998)

d- Mussels (*Perna viridis*) from Inner gulf of Thailand (dry wt.); Cheevaporn and Menasveta (2003)

e-This study

Figure 4-11 Mean concentrations (µg/g) of Cu (a) and Zn (b) in soft tissues of mussels in many part of the world

The mean of concentration of Cu and Zn in the oyster of this study and the many part of the world were higher than the permission standard limit in food, Thailand, whereas Cu and Zn in the mussels showed the lower than the standard (Figure 4-10 and Figure 4-11).

Table 4-13 Mean ± S.D. of heavy metal concentrations (µg/g) in oysters and mussels at different hour after passed depuration process (µg/g wet weight)

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

Concentration of Cu and Zn in mussels before the depuration (0 hour) were 9.853 ± 0.447 and 32.207 ± 1.767 µg/g wet weight and concentration of Cu and Zn in oysters before the depuration (0 hour) were 39.080 ± 8.911 and 178.767 ± 5.160 µg/g wet weight which higher than the permission standard limit in food, Thailand. After 72 hours of depuration period passed Cu and Zn in mussels were 8.137 ± 0.619 and 19.580 ± 1.040 µg/g wet weight and Cu and Zn in oysters were 8.137 ± 0.619 and 19.580 ± 1.040 µg/g wet weight which found that lower than the permission standard (Table 4-13).

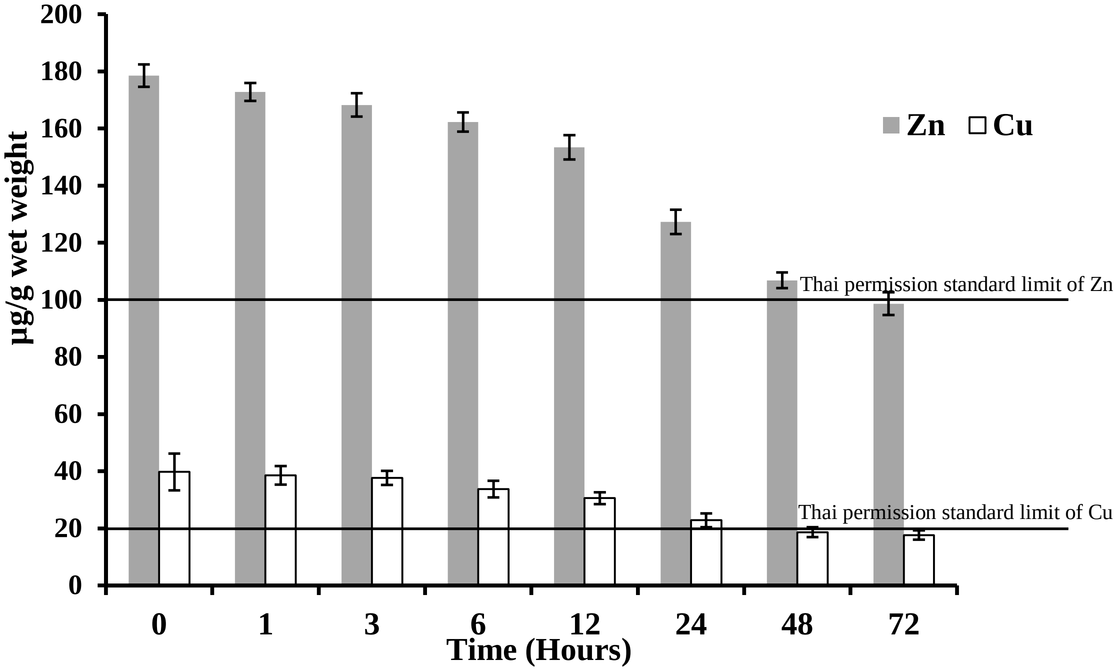


Figure 4-12 Comparison heavy metal concentrations (μg/g) in the oysters (*Saccostrea cucullata*) at different hour after passed depuration process (μg/g wet weight)

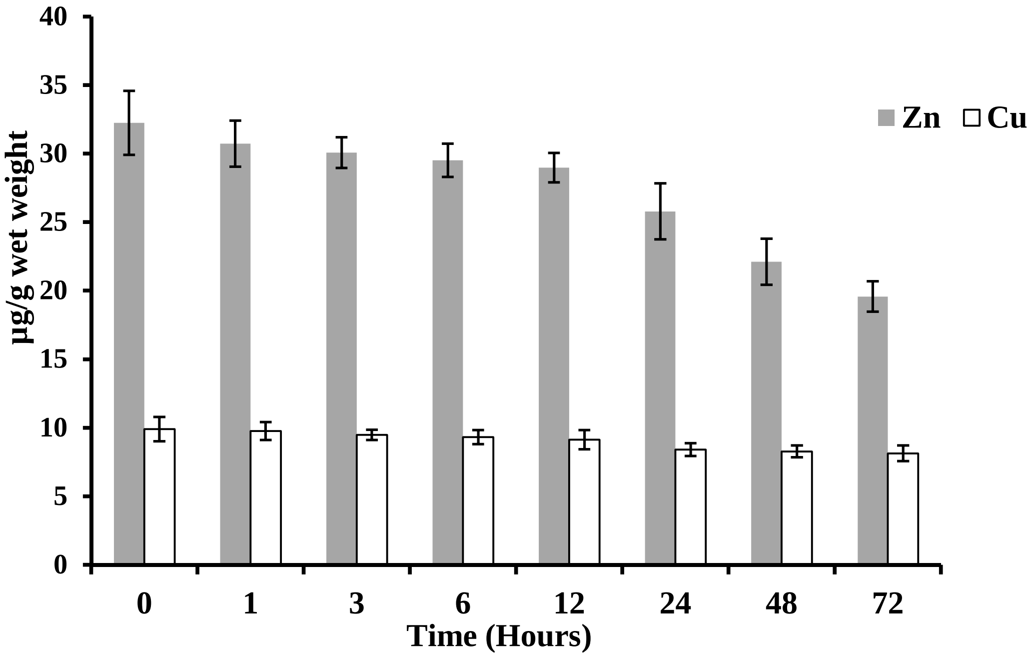
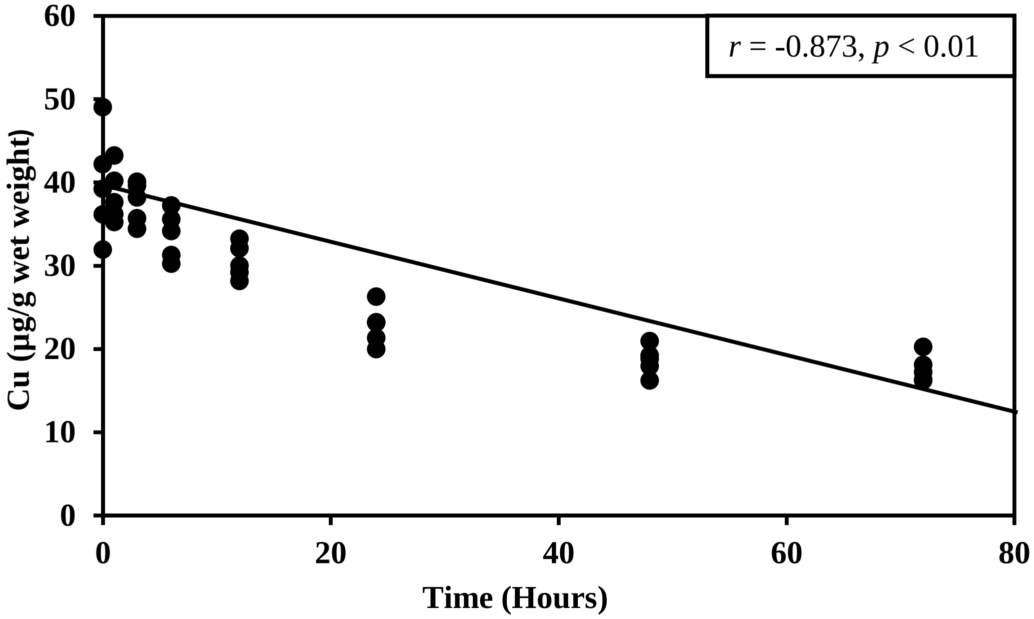


Figure 4-13 Comparison heavy metal concentrations (μg/g) in the mussels (*Perna viridis*) at different hour after passed depuration process (μg/g wet weight)

(a)



(b)

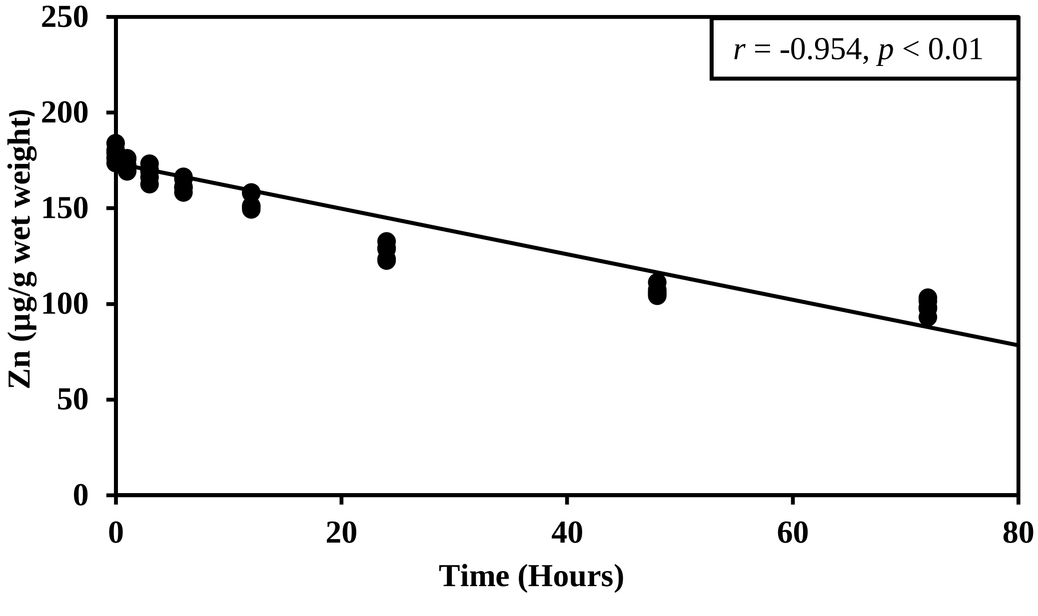
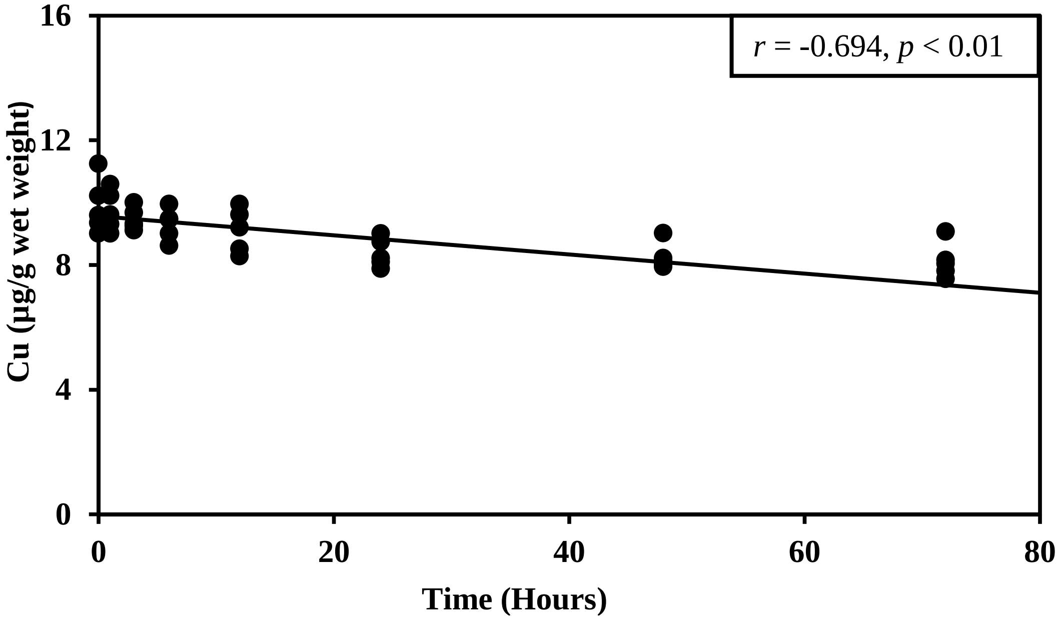


Figure 4-14 Variation of Cu (a) and Zn (b) concentrations (µg/g wet weight) in the oysters (*Saccostrea cucullata*) during the depuration periods. Pearson correlation significant at $p<0.01$.

