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# Bioaccumulation of heavy metals in Nha Trang bay, Khanh Hoa, Viet Nam

Tran Thi Mai Phuong

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**UNIVERSITE DE NICE-SOPHIA ANTIPOLIS - UFR Sciences**

Ecole Doctorale Sciences Fondamentales et Appliqués N°364

# **T H E S E**

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**Tran Thi Mai PHUONG**

TITRE

## **BIOACCUMULATION DES MÉTAUX LOURDS DANS LA BAIE DE NHA TRANG, KHANH HOA, VIET NAM**

Thèse dirigée par Nguyen Ky PHUNG et MARMIER Nicolas

soutenue le 24 Septembre 2014

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**IN NHA TRANG BAY, KHANH HOA PROVINCE**  
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## ABSTRACT

Over the last three decades, the pollution of heavy metals in Nha Trang coast and the environs comes from many human activity sources, in which 90% of wastewater discharged directly into the rivers then coastal zone without treatment. Other major sources from industrial, agriculture, aquaculture activities, oil drilling, tourism and port activities may also cause direct contamination of heavy metal in this zone. The metals body burden in mollusks may reflect the concentrations of metals in surrounding water and sediment, and may thus be an indication of quality of the surrounding environment.

The study on the potential bioaccumulation of mollusks as bioindicator is an important effort that contributes to the findings of method in monitoring pollution in an environment of tropical regions. This thesis reports the results of our investigation of heavy metals in mollusks and sediment samples collected from four coastal sites of different environmentally background in Nha Trang bay and adjacent areas, Khanh Hoa province, Viet Nam during 2 years from 2012 to 2013. The study used suitable analyzing methods update to find the bioaccumulation capacity of trace metals in marine ecosystems, evaluation of the bioavailability of potentially toxic substances on human health risks and available for predicting the environmental fat and effects of pollutions. Results from this study demonstrated that the 5 metals As, Cr, Cd, Cu and Zn concentrations of sediments were acceptable or moderate biological effects. These studied mollusk species including *Laternula anatine*, *Glauconome virens*, *Katelsia hiantina* and *Geloina coaxans* have high potential factors in term of metal BSAF in their tissues. They have served as good bioindicator organisms, in which *K.hiantina* might be the best indicator of metal pollution as it is high presence, abundance and wide geographical distribution in the study area.

The obtained results of the research not only provided the environmental status in term of heavy metal pollution and the bioaccumulation in Nha Trang bay, they are also scientific bases for further studies regarding bio-monitoring for marine environmental protection and natural conservation in Nha Trang bay and southern coast of Viet Nam.

**Key words:** Coastal pollution, sediment, mollusk, heavy metals, bioaccumulation, Nha Trang Bay

## RÉSUMÉ

Au cours des trois dernières décennies, la baie de Nha Trang et ses environs sont pollués par diverses activités humaines. 90% des eaux usées non traitées sont déversées directement dans les rivières sur la zone côtière. L'industrie, l'agriculture, l'aquaculture, le forage pétrolier, le tourisme ainsi que les activités portuaires peuvent également provoquer une contamination directe par des métaux lourds dans cette zone. La charge corporelle des métaux dans les mollusques reflète les concentrations de métaux dans l'eau et dans les sédiments environnants, telle est l'indication de la qualité de l'environnement.

L'étude sur le potentiel de bioaccumulation des mollusques comme bioindicateur est un effort important à appliquer rigoureusement dans les régions tropicales. Cette thèse présente les résultats de notre enquête pendant deux ans (2012-2013) dans 4 sites côtiers de la baie de Nha Trang de la province de Khanh Hoa (Viet Nam). L'étude a utilisé des méthodes d'analyse appropriées pour déterminer la pollution en métaux lourds des sédiments et interpréter la capacité de bioaccumulation ces métaux lourds dans plusieurs types de mollusques pour la proposer des bioindicateur et évaluer les risques liés à la consommation de ces mollusques provient des substances toxiques. Les résultats de cette étude ont démontré que la concentration de métaux lourds comme Cr, Cd, Cu et Zn ne présentent pas les effets biologiques. Un traitement des résultats sur le choix d'une espèce comme bioindicateurs, y compris *Laternula anatine*, *Glaucanome virens*, *Katelsia hiantina* et *Geloina coaxans* en terme de bioaccumulation BSAF de métal dans leurs tissus. Les facteurs de bioaccumulation conduisant au choix d'une espèce particulière, la quelle *K.hiantina* pourrait être le meilleur indicateur de la pollution par les métaux.

Enfin, la recherche présente des résultats scientifiques sur la contamination des sédiments, à la bioaccumulation des métaux lourds, puis au choix de bioindicateur que pour la surveillance biologique et la protection de l'environnement marin en baie de Nha Trang et sur la côte sud-est du Viet Nam.

**Mots clés:** pollution des zones côtières, les sédiments, les mollusques, les métaux lourds, la bioaccumulation, baie de Nha Trang.

## **AIM AND STEPS OF THE STUDY**

This study is to identify heavy metals in coastal sediments and in mollusks from four principal areas inside the Nha Trang bay and adjacent areas, Khanh Hoa province, Viet Nam during 2 year: 2012-2013 and would provide important evidence of bioaccumulation potential of heavy metals as well as a whole of ecosystem ecological risk impacts of selected trace elements in the health of the marine ecosystem in this area.

To achieve these objectives, the study conducted on the 3 main steps of research:

Step 1: Definition the ranges and state of contamination of these seven metals of sediments as As, Cd, Cr, Cu, Zn, Fe and Al. This step including collected and analyzed coastal surface sediments to determine the physico-chemical properties and heavy metal contents of the surface sediment; comparison with results from other geographical regions and to assess relationship between heavy metal contents and physicochemical characteristics of the sediments.

Step 2: Assessments important evidence of bioaccumulation potential of heavy metals in this area: To investigate the relation of body size to total body burdens of metals in organisms as well as their parameters such as TOC, pH and particle size distribution possibly controlling the degree of contaminations are also discussed

Step 3: Investigate the effects of some heavy metals from human activities on the marine ecosystems. This integrated approach allows a better understanding of the fate of trace elements in different components of the coastal ecosystem, and an evaluation of the bioavailability of potentially toxic substances and of human health risks.

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## LIST OF ACRONYMS

Al	Aluminium (metal)
AT	Average time for non-carcinogen in day
ATLh	Acceptable tissue levels for humans
ATLhC	Acceptable tissue levels of carcinogens for humans
ATLhN	Acceptable tissue levels of noncarcinogens for humans
ATL	Acceptable tissue level
ATLw	Acceptable tissue levels for wildlife
As	Arsenic (metal)
ASTM	American Standard testing method
BAF	Bioaccumulation factor: threshold for soil or sediment
BCF	Bioconcentration factor
BCOI	Bioaccumulative contaminant of interest
BMF	Biomagnification factor
BSAF	Biota-sediment accumulation factor (for organic chemicals)
BT	Binh Tan (sampling site)
BW	Average body weigh
CASRN	Chemical Abstracts Service Registry Number
CBA	Coefficient of biological accumulation
CBAC	Cumulative bioaccumulation index for an individual chemical;
Cd	Cadmium (metal)
Cd	Degree of contamination
CE	Coefficient of enrichment
CI	Condition index
Cinorg	Concentration in shellfish (mg/kg fresh weight)

CL	Cam Lam (sampling site)
Co	Coban (metal)
COD	Chemical oxygen demand
COI	Contaminant of interest
COPC	Contaminant of potential concern
CPSo	Oral carcinogenic potency slope
CTL	Critical tissue level (for fish)
Cu	Copper (metal)
Cr	Chromium
D	Dry season
Db	Bulk density
DEQ	Department of Environmental Quality
DL	Detection limit
DNA	Deoxyribose Nucleotide acid
DO	Dissolved oxygen
DQO	Data quality objectives
ECTissue	Equilibrium contaminant tissue concentration in fish
EF	Enrichment factor
EFr	Exposure frequency (365 days/year)
ED	Exposure duration (70year)
EDI	Estimated daily intake
EPA	Environmental protection agency (American)
EPC	Exposure point concentration
ERA	Ecological risk analysis
ER-M	Effect range median

ER-L	Effect range low
GPS	Global positioning system
FAO	Food and agriculture organization of the United Nations
FDA	Food and Drug administration
Fe	Iron (metal)
Foc	Fraction of organic carbon in the sediment
Fl	Fraction of lipid content in the organism
Fs	Feasibility study
HCMUS	Ho Chi Minh University of Science
HO	Hydroxyl radical
IAEA	International Atomic Energy Agency
ICP – OES	Inductively coupled plasma optical emission spectrometry
Igeo	Geoaccumulation index
IR	Ingestion rate of shell fish (g/day)
IRIS	Integrated Risk Information system (1,5mg/kg/day)
ISQVs	Interim Sediment Quality Values
Kd	Distribution coefficient for inorganics
LEL	Lowest Effect Level
LOD	Limit of detection
LOQ	Limit of quality
MC	Moisture content
MFA	Malaysian Food Act
MDL	Method detection limit
mg/kg WW	The mg/kg concentration is based on the wet weight of the sample
mg/kg DW	The mg/kg concentration is based on the dry weight of the sample

Mn	Manganese (metal)
Mo	Molibdenium (metal)
MOSTE	Ministry of science and technology of Viet Nam
MPI	Metal pollution index
MPL	Maximum permissible levels
MT	Metallothienseins
MSWI	Metal/shell weight indices
N	Total number of contaminants in sediment at the site
ND	Non detection
ND	Ngoc Diem (sampling site)
Ni	Nikel
NFA	No further action
NOAA	National Oceanic and Atmospheric Administration
NW	North West
OAR	Oregon Administrative Rule
OC	Organic carbon
ODEQ	Oregon Department of Environmental Quality
OECD	Organisation for Economic Co-operation and Development
OM	Organic material
Pb	Lead (metal)
PERI	Potential ecological risk index
R	Rainy season
RBAC	Bioaccumulation index for each individual chemical in the sediment
RI	Risk index
ROS	Reactive oxygen species