(a)



(b)

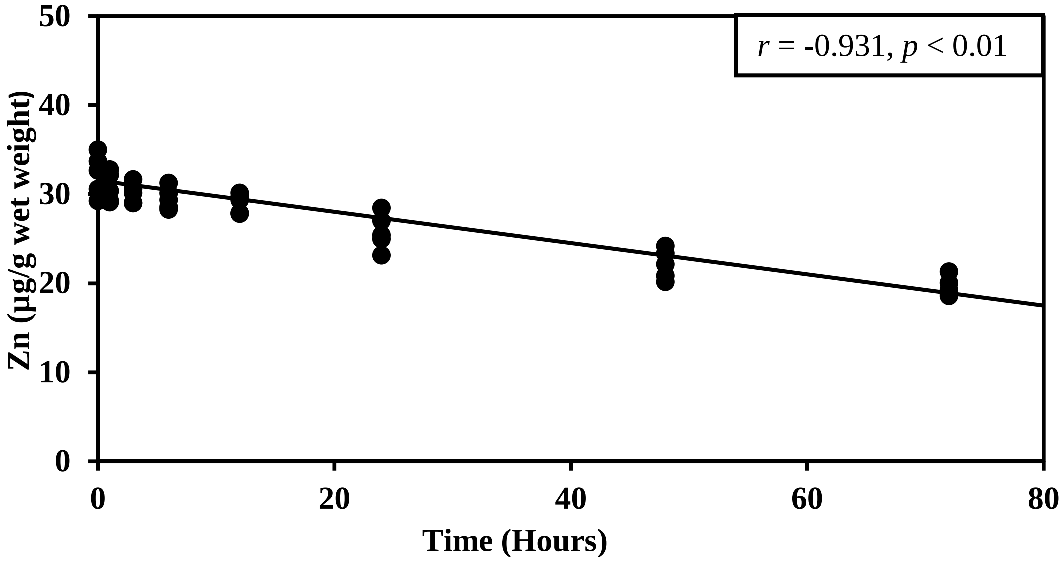


Figure 4-15 Variation of Cu (a) and Zn (b) concentrations (µg/g wet weight) in the mussels (*Perna viridis*) during the depuration periods. Pearson correlation significant at $p<0.01$.

The Cu and Zn concentrations in oysters passed the depuration exhibited a significant linear decrease: r= -0.873, p<0.001 for Cu and r= -0.954, p<0.001 for Zn (Figure 4-14). The Cu and Zn concentrations in mussels passed the depuration exhibited a significant linear decrease: r= -0.694, p<0.001 for Cu and r= -0.931, p<0.001 for Zn (Figure 4-15).

Multiple regression analysis of depuration

Table 4-14 showed the multiple regression equations of the relationship between the depuration rate of Cu, Zn and the depuration variation factor (depuration period; hour and weight of bivalves). These equations revealed that Cu and Zn in the oyster and the mussels have significantly positive relationship with the time (hour) and the weight (g).

Table 4-14 Multiple regression equations of depuration Cu and Zn in oysters (*Saccostrea cucullata*) and mussels (*Perna viridis*)

Bivalves	Depuration rate (µg/g)	Multiple regression equation	R^2	Significance of regression
Oysters (*Saccostrea cucullata*)	Cu	$\mathrm{Log}Y_{Cu}$ = -4.0905 + 0.001(Time; hour) + 0.100(Weight; g)	0.961	0.000
	Zn	$\mathrm{Log}Y_{Zn}$ = -1.020 + 0.002(Time; hour) + 0.050(Weight; g)	0.983	0.000
Mussels (*Perna viridis*)	Cu	Y_{Cu} = -15.849 + 0.020(Time; hour) + 1.684(Weight; g)	0.904	0.003
	Zn	Y_{Zn} = -14.853 + 0.087(Time; hour) + 3.036(Weight; g)	0.977	0.000

CHAPTER 5
DISCUSSIONS AND CONCLUSION

Part 1. The investigation of distribution of selected heavy metals in the river basin of Chanthaburi coastal areas

The distribution of heavy metals in bivalves

The bivalve species (*Anadara granosa, Perna viridis,* and *Saccostrea cucullata*) in this study are the abundance species and the commercially popular bivalves in the Gulf of Thailand by the investigation of the international Mussel Watch Program (Cheevaporn & Menasveta, 2003). The distribution of heavy metals in bivalves of this study area exhibited low concentrations as suggested by comparing with Permission standard limit in food of Thailand and WHO. The heavy metal contents appear quite low by comparison to these same species from elsewhere in the world (Szefer, Wolowicz, Kusak, Deslous-Paoli, Czarnowski, Frelek, & Belzunce, 1999; Figueira, Lima, Branco, Quintino, Rodrigues, & Freitas, 2011; Alkarkhi, Ismail, & Easa, 2008; Phillips & Muttarasin, 1985; Otchere, 2003; Ibrahim, 1995; Yap, Muhamad Azlan, Cheng, & Tan, 2011; Cantillo, 1998; Kwon & Lee, 2001; Ruangwises & Ruangwises, 1998; Yap, Ismail, & Tan, 2004; Catsiki & Florou, 2006; Jiann & Presley, 1997; Gawade, Harikrishna, Sarma, & Ingole, 2013; Chanharlang, Bakhtiari, & Yavari, 2012) as shown in Figure 5-1, Figure 5-2 and Figure 5-3.

The concentration of Cu and Zn in oyster of this study and the several surveys higher than the permission standard limit in food of Thailand. Heidari, Bakhtiari and Shirneshan (2013) confirmed the Cu and Zn contents in the soft tissue of the *Saccostrea cucullata* and global guidelines in many cases higher than the permissible amount for human consumption. In general Cu and Zn are generally known as essential elements for aquatic organisms needed for the cell growth and coenzyme-catalyzed reactions. The higher contents of Cu and Zn in the soft tissue due to Cu and Zn serve as structural ion and the high affinity to interact with metallothioneins proteins, therefore metallothioneins likely play important roles in transporting both essential and toxic metals in oyster (Burger & Gochfeld, 2006). Cu and Zn are an essential requirement for the nutrition, but the excess Cu and Zn can be harmful to the health (Fosmire, 1990). This study found that the almost heavy metal contents great accumulated in the oyster. The ability of oyster species to accumulate trace metals was found by various reports especially the public health point of view (Otchere, 2003; Birch, Melwani, Lee, & Apostolatos, 2014; Chaharlang, Bakhtiari, & Yavari, 2012; Gawade et al., 2013)

The distribution of heavy metals in sediment and seawater

The results of heavy metal contents in sediment and seawater in the river basin of Chanthaburi coastal area and several researches as shown in Figure 5-4 and Figure 5-5. Considering the data obtained from the various surveys, it can be found that the heavy metal concentrations in sediment and seawater during the study period are comparable to natural level as suggested by comparing with the world average and the standard level of Thailand. Higher some metals contents in sediment and seawater of Gulf of Thailand due to the contamination from urban and industrial areas, especially the effect from Map Ta Phut industrial estate in Rayong Province (Thongra-ar et al., 2008)

Fe and Zn are highest concentration in sediment and seawater as shown in Table 4-2 and Table 4-3. Glasby, Szefer, Geldon and Warzocha (2004) indicating that the crustal weathering may be the main source of Fe and Zn in the environmental coast which major element in the earth's crust have highest values. It is known that floods enhance the transportation of natural and anthropogenic sediment into the river and subsequently deposit in the environment of the river mouth. Chanthaburi Province was classified as a high risk area of landslide, water flow and flood due to the landslide disaster of Kitchakood Mountain in 2000 and flooding crisis in 2006 (Anecksamphant, 2004). Furthermore, Fe and Zn are micronutrients in a fertilizer and an addition to the ingredient of supplementary food in shrimp farms whereas in 2003 the shrimp farm land, urban land and agriculture developing area are continuously increasing in Chanthaburi Province up to these days (Runping & Kheoruenromne, 2003).

The sediment quality index (SQI: I_{geo} and EF)

The results of the geoaccumulation index and the enrichment factor values of all heavy metals content in the sediments are still below those unpolluted (I_{geo}<0) and not enriched (EF<1) levels, respectively. It can be concluded that the heavy metals uncontamination level in Chanthaburi coastal area. The EF and *Igeo* values of almost heavy metals in the Klang river basin, Malaysia (Najia & Ismail, 2011) higher than another area as show in Figure 5-8 and Figure 5-9. The high contamination of these heavy metals could be related to the local point source which the industry-affected are than urban-affected area.

Zn is a natural element of highest enrichment factor and high geoaccumulation index in the river basin is a result of derivation and accelerated erosion on land (Baptista Neto, Smith, & McAllister, 2000). Furthermore, the widespread of urbanization, the use of fertilizers and pesticides in agricultural activities are minor sources of Zn (Ghrefat &Yusuf, 2006). The results of an analysis of the I_{geo} and EF values (Figure 4-3 and Figure 4-4) showed that the distribution of the Essential Elements (Fe, Cu and Zn) were higher than that of the Non Essential Elements (Pb and Cd except Cr). Hem (1985) reported that Cr was the 17th most abundant metal in the earth’s crust and it was found that the amount in sedimentary rock was higher than those of Pb and Cd. In addition, Cr is a major component of steel alloys furnitures (10-26%) and it is used in many products in daily life more than Pb and Cd (Bielicka, Bojanowska, & Wiśniewski, 2005).

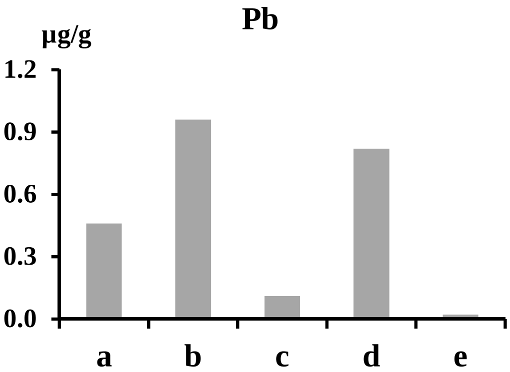
Figure 5-6 shows the scatter-plot for heavy metals (Pb, Cd, Cr, Fe, Cu and Zn) vs. organic matter, with the resultant regression line. The all heavy metals were shown a very strong positive correlation with organic matter, which have R^2 values of 0.973 for Pb, 0.916 for Cd, 0.873 for Cr, 0.768 for Fe, 0.927 for Cu and 0.815 for Zn. The scatter-plot and the regression lines of heavy metals (Pb, Cd, Cr, Fe, Cu and Zn) and the clay particle were shown in Figure 5-7 as shown with R^2 values of 0.945 for Pb, 0.957 for Cd, 0.919 for Cr, 0.808 for Fe, 0.941 for Cu and 0.866 for Zn. The study apparently indicated that for sediment samples along the Chanthaburi coastal area including: Pb, Cd, Cr, Fe, Cu and Zn strong correlation with organic matter and clay particle.

The result of this study indicated that the OM and clay particle were relatively more statistically significant for controlling the distribution of Pb, Cd, Cr, Fe, Cu and Zn in the sediments of high *r* values. Chen, Kao, Chen, and Dong (2007) demonstrated that the OM and clay were more important factor in affecting the heavy metals distribution than other characteristics in the studied sediment of high correlation coefficient values. An organic compound in the sediment plays an important role in heavy metals distribution because heavy metals are generally bounded in largest fraction to the OM (Peng, Song, Yuan, Cui, & Qiu, 2009) Moreover, the grain size is the one of the most investigated supported indicative of heavy metals distribution when the grain size decreases and the metal contents increases.

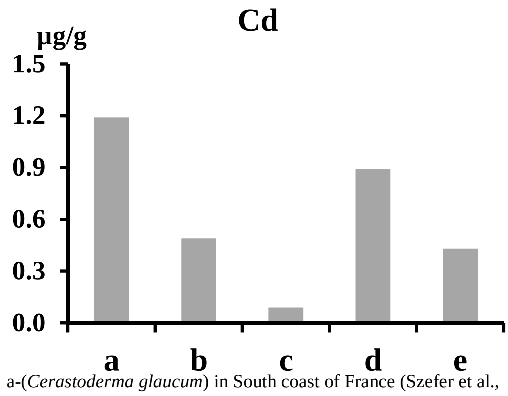
The bioconcentration factor (BCF)

Bioconcentration factor in were calculated and showed the ability of heavy metals accumulation from environment to bivalves. $BCF_{Bivalves/Seawater}$ values of all heavy metals (Pb, Cd, Cr, Fe, Cu and Zn) are higher than 1 in cockles, mussels and oysters indicated that the ability to accumulate heavy metals from seawater. For $BCF_{Bivalves/Sediment}$ indicated that limited ability of selected heavy metals cockles in accumulation from sediment except Cd and Zn. $BCF_{Bivalves/Sediment}$ values of the mussels and oysters showed the ability of accumulation for Cd, Cu and Zn whereas Pb, Cr and Fe showed the limited of accumulation (Table 4-5, Table 4-6 and Table 4-7). The results of $BCF_{Bivalves/Seawater}$ and $BCF_{Bivalves/Sediment}$ showed the ability of *Saccostrea cucullata* to accumulation the Cd, Cr, Cu and Zn with the highest BCF values, while Pb and Fe were found that highest in the *Anadara granosa*.

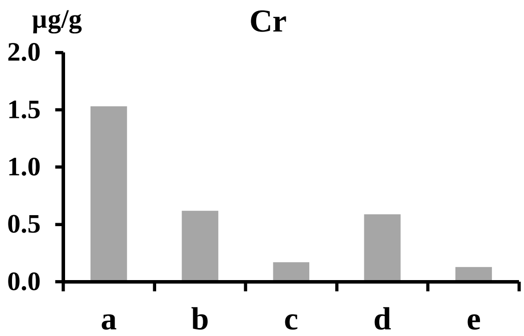
The $BCF_{Bivalves/Seawater}$ and $BCF_{Bivalves/Sediment}$ values of Cd were the highest in all river basin and all bivalve species, whereas the Cd concentration was found that lowest in seawater and sediment of the river basin of Chanthaburi coastal area (Table 4-2 and Table 4-3). Abdullah et al. (2007) reported the $BCF_{Bivalves/Seawater}$ value of Cd was highest value, but Cd concentration in seawater was lowest value. On the contrary, the Pb was lowest the BCF value, while the Pb concentration was the highest in seawater. The uptake and the depuration of Cd were not observed in the oyster samples, whereas Zn had significant depuration (Amaral et al., 2005)



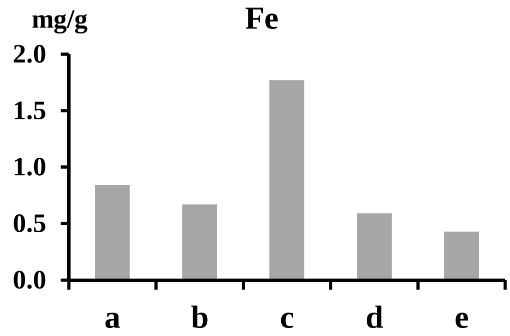
a-(*Cerastoderma glaucum*) in South coast of France (Szefer et al., 1999), b-(*Cerastoderma edule*) in Northwest coast of Portugal (Figueira et al., 2011), c-(*Anadara granosa*) in North west coast of Peninsular, Malaysia (Alkarkhi et al., 2008), d-(*Anadara granosa*) in gulf of Thailand (Phillips and Muttarasin, 1985) and e-This study



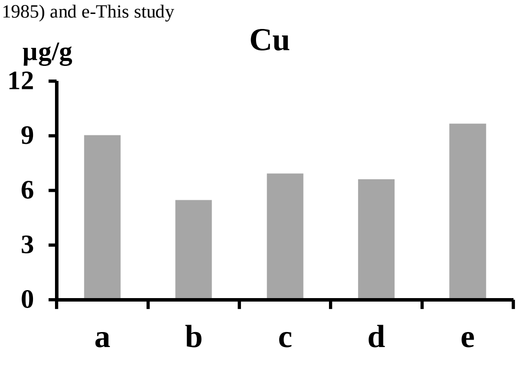
a-(*Cerastoderma glaucum*) in South coast of France (Szefer et al., 1999), b-(*Anadara senilis*) in Lagoons in Ghana (Otchere, 2003), c-(*Cerastoderma edule*) in Northwest coast of Portugal (Figueira et al., 2011), d-(*Anadara granosa*) in North west coast of Peninsular, Malaysia (Alkarkhi et al., 2008), and e-This study



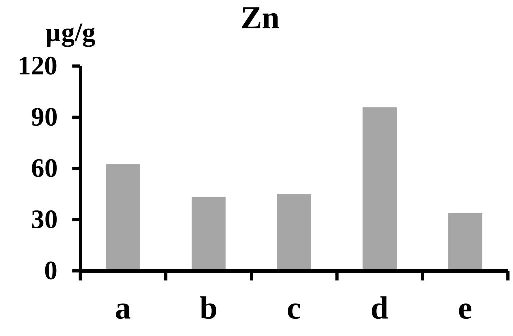
a-(*Cerastoderma glaucum*) in South coast of France (Szefer et al., 1999), b-(*Cerastoderma edule*) in Northwest coast of Portugal (Figueira et al., 2011), c-(*Anadara granosa*) in North west coast of Peninsular, Malaysia (Alkarkhi et al., 2008), d-(*Anadara granosa*) in gulf of Thailand (Phillips and Muttarasin, 1985) and e-This study



a-(*Cerastoderma glaucum*) in South coast of France (Szefer et al., 1999), b-(*Anadara senilis*) in Lagoons in Ghana (Otchere, 2003), c-(*Anadara granosa*) in Aquaculture areas of Peninsular, Malaysia (Ibrahim, 1995), d-(*Anadara granosa*) gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

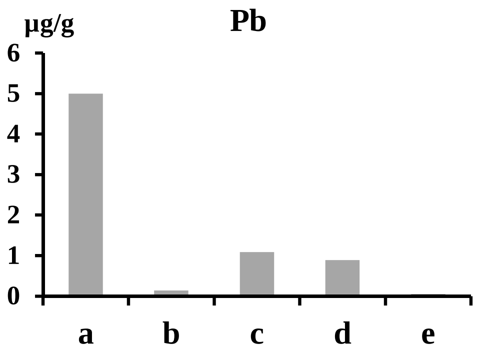


a-(*Cerastoderma glaucum*) in South coast of France (Szefer et al., 1999), b-(*Anadara senilis*) in Lagoons in Ghana (Otchere, 2003), c-(*Anadara granosa*) in Perak, Malaysia (Yap et al., 2011), d-(*Anadara granosa*) gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

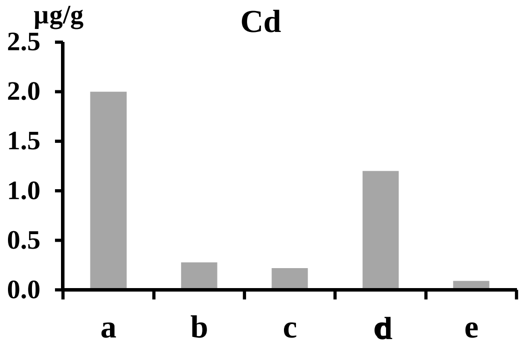


a-(*Cerastoderma glaucum*) in South coast of France (Szefer et al., 1999), b-(*Anadara senilis*) in Lagoons in Ghana (Otchere, 2003), c-(*Cerastoderma edule*) in Northwest coast of Portugal (Figueira et al., 2011), d-(*Anadara granosa*) gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

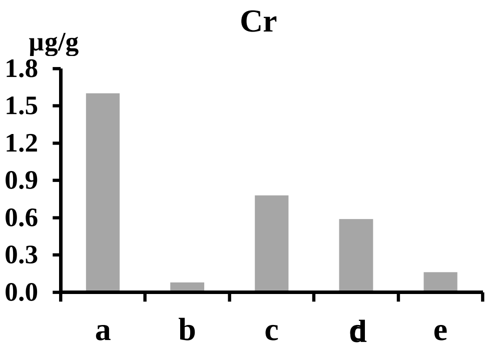
Figure 5-1 Comparison heavy metals in cockles between this study and other researches



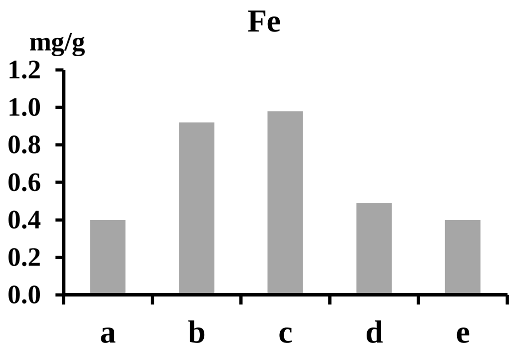
a-(*Mytilus edulis*) of World average (Cantillo, 1998), b-(*Perna viridis*) in Masan bay, Korea (Kwon and Lee, 2001), c-(*Perna viridis*) in Coastal area of the gulf of Thailand (Ruangwises and Ruangwises, 1998), d-(*Perna viridis*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study



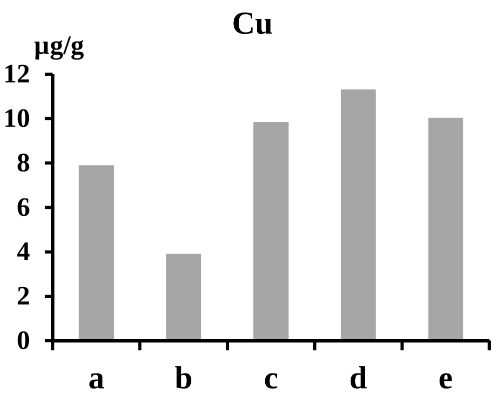
a-(*Mytilus edulis*) of World average (Cantillo, 1998), b-(*Perna viridis*) in Masan bay, Korea (Kwon and Lee, 2001), c-(*Perna viridis*) in West coast of Peninsular, Malaysia (Yap et al., 2004), d-(*Perna viridis*) in Coastal area of the gulf of Thailand (Ruangwises and Ruangwises, 1998), and e-This study



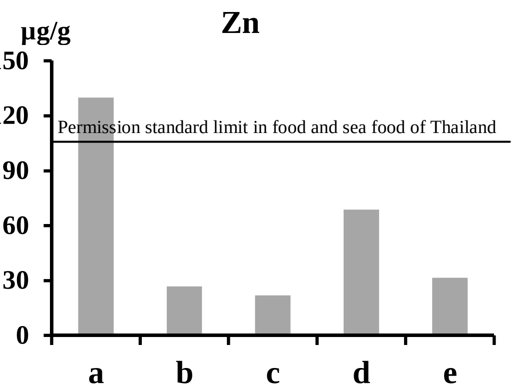
a-(*Mytilus edulis*) of World average (Cantillo, 1998), b-(*Perna viridis*) in Masan bay, Korea (Kwon and Lee, 2001), c-(*Perna viridis*) in Coastal area of the gulf of Thailand (Ruangwises and Ruangwises, 1998), d-(*Perna viridis*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study



a-(*Mytilus galloprovincialis*) in Thermaikos gulf, Greece (Catsiki and Florou, 2006), b-(*Perna viridis*) in Lagoons in Ghana (Otchere, 2003), c-(*Perna viridis*) in West coast of Peninsular, Malaysia (Yap et al., 2004), d-(*Perna viridis*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

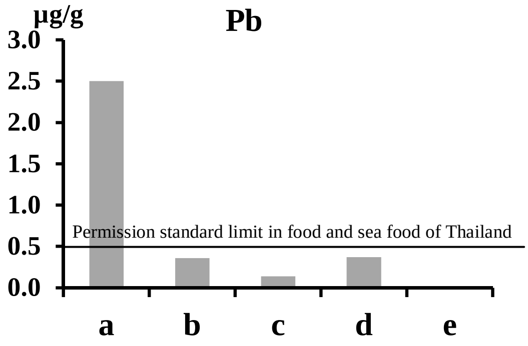


a-(*Mytilus edulis*) of World average (Cantillo, 1998), b-(*Mytilus galloprovincialis*) in Thermaikos gulf, Greece (Catsiki and Florou, 2006), c-(*Perna viridis*) in Coastal area of the gulf of Thailand (Ruangwises and Ruangwises, 1998), d-(*Perna viridis*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

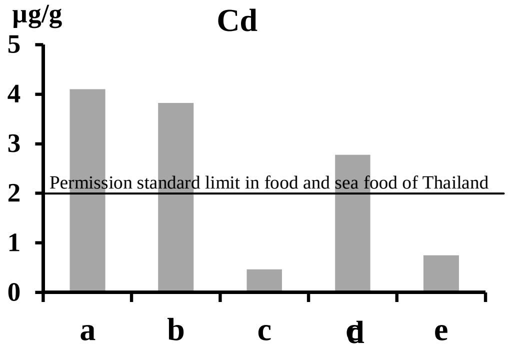


a-(*Mytilus edulis*) of World average (Cantillo, 1998), b-(*Perna viridis*) in Masan bay, Korea (Kwon and Lee, 2001), c-(*Perna viridis*) in West coast of Peninsular, Malaysia (Yap et al., 2004), d-(*Perna viridis*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

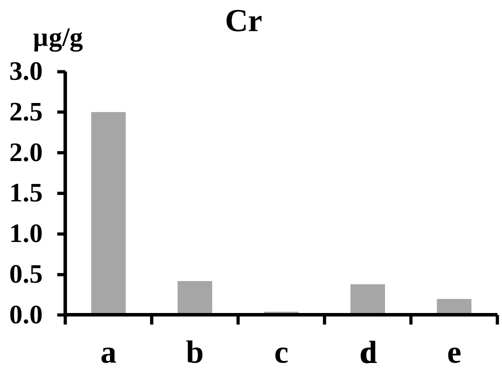
Figure 5-2 Comparison heavy metals in mussels between this study and other researches



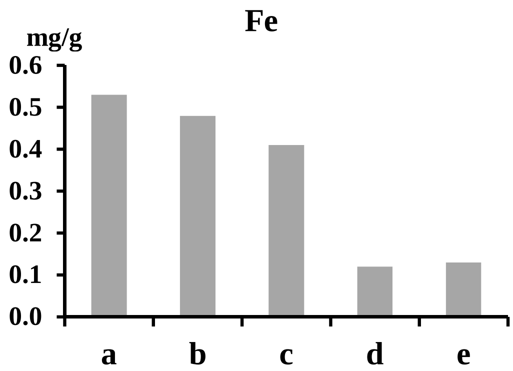
a-(*Crassostrea virginica*) of World average (Cantillo, 1998), b-(*Crassostrea virginica*) in Galveston bay, Texas (Jiann and Presley, 1997), c-(*Saccostrea cucullata*) in Masan bay, Korea (Kwon and Lee, 2001), d-(*Saccostrea cucullata*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study



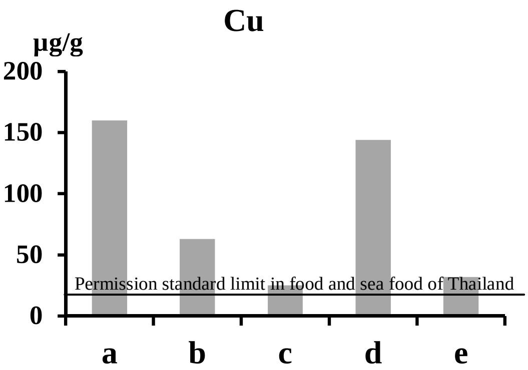
a-(*Crassostrea virginica*) of World average (Cantillo, 1998), b-(*Crassostrea virginica*) in Galveston bay, Texas (Jiann and Presley, 1997), c-(*Saccostrea cucullata*) in Masan bay, Korea (Kwon and Lee, 2001), d-(*Saccostrea cucullata*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study