Zn	Zinc (metal)
Se	Selenium (metal)
SEL	Severe Effect Level
Sn	Tin (metal)
SH	Thiol group
SLV	Screening level value
SLVBH	Sediment bioaccumulation screening level for humans
SLVBW	Sediment bioaccumulation screening level for wildlife
SQGs	Sediment Quality Guidelines
SSD	Species sensitivity distribution
SW	South West
TD	Tan Dao (sampling site)
TMF	Trophic Magnification factor
THQ	Target hazard quotient
TR	Target cancer risk
T&E	Threatened or endangered
TOC	Total organic carbon
TSS	Total suspended solid
TRV	Toxicity Reference Value
WHO	World Health Organisation
WQC	National Recommended Water Quality Criteria
UCL	Upper confidence limit of the arithmetic mean
UNS	University of Nice
UNEP	United Nations environment programme
WDOE	Washington Department of Ecology



# CHAPTER 1

## LITERATURE REVIEW

### 1.1 PROBLEM STATUS

Pollution has considerably degraded the coastal and marine environment, including estuaries over the past 30 years. Increasing urbanization, industrialization and tourism, coupled with a growing coastal population, have degraded coastal areas, reduced water quality and increased pressures on marine resources. There have, however, been significant changes in perspective, and new concerns have emerged (MOSTE, 1999). Elevated concentrations of trace metals in aquatic bodies as a result of human activities have been recorded since ancient times. However, excessive releases of toxic trace metals into the urban environment and the associated health implications only became apparent in the 1960s when anthropogenic metal contamination of the environment was denoted. From an environmental and health perspective, this profound geographical development will have a critical influence on our immediate environment and its quality for human health. On a daily basis, numerous human activities including municipal, industrial, commercial and agricultural operations release a variety of toxic and potentially toxic pollutants into the environment (Cheung et al, 2003).

All these elements reach the ocean floor through coastal region, which connects pollutions to the marine ecosystem. Though coastal region are highly productive, dynamic and much diversified regions, the entry of these elements in the biotic system is much easier. From the marine biotic communities the bottom dwelling mollusks have a tremendous capacity of bioaccumulation. Since the coasts are more prone to the accumulation of all toxic elements and chemicals there is a higher chance of accumulation in the body of mollusks. In the dynamic lotic riverine ecosystem deposition and accumulation of such elements are rare because of its fluvial dynamics. But due to the continuous and alternate tidal actions, the retention times for such elements are high in the coastal region, which results in the better chance for their entry in to the biotic communities. The fast growing and highly edible green mussel in the coastal region are

more susceptible to heavy metal accumulation and act as a route of toxicity to human population.

It is widely accepted that anthropogenic activity makes a significant contribution to the total aquatic burden of toxic metals by both point source and non point source contamination can occur. Non point source contamination usually arises from agricultural, industrial, and urban effluents that reach the coast by way of waterways, surface runoff, and precipitation. Both benthic and pelagic species may thus become contaminated by direct uptake and or through biomagnifications. Nevertheless, a permanent control of water quality is indispensable. To reveal the presence of pollutants and to measure their toxic effect biological indicators can be used, which are suitable for prediction of the expectable toxic influence of known or unknown substances. (Sanjay et al, 2011)

The pollution of heavy metals in Nha Trang coastal comes from many human activity sources. About 90 percents of wastewater from Nha trang city discharged directly into the rivers without treatment then make their way to estuaries and Nha trang bay. Other major sources from industrial, agriculture activities, oil drilling, tourism and port activities may also cause the direct contamination of heavy metals in this zone. Aquaculture fish in the sea with nearly 7,000 carges caused more serious affecting to the bay nowadays. In fact, approximately 10 tons per day of solid waster were discharged into the sea by 5.000 people living in islands. In the other hand, Nha Trang bay is a tourism city, however, they are more than 40 tourist boats transports every day. (Phung et al, 2009).

In the recent year, contamination of heavy metals has been great problems to the natural environment, especially to marine ecosystems in coastal Viet Nam. Since 1996, in Viet Nam, the increase of metal concentration in the sediment had been observed in Quang Ninh, Hai Phong, Da Nang and Khanh Hoa (Phuong et al, 2012), but there have never been any published reports on the background of heavy metals in such a mollusk species, especially in Khanh Hoa coastal zone. In Nha Trang bay, the concentrations of heavy metal in surface sediments in period 1996-2011 were ranged between 4.58-43.2; 5.51-13.6; 0.32-34.1; 10.23-2,7; 0,15-0,33, 0,09-0,43 ( $\mu\text{g/g DW}$ ) for Zn, Cu, Pb, As, Cd and Hg respectively. The fine fraction of sediment in Nha Trang bay goes from moderately to strongly contamination with respect to the analysis of 4 heavy metals (Zn, As, Cu and Pb) (Phuong et al, 2012) and receives attention from local managers.

The heavy metals can be either adsorbed into sediments or accumulated in benthic organism, sometimes to toxic levels. Studies on heavy metal pollution especially in coastal zones increased over the last few decades at global scale. Therefore, the mobility, bioavailability and subsequent toxicity of metals have been a major research area (Ghabbour et al, 2006). Generally, the presence of contamination by metals have been considered only in important harbours such as New York (Feng et al, 1998), Boston (Manheim F.T and others, 1998), the Atlantic French harbours (Fichet et al, 1999) and, more recently, in Baltimore (Mason et al, 2004), Montevideo (Muniz et al, 2004) and Naples (Adamo et al, 2005). However, very few studies deal with the effect of levels of toxic elements on the health of mollusks in tropical and subtropical regions such as Vietnam and other Southeast Asian countries, particularly in Khanh Hoa province, where, in addition to human activities such as harbor activities within the estuary, industrial, agricultural and residential activities around the coastal can release heavy metals to the environment.

## **1.2 HEAVY METAL POLLUTIONS**

### **1.2.1 Metals in environment**

Metals are considered as important toxic pollutants and there is extensive literature concerning their accumulation in ecosystems. Some metals enter the sea from the atmosphere, by volcanoes, natural weathering of rocks, e.g. natural inputs of metals, such as Aluminum in wind-blowing dust of rocks and shales, but also by numerous anthropogenic activities, such as mining, combustion of fuels, industrial and urban sewage and agricultural practices. On a global scale there is now abundant evidence that anthropogenic activities have polluted the environment with heavy metals from the poles to the tropics and from the mountains to the depths of the oceans. Some metals are deposited by gas exchange at the sea surface, by fallout of particles (dry deposition) or are scavenged from the air column by precipitation (rain) which is called wet deposition. For example, Lead inputs in the atmosphere from industrial and vehicular exhaust are much greater than natural inputs. The natural levels of heavy metals in the environment had never been a threat to health but in the recent years increased industrial activities leading to air born emissions, auto exhausts, effluents from industries as well as solid waste dumping have

become the sources of large quantities of heavy metals into the environment (Mhatre G.N, 1991).

Metals enter the environment by means of natural processes or are derived from human activities. For some metals, natural and anthropogenic inputs may be of the same order (Zn), whereas for others (Pb) inputs due to human activities dwarf natural inputs (Clark R.B, 2001). Much of those human activities are located in the fluvial watersheds and in the margins of estuaries (Salomons and others, 1984), being important areas for the concentration of contaminants, due to coastal industrial activity and human settlement. There, trade is growing rapidly and much of it depends on shipping, most of import and export travel by sea, and the marine harbour environment is degraded. The increase of metal concentrations in the sediment is a symptom of this process as has been observed in the Pacific harbours (Wolanski E, 2006). Rivers make a major contribution of metals in the marine environment. The nature of metals depends on bearing deposits in the catchments area and the discharge of human waste and discharges when the river passes through urban areas. Dredging of shipping channels produces large quantities of metal pollution. Much smaller quantities of metals are added to the sea by direct discharges of industrial and other waste and the dumping of sewage sludge. (Clark et al, 1997; Depledge et al, 1998). Atmospheric and river inputs, dredging soil, direct discharges, industrial dumping and sewage sludge are some of the important contributors to metal pollution, which lead to the release of metals to the marine environment (Valavanidis A et al, 1999).

The oceans provide a vital sink for many heavy metals and their compounds. There is a growing concern that the natural cycling rates of many metals are being disturbed by anthropogenic activities, especially the release from industrial, domestic and urban effluents of increasing amounts of Pb, Zn, Cd, Hg and Cu.(Schindler P.W,1991). Atmospheric metal pollution is responsible from most of the dissolved Cd, Cu, Fe, Zn, Ni and As in the oceans. (GESAMP, 1990). The world wide emissions of metals to the atmosphere (thousands of tons per year) by natural sources is estimated as: Ni: 26, Pb: 19, Cu: 19, As: 7.8, Zn: 4, Cd: 1.0, Se: 0.4 (tons  $\times 10^3$ /yr). Whereas, from anthropogenic sources: Pb: 450, Zn: 320, Ni: 47, Cu: 56, As: 24, Cd: 7.5, Se: 1.1 (tons  $\times 10^3$ /yr). (Clark et al, 1997). It is obvious from these numbers that Pb, Zn, As, Cd and Cu are the most important metal pollutants from human activities.

Metals of major interest in bioavailability studies, as listed by the U.S. Environmental Protection Agency, are Al, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Sb (EPA, 1978). Other metals that are presently of lesser interest are Ag, Ba, Co, Mn, Mo, Na, Ti, V, and Zn. (McKinney et al, 1992). These metals were selected because of their highly toxic properties, their effects on the environment and the living organisms, their potential for human exposure and increased health risk. Some highlights concerning the bioavailability of As, Cd, Cr, Cu, Mo, Ni, Pb, and Zn in sediment are discussed in this study. An additional data are available in the references listed for some major such heavy metals are:

### **Arsenic (As)**

Arsenic mobility, bioavailability, and toxicity are dependent on speciation: arsenite ( $\text{AsO}_3^{-3}$ ) forms are much more toxic to biological species and are more mobile than arsenate ( $\text{AsO}_4^{-3}$ ) forms (Kersten, 1988). Arsenic is chemically similar to phosphorous. Arsenate interferes with phosphate metabolism that is widespread in the biosphere. Metallo-organic forms of arsenic also may be much more bioavailable than inorganic forms; however, organic-bound arsenic is excreted by most species and does not appear to be highly toxic (Luoma S.N, 1983). Adsorption and desorption on iron and aluminum oxide minerals is the main factor controlling arsenic behavior in soil and sediment. Maximal adsorption occurs at different pH for As {III} (pH 9.2) and As {V} (pH 5.5) as a function of the adsorbing mineral;  $\text{As}^{+3}$  mobility is enhanced under oxic conditions. Arsenic is apparently highly mobile in anoxic sediment-water systems. Development of acidic and oxidizing conditions tends to release large amounts of arsenic into solution due to decreased sorption capacity of both forms of arsenic (Léonard A, 1991). Arsenic can be found in some film chemistry, but is not very common.