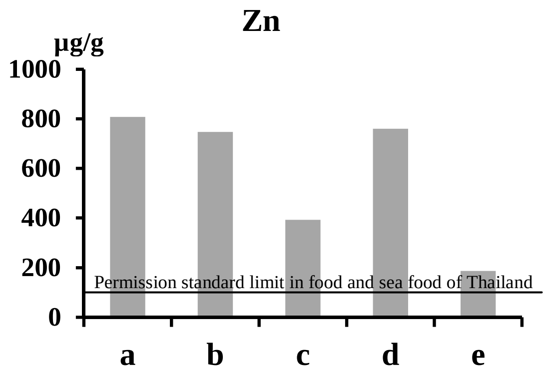
a-(*Crassostrea virginica*) of World average (Cantillo, 1998), b-(*Crassostrea virginica*) in Galveston bay, Texas (Jiann and Presley, 1997), c-(*Saccostrea cucullata*) in Masan bay, Korea (Kwon and Lee, 2001), d-(*Saccostrea cucullata*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study



a-(*Crassostrea virginica*) in Galveston bay, Texas (Jiann and Presley, 1997), b-(*Crassostrea tulipa*) in Lagoons, Ghana (Otchere, 2003), c-(*Crassostrea madrasensis*) in Gosthani estuary, East coast of India (Gawade et al., 2013), d-(*Saccostrea cucullata*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

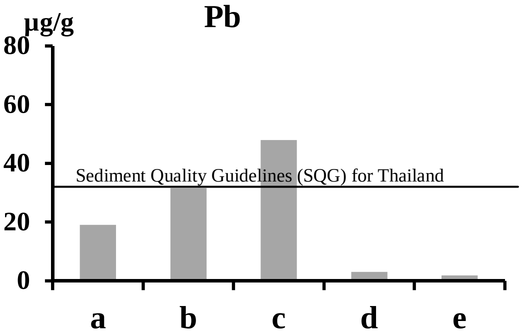


a-(*Crassostrea virginica*) of World average (Cantillo, 1998), b-(*Crassostrea virginica*) in Galveston bay, Texas (Jiann and Presley, 1997), c-(*Saccostrea cucullata*) in Masan bay, Korea (Kwon and Lee, 2001), d-(*Saccostrea cucullata*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

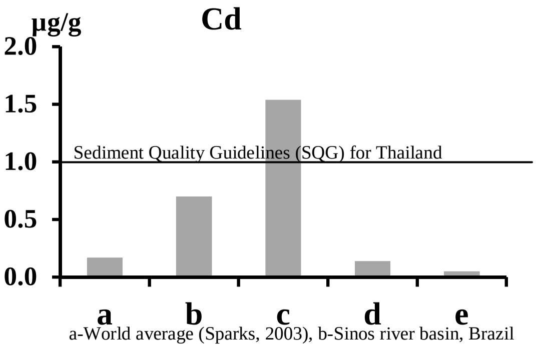


a-(*Crassostrea virginica*) in Galveston bay, Texas (Jiann and Presley, 1997), b-(*Saccostrea cucullata*) in Persian gulf, Iran(Chaharlang et al., 2012), c-(*Saccostrea cucullata*) in Masan bay, Korea (Kwon and Lee, 2001), d-(*Saccostrea cucullata*) in Gulf of Thailand (Phillips and Muttarasin, 1985), and e-This study

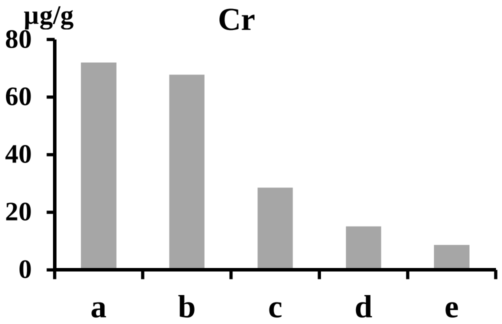
Figure 5-3 Comparison heavy metals in oysters between this study and other researches



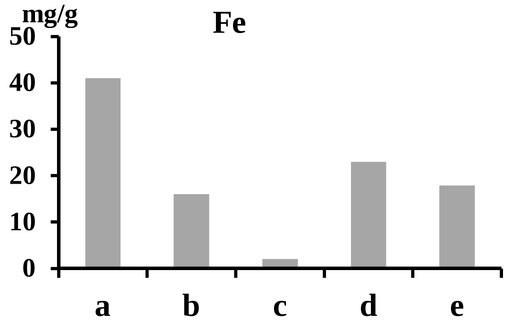
a-World average (Sparks, 2003), b-Coast of the South Sea of Korea (Song et al., 2014), c-Klang river basin, Malaysia (Naji & Ismail, 2011), d-Eastern coast of the gulf of Thailand (Thongra-ar et al., 2008) and e-This study



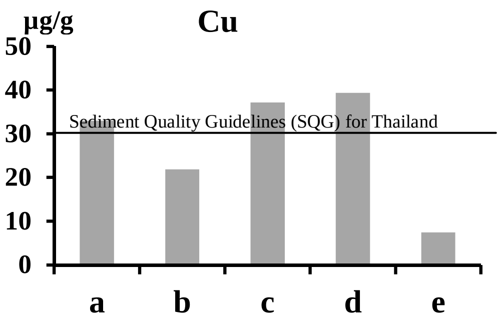
a-World average (Sparks, 2003), b-Sinos river basin, Brazil (Weber et al., 2013), c-Klang river basin, Malaysia (Naji & Ismail, 2011), d-Eastern coast of the gulf of Thailand (Thongra-ar et al., 2008) and e-This study



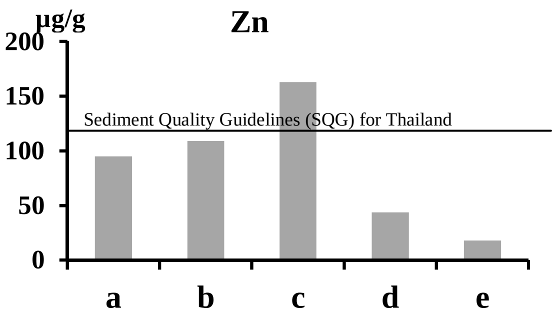
a-World average (Sparks, 2003), b-Coast of the South sea of Korea (Song et al., 2014), c-Pulicat lake (brackish water lagoon), India (Kamal-Kannan et al., 2008), d-Southern coast of the gulf of Thailand (Pumijumnong et al., 2012) and e-This study



a-World average (Sparks, 2003), b-Sinos river basin, Brazil (Weber et al., 2013), c-Klang river basin, Malaysia (Naji & Ismail, 2011), d-Eastern coast of the gulf of Thailand (Thongra-ar et al., 2008) and e-This study

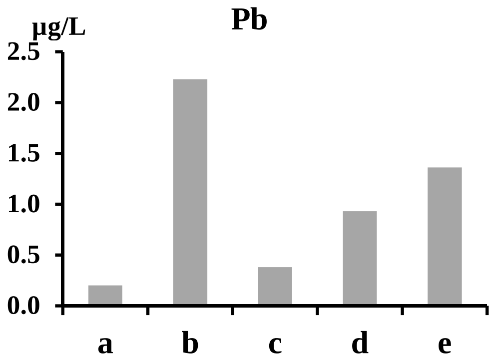


a-World average (Sparks, 2003), b-Coast of the South Sea of Korea (Song et al., 2014), c-Klang river basin, Malaysia (Naji & Ismail, 2011), d-Eastern coast of the gulf of Thailand (Thongra-ar et al., 2008) and e-This study

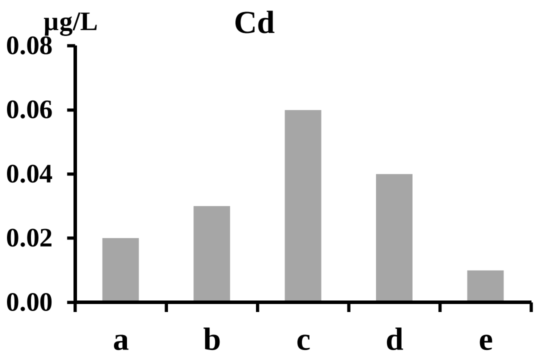


a-World average (Sparks, 2003), b-Coast of the South Sea of Korea (Song et al., 2014), c-Klang river basin, Malaysia (Naji & Ismail, 2011), d-Eastern coast of the gulf of Thailand (Thongra-ar et al., 2008) and e-This study

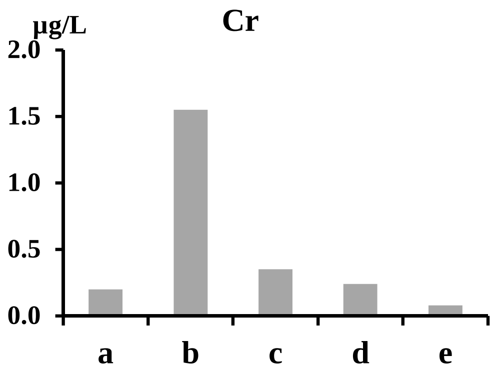
Figure 5-4 Comparison heavy metals in sediment between this study and other researches



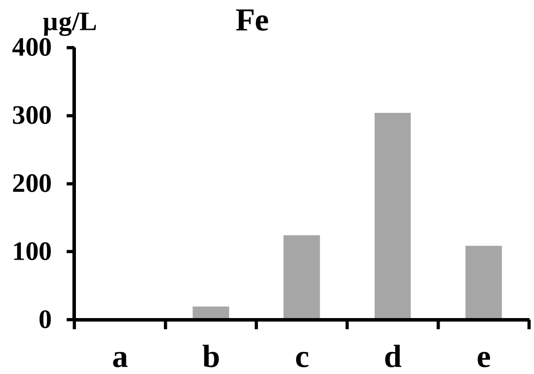
a-World (background) average (Turekian, 1969), b-Gulf of Chabahar, Oman sea (Bazzi, 2014), c-Gulf of Thailand (Hungspreugs, 1982), d-Gulf of Thailand (Rattanachongkiat, 1998) and e-This study



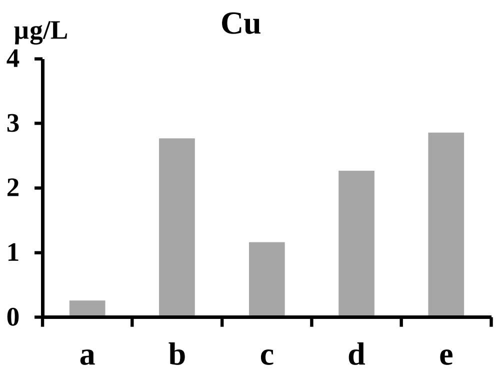
a-World (background) average (Chester, 1990), b-Gulf of Chabahar, Oman sea (Bazzi, 2014), c-Saemangeum coastal area, Korea (Kim et al., 2010), d-Gulf of Thailand (Rattanachongkiat, 1998) and e-This study



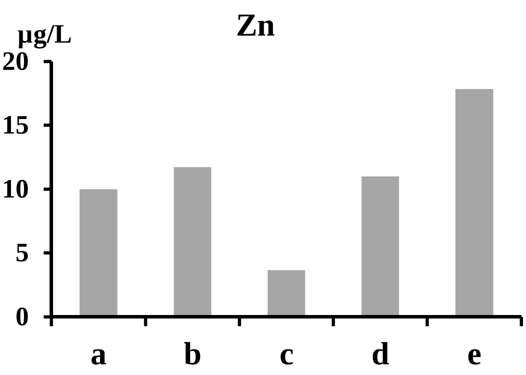
a-World (background) average (Chester, 1990), b-Gulf of Chabahar, Oman sea (Bazzi, 2014), c-Coast of Singapore (Cuong et al., 2008), d-Gulf of Thailand (Hungspreugs, 1982) and e-This study



a-World (background) average (Turekian, 1969), b-Gulf of Chabahar, Oman sea (Bazzi, 2014), c-Gulf of Thailand (Hungspreugs, 1982), d-Gulf of Thailand (Rattanachongkiat, 1998) and e-This study



a-World (background) average (Chester, 1990), b-Gulf of Chabahar, Oman sea (Bazzi, 2014), c-Coast of Singapore (Cuong et al., 2008), d-Gulf of Thailand (Hungspreugs, 1982) and e-This study



a-World (background) average (Chester, 1990), b-Gulf of Chabahar, Oman sea (Bazzi, 2014), c-Coast of Singapore (Cuong et al., 2008), d-Gulf of Thailand (Hungspreugs, 1982) and e-This study

Figure 5-5 Comparison heavy metals in seawater between this study and other researches

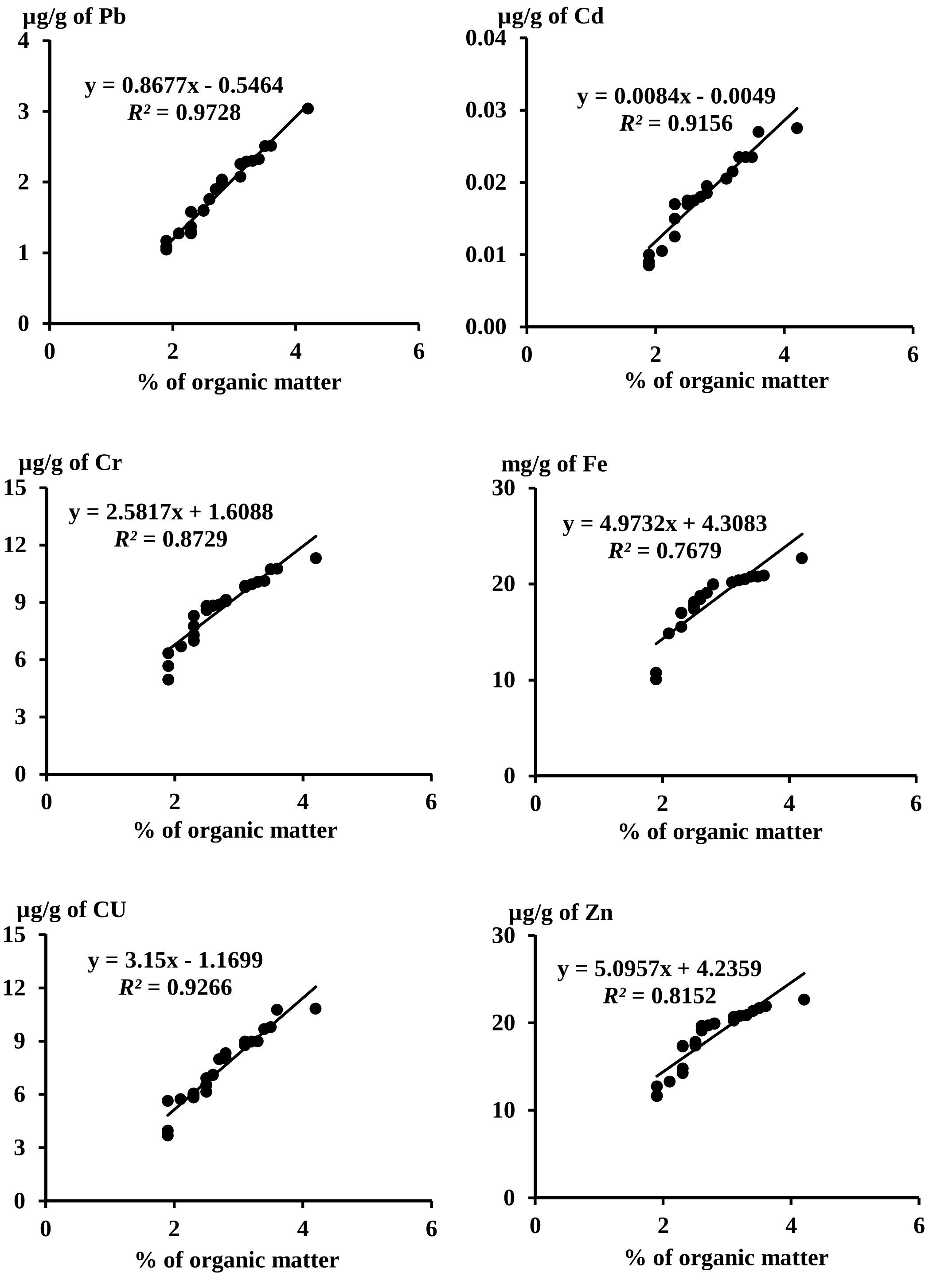


Figure 5-6 Heavy metals concentration vs. organic matter (OM) scatter plots of the sediment in the river basin Chanthaburi coastal areas

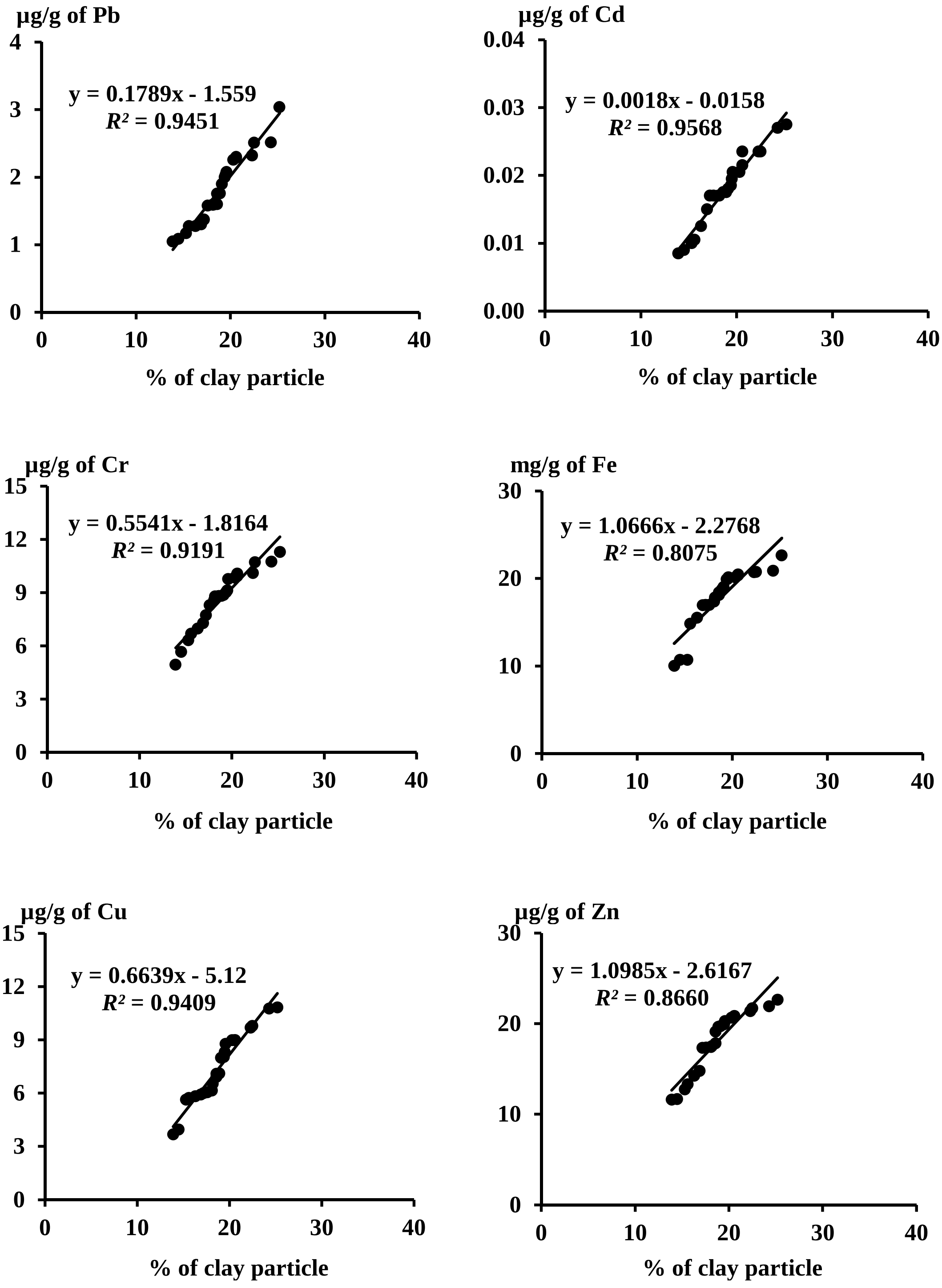
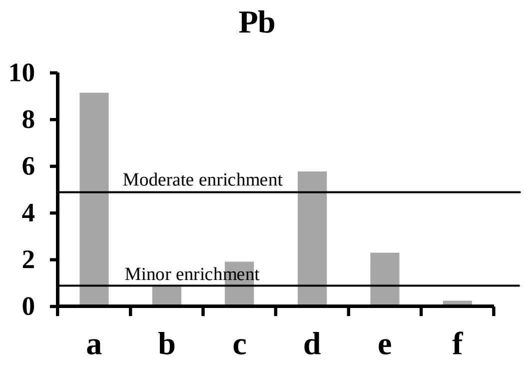
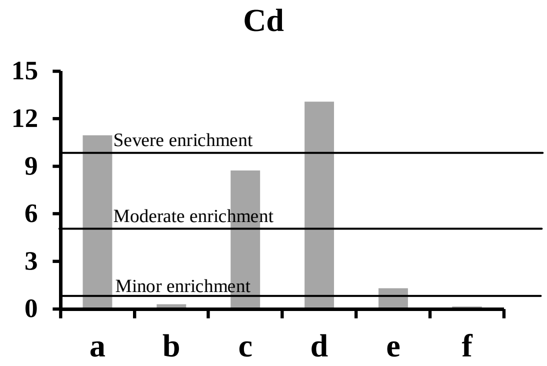


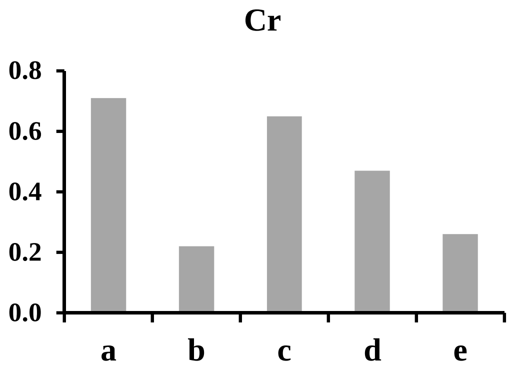
Figure 5-7 Heavy metals concentration vs. clay particle scatter plots of the sediment in the river basin Chanthaburi coastal areas



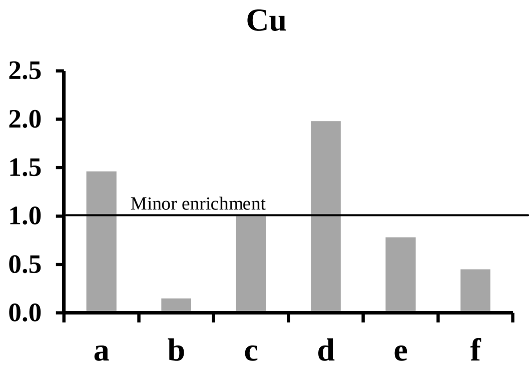
a-Chesapeake bay, USA. (Rule, 1986), b-Ala river in southwestern, Nigeria (Abata et al., 2013), c-Northeast coast of India (Banerjee et al., 2012), d-Klang river basin, Malaysia (Naji and Ismail, 2011), e-Bang-Pakong river estuary, Thailand (Cheevaporn, 1995) and f-This study



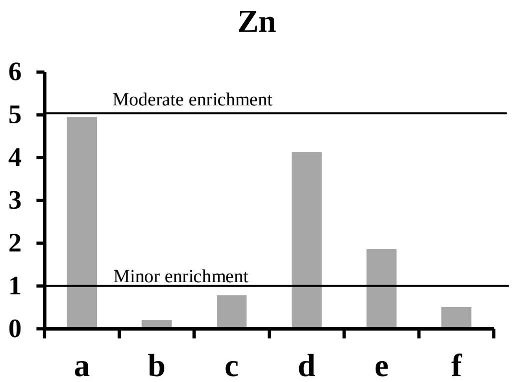
a-Chesapeake bay, USA. (Rule, 1986), b-Ala river in southwestern, Nigeria (Abata et al., 2013), c-Northeast coast of India (Banerjee et al., 2012), d-Klang river basin, Malaysia (Naji and Ismail, 2011), e-Bang-Pakong river estuary, Thailand (Cheevaporn, 1995) and f-This study



a-Chesapeake bay, USA. (Rule, 1986), b-Ala river in southwestern, Nigeria (Abata et al., 2013), c-Northeast coast of India (Banerjee et al., 2012), d-Gulf of Thailand (Censi et al., 2006), e-This study

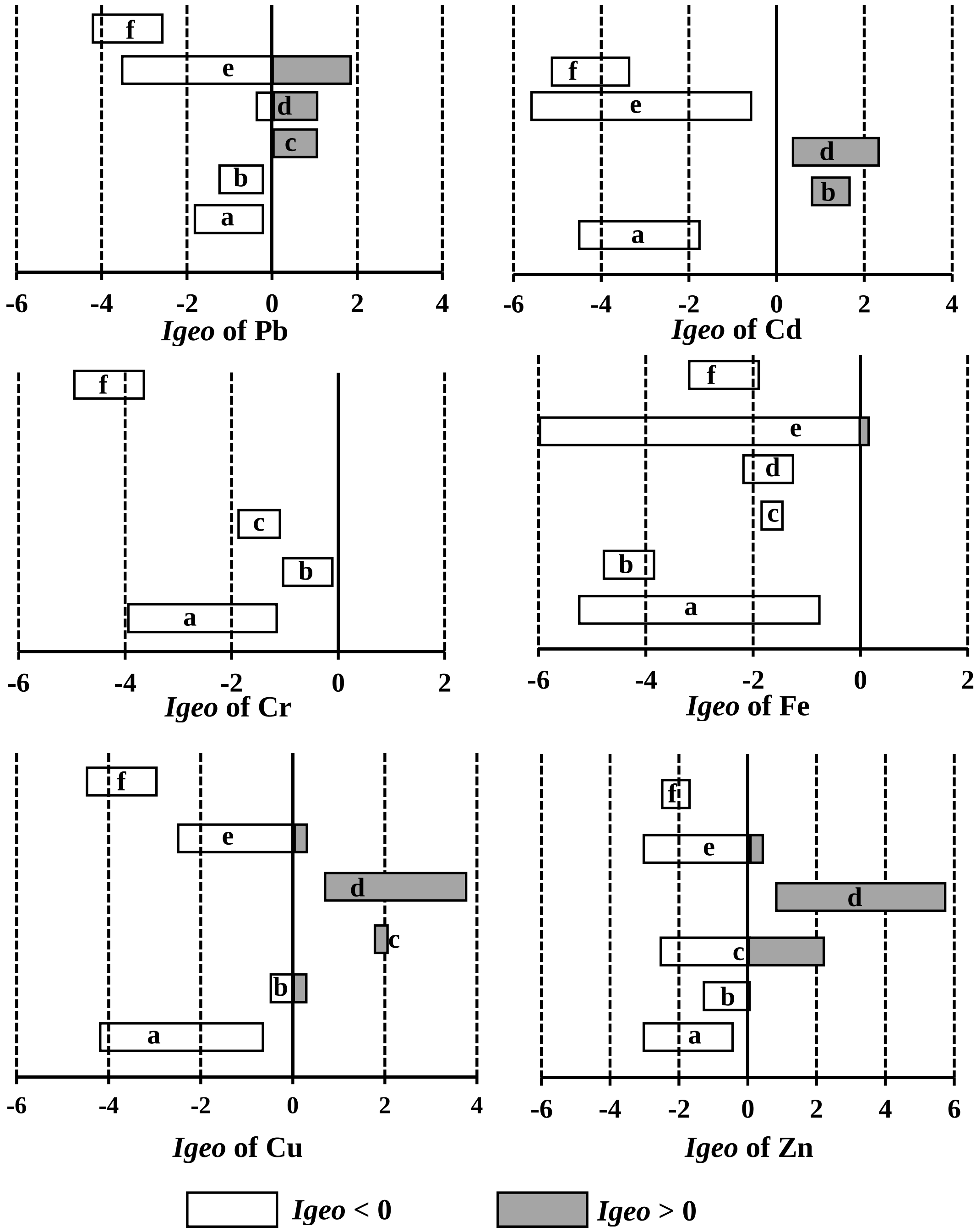


a-Chesapeake bay, USA. (Rule, 1986), b-Ala river in southwestern, Nigeria (Abata et al., 2013), c-Northeast coast of India (Banerjee et al., 2012), d-Klang river basin, Malaysia (Naji and Ismail, 2011), e- Gulf of Thailand (Censi et al., 2006) and f-This study



a-Chesapeake bay, USA. (Rule, 1986), b-Ala river in southwestern, Nigeria (Abata et al., 2013), c-Northeast coast of India (Banerjee et al., 2012), d-Klang river basin, Malaysia (Naji and Ismail, 2011), e- Gulf of Thailand (Censi et al., 2006) and f-This study