### **Cadmium (Cd)**

The redox potential of sediment-water systems exerts controlling regulation on the chemical association of particulate cadmium, whereas pH and salinity affect the stability of its various forms (Kersten M, 1988). Elevated chloride contents tend to enhance chloride complex formation, which decreases the adsorption of cadmium on sediment, thereby increasing cadmium mobility (Bourg A.C, 1988) and decreasing the concentration of dissolved  $\text{Cd}^{+2}$  and bioavailability (Luoma S.N, 1983). In anoxic environments, nearly all

particulate cadmium is complexed by insoluble organic matter or bound to sulfide minerals. Greenockite (CdS) has extremely low solubility under reducing conditions thereby decreasing cadmium bioavailability. Oxidation of reduced sediment or exposure to an acidic environment results in transformation of insoluble sulfide-bound cadmium into more mobile and potentially bioavailable hydroxide, carbonate, and exchangeable forms (Kersten, 1988). Studies of lake and fluvial sediment indicate that most cadmium is bound to exchangeable site, carbonate fraction, and iron-manganese oxide minerals, which can be exposed to chemical changes at the sediment-water interface, and are susceptible to remobilization in water (Schintu et al, 1991). Cadmium is a common metal found in anthropogenically contaminated aquatic environments and is toxic to aquatic biota at elevated levels. Cadmium can be found in some pigments, especially orange, red, and yellow colors. In oxidized, near neutral water,  $\text{CdCO}_3$  limits the solubility of  $\text{Cd}^{2+}$  (Kersten M, 1988). In a river polluted by base-metal mining, cadmium was the most mobile and potentially bioavailable metal and was primarily scavenged by non-detrital carbonate minerals, organic matter, and iron-manganese oxide minerals (Prusty et al, 1994).

Mollusks accumulate large concentration of calcium ranging from 1900 – 2000 ppm dry weight (Clark, 1992). Highest concentration in cadmium causes several health problems in human. Cadmium and its compounds along with mercury and some other dangerous metals are, however, included in the blacklist. It is being used routinely in different industrial processes and its potential hazard to life form is predominant. Eating food or drinking water with very high cadmium levels severely irritates the stomach, leading to vomiting and diarrhea, and sometimes death. Eating lower levels of cadmium over a long period of time can lead to a build-up of cadmium in the kidneys. If the levels reach a high enough level, the cadmium in the kidney will cause kidney damage, and also causes bones to become fragile and break easily. As a conservative approach, and based on the limited human data and the studies in rats, the United States Department of Health and Human Services (DHHS, 1999) has determined that cadmium and cadmium compounds may reasonably be anticipated to be carcinogens.

### **Chromium (Cr)**

The major source of chromium emission in to the environment from the chemical manufacturing industry, combustion of fossil fuel, cement producing plants, waste from

electroplating, leather tanning, textile industry and consumer products such as inks, paints, papers, toner powder used in copying machine... Chromium is the naturally occurring compound found in soil, rocks and plants. It normally exists in oxidation states ranging from chromium (II) to Chromium (VI). However, two major forms trivalent (III) and hexavalent (VI) forms have biological significance.

Physiologically chromium is considered as a trace element and it is required for the optimum function of insulin in mammalian tissues and the maintenance of normal metabolism of glucose, cholesterol and fat. The normal level of blood chromium concentration in human beings is between 20-30µg/l. It is found that the intake of chromium is about 50-200 µg/day is regarded to be safe and adequate.

Hexavalent chromium is an extremely toxic metal, which exist as an anion ( $\text{CrO}_4^{2-}$ ) and most readily absorbed from the gastrointestinal tract, skin and lungs. Most reports describe the toxicity of chromium (VI) in the form of chromate or dichromate. It can cause chronic ulceration of skin surface, denaturation of tissue proteins, asthma, kidney failure, discoloration of teeth and inflammation of skin. Acute poisoning results in symptoms such as dizziness, intense thirst, abdominal pain, vomiting and shock and sometimes death may occur due to the presence of urea in blood.

### **Copper (Cu)**

Copper is most efficiently scavenged by carbonate minerals and iron-manganese oxide minerals and coatings and is less mobile than cadmium, lead, and zinc (Prusty et al,1994); in most other situations lead is less mobile than copper. Elevated chloride contents decrease adsorption of copper on sediment, due to chloride complexation, which results in greater solubility and mobility (Bourg A.C, 1988); (Gambrell et al, 1991). In systems with high total copper contents, precipitation of malachite controls dissolved copper contents at low pH (Bourg A.C, 1988; Salomons W, 1995). Sometimes, elemental substitution is more complex; for example, copper toxicity is related to low abundances of zinc, iron, molybdenum, and (or) sulfate (Chaney R.L, 1988).

The natural input of copper to the marine environment from erosion of mineralized rocks. Anthropogenic inputs of copper are from the production of electrical equipments, as chemical catalysts, as antifouling agents in paints, as algicides, in alloys, and as wood preservatives. Copper dissolved in seawater is chiefly in the form of  $\text{CuCO}_3$  or in reduced

salinity as  $\text{CuOH}^+$ . It also forms complexes with organic molecules. Mollusks have a tremendous capacity to accumulate copper from contaminated waters. Reports are saying the copper concentration factor for oysters growing in contaminated waters is 7500 and they may accumulate 2000 ppm of copper in their blood (Clark, 1992).

### **Lead (Pb)**

The main sources of lead in the aquatic environment are leaded gasoline and mining (Prosi F, 1989). Leaded gasoline results in introduction of organometallic lead compounds, which eventually reach surface water, into the atmosphere. Mining releases inorganic lead compounds. Both organic and inorganic forms of lead pose serious health risks to all forms of life (Ewers U et al, 1990). Inorganic lead compounds (sulfide, carbonate, and sulfate minerals) are commonly abundant in sediment but have low solubilities in natural water. Naturally occurring lead in mineral deposits is not very mobile under normal environmental conditions, but becomes slightly more soluble under moderately acidic conditions. Soluble lead is little affected by redox potential (Gambrell et al, 1991). Lead is tightly bound under strongly reducing conditions by sulfide mineral precipitation and complexation with insoluble organic matter, and is very effectively immobilized by precipitated iron oxide minerals under well oxidized conditions (Gambrell et al, 1991). In the aquatic environment, total dissolved lead abundances in water and pore water control primary uptake by organisms. Lead bioaccumulation is primarily dependent on the amount of active lead compounds (predominantly aqueous species) in the environment and the capacity of animal species to store lead (Prosi F, 1989). Particulate lead may contribute to bioaccumulation in organisms. For humans, particles that are inhaled but not exhaled are especially important. Variations in physiological and ecological characteristics of individual species lead to different enrichment factors and tolerances for each organism. A study of bottom dwelling organisms suggests that iron rich sediment inhibits lead bioavailability (Luoma S.N, 1989). In a study of lake and fluvial sediment, most lead was bound to a carbonate fraction or to iron-manganese oxide minerals, both of which respond to chemical changes at the sediment-water interface, and are susceptible to remobilization in water (Schintu M et al, 1991). In a polluted river environment, lead is most efficiently scavenged by non-detrital carbonate and iron-manganese oxide minerals and is less mobile than cadmium (Prusty et al, 1994). Lead that can be found in some inks and coatings pigments (although not common), electrodes, solder, battery plates (if



maintenance is performed on batteries), and paper but is reportable only if 0.5 lbs or more of lead is released in the form of dust.

Lead is a highly toxic substance, exposure to which can produce a wide range of adverse health effects. Both adults and children can suffer from the effects of lead poisoning, but childhood lead poisoning is much more frequent. Even today, at minimum more than four hundred thousand children under the age of six who have too much lead in their blood. Lead (Pb) is typical example of anthropogenic metal pollution. Beginning with very low levels at about 2.700 years ago, Pb concentration increased during the industrial age and has risen rapidly since Pb was added to gasoline fuel of vehicles. Pb levels in Greenland ice have risen 200-fold from the natural level. (Harrison et al, 1991).

There are many ways in which humans are exposed to lead: through deteriorating paint, household dust, bare soil, air, drinking water, food, ceramics, home remedies, hair dyes and other cosmetics. Much of this lead is of microscopic size, invisible to the naked eye. More often than not, children with elevated blood lead levels are exposed to lead in their own home. Young children under the age of six are especially vulnerable to lead's harmful health effects, because their brains and central nervous system are still being formed. For them, even very low levels of exposure can result in reduced IQ, learning disabilities, attention deficit disorders, behavioral problems, stunted growth, impaired hearing, and kidney damage. At high levels of exposure, a child may become mentally retarded, fall into a coma, and even die from lead poisoning. In adults, lead can increase blood pressure and cause fertility problems, nerve disorders, muscle and joint pain, irritability, and memory or concentration problems. When a pregnant woman has an elevated blood lead level, that lead can easily be transferred to the fetus, as lead crosses the placenta. In fact, pregnancy itself can cause lead to be released from the bone, where lead is stored often for decades after it first enters the blood stream.

### **Nickel (Ni)**

Nickel is a silver-white metal with siderophilic properties that facilitate the formation of nickel-iron alloys. In contrast to the soluble nickel salts (chloride, nitrate, and sulfate), metallic nickel, nickel sulfides, and nickel oxides are poorly water-soluble. Nickel carbonyl is a volatile liquid at room temperature that decomposes rapidly into carbon monoxide and nickel. Drinking water and food are the main sources of exposure for the

general population with the average American diet containing about 300 µg Ni/d. Nickel is highly mobile in soil, particularly in acid soils. There is little evidence that nickel compounds accumulate in the food chain. Nickel is not a cumulative toxin in animals or in humans. The initial effects involve irritation of the respiratory tract and nonspecific symptoms. Patients with severe poisoning develop intense pulmonary and gastrointestinal toxicity. Diffuse interstitial pneumonitis and cerebral edema are the main cause of death.