Figure 5-8 Comparison enrichment factor (EF) in the sediment between this study and other researches



a-Quiberon bay, South Brittany, France (Ong et al., 2013), b-Mediterranean sea in western of Egypt (Ahdy and Khaked, 2009), c-Dikrong river, India (Chakravarty and Patgiri, 2009), d-Klang river basin, Malaysia (Naji and Ismail, 2011), e-Gulf of Thailand (Thongra-ar et al., 2008) and f-This study

Figure 5-9 The range geoaccumulation (*Igeo*) values in the sediment between this study and other researches

Part 2. The experimental depuration of essential elements (Cu and Zn) in the oysters (*Saccostrea cucullata*) and mussels (*Perna viridis*) in the river basin of Chanthaburi coastal areas

Concentrations of Cu and Zn in oyster after 72 hours depuration were still closely the permission standard limit in food of Thailand and there was no difference of Cu at 48 and 72 hours. Amaral et al. (2005) reported incomplete metal elimination in the long period because the metals in the dissolved form are quickly depurated, while metals in amorphous granules are kept for the long period in the tissues. Bivalves have depuration mechanism to reduce accumulation of metals by the effectiveness of depuration process is depend on various factors such as the physiology of bivalves, time of depuration and environmental conditions in depuration experiment (Anacleto, Maulvault, Nunes, Carvalho, Rosa, & Marques, 2015). This study showed the relationship of the depuration variation factor including: the depuration period (hour) and weight of bivalves with the multiple regression equations as shown in Table 4-14.

Conclusion

The heavy metals contamination were not appeared in the river basin of Chanthaburi coastal area, except Cu and Zn in the oyster (*Saccostrea cucullata*) which higher than the permission standard limit in food, Thailand. The results of the geoaccumulation index and the enrichment factor values of the heavy metals content in the sediments revealed that the study area was unpolluted ($I_{geo}<0$) and not enriched (EF<1), respectively. The Cu and Zn concentrations in the oysters were lower than the permission standard limit in food, Thailand by the depuration process at 72 hours.

Suggestion for further studies

1. The investigation and monitoring should be continuously performance to assess the long term effect of heavy metals in Chanthaburi coastline.
2. The depuration study should be expanded to nonessential elements and to the abundance species in the located area such as gastropod groups, white shrimp and mud crab.

REFERENCES

Abata, E.O., Aiyesanmi, A.F., Adebayo, A.O., & Ajayi, O.O. (2013). Assessment of heavy metal contamination and sediment quality in the urban river: a case of Ala river in southwestern, Nigeria. *Journal of Applied Chemistry,4*(3), 56-63.

Abdullah, M.H., Sidi, J., & Aris, A.Z. (2007). Heavy metals (Cd, Cu, Cr, Pb and Zn) in Meretrix meretrix Roding, water and sediments from estuaries in Sabah, North Borneo. *International Journal of Environmental & Science Education, 2*(3), 69-74.

Adu, O.K. (2010). *An assessment of heavy metal contamination of sediments and tissues of the clam in the Volta estuary, Ghana*. Master's thesis, Faculty of Renewable Natural Resources, Kumasi University.

Ahdy, H.H.H., & Khaked, A. (2009). Heavy metals contamination in sediments of the western part of Egyptian Mediterraneansea. *Australian Journal of Basic and Applied Sciences, 3*(4), 3330-3336.

Alkarkhi, F.M.A., Ismail, N., & Easa, A.M. (2008). Assessment of arsenic and heavy metal contents in cockles (*Anadara granosa*) using multivariate statistical techniques. *Journal of Hazardous Materials, 150*, 783-789.

Amaral, M.C., Rebelo, M.F., Torres, J.P.M., & Pfeiffer, W.C. (2005). Bioaccumulation and depuration of Zn and Cd in mangrove oysters (Crassostrea rhizophorae, Guilding, 1828) transplanted to and from a contaminated tropical coastal lagoon. *Marine Environmental Research*, *59*, 277-285.

American Society for Testing Materials (ASTM). (2007). *Standard test method for particle-size analysis of soil: Test method ASTM D422 (Sieve analysis).* ASTM international.

Anacleto, P., Maulvault, A.L., Nunes, M.L., Carvalho, M.L., Rosa, R., & Marques, A. (2015). Effects of depuration on metal levels and health status of bivalve molluscs. *Food Control, 47*, 493-501.

Anecksamphant, S. (2004). *Database of landslide hazard case study: Kitchakood mountain, Chanthaburi Province*. Master's thesis of Science, Technology of Information System Management, Faculty of Graduate Studies, Mahidol University.

Astudillo Rojas de, L., Chang Yen, I., & Bekele, I. (2005). Heavy metals in sediments, mussels and oysters from Trinidad and Venezuela. *International Journal of Tropical Biology and Conservation, 53*(1), 277-285.

Bakan, G., & Balkas, T.I. (1999). Enrichment of metals in the surface sediments of Sapanca Lake. *Water Environment Research, 71*, 71-74.

Banerjee, K., Senthilkumar, B., Purvaja, R., & Ramesh, R. (2012). Sedimentation and trace metal distribution in selected locations of Sundarbans mangroves and Hooghly estuary, Northeast coast of India. *Environmental Geochemistry and Health,34*(1), 27-42.

Baptista Neto, J.A., Smith, B.J., & McAllister, J.J. (2000). Heavy metal concentrations in surface sediments in a nearshore environment, Jurujuba Sound, Southeast Brazil. *Environmental Pollution, 109*, 1-9.

Bazzi, A.O. (2014). Heavy metals in seawater, sediments and marine organisms in the gulf of Chabahar, Oman sea. *Journal of Oceanography and Marine Science, 5*(3), 20-29.

Berandah, F.E., Kong, Y.C., & Ismail, A. (2010). 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 Langrat, Malaysia. *Environment Asia, 3*(1), 79-83.

Bernd, B. (2000). *Bioaccumulation.* Berlin: Springer-Verlag.

Bielicka, A., Bojanowska, I., & Wiśniewski, A. (2005). Two faces of chromium-pollutant and bioelement. *Polish Journal of Environmental Studies, 14*(1), 5-10.

Birch, G.F., Melwani, A., Lee, J.H., & Apostolatos, C. (2014). The discrepancy in concentration of metals (Cu, Pb and Zn) in oyster tissue (*Saccostrea glomerata*) and ambient sediment (Sydney estuary, Australia). *Marine Pollution Bulletin, 36*, 236-274.

Burger, J., & Gochfeld, M. (2006). Locational differences in heavy metals and metalloids in pacific blue *Mytilus edulis* from Adak Island in the Aleutian Chain, Alaska. *Science of Total Environment*, *368*, 937-950.

Cantillo, A.Y. (1998). Comparison of results of mussel watch programs of the United States and France with worldwide mussel watch studies. *Marine Pollution Bulletin, 36*(9), 712-717.

Catsiki, V.A., & Florou, H. (2006). Study on the behavior of the heavy metals Cu, Cr, Ni, Zn, Fe, Mn and ^{137}Cs in an estuarine ecosystem using *Mytilus gallopprovincialis* as a bioindicator species: the case of Thermaikos gulf, Greece. *Journal of Environmental Radioactivity, 86*, 31-44.

Censi, P., Spoto, S.E., Saiano, F., Sprovieri, M., Mazzola, S., Nardone, G., Di Geronimo, S.I., Punturo, R., & Ottonello, D. (2006). Heavy metals in coastal water systems a case study from the northwestern gulf of Thailand. *Chemosphere, 64*(7), 1167-1176.

Chaharlang, B.H., Bakhtiari, A.R., & Yavari, V. (2012). Assessment of cadmium, copper, lead and zinc contamination using oysters (*Saccostrea cucullata*) as biomonitors on the coast of the Persian Gulf, Iran. *Bulletin of Environmental Contamination and Toxicology, 88*(6), 956-961.

Chakravarty, M., & Patgiri, A.D. (2009). Metal pollution assessment in sediment of the Dikrong river, N.E. India. *Journal of Human Ecology, 27*(1), 63-67.

Cheevaporn, V. (1995). *History of heavy metal contamination in Bang Pakong RiverEstuary, Thailand.* Doctoral dissertation, Marine Science Institute, University of the Philippines.

Cheevaporn, V., & Menasveta, P. (2003). Water pollution and habitat degradation in the Gulf of Thailand. *Marine Pollution Bulletin, 47,* 43-51.

Cheevaporn, V., & San Diego-McGlone, M.L. (1997). Aluminium normalization of heavy-metal data from estuarine and coastal sediments of the gulf of Thailand. *Thammasat International Journal of Science and Technology, 2*(2), 37-46.

Chen, C.W., Kao, C.M., Chen, C.F., & Dong, C.D. (2007). Distribution and accumulation heavy metals in sediments of Kaohsiung Harbor, Taiwan. *Chemosphere, 66*, 1431-1440.

Chester, R. (1990). *Marine Geochemistry.* London: Unwin Hyman.

Combs, S.M., & Nathan, M,V. (1998). Soil organic matter. In Brown, J.R. (Ed.), *Recommended chemical soil test procedure for the north central region*. Columbia, USA: North Central Regional Research Publication No. 221.

Cuong, D.T., Karuppiah, S., & Obbard, J.P. (2008). Distribution of heavy metals in the in the dissolved and suspended phase of the sea-surface microlayer, seawater column and in sediments of Singapore's coastal environment. *Environmental Monitoring and Assessment, 138*(1-3), 255-272.

Dean, J.R. (2003). *Methods for environmental trace analysis.* West Sussex: John Wiley & Sons.

Din, Z.B. (1992). Use of aluminium to normalize heavy-metal data from estuarine and coastal sediments of strait of Melaka. *Marine Pollution Bulletin,44*(10), 484-491.

Figueira, E., Lima, A., Branco, D., Quintino, V., Rodrigues, A.M., & Freitas, R. (2011). Health concerns of consuming cockles (*Cerastoderma edule* L.) from a low contaminated coastal system. *Environment International, 37*, 965-972.

Förstner, U., Ahlf, W., & Calmono, W. (1993). Sediment quality objectives and criteria development in Germany. *Water Science and Technology, 28*, 307-316.

Fosmire, G.J. (1990). Zinc toxicity. *American journal of Clinical Nutrition, 51*, 225-227.

Gawade, L., Harikrishna, C.N.V., Sarma, V.V., & Ingole, B.S. (2013). Variations in heavy metals concentration in the edible oyster *Crassostrea madrasensis,* clam *Polymesoda erosa* and grey mullet *Liza aurata* from coastline of India. *Indian Journal of Science, 2*(4), 59-63.

Geffard, A., Amiard, J.C., & Amiard-Triquet, C. (2002). Kinetics of metal elimination in oysters from a contaminated estuary. *Comparative Biochemistry and Physiology, 131*, 281-293.

Ghrefat, H., & Yusuf, N. (2006). Assessing Mn, Fe, Cu, Zn, and Cd pollution in bottom sediments of Wadi Al-Arab Dam, Jordan. *Chemosphere, 65*, 2114-2121.

Glasby, G.P., Szefer, P., Geldon, J., & Warzocha, J. (2004). Heavy metal pollution of sediments from Szczecin lagoon and the Gdansk basin, Poland. *Science of the Total Environment*, *330*, 249-269.

Heidari, B., Bakhtiari, A.R., & Shirneshan, G. (2013). Concentration of Cd, Cu, Pb and Zn in soft tissue of oyster (*Saccostrea cucullata*) collected from the Lengeh Port coast, Persian Gulf, Iran: A comparison with the permissible limit for public health. *Food Chemistry, 141*, 3014-3019.

Hem, J.D. (1985). *Study and interpretation of the chemical characteristics of natural water* (3rd ed.). Virginia, USA: United states government printing office.

Herut, B., & Sandler, A. (2006). *Final report normalization methods for pollutants in marine sediments: review and recommendations for the Mediterranean.* Jerusalem: Isael Oceanographic and Limnological Research.

Hungspreugs, M. (1982). Baseline levels of Cadmium, Chromium, Copper, Lead and Zinc in seawater from the gulf of Thailand. *ScienceAsia, 8*(4), 193-204.

Ibrahim, N. (1995). Trace element content of Malaysian cockles (*Anadara granosa*). *Food Chemistry, 54*, 133-135.

Jiann, K.T., & Presley, B.J. (1997). Variations in trace metal concentrations in american oysters (*Crassostrea virginica*) collected from Galveston bay, Texas. *Estuaries, 20*(4), 710-724.

Kamala-Kannan, S., Batvari, B.P.D., Lee, K.J., Kannan, N., Krishnamoorthy, R., Shanthi, K., & Jayaprakash, M. (2008). Assessment of heavy metals (Cd, Cr and Pb) in water, sediment and Seaweed (*Ulva lactuca*) in the Pulicat Lake, South East India. *Chemosphere, 71*, 1233-1240.

Kim, K.T., Kim, E.S., Cho, S.R., Park, J.K., Ra, K.T., & Lee, J.M. (2010). Distribution of heavy metals in the environmental samples of the Saemangeum coastal area, Korea. *Coastal Environmental and Ecosystem Issues of the East China Sea, Environmetal Issues*, 71-90.

Kwon, Y.T., & Lee, C.W. (2001). Sediment metal speciation for the ecological risk assessment. *Analytical Sciences, 17*, i1015-i1017.

Lares, M.L., Flores-Muñoz, M.G., & Lara-Lara, R. (2002). Temporal variability of bioavailable Cd, Hg, Zn, Mn and Al in an upwelling regime. *EnvironmentalPollution, 120*(3), 595-608.

Lee, R., Lovatelli, A., & Ababouch, L. (2008). *Bivalve depuration: fundamental and practical aspects.* Rome: Food and Agriculture Organization of the United Nation.

Naji, A., & Ismail, A. (2011). Assessment of metals contamination in Klang river surface sediments by using different indexes. *Environment Asia, 4*(1), 30-38.

Ong, M.C., Menier, D., Shazili, N.A.M., & Kamaruzzaman, B.Y. (2013). Geochemical characteristics of heavy metals concentration in sediments of Quiberon bay waters, South Brittany, France. *Oriental Journal of Chemistry,29*(1), 39-45.

Otchere, F.A. (2003). Heavy metals concentrations and burden in the bivalves (*Anadara* (*Senilia*) *senilis*, *Crossostrea tulipa* and *Perna perna*) from lagoons in Ghana: Model to describe mechanism of accumulation/eccretion. *African Journal of Biotechnology*, *2*(9), 280-287.

Panutrakul, S., Khaesejan, T., & Thungkao, S. (2007). Risk assessment of consumed oyster from aquacultural sites in Sub-district of Ang Sila, Chonburi province. In *The 45th National Kasetsart University Conference. Scientific program No.6* (pp. 29-36).

Panutrakul, S., & Khamdee, A. (2008). Reduction of heavy metal in live oyster (Saccostrea cucullata) using small re-circulating depuration unit. In *The Annual Conference on Fisheries 2008 (pp. 19-27)*. Bangkok: Department of Fisheries.

PCD (Pollution Control Department). (2006). *Proposed marine and coastal sediment quality guidelines*. Final report, UNEP GEF Project “Reversing environmental degradation trends in the South China Sea and Gulf of Thailand”.

Peng, J.F., Song, Y.H., Yuan, P., Cui, X.Y., & Qiu, G.L. (2009). The remediation of heavy metals contaminated sediment. *Journal of Hazardous Materials, 161*, 633-640.

Phillips, D.J.H, & Muttarasin, K. (1985). Trace metals in bivalve mollusks from Thailand. *Marine Environmental Research, 15*(3), 215-234.

Pumijumnong, N., & Uppadit, B. (2012). Accumulation of heavy metals in mangrove sediments of Chumphon province, Thailand. *Journal of Environmental Research, 34*(2), 21-38.

Rattanachongkiat, S. (1998). *Distribution of trace metals in the Gulf of Thailand and East Coast of Malay Peninsula*. Master's thesis, Department of Marine Science, Chulalongkorn University.

Ruangwises, N., & Ruangwises S. (1998). Heavy metals in green mussels (*Perna viridis*) from the Gulf of Thailand. *Journal of Food Protection, 61*(1), 94-97.

Rule, J.H. (1986). Assessment of trace element geochemistry of Hampton roads harbor and lower Chesapeake bay area sediments. *Environmental Geology and Water Sciences,8*(4), 209-219.

Runping, S., & Kheoruenromne, I. (2003). Monitoring land use dynamics in Chanthaburi Province of Thailand using digital remotely sensed images. *Pedosphere*, *13*(2), 157-164.

Song, Y., Choi, M.S., Lee, J.Y., & Jang, D.J. (2014). Regional background concentrations of heavy metals (Cr, Co, Ni, Cu, Zn, Pb) in coastal sediments of the South Sea of Korea. *Science of the Total Environment, 482-483*, 80-91.

Sparks, D.L. (2003). *Environmental soil chemistry* (2^{nd} ed.). U.S.A.: China Translation & Printing Services.

Spooner, D.R., Maher, W., & Otway, N. (2003). Trace metal concentrations in sediments and oysters of Botany bay, NSW, Australia. *Archives of Environmental Contamination and Toxicology, 45,* 92-100.

Suthar, S., Nema, A.K., Chabukdhara, M., & Gupta, S.K. (2009). Assessment of metals in water and sediments of Hindon River, India: Impact of industrial and urban discharges. *Journal of Hazardous Material, 171*, 1088-1095.

Szefer, P., Wolowicz, M., Kusak, A., Deslous-Paoli, J.M., Czarnowski, W., Frelek, K., & Belzunce, M.J. (1999). Distribution of mercury and other trace metals in the cockle *Cerastoderma glaucum* from the Mediterranean lagoon Etang de Thau. *Archives of Environmental Contamination and Toxicology, 36*, 56-63.

Taylor, S.R. (1964). Abundance of chemical elements in the continental crust: a new table. *Geochimica et Cosmochimica Acta, 28*, 1273-1285.

The Perkin Elmer Corporation. (1994). *Analytical Methods for Atomic Absorption Spectrophotometry.* U.S.A.: Perkin Elmer Corporation.

Thongra-ar, W., Musika, C., Wongsudawan, W., & Munhapol, A. (2008). Heavy metals contamination in sediments along the eastern coast of the gulf of Thailand. *Environment Asia, 1*(1), 37-45

Turekian, K.K. (1969). *The oceans, streams and atmosphere.* Berlin: Springer.

Turekian, K.K., & Wedepohl, K.H. (1961). Distribution of the elements in some major units of the earth's crust. *The Geological Society of America, 72*(2), 175-192.

Vallee, B.L., & Wacker, W.E.L. (1970). Metalloproteins. In Neurath, H. (Ed.), *The Proteins Volume 5*. New York and London: Academic Press.

Walker, C.H., Hopkin, S.P., Sibly, R.M., & Peakall, D.B. (2006). *Principles of Ecotoxicology* (3rd ed.). United State of America: Taylor & Francis.

Weber, P., Behr, E.R., Knorr, C.De L., Vendruscolo, D.S., Flores, E.M.M., Dressler, V.L., & Baldisserotto, B.(2013). Metal in the water, sediment, and tissues of two fish species from different trophic level in a subtropical Brazilian river. *Microchemical Journal, 106*, 61-66.

Yap, C.H., & EDWARD, F.B. (2010). Distribution of heavy metals in the different parts of *Cerrithidae Obtusa* and the relationships between metal distribution and allometric parameters of the snail. *Environment Asia, 3*(2), 38-48.

Yap, C.K., Ismail, A., & Tan, S.G. (2004). Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. *Food Chemistry, 84*, 569-575.

Yap, C.K., Muhamad Azlan, A.G., Cheng, W.H., & Tan, S.G. (2011). Accumulation and Depuration of Cu and Zn in the Blood Cockle *Anadara granosa* under Laboratory Conditions. *Tropical Agriculture Science, 34*(1), 75-82.

APPENDIX

APPENDIX A

The procedure of samples preparation

The procedure for nitric acid digestion of bivalve tissues (Yap & Edward, 2010)

1) Prepare about 0.5 g of bivalve tissues placed in beaker.

2) Add 10 ml of concentrated nitric acid (Superpure Grade, Merck Chemicals, HNO_3 65%, Germany)

3) Place in a hot block digester at low temperature (40°C) for one hour after that fully digested at high temperature (140°C) for at least three hours.

4) The digested samples were then diluted to a volume of 40 ml with double distilled water

5) The sample was then filtered through Whatman No.1 filter paper

6) The sample completed for determination with Atomic Absorption Spectrophotometer (AAS)

The procedure for the acid digestion of sediment using a hot-plate (Dean, 2003)

1) Prepare about 1 g of sediment samples placed in beaker.

2) Add 10 ml 1:1 nitric acid, cover with a watch glass and heat for 15 minutes

3) Cool the sample and add 5 ml concentrated nitric acid, cover with a watch glass and heat for 30 minutes (Additional nitric acid added until no brown fumes given off)

4) Reduce volume to < 5 ml and cool, add 2 ml water and 3 ml 30% H_2O_2 and heat. (Additional H_2O_2 added until effervescence ceased) Processes continued for 2 hours and reduce volume to less than 5 ml.

5) Add 10 ml HCl and heat for 15 minutes.

6) Filter and quantitatively transfer to a 100 ml volumetric flask.

The procedure for sea water preparation with concentrated HNO_3

This procedure was modified from The Perkin Elmer Corporation (1994)

1) The samples were filtered through GF/C Whatman filter paper 0.45 μm.

2) Pipette 50 ml of samples in Erlenmeyer flask

3) Add 1 ml of concentrated HNO_3

4) Place in a hot plate at temperature 100-110°C for at least 2-3 hours

5) Make volume with deionized water in 25 ml volumetric flasks.

Determination of organic carbon in sediments by Weight Loss on Ignition technique (Combs & Nathan, 1998)

1) Scoop 5 to 10 g of dried, ground (10 mesh) sediment into tarred crucibles.

2) Dry for 2 hours at 105°C (for gypsiferous and low organic matter sediments, heat for 2 hours at 150°C).

3) Record weight to plus or minus 0.001 g.

4) Heat at 360°C for 2 hours (after temperature reaches 360°C).

5) Cool to 150°C.

6) Weight in a draft-free environment to plus or minus 0.001 g.

7) Calculate percentage weight loss on ignition (LOI):

$$LOI = \frac{(\text{weight at } 105^{\circ}C) - (\text{weight at } 360^{\circ}C) \times 100}{\text{Weight at } 105^{\circ}C}$$

Grain size determination of sediment by Sieve analysis (American Society for Testing and Materials, 1990)

1) Write down the weight of each sieve and record the weight of given dry sediment sample.

2) Place the sediment sample in each sieves by begin from large to thin sieves (including: 250 Meah; 63 μm and 325 Meah; 45 μm)

3) Shake all sieves for 10 minutes and record the weight of each sieve with its retained samples.

4) Calculate the percent retained on each sieve by dividing the weight retained on each sieve by the original sample mass. The calculation the percent passing can be started with 100 percent and subtracting the percent retained on each sieve as a cumulative procedure.

APPENDIX B

The quality control of analysis

Table appendix B-1 Appropriated condition for heavy metals analysis (Cd, Pb, Cr, Fe, Cu and Zn)

Appropriated condition	Species of heavy metal					
	Cd	Pb	Cr	Fe	Cu	Zn
Mode	Graphite	Graphite	Graphite	Flame	Flame	Flame
Wavelength: nm	228.7	283.3	425.4	372.0	324.7	213.9
Spectra slit width: nm	0.5	0.5	0.5	0.2	0.5	0.5
HCL (Current: mA)	5.0	10.0	10.0	10.0	10.0	7.0
Fuel	-	-	-	Air-Acetylene	Air-Acetylene	Air-Acetylene
Flame	GF*	GF*	GF*	Lean-Blue	Lean-Blue	Lean-Blue

*GF-Graphite Furnace

Table appendix B-2 Analysis certified reference material and the percentages of recovery of National Research Council, Canada: Marine Dogfish Reference Materials (DORM-3) and Marine Sediment Reference Materials (MESS-3)

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

Table appendix B-3 Seawater quality along the Chanthaburi coastal area

Parameter/ Location	Wang-Ta-Nord (A)		Chanthaburi (B)		Welu (C)	
	Wet	Dry	Wet	Dry	Wet	Dry
Temperature (°C)	31	34	30	33	31	33
Salinity (g/L)	8.0	12.0	10.0	15.0	11.0	18.0
pH	7.35	7.22	7.95	7.23	7.04	7.15
Conductivity (μS/cm)	10.92	8.65	5.26	4.24	12.01	10.74
Dissolved Oxygen (mg/L)	6.65	6.12	6.00	6.36	5.45	6.30
Phosphate (PO_4^{3-}) (μg/L)	15.99	19.43	15.14	17.23	16.57	14.58

*Wet (May 2012-October 2012), Dry (November 2012-April 2013)

APPENDIX C

The results of the statistical analysis

Table appendix C-1 Correlation matrix of heavy metals in Cockles (*Anadara granosa*) and selected physicochemical and physiological characteristic (n=24)