Nickel is a common sensitizing agent with a high prevalence of allergic contact dermatitis. Nickel and nickel compounds are well recognized carcinogens. However, the identity of the nickel compound or compounds, which cause the increased risk of cancer, remains unclear. Currently, there are little epidemiological data to indicate that exposure to metallic nickel increases the risk of cancer, or that exposure to the carcinogenic forms of nickel causes cancer outside the lung and the nasal cavity.

### **Molybdenum (Mo)**

Molybdenum is an essential element for many animals and plants as it is required in their enzyme system. Molybdenum can be present in molybdate anions,  $\text{MoO}_4^{2-}$ , in soil where it can be mobile and bioavailable, because it is geochemically similar to sulfate. Molybdate ion is often associated with iron oxyhydroxide minerals, where it competes with phosphate and organic matter. Molybdenosis in animals is associated with soil that contains large amounts of available molybdenum, especially in forage plants with low sulfur and copper contents (Neuman et al, 1987).

### **Zinc (Zn)**

In slightly basic, anoxic marsh sediment environments, zinc is effectively immobilized and not bioavailable (Gambrell et al, 1991). Substantial amounts of zinc are released to solution if this sediment is oxidized or exposed to an acidic environment. Very high abundances of soluble zinc are present under well oxidized conditions and at pH 5.0 to 6.5, whereas low abundances of soluble zinc are present at pH 8 under all redox conditions and at pH 5.0 to 6.5 under moderately and strongly reducing conditions (Gambrell et al, 1991). In polluted river environments, most zinc is scavenged by non-detrital carbonate minerals, organic matter and oxide minerals and is less mobile than cadmium (and perhaps less mobile than lead) (Prusty et al, 1994). Elevated chloride contents decrease adsorption of zinc on sediment (Bourg A.C, 1988).

Zn, as other transition metals, is essential for the health and growth of most organisms. It is a cofactor of nearly 300 enzymes. In phytoplankton, carbonic anhydrase, zinc-based metalloenzyme, is involved in the inorganic carbon acquisition from seawater. The activity of this enzyme has been shown to be dependent on the level of CO<sub>2</sub> and on the availability of Zn, thus conferring on Zn a key role in oceanic carbon cycling.

The Zn enrichment in the marine environment stimulates the calcification capacity of the mollusk hence the calcification of them. In natural seawater, concentrations of dissolved zinc are often very low (ca. 21.7 nmol.l<sup>-1</sup>). However, while zinc is an important at trace concentrations, it can also be very toxic when in excess, forming dangerous free radicals. A mollusk has an opportunity to be exposed to high metal concentrations as a result of human activities or natural disaster.

Heavy metals are among the most common environmental pollutants and their occurrence in waters and biota indicate the presence of natural or anthropogenic sources. Numerous studies have demonstrated that the concentrations of heavy metals in suspended and bed sediments can be sensitive indicators of contaminants in hydrological systems (Diagomanolin et al, 2004; Idris et al, 2007; Jain C.K, 2004)

Zinc is an essential element to human being. Zinc is widely seen in nature. The natural concentration of zinc in soil estimated to be 10-30 mg/kg. Zinc is used in coating of other metals, in alloys and many common goods. Besides this zinc is used for wood preservation, catalyst, ceramic, fertilizers, batteries, paints, explosives household and medical appliances. According to WHO, 1996 the dietary requirement of zinc up to 22mg/day, which is equivalent to 0.3mg/kg bw/day. Gastrointestinal absorption of zinc varied substantially from 8-80%. The absorption decreases after ingestion with calcium and phosphorus. This is due to the precipitation of zinc in the intestine. Dermal absorption of zinc also noticed. There is little information about the toxicity of zinc exposure. Chronic exposure of zinc leads to anemia.

### **1.2.2 Pollution of heavy metal in marine sediments**

Because sediments can contain significantly higher concentrations of some chemicals than the overlying water, it is important to evaluate the potential for such chemicals to accumulate in aquatic organisms (Renier et al, 2001). Among aquatic pollutants, heavy metals are the most appropriate indicator of pollution, because of their

stability in sediments and scarcity in natural environments (Saeki et al, 1993). Heavy metals introduced to aquatic environments by industrial, domestic and mining activities are ultimately absorbed by deposits and incorporated into sediments. Hence sediments are the most concentrated physical pool of metals in aquatic systems.

Heavy metal concentrations in sediment are many times greater than the same metals in the water column. Sediments can act as a scavenger agent for heavy metal and an adsorptive sink in aquatic environment. It is therefore considered to be an appropriate indicator of heavy metal pollution (Idris et al, 2004). Surface sediments are a feeding source for biological life, a transporting agent for pollutants and an ultimate sink for organic and inorganic matter settling. In heavily polluted sediments, the anthropogenically introduced components by far exceed the natural components and pose a risk to the marine ecosystem (Algan et al, 1999; Waldichuk et al, 1985). Metals in contaminated sediments may persist and impact upon ecosystems for decades, or remain largely dormant until desorption via a resuspension event releases the toxicants to seawater, greatly increasing their potential impact. In general, environmental pollutants such as metals pose serious risks to many aquatic organisms.

Trace metals, when entering into natural water become part of the water-sediment system and their distribution processes are controlled by a dynamic set of physical-chemical interactions and equilibrium. Sediments are basic components of the environment as it provides nutrients for living organisms and serves as sink for deleterious chemical species, reflect the history of the pollution (Singh et al, 2003). Heavy metals are mainly distributed between the aqueous phase and the suspended sediments during their transport. Riverine suspended load and sediments have important function of buffering heavy metal concentrations particularly by adsorption or precipitation. More than 97% of the mass transport of heavy metals to the oceans is associated with river sediments (Jain C.K, 2004).

The presence of heavy metals in sediments is affected by the particle size, composition of the sediments and other organic substances. (Ghabbour et al, 2006). Physical mixing of fluvial and marine particulates leads to a continuous decrease in the trace metal content of the suspended matter with increasing salinity. (Cheung et al, 2003). Metals are natural constituents of the sediments in coastal zones and they may be oligoelements, such as zinc for example, which is a natural element essential for living

organisms when present in small amounts, or do not have any known biological role, as is the case for lead. However, both can become toxic for living organisms at high concentrations (Ewers et al, 1991; Ohnesorge et al, 1991).

Sediments may contribute significantly to concentrations of metals in benthic invertebrates, either by absorption/adsorption from interstitial water or by direct ingestion within food chains and the potential risk of human exposure, makes it necessary to monitor the levels of these contaminants in marine organisms. This information is needed to help predict potentially adverse effects on fish, shellfish, and other aquatic prey animals, or on wildlife or humans that consume them (ODEQ, 2007). In the Atlantic and Mediterranean harbours these changes in the sediment reservoir have been occasionally studied and some researches were made as information show in Table 1.1

Table 1.1 Trace metal concentrations ( $\mu\text{g}/\text{kg DW}$ ) in marine sediments with different world areas

	<b>Location</b>	<b>Cu</b>	<b>Zn</b>	<b>Pb</b>	<b>Cd</b>	<b>Cr</b>	<b>References</b>
1	Gulf of Aden	3.1-27	13-51	5.3-23	0.3-2.6	5.7-25	Mostaf et al, 2009
2	Marmara Sea	12-30	34-50	21-31	0.02-0.5	27-61	Topcuozlu et al, 2004
3	Suez gulf	2.7-12	25-58	12-42	1.2-3.9	2.5-12	Hamed et al,2006
4	Moroccan estuarines	24-66	113-217		0.9-3.3		Cheggour et al, 2005
5	Naples harbor	12-5743	17-7234	19-30	0.01-3	7-1798	Sprovieri et al, 2007
6	Southern black Sea	15-119	24-141	12-69	-	13-238	Yucesoy et al,1992
7	Jiaozhou Bay	17-34	80-110	24-49	0.1-0.3	41-88	Xyaoyu et al, 2010
8	Hangzhou Bay	24.1		22.6	0.09		Che et al, 2003
9	Changjiang Estuary	21.2		21.7	0.12		Che et al,2003
10	Gulluk Bay	25	81	20	0.56		Dalman et al, 2005
11	Texas Estuarines	1-20	40-295	8-150	0.2-45	3.5-1332	Sharma et al,1999
12	Scheldt estuarines	1-2600	9-1500	4-455	0.1-20.2	7-202	Zwolsman et al, 1996
13	Izmir bay	13-49	45-114	23-52			Balci et al, 1993
14	Boston harbor	67	118	86		131	Bothner et al,1998

## **1.3 BIOACCUMULATION OF METALS IN LIVING ORGANISMS**

### **1.3.1 Heavy metals on benthic organisms**

#### *1.3.1.1 Metabolism and biokinetic of metal in organisms*

Metals, being elements, cannot be broken down, although their chemical state may be altered and form compounds of varying toxicity to aquatic organisms depending upon the environmental conditions. Understanding the mechanisms that control bioavailability to benthic organisms has important implications for the cycling of metals through different compartments of coastal ecosystems. So far no one principle or method has proved universal in accurately predicting the bioavailability of metals accumulated by animals living in contaminated sediments although some methods show promise (Luoma et al, 1983).

Unlike organic pollutants, natural processes of decomposition do not remove heavy metals. On the contrary, they may be enriched by organisms and can be converted to organic complexes, which may be even more toxic. The metal solubility is principally controlled by pH, concentration and type of ligands and chelating agents, oxidation-state of the mineral components and the redox environment of the system. Since each form may have different bioavailability and toxicity, the environmentalists are rightly concerned about the exact forms of metal present in the aquatic environment. The toxicity and fate of the water borne metal is dependent on its chemical form and therefore quantification of the different forms of metal is more meaningful than the estimation of its total metal concentrations. Critical assessment of the endpoints of determination for potentially and actually available and accessible metal fractions in the environmental matrices of water, soil, and sediment become the basis for a need specific monitoring strategy.

Some research for mechanisms of metal toxicity on organisms is indicated that:

The mechanisms by which metals exert their toxicity in living organisms is very diverse, especially their involvement in oxidative biochemical reactions through the formation of reactive oxygen species (ROS). (Goyer R.A, 1991). Molecular mechanisms of heavy metal cytotoxicity include damage to plasma membranes, following binding to proteins and phospholipids, inhibition of Na, K dependent ATPases, inhibition of transmembrane amino acid transport, enzyme inhibition, lipid peroxidation and oxidative

DNA damage, depletion of antioxidant enzymes (such as glutathione) through the generation of ROS. (Stohs S, 1995; Sigel A et al, 1992). Metal ions can penetrate inside the cell, interrupting cellular metabolism and in some cases can enter the nucleus. Metals cations can bind to DNA through ionic and coordinated bonds in a reversible way, but cannot produce all the lesions observed in chromatin of cells. Hence, not only the direct, but mostly indirect effects of metals on nuclear chromatin must be considered more important in DNA damage. In the last decade it has been proved that metal carcinogenicity is mediated by the generation of reactive free radicals (especially hydroxyl radicals, HO•) and ROS. (Kasprzak K, 1995).

The entrance of certain metals into the nucleus can enhance the synthesis of RNA that codes for metallothioneins. Metallothioneins (MT) are peptides found mainly in the cytosol, lysosomes and in the nucleus, low molecular weight peptides, high in the amino acid cysteine which contains a thiol group (-SH). The thiol group enables MTs to bind heavy metals. Metallothioneins can be induced by essential and non-essential metals in aquatic organisms (mollusks, crustaceans). The MT induction is leading to changes in several biochemical processes that have the potential to be used as biomarkers of exposure and evaluation of pollution in the marine environment (Hamer D.H, 1986). In bivalves, metallothioneins may be trapped in whole soft tissues. Induction of metallothioneins binding cadmium in various soft tissues, gills, labial pulps, digestive gland as well as in remaining tissues, have been studied by several researchers (Biliaff B.O et al, 1997; Bebianno M.J et al, 1993).

Trace metal exposure may induce specific metal-binding ligands. Other ligands such as sulfide are important for Ag biokinetic changes in bivalves. Metals also interact strongly in their accumulation by aquatic animals. Generally, dissolved Hg uptake is reduced following exposure to other metals such as Ag, Cd, Cu, and Zn in mollusks and invertebrates. The tissue body burden and the detoxificatory fate of metals in animals seem to be more important in affecting metal accumulation than the nature of the exposure routes (aqueous vs. dietary) or of the exposure regimes. Trace metal accumulation may also be variable in different natural populations of bivalves as a result of different physicochemical environments and histories of exposure (Jain C.K, 2004). Large amounts of dissolved organic complexes and particulate matter with heavy metals are transported great distances to end up in the sediments of the estuaries. Some metals, such as Cd, can be



released from their organic complexes by increasing Cl (chlorine) concentrations, which form chloride complexes. (Elbay et al, 1987).

Some metals are available for uptake into organisms from solution only as free ions, whereas others are transported over biological membranes as inorganic complexes. In experiments with Cu and Cd their toxicity (and their bioavailability) is correlated with the concentration of free metal concentration (Zamuda C, 1982; Sunda et al, 1978).

### ***1.3.1.2 Effect of metal on mollusks***

Mollusks have the ability to efficiently bioaccumulate in the high concentrations of dissolved metals and its effects on enzymatic activity determining their growth. They are a major seafood source of protein and nutrition, therefore, becomes a potential carrier of contaminants from aquatic environment to man (Malek et al, 2004).

Many metals are essential to living organisms but some of them are highly toxic or become toxic at high concentrations. Fe (hemoglobin), Cu (respiratory pigments), Zn (enzymes), Co (Vitamin B12), Mo and Mn (enzyme). Light metals Sodium (Na), Potassium (K) and Calcium (Ca) which plays important biological roles. Transition metals Fe, Cu, Co and Mn which are essential but may be toxic at high concentrations. Metals such as arsenic (As), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), manganese (Mn), zinc (Zn) etc. which are generally not required for metabolic activity and are toxic to living organisms at quite low concentrations. (Forster U, 1983; Meria E, 1991).

Metals such as Hg, Pb, Sn, Ni, Se, Cr and As do not degrade in general; therefore, they accumulate throughout the trophic chain. Marine organisms are able to accumulate certain amount of toxic elements naturally through continuous exposure to pollutants present in seawater and food (Malek et al, 2004). Accumulation in living organisms leads to concentrations several orders of magnitude higher than those of the surrounding water (Casas et al, 2008). Despite this the relationship between the concentration of a metal in the environment and in an organism is far from straight forward as the accumulation ratio depends on many factors; some of them have an environmental origin (temperature, pH, salinity, etc.), whereas others are related to biological factors like age, sex, sexual maturity stage, etc. (Mubiana et al, 2000). Toxic elements accumulation by marine organisms is a complex dynamic process, determined by both environmental and physiological factors i.e.

water chemistry, size, contamination of feedstuffs, feeding intensity, position in the food chain etc. (Kryshev et al, 2000; Smith et al, 2002). However, marine organisms eventually lose much of their activity in the contaminated area then heavy metal toxicity in aquatic organisms, in association with the long residence time.

In the last few decades increasing attention has been paid to the relationship between the conformation of heavy metals and their impact on aquatic organisms. Relationship of Cd, Pb, Zn, Cu, Ni, Co, Cr, Mn and Fe in the soft tissue of *Turbo coronatus*, *Acanthopleura haddoni*, *Ostrea cucullata* and *Pitar sp.*, as well as in associated surface sediments (bulk and bioavailable metal concentrations) from the Gulf of Aden, Yemen were showed the significant spatial differences in metal concentrations in the mollusks and associated sediments. A slope of the linear regression was noted significantly higher than unity for Fe (9.91) and Cd (3.45) in *A. haddoni* and for Ni (4.15) in *T. coronatus*, suggesting that the bioavailability of these metals is disproportionably increased with a degree of enrichment of the sediments in Fe, Cd and Ni, respectively. A slope constant approximating to unity (1.14) for Cu in *A. haddoni* relative to its concentration in sediment extract implies that bioavailability of this metal proportionally increased with growing concentrations of its labile forms in the associated sediment (Szefer et al, 1999).

### ***1.3.1.3 Content of heavy metals in mollusks***

Metals can be accumulated by biota from the water column, sediment or diet, and transferred through the food chain to eventually impact on human health (Forstner et al, 1983). Uptake pathways in mollusks may include exposure through diet (prey items living among contaminated sediments), water (direct exposure of metals via the gills) and sediments (ingestion of sediments). Mollusks are filter feeders, which feed on algae, zooplanktons and excreta of all aquatic vertebrates mainly present in the bottom sediments of the water bodies. Mollusks are the major bottom feeders in the marine ecosystem, which also have tremendous capacity to accumulate all the microelements present in their food. Mollusks are considered as the main bioaccumulators of toxic chemicals as pesticides, heavy metals... Heavy metals are the class of highly toxic elements, causing great health problem to human life through bioaccumulation from the edible bivalves.

Sediments act as both carriers and sinks for contaminants in aquatic environments. Sediments directly stress marine ecosystem by reducing available reproduction or light

energy, impeding recruitment and smothering, which also leads to more organism's disease. Metals in contaminated sediments are persistent and have the potential to impact upon coastal ecosystems for decades. They may remain largely dormant until desorption during a coastal hazard releases the toxicants to seawater, greatly increasing their impact. In general, metals pose a significant risk to mollusk ecosystems. Unfortunately, little information is presently available on the toxic impact of contaminant metals on organisms.

Numerous studies have demonstrated that the concentrations of the majority of heavy metal emissions from anthropogenic activities accumulates in river and ocean sediments, where they are absorbed onto clays and other fine-grained materials. However, the sediments can release heavy metals to the overlying water via natural or anthropogenic processes, causing potential adverse effects to the ecosystems. Moreover, benthic organisms can take up metals directly from the sediments, which in turn enhance the potential of some metals entering into the food chain (Adamo et al, 2005; Chen et al, 2007).

The pollution levels of the marine environment by heavy metals can be estimated by analyzing sediments and marine organisms. The levels of heavy metals in mollusks and other invertebrates are often considerably higher than in other constituents of the marine environment. Compared to sediments, mollusks exhibit greater spatial sensitivity and therefore, are the most reliable tool for identifying sources of biologically available heavy metal contamination (Szefer, 1986). Heavy metals occur naturally and from anthropogenic sources in the ecosystems with large variations in concentrations. They can be bioaccumulated through the food chain posing a toxic risk to species higher in the food chain and to humans (Stankovic et al, 2013)

In sediments, very little of the total metal content is present in free ionic or chelated form in the interstitial solution. Most of the metal content occurs in complex form with insoluble inorganic and organic ligands. Particularly, heavy metals can be absorbed on negatively charged surfaces of clay minerals, organic matter or iron and manganese hydrous oxides from which they can be removed by ion-exchange with hydrogen ions and other cation species. This labile or exchangeable fraction of heavy metals is largely bioavailable. Heavy metals are also occluded in precipitated iron and manganese hydrous oxides or present as carbonates or sulfides. In addition, metal atoms may also be present in

the lattices of primary and secondary minerals. In the latter forms, metals are largely unavailable to organisms, therefore bioavailability is low (Thornton I, 1995).

High sediment load is known to negatively impact organisms but the effect of toxic elements attached to these sediments on mollusk has not been determined. The impact of heavy metals on mollusks is largely unexplored, but potential long-term damage includes: reduced population, increased susceptibility to disease, reduced fertility/reproduction and reduced survival of larvae/juveniles. The accumulation of trace elements in aquatic consumers is of interest to environmental scientists concerned with the fate and effect of contaminants, as well as to ecologists interested in food web dynamics and trace metal biogeochemical cycles to assess the toxic impact or distribution of contaminants (Nguyen H.L et al, 2005).

So far, the individual effects of these metal contaminants have not been defined. In recent years, researchers have focused their attention on the identification of other possible bioindicators for trace metal pollution, such as the mollusks. The available data are of particular interest concerning filter-feeding bivalves which are well known for their ability to reflect environmental levels of trace metal contaminants in marine ecosystems (Chassard B, 1989). Several biomonitoring studies for the heavy metals pollution in marine ecosystem, have been carried out in past two decades using different mollusk like examples as show in table 1.2.

Table 1.2 Trace metal concentrations in dried soft tissue of clams from different environment

N	Locations	Species	Cu	Zn	Pb	Cd	References
1	Jiaozhou Bay	<i>R.philippinarum</i>	0.6-23	8-120	0.33-1.66	0.2-2.3	Lui et al, 1983
2	Moroccan estuarines	<i>S.plana</i>	16-20	242-222	ND	0.2-1.6	Cheggour et al,2005
3	Edo Miranda Venezuela	<i>T.mactroidea</i>	11-49	55-166	1.5-4.9	1-1.9	LaBrecque et al, 2004
4	Ras Al nouf Qatar	<i>C.callipyga</i>	8.35	69.1	1.45	1.17	Mora et al, 2004
5	Bohai Sea	<i>R.philippinarum</i>	1.2-4.3	9.9-20	0.1-0.3	0.1-0.6	Liang et al,2004
6	Gulf of California	<i>V.gigas</i>	8.26	844.8	2.89	115.2	Ruelas I et al, 2003
7	Rio dela plata Argentina	<i>C.fluminea</i>	28-89	118-316	ND	0.5-1.9	Bilos et al, 1998
8	Atlantic coast of Spain and Portugal	<i>Cardium</i>	15	210	32	1.5	Stenner et al, 1971
9	Nha Phu Bay, Viet nam	<i>P.viridis</i>	0.54-1.81		0.14-1.13	0.03-0.21	Ha D V et al, 2007
10	Han and De estuarines	<i>Corbicula sp</i>	0.37-0.51		1.67-2.1		Khanh N.V et al, 2009
11	Nam O, Da nang coast	<i>O. Rivulasis</i>	10.35		1.35		Mui L.T et al, 2008
12	Nam O, Da nang coast	<i>C. Sinensis</i>	14.72		1.85		Mui L.T et al, 2008
13	Nam O, Da nang coast	<i>A. subcrennata</i>	16.52		2.12		Mui L.T et al, 2008

14	Nam O, Da nang coast	<i>Perna viridis</i>	12.23		1.65		Mui L.T et al, 2008
15	Xuan Trieu, Danang Coast	<i>M.guadragular</i>	7.15		1.13		Mui L.T et al, 2008
16	Xuan Trieu, Danang Coast	<i>A. subcrennata</i>	12.21		1.87		Mui L.T et al, 2008
17	Son Tra, Danang Coast	<i>C. sumatrensis</i>	10.15		1.39		Mui L.T et al, 2008
18	Son Tra, Danang Coast	<i>P.undulata</i>	9.17		1.23		Mui L.T et al, 2008
19	Son Tra, Danang Coast	<i>Perna viridis</i>	8.75		1.15		Mui L. et al, 2008

Note: Concentrations of metals in mg/kg DW

### **1.3.2 Bioaccumulation**

#### ***1.3.2.1 Definition***

Bioaccumulation is a general term applied when there is a net accumulation of a chemical by an organism as a result of uptake from all routes of exposure. Bioaccumulation of contaminants from sediments to benthic organisms and their subsequent transfer through the food web provides an exposure pathway to higher-level organisms (ODEQ, 2007)

The term bioaccumulation includes bioconcentration, which is the net accumulation of a chemical directly from living environment by an organism, and biomagnifications, which refers to the process by which chemicals tend to accumulate to higher concentrations at higher levels in the food web due to dietary accumulation (ODEQ, 2007)

Biomagnification is generally defined as the process of bioaccumulation along food chains or more precisely: within food webs, following various pathways on different trophic levels. However, this process must not necessarily end up in a magnification, leading to a stepwise increase with highest concentrations in organisms being in terminal positions of food webs e.g. whales, crocodiles, humans (Bernd Beek et al, 2000)

The terms bioconcentration and bioconcentration factor (BCF) as defined by OECD guidelines should be limited to laboratory test systems (OECD, 2000) where the uptake of a chemical is nearly exclusively restricted to the soluble fraction and any other uptake routes can be neglected e.g. by minimizing the particle bound fraction of suspended matter. But even under controlled laboratory conditions a certain uptake of adsorbed fractions onto food may occur. Generally all fractions must be considered potentially bioavailable regardless of the route of uptake.

The extent of bioaccumulation of metals is dependent on the total amount, the bioavailability of each metal in the environmental medium and the route of uptake, storage and excretion mechanisms. The requirements of different organisms for essential metals vary substantially but optimal concentration ranges are narrow and frequently under careful homeostatic control. Excess metal concentration in an organism must be actively excreted, compartmentalized in cells or tissues, or metabolically immobilized. Some metal escape all these actions causing toxic and other adverse effects.(Chapman et al, 1996; Rand

et al, 1985) Metal in the aquatic environment are bioaccumulated by organisms either passively from water or by facilitated uptake. Essential metals are maintained by binding to organic molecules at a variety of biochemical sites where they function mainly as catalysts to induce or enhance enzymatic activity (Regan L, 1993)

Essential metals at high concentrations can have sublethal toxicity effects to some organisms or lethal consequences to others. Also, metals at deficient concentrations can have again adverse health effects. Thus essential metals can have a double “toxic” threshold (Rainbow P.S, 2007)

Living organisms exposed environmentally to high metal concentrations follow various mechanisms to counter potential toxicity. They reduce intake, enhance excretion and/or sequestration of the metals within tissues to render them non toxic. Metals can be sequestered through storage by metal binding proteins, such as metallothioneins, in cellular vesicles and granules. Some storage mechanisms can be related in some organisms of providing essential metals for future needs (Malins D.C, 1993)

Futhermore, once heavy metals are in the sediment, it can easily up take and accumulate in living tissues of benthic organisms as well as mollusk community. And when humans eat these organisms, that metals can be built up large concentration of them in human and finally can put human health at risk, because they cannot be metabolized. The subsequent remobilization of these metals from sediments and their transfer to food web can impact local communities, sources of food supply as well as their sources of livelihood. A need to assess the impacts of contamination of heavy metals on the health and function of the vulnerable components of coastal ecosystems in Viet Nam is crucial. This knowledge is a key to manage coastal environments for sustainable and wise use, and to assure the public health and safety of the population consuming seafood in Viet Nam.

#### ***1.3.2.2 Bioaccumulation factor (BAF)***

Bioaccumulation is quantitatively expressed by the bioaccumulation factor (BAF), the ratio of the concentration reached in the organism under steady state condition and the concentration of the surrounding medium. This factor can be related to the whole organism or tissues and organs there of on a wet, dry or lipid weight basis depending on the context.

Usage of BAF offers unique approach for investigating and understanding the behavior and fate of these toxic substances in coastal ecosystems. Transfer factors, also



referred to as bioaccumulation or concentration factors, indicate the most likely metals concentration in an organism due to its exposure. Transfer factors for a given element may differ by several orders of magnitude in freshwater compared to marine biota. Since critical pathway for contaminant to enter our body systems are generally from food chains, particular emphasis must be given to study the transfer factors and dose assessment in marine biota which later can be used to estimate health risk from consumption of contaminated seafood. Therefore, this study will establish the transfer factors and dose response of marine biota found in the region and later be used to estimate health risk from consumption of contaminated seafood.

### ***1.3.2.3 Bioaccumulation of heavy metal in mollusks***

Bioaccumulation in mollusks and most of the other aquatic animals involves the uptake of chemical from the water and sediments. Bioaccumulation is the process, which causes an increased chemical concentration in organisms compared to that in environment, due to uptake by all exposure routes including dietary absorption, transport across respiratory surfaces and dermal absorption. Bioaccumulation can thus be viewed as a combination of bioconcentration and food uptake. Biomagnification can be regarded as a special case of bioaccumulation in which the chemical concentration in the organism exceeds that in the organism's diet due to dietary absorption. The extent of bioaccumulation thus can play key role in determining water and sediment quality criteria. The assessment of the levels of heavy metals pollution in aquatic mollusks which are used as bioaccumulation indicators has become an important task in preventing risks to public health. It is to be pointed out that for a living species to be used as bioaccumulator some essential characteristics are necessary (Cheung et al, 2003; Bebianno M.J, 1993).

- It must be typical of the ecosystem studied (eg non-migratory), ubiquitous and abundant,
- Its size, biotype and behavior must be such as to make sampling easy.
- It must bioconcentrate xenobiotics substances to a level sufficient to perform a direct analysis without pre-concentrations.
- It must be able to stand high concentrations of different toxic substances so as to survive the pollutant studied.

- It lives in a sessile style, thus definitely representing the local pollution.
- Its life long enough for the comparisons between various ages.
- It occupies the important position in food chain.
- Dose effect relationship can be observed in it

Assessing bioaccumulation is also a component of international efforts to identify and control chemicals of environmental concern. It is now generally accepted that substances, which are persistent, bioaccumulative, and toxic and are subject to long range transport are of particular concern. There is a need to establish reliable procedures for estimating bioaccumulation potential from knowledge of molecular structure or from readily measurable properties of the substance. There is a further incentive to adopt a tiered assessment system in order that those substances which are not bioaccumulative can be rejected from the assessment process at an early stage with minimal expense and effort. Even toxic effects on ecosystems start with these chemical reactions in individuals. In past few years monitoring programs, conducted to evaluate water quality, usually include chemical and common biological parameters, the use of biochemical markers for surveys is less frequent, but recently more efforts have been given to propose these biomarkers of exposure and effect, in toxicity testing aiming an application in pollution monitoring (Christensen JM, 1995)

Both essential and non-essential trace elements are known to be highly accumulated by invertebrates, in particular by a variety of mollusks species. Aquatic mollusks seem to reflect ambient metal contamination and are therefore widely used as bioindicator organisms. Undoubtedly, aquatic mollusks are amongst the most thoroughly investigated bioindicator organisms. The translocation of sentinel species, mainly mussels from a reference site to the study areas has been demonstrated as a useful strategy for the assessment of water quality in coastal and estuarine environments, either through bioaccumulation or biomarkers analysis Gundacker C, 2000; Lee et al, 2006; Nakhle et al, 2006).

In an earlier chemical and biological study of a marine bay in Washington State (EPA, 1976), Pacific oysters *Crassostrea gigas* and *Ostrea edulis* from the upper bay contained the highest concentrations of cadmium (1.1 mg/kg DW), copper (7.6 mg/kg

DW), and zinc (150 mg/kg DW), while exhibiting the lowest levels of arsenic (0.20 mg/kg DW) and mercury (0.008 mg/kg DW).

Last some years, the concentrations of some heavy metals in the tissues of mussels, oysters and cockles have been observed in some samples collected along the coastal water of Khanh Hoa province. Concerning seafood safety, the average metal contents (Zn, Cu, Cr) in the samples from Van Phong bay, especially My Giang Island already exceeded the maximum permissible levels set up by Ministry of Public Health of Vietnam. The mean concentrations of heavy metals for mollusk *Turbo bruneus*, *Lambis lambis* and oyster *Saccostrea cucullata* were 1,2 - 9,5 mg/kg WW for Pb; 0,4 - 3,1 µg/g WW for Cd; and 1,0 - 5,8 mg/kg WW for Cr (Vinh L.T, 2005). All of these results show that the contaminants of the heavy metals (e.g. Cu, Cd, As, Hg...) that can bioaccumulate to the mussels community in the coastal.

There is very little information available on the impacts of heavy metals pollutants in the coastal areas of Khanh Hoa although their presence is noticeable particularly in solid wastes disposal. The heavy metals in the coastal waters of Nha Trang are being accumulated in the sediments and marine organisms particularly those resident in the polluted areas. The accumulation of eight heavy metals (As, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn) in the resident fauna from polluted coastal areas of Nha Trang has not been reported. The heavy metals are being accumulated in considerably higher concentrations in marine organisms of the polluted localities. The accumulation of five heavy metals (Cu, Co, Mn, Zn, and Fe) in the resident fauna from Van Phong and Cam Ranh bay in considerably higher concentrations has been reported in marine organisms comprising of resident fauna of fishes including edible fishes, shrimps, some benthic organisms (bivalves and barnacles) from these areas. The concentrations of iron and zinc were found higher than the corresponding values for Mn, Cu and Co.

Previous studies have indicated that lead, zinc, cadmium, and copper were major heavy metal contaminants in the Nha Phu region (Vinh L.T, 2003). For this time, they are not research with combined analysis for the coastal sediment and mollusks species to recognize relationship between them and their bioaccumulation in this coastal region. The first aspect of this study was therefore to utilize mollusks to assess the range and distribution of the heavy metal contaminants at four strategic marine sites noted for

different potential environmental contamination in the Nha Trang bay. The second aspect was to investigate whether the extent of contaminations in mollusks in each location were associated with differences observed in population densities and banding pattern attributes which were previously linked to nutritional factors in the Khanh Hoa region.

### **1.3.3 Factors affecting bioaccumulation of heavy metal in mollusks**

Knowledge on the factors affecting bioaccumulation of heavy metal in mollusks may help us to understand the processes involved in the uptake of metals by the organisms. Numerous factors influence the accumulation of heavy metals in mollusks tissue. It was observed that the concentration of metal in the mollusks depends not only on the level of the element in the environmental factors but also on other biological factors such as size, age, speed of growth, sex and reproductive conditions of the mollusks, season, salinity, chemical species and interaction with other pollutants (Sanjay et al, 2011)

Many studies have shown that the concentrations of heavy metals accumulated by marine organisms are depending on the environmental factors: Geochemical and biological factors. Different geochemical factors such as water current, water flow, renewal of water, pH, hardness, salinity etc greatly affects the distribution of heavy metals in the mollusks. The greater metabolic rate of small organisms may partially account for the higher concentration of the essential elements as Cu and Zn (Williamson P, 1980). Uptake of metals and subsequent bioavailability are not only highly dependent on geochemical but also biological factors.

#### ***1.3.3.1 Geochemical factors***

Geochemical factors that influence bioaccumulation are organic carbon, water hardness, temperature, pH, dissolved oxygen, sediment grain size, and hydrologic features of the system (Elder J.F et al, 1991).

Uptake and accumulation in deposit feeders would be expected to correlate to metal concentrations in sediments, whereas accumulation in filter feeders would most likely reflect metal concentrations in water (Newman et al, 1982) and high environmental phosphate concentration facilitates the uptake of cadmium by organisms (Romeo et al, 2000). Several of these factors are described above and others are discussed in the following paragraphs.

## **pH**

Hydrogen ion activity (pH) is probably the most important factor governing metal speciation, solubility from mineral surfaces, transport, and eventual bioavailability of metals in aqueous solutions. pH affects both solubility of metal hydroxide minerals and adsorption-desorption processes. Most metal hydroxide minerals have very low solubilities under pH conditions in natural water. Because hydroxide ion activity is directly related to pH, the solubility of metal hydroxide minerals increases with decreasing pH, and more dissolved metals become potentially available for incorporation in biological processes as pH decreases. Ionic metal species also are commonly the most toxic form to aquatic organisms (Salomons, 1995).

Adsorption, which occurs when dissolved metals are attached to surfaces of particulate matter (notably iron, manganese, and aluminum oxide minerals, clay, and organic matter), is also strongly dependent on pH and, of course, the availability of particulate surfaces and total dissolved metal content (Bourg, 1988; Elder, 1989). Metals tend to be adsorbed at different pH values, and sorption capacity of oxide surfaces generally varies from near 0 percent to near 100 percent over a range of about 2 pH units.

The adsorption edge, the pH range over which the rapid change in sorption capacity occurs, varies among metals, which results in precipitation of different metals over a large range of pH units. Consequently, mixing metal-rich, acidic water with higher pH, metal-poor water may result in dispersion and separation of metals as different metals are adsorbed onto various media over a range of pH values. Cadmium and zinc tend to have adsorption edges at higher pH than iron and copper, and consequently they are likely to be more mobile and more widely dispersed. Adsorption edges also vary with concentration of the complexing agent; thus, increasing concentrations of complexing agent increases pH of the adsorption edge (Bourg, 1988). Major cations such as  $Mg^{+2}$  and  $Ca^{+2}$  also compete for adsorption sites with metals and can reduce the amount of metal adsorption (Salomons, 1995). Davies C. and others, 1982 found adsorption of Cd to seawater sediment increased with increasing pH levels between 6.5 and 9.0.

## **Metals**

The efficiency of bioaccumulation via sediment ingestion is dependent on geochemical characteristics of the sediment. Luoma (1989) describes variation of cadmium

uptake from sediment by clams as a function of sediment iron content; cadmium uptake from iron oxide-rich sediment was not detected, but a significant proportion of total cadmium uptake was a consequence of iron poor sediment ingestion by clams.

Interaction among metals can result both in stimulation and antagonism, as described by Luoma (1989). Stimulation occurs when uptake of one metal induces synthesis of binding sites that affect accumulation of both metals. Stimulation of cadmium uptake by lead exposure and of zinc by cadmium exposure has been observed in rats. Antagonism is commonly observed in simultaneous exposure to several metals. Antagonism to zinc uptake occurs during exposure to cobalt in clams and to copper in phytoplankton and microalgae. Antagonism between zinc and cadmium occurs in phytoplankton and macroalgae. Antagonism between copper and lead and between cadmium and mercury has also been reported in some types of organisms. In addition, more widespread elements, such as calcium and magnesium, can inhibit some types of metal-organic interactions. Zinc uptake by fish is influenced by this phenomenon but uptake of copper and cadmium are not affected.

Bioavailability studies indicate that aquatic organisms uptake free metal ions (metal hydroxides) from solution quite efficiently; similarly, terrestrial animals uptake metal from solutions more efficiently than via direct particulate matter ingestion (Luoma, 1983).

The data suggest that, in low and moderate heavy metal medium gradients, Fe ions, which are antagonistic of Cu and Zn ions, inhibit their assimilation and stimulate the accumulation of Cr, Ni, Co, Cd, and Pb, which have strong positive association with Fe ions. Extremely high concentrations of Cr, Ni, Co, Cd, and Pb in the individuals displaying the signs of pathology and mutagenesis are indicative of “excess” or toxic accumulation of these elements. In high heavy metal medium gradients, similar signs may be related to the toxic concentration of Zn, Cu, Cd, and Pb at a reduced Fe and predominant Mn accumulation. Synergistic interaction of Zn and Cu ions inhibits the accumulation of Ni, Cr, and Co, as their antagonists. No mollusks with signs of pathology were found in the low heavy metal medium gradients. The deficient or optimal heavy metal accumulation can be assessed from the concentrations of the above metal indicative of an intense or relatively low accumulation, as estimated by the coefficient of biological accumulation (CBA). Heavy metal tissue enrichment was calculated with respect to Fe, which is the most

vitaly important element for metabolic processes; the coefficient of enrichment (CE) was calculated with modifications for the aquatic medium.

### **Particulate size**

Particulate size and resulting total surface area available for adsorption are both important factors in adsorption processes and can affect metal bioavailability (Luoma, 1989). Small particles with large surface area to mass ratios allow more adsorption than an equivalent mass of large particles with small surface area to mass ratios.

Reduced adsorption can increase metal bioavailability by increasing concentrations of dissolved metals in associated water. The size of particles released during mining depends on mining and beneficiation methods. Finely milled ore may release much smaller particles that can both be more widely dispersed by water and wind, and which can also serve as sites of enhanced adsorption. Consequently, mine tailings released into fine grained sediment such as silty clays found in many playas can have much lower environmental impact than those released into sand or coarse grained sediment with lower surface area and adsorption. Consequently, geologic and (or) environmental conditions that enhance dissolved metal abundances (for example, lower pH) result in greater metal bioavailability. Indirect controls, such as larger particle or sediment size, also can result in greater bioavailability of metals by reducing adsorption and increasing dissolved metal contents. Metal assimilation from ingested particulate matter is also important, however, because metals are highly concentrated in this form (Luoma, 1989).

Moreover, several sediment types possess an adsorption affinity for zinc (Zn) ions, thereby decreasing Cd availability to benthic invertebrates (Jackim et al., 1977).

### **Organic carbon**

In recent organic carbon-rich sediments, trapped interstitial fluids can commonly form a strongly reducing (anoxic) environment. Low redox potential in this environment can promote sulfate reduction and sulfide mineral deposition. During diagenesis, much of the non silicate bound fraction of potentially toxic metals such as arsenic, cadmium, copper, mercury, lead, and zinc, can be coprecipitated with pyrite, form insoluble sulfides, and become unavailable to biota (Morse, 1994). Seasonal variation in flow rates or storms that induce an influx of oxygenated sea water can result in rapid reaction of this anoxic

sediment and thereby release significant proportions of these metals. Pyritization and depyritization of trace metals probably can be an important process in controlling bioavailability of many trace metals, especially in the marine environment (Morse, 1994).

### **Temperature**

Accumulation by marine bivalves can increase with a rise in temperature (Phillips et al., 1993). As noted above, temperature can strongly influence the rate of biological processes; rates double for every 10°C temperature increment. The increase in biological process rates does not necessarily result in increased bioaccumulation of metals, because both influx and efflux rates of metals may increase.

In Greenland, blue mussels *Mytilus edulis* have been employed as indicators in areas influenced by mining activities. It is known that metal levels in mussels not only reflect the heavy metal concentration in the surrounding water, but element accumulation is also influenced by sampling season, location within the intertidal zone, and mussel size (Riget et al., 1996). Cadmium accumulation increases with temperature because of its effect on the metabolic activity of the host (Ray, 1986).

### **Salinity**

Accumulation by marine bivalves can decrease with increasing salinity (Gibb et al., 1996; Eisler, 1981). Moreover, some studies have shown that increasing salinity will tend to decrease Cd uptake (Davies C. et al., 1982; Luoma et al., 1990). Salinity influences availability of contaminants whereas temperature changes can alter bivalve spawning, which will affect the soft tissue mass and lipid composition of the mollusks (Biliaff et al., 1997).

#### ***1.3.3.2 Biological factors***

Among biological factors, there are major differences in bioaccumulation between mollusks species or metal concentration may differ according to the species. Body size and weight also play an important role in bioaccumulation of metals. Within a single species accumulation can be a function of age, size, sex, genotype, phenotype, feeding activity and reproductive state (Boening D.W, 1997).

Sex plays a significant role in metals accumulation in mollusks. Significantly lower concentrations of cadmium and zinc concentrations were found in males as compared to



females in *Perna perna* species and high concentration of cadmium was reported in *Pecten maximus* (Romeo et al., 2000; Boyden C.R, 1974).

Body size, condition index and tidal height also affects the concentrations of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in *Mytilus edulis* and findings suggested that the body weight was inversely related to metal concentrations and for Cd, Mn, Pb and Zn the regression was affected by tidal height. Except for As, Fe and Mn metal concentrations were inversely related to physiological status though no differences between essential and non-essential metals were recorded. Thus it is recommended that stringent measures during sampling for biomonitoring or metal concentrations at each location must be normalized to a common body size, condition index and tidal height (Mubiana et al., 2006).

However, the magnitude to this accumulation can vary widely between species. Talbot and Magee (1978) measured Cd levels in oysters and mussels from Philip Bay and Corio Bay near Melbourne, Australia. Both shellfishes were highly contaminated, with oysters containing more Cd than mussels (35.5–174.3mg/kg DW and 2.8–17.0mg/kg DW, respectively). Mussels collected from piers contained less Cd than mussels living on sediment, implicating sediments as the ‘sink’ for the metal, and therefore the matrix contributing most to uptake. Oysters, abalone and mussels are capable of accumulating extremely high levels of Cd in edible portions and therefore represent a greater hazard to human consumers than other marine organisms (EPA, 1978).

### **Influence of length and weight**

Aside from the species, the body size of oysters is one of the most important factors that affect the metal accumulation rates in biological tissues (Meyer et al., 1998); however, the soft tissue weight was higher in samples from the dry season, when the condition index is greater. Condition Index is normal if CI =90; high if CI = 120 and low if CI = 70 - 80.

During the present study, consistent with as light increase in length and weight of mollusks from dry to rainy periods, metal concentrations also showed an increase from dry to rainy periods suggesting that metals accumulated in mollusks are not totally excreted to baseline concentrations (Ju et al., 2011). This is further supported by significant correlations between all the metals and both length and weight of mollusks. Borchardt and others, 1988 suggested that an increase in bivalves’ biomass in the unpolluted estuarine areas of the North Sea (Atlantic Ocean) from monsoon to non-monsoon periods lead to an

increase in metal concentrations and, when expressed graphically, metal concentrations in bivalves always follow a sinusoidal curve with respect to biomass.

Metal concentrations in mollusks during the dry and rainy seasons were plotted against their respective lengths and irregular patterns were observed, indicating the bioavailability of metals and an efficient metabolism. In certain cases, a short shell length or long shell length does not correlate with a low metal concentration or high metal concentration, thus disrupting a sinusoidal curve pattern and suggesting that levels of metals present in mollusks are strongly dependent on the anthropogenic input into the estuary.

Based on the results of Spearman's correlation coefficients between the biological parameters of the specimens and the mean levels of the elements in the prismatic layer, significant positive relationships were found for Mg with weight and length and also for Al with width and height, but significant negative correlations were observed for Fe and Mn with height. In terms of nacreous layer, significant positive correlations were detected for Al with width and height, while the negative relationships were found for Mn with weight and length and for Ba and Li with hinge length.

### **Organ of organism**

Hard clams *Mercenaria mercenaria* were exposed to 0.1µg Cd/ml seawater for either a period of 31 days, or 3 days, each followed by depuration in clean seawater for 2 to 64 days. Cadmium accumulated at increasing rates in the kidney, but at decreasing rates in all other organs during the exposure period. The overall rate of accumulation was significantly greater for the kidney than for the gill, mantle, digestive gland, and remaining viscera. No loss of Cd or other detectable metals occurred even after 64 days depuration. The degree to which Cd accumulated in the kidney, gill, and digestive gland did not correlate with the concentrations of other metals present in these organs (Robinson et al., 1986).

### ***1.3.3.3 Mechanisms of bioaccumulation***

These mechanisms may also vary with physiological and environmental factors (Bryan, 1973) or even with the sexual state of the animal (Alexander et al., 1976). So the observed variation in metal levels in mollusks at different stations may be related to one or both of the following mechanisms:

The availability of different metals to the animal varies with different stations, and the animal involves different uptake and retention mechanisms for the same metal at different stations. Since these mollusks inhabit such different environs, one cannot expect the same level of exposure to a metal of potential concern. The littleneck clam *P. staminea* and butter clam *S. giganteus* differ in their rate of bioaccumulation, based on their metabolic rate, living environment, and feeding habits (Wayne D, 1994). Butter clams have a higher metabolic rate, and a more accelerated growth rate than the littleneck. The feeding behavior of the butter clam relies on deeper solids. It is often found near the lower littoral zone deeply buried in sand and in bays or off rocky coasts in water depths up to 50 meters (Ricketts et al., 1962). For instance, littlenecks might be more sensitive to compounds in surface deposits and those suspended in fluids. In contrast, butter clams and other *Protobranchia* would be more sensitive to the bulk chemistry of the subsurface sediment matrix. In contrast, littleneck clams are more sessile. The littleneck is a poor digger and never lives in shifting sand where rapid digging is essential. It inhabits bays and estuaries in coarse, sandy mud. This species is also widely harvested for food, and is most often found in the upper littoral zone (Audubon, 1990). Therefore, the order of metal accumulation in the animal was Fe>Zn>Mn>Pb>Cu>Ni>Cr>Cd, where, Fe was the highest followed by Zn, Mn and Pb then Cd, while Cu, Ni and Cr were changeable in their order at different stations. This order may be due to variation in the levels of discharged metals at different stations and also the chemical changes of metal before being taken up by the tested limpet.

Ayling, 1974 suggested that different mechanisms exist for the up take of Cu, Cr, Zn and Cd within the oyster *Crassostrea agigas*. Cadmium uptake is believed to be a passive mechanism (Spacie et al., 1995; Daskalakis, 1996). *Crassostrea virginica* exposed to Cd rapidly accumulated high levels of the metal in soft tissues. Maximum concentrations of Cd were independent of exposure concentrations (Daskalakis, 1996). The target organelle of Cr accumulation was shown to be the lysosome where the metal was associated with phosphorus and sulfur and trapped in an insoluble form. In *M. edulis*, the metabolism and transport of Cr was compared with other metals, and led to the conclusion that Cr is metabolized and distributed differently from most toxic metals (Usero et al., 1996; Phillips et al., 1993). Cadmium and lead concentrations in sediment and tissues tend to be of similar magnitude, suggesting that they are not regulated by the organism (Phillips

et al., 1993). These metals have not been demonstrated to be utilized in metabolism (Eisler, 1981). Tissue Cu and Zn concentrations were higher than the sediments, indicating that these metals are probably taken up actively possibly reflecting their use in metabolism (Genest et al., 1981). Some investigators have reported significant depuration of Cd by some bivalves but other organisms show very effective retention of Cd. Genest and Hatch, 1981 found that Zn accelerated Cd transport from gills to other organs. Prior exposure to Zn retarded Cd uptake in the excised gill of *A. anatina*. Much of what is known about Cd bioaccumulation by marine organisms has come from laboratory studies and therefore extrapolation to certain field conditions may be inherently uncertain (Ray, 1986).

The second mechanism could operate for one or two reasons, either the different animal populations have various abilities to accumulate metals at the different stations or the uptake of other metals. The second alternative has been extensively studied with other molluscs (Romeril, 1971; Martin et al., 1975; Howard, 1975; Coughtrey et al., 1977). It is also suggested by the results of the present data that a significant correlation exists between Cu, Cr and Ni at all stations. Coughtrey et al., 1977) showed that the correlation between Cd, Pb, Zn and Cu for *Helix aspersa* was significant at the less contaminant site and non significant and marginally significant at the more contaminant site.

Considering that many factors could affect the trace metals bioaccumulation in mollusks, e.g. physiological state, size and season (Romeo et al., 1994) our study investigated the metallic concentrations according to the season, animal size and distribution in organs. It was realized in two different sites of the Nha Trang bay.

## **1.4 BIOMONITORING**

### **1.4.1 Biomonitoring**

Chemical analysis of the environment matrix such as water, sediment is the most direct approach to reveal the heavy metal pollution status in the environment, while it cannot afford the powerful evidence on the integrated influence and possible toxicity of such pollution on the organisms and ecosystem. Biomonitoring is a scientific technique for assessing environment including human exposures to natural and synthetic chemicals, based on sampling and analysis of an individual organism's tissues and fluids. The results of these measurements provide information about the amounts of natural and manmade chemicals that have entered and remained in the organisms and the corresponding effects

induced. Due to consistency between the selected organisms and the corresponding living space, biomonitoring can directly