	Pb	Cd	Cr	Fe	Cu	Zn	OM	Sand	Silt	Clay	pH	EC	Salinity	Weight
Pb	1.000													
Cd	0.584**	1.000												
Cr	0.093	0.220	1.000											
Fe	0.156	-0.139	0.110	1.000										
Cu	0.017	-0.008	0.374	-0.011	1.000									
Zn	0.085	0.026	0.367	0.098	0.815**	1.000								
OM	-0.451*	-0.511*	-0.423*	-0.235	-0.336	-0.453*	1.000							
Sand	0.336	0.320	-0.198	0.025	-0.128	-0.211	-0.486*	1.000						
Silt	0.025	0.163	0.330	-0.033	0.135	0.137	-0.327	-0.384	1.000					
Clay	-0.463*	-0.451*	-0.066	-0.005	-0.016	-0.093	0.740**	-0.688**	-0.405*	1.000				
pH	0.214	0.006	0.423*	0.459*	0.463*	0.421*	-0.462*	0.131	0.157	-0.246	1.000			
EC	0.423*	0.441*	0.029	0.049	-0.290	-0.283	0.007	0.370	-0.075	-0.299	0.258	1.000		
Salinity	0.465*	0.209	0.015	0.001	0.117	0.037	0.218	0.026	-0.288	0.206	0.248	0.317	1.000	
Weight	0.143	0.104	0.498*	0.063	0.449*	0.149	0.024	-0.038	-0.117	0.134	0.430*	0.085	0.261	1.000

*, **: Correlation is significant at the 0.05 and 0.01 level, respectively

Table appendix C-2 Correlation matrix of heavy metals in Mussels (*Perna viridis*) and selected physicochemical and physiological characteristic (n=24)

	Pb	Cd	Cr	Fe	Cu	Zn	OM	Sand	Silt	Clay	pH	EC	Salinity	Weight
Pb	1.000													
Cd	0.220	1.000												
Cr	-0.074	0.149	1.000											
Fe	0.368	0.493*	0.146	1.000										
Cu	0.297	0.279	0.069	0.435*	1.000									
Zn	0.372	0.121	-0.104	0.419*	0.678**	1.000								
OM	-0.409*	-0.459*	-0.489*	-0.117	-0.442*	-0.078	1.000							
Sand	0.114	0.092	-0.012	-0.108	-0.018	-0.112	-0.486*	1.000						
Silt	-0.057	0.276	0.247	0.160	0.046	-0.217	-0.327*	-0.384	1.000					
Clay	-0.072	-0.456*	-0.187	-0.015	-0.021	-0.408*	0.740**	-0.688**	-0.405*	1.000				
pH	0.037	0.174	0.007	0.421*	0.470*	0.083	-0.462*	0.131	0.157	-0.246	1.000			
EC	0.026	0.140	0.099	0.125	-0.159	-0.009	0.007	0.370	-0.075	-0.299	0.258	1.000		
Salinity	0.201	0.089	0.511*	0.526*	0.289	0.443*	0.218	0.026	-0.288	0.206	0.248	0.317	1.000	
Weight	0.048	0.149	0.017	0.085	0.014	0.154	0.063	0.219	-0.118	-0.119	0.261	0.716**	0.139	1.000

*, **: Correlation is significant at the 0.05 and 0.01 level, respectively

Table appendix C-3 Correlation matrix of heavy metals in Oysters (*Saccostrea cucullata*) and selected physicochemical and physiological characteristic (n=24)

	Pb	Cd	Cr	Fe	Cu	Zn	OM	Sand	Silt	Clay	pH	EC	Salinity	Weight
Pb	1.000													
Cd	0.466*	1.000												
Cr	0.596**	0.280	1.000											
Fe	0.416*	0.755**	0.488*	1.000										
Cu	-0.110	0.233	-0.136	0.212	1.000									
Zn	-0.041	0.309	0.073	0.325	0.615**	1.000								
OM	-0.419*	-0.056	-0.411*	-0.122	-0.178	-0.007	1.000							
Sand	-0.156	0.044	0.115	0.006	0.291	-0.137	-0.486*	1.000						
Silt	0.214	-0.050	0.186	0.144	0.081	0.198	-0.327	-0.384	1.000					
Clay	-0.012	-0.004	-0.421*	-0.120	-0.418*	-0.020	0.740**	-0.688**	-0.405*	1.000				
pH	0.423*	0.435*	0.161	0.430*	0.026	0.034	-0.462*	0.131	0.157	-0.246	1.000			
EC	0.140	0.250	0.074	0.221	0.464*	0.423*	0.007	0.370	-0.075	-0.299	0.258	1.000		
Salinity	0.443*	0.408*	0.198	0.420*	0.221	0.078	0.218	0.026	-0.288	0.206	0.248	0.317	1.000	
Weight	0.136	0.117	0.441*	0.407*	0.070	0.411*	0.077	-0.166	0.135	0.063	0.220	-0.085	-0.070	1.000

*, **: Correlation is significant at the 0.05 and 0.01 level, respectively

Table appendix C-4 Correlation matrix of heavy metals and selected sediment characteristic (n=24)

	Pb	Cd	Cr	Fe	Cu	Zn	OM	Sand	Silt	Clay	pH	EC
Pb	1.000											
Cd	0.687**	1.000										
Cr	0.484*	0.636**	1.000									
Fe	0.409*	0.553**	0.638**	1.000								
Cu	-0.009	0.339	0.249	0.122	1.000							
Zn	0.331	0.682**	0.608**	0.645**	0.312	1.000						
OM	0.556**	0.613**	0.422*	0.443*	0.408*	0.558*	1.000					
Sand	-0.103	0.105	0.072	-0.160	0.254	0.144	-0.486*	1.000				
Silt	0.099	0.063	0.253	0.066	0.268	0.164	-0.327	-0.384	1.000			
Clay	0.602**	0.553**	0.412*	0.473*	0.562**	0.575**	0.740**	-0.688**	-0.405*	1.000		
pH	0.033	0.234	0.327	-0.697**	0.308	-0.619**	-0.462*	0.131	0.157	-0.246	1.000	
EC	0.371	0.033	0.250	0.221	0.142	0.153	0.007	0.370	-0.075	-0.299	0.258	1.000

*, **: Correlation is significant at the 0.05 and 0.01 level, respectively

Table appendix C-5 Correlation matrix of heavy metals and selected seawater characteristic (*n*=24)

	Pb	Cd	Cr	Fe	Cu	Zn	pH	EC	Salinity
Pb	1.000								
Cd	0.114	1.000							
Cr	0.217	-0.362	1.000						
Fe	-0.031	0.705**	-0.123	1.000					
Cu	0.386	-0.169	0.394	-0.439*	1.000				
Zn	0.090	-0.350	0.339	-0.443*	0.383	1.000			
pH	-0.285	-0.570**	-0.459*	-0.251	-0.095	-0.117	1.000		
EC	0.320	0.047	0.195	0.516*	0.579**	0.505*	0.258	1.000	
Salinity	0.408*	0.418*	0.113	0.071	0.412*	0.248	0.248	0.317	1.000

*, **: Correlation is significant at the 0.05 and 0.01 level, respectively

Table appendix C-6 ANOVA analysis of heavy metal contents in bivalves

mean of variances		sum of squares	df	mean squares	*F*-ratio	significant
Pb	station	0.000	2	6.278×10^{-5}	5.785	0.005*
	species	0.002	2	0.001	101.227	0.000*
Cd	station	0.255	2	0.128	6.891	0.002*
	species	4.874	2	2.437	131.492	0.000*
Cr	station	0.004	2	0.002	1.581	0.214
	species	0.051	2	0.026	20.824	0.000*
Fe	station	6471.948	2	3235.974	0.915	0.406
	species	1.821×10^{6}	2	9.105×10^{5}	257.510	0.000*
Cu	station	461.331	2	230.665	21.244	0.000*
	species	8821.003	2	4410.501	406.195	0.000*
Zn	station	1625.162	2	812.581	16.672	0.000*
	species	3.737×10^{5}	2	1.868×10^{5}	3833.802	0.000*

* the difference is significant at the 0.05

Table appendix C-7 ANOVA analysis of heavy metal contents in sediments

mean of variances		sum of squares	df	mean squares	F-ratio	significant
Pb	station	2.620	2	1.310	7.401	0.004*
Cd	station	9.813×10^{-5}	2	4.906×10^{-5}	1.902	0.174
Cr	station	7.888	2	3.944	1.517	0.242
Fe	station	5.284×10^{7}	2	2.642×10^{7}	2.632	0.095
Cu	station	12.326	2	6.163	1.718	0.204
Zn	station	24.585	2	12.292	1.093	0.353

* the difference is significant at the 0.05

Table appendix C-8 ANOVA analysis of heavy metal contents in seawater

mean of variances		sum of squares	df	mean squares	F-ratio	significant
Pb	station	0.042	2	0.021	0.234	0.793
Cd	station	0.051	2	0.026	19.519	0.002*
Cr	station	0.002	2	0.001	2.811	0.083
Fe	station	6461.344	2	3230.672	65.957	0.000*
Cu	station	0.688	2	0.344	8.234	0.002*
Zn	station	36.454	2	18.227	5.370	0.013*

* the difference is significant at the 0.05

Table appendix C-9 ANOVA analysis of geoaccumulation index

mean of variances		sum of squares	df	mean squares	*F*-ratio	significant
Pb	station	1.742	2	0.871	7.707	0.003*
Cd	station	0.897	2	0.448	2.310	0.124
Cr	station	0.286	2	0.143	1.702	0.206
Fe	station	0.448	2	0.224	2.464	0.109
Cu	station	0.541	2	0.270	1.737	0.200
Zn	station	0.228	2	0.114	1.357	0.279

* the difference is significant at the 0.05

Table appendix C-10 ANOVA analysis of enrichment factor

mean of variances		sum of squares	df	mean squares	*F*-ratio	significant
Pb	station	0.010	2	0.005	1.063	0.363
Cd	station	0.001	2	0.000	0.181	0.836
Cr	station	0.001	2	0.000	0.194	0.825
Cu	station	0.140	2	0.070	5.682	0.011*
Zn	station	0.012	2	0.006	0.797	0.464

* the difference is significant at the 0.05

Table appendix C-11 ANOVA analysis of $BCF_{Bivalves/Sediment}$

mean of variances		sum of squares	df	mean squares	*F*-ratio	significant
Pb	station	1.756×10^{-5}	2	8.778×10^{-6}	0.434	0.667
	species	9.356×10^{-5}	2	4.678×10^{-5}	6.191	0.035*
Cd	station	157.866	2	78.933	0.205	0.820
	species	2090.293	2	1045.146	16.685	0.004*
Cr	station	2.889×10^{-6}	2	1.444×10^{-6}	0.092	0.914
	species	8.089×10^{-5}	2	4.044×10^{-5}	14.560	0.005*
Fe	station	8.667×10^{-6}	2	4.333×10^{-6}	0.033	0.968
	species	0.001	2	0.000	121.138	0.000*
Cu	station	2.701	2	1.351	0.380	0.699
	species	19.757	2	9.878	13.822	0.006*
Zn	station	1.256	2	0.659	0.269	0.465
	species	150.373	2	75.187	239.288	0.000*

* the difference is significant at the 0.05

Table appendix C-12 ANOVA analysis of $BCF_{Bivalves/Seawater}$

mean of variances		sum of squares	df	mean squares	*F*-ratio	significant
Pb	station	8.542	2	4.271	0.148	0.865
	species	143.049	2	71.524	11.133	0.010*
Cd	station	3.515×10^{9}	2	1.757×10^{9}	0.903	0.454
	species	9.848×10^{9}	2	4.924×10^{9}	5.528	0.044*
Cr	station	8.779×10^{5}	2	4.389×10^{5}	1.755	0.251
	species	1.208×10^{6}	2	6.044×10^{5}	3.099	0.119
Fe	station	1.799×10^{6}	2	8.995×10^{5}	0.239	0.795
	species	2.192×10^{7}	2	1.096×10^{7}	26.397	0.001*
Cu	station	1.300×10^{7}	2	6.503×10^{6}	0.261	0.779
	species	1.365×10^{8}	2	6.827×10^{7}	15.770	0.004*
Zn	station	2.921×10^{6}	2	1.460×10^{6}	0.059	0.943
	species	1.477×10^{8}	2	7.386×10^{7}	129.662	0.000*

* the difference is significant at the 0.05

Table appendix C-13 ANOVA analysis of the depuration Cu and Zn in oysters and mussels

Heavy metals	Factor	Sum of squares	df	Mean Squares	*F*-ratio	Sig.
Cu	hours	1658.293	7	236.899	44.447	0.000*
	bivalves	8709.260	1	8709.260	1634.025	0.000*
Zn	hours	21935.193	7	3133.599	378.003	0.000*
	bivalves	281565.705	1	281565.705	33965.016	0.000*

* the difference is significant at the 0.05

Table appendix C-14 The multiple comparison of the Cu depuration of oysters (*Saccostrea cucullata*) in each depuration hour with post hoc analysis (Turkey technique: homogenous subset)

Hour	subset		
	1	2	3
72	17.645		
48	18.650		
24	22.832		
12		30.572	
6		33.736	33.736
3			37.636
1			38.528
0			39.748
Sig.	0.210	0.770	0.093

Table appendix C-15 The multiple comparison of the Zn depuration of oysters (*Saccostrea cucullata*) in each depuration hour with post hoc analysis (Turkey technique: homogenous subset)

Hour	subset						
	1	2	3	4	5	6	7
72	98.687						
48		106.861					
24			127.306				
12				153.436			
6					162.318		
3					168.261	168.261	
1						172.787	172.787
0							178.554
Sig.	1.000	1.000	1.000	1.000	0.228	0.554	0.260

Table appendix C-16 The multiple comparison of the Cu and Zn depuration of mussels (*Perna viridis*) in each depuration hour with post hoc analysis (Turkey technique: homogenous subset)

Hour	Cu			Zn		
	subset			subset		
	1	2	3	1	2	3
72	8.137			19.572		
48	8.275	8.275		22.114		
24	8.403	8.403			25.786	
12	9.129	9.129	9.129		28.970	28.970
6	9.317	9.317	9.317			29.513
3		9.484	9.484			30.076
1			9.761			30.728
0			9.898			32.234
Sig.	0.063	0.053	0.470	0.224	0.061	0.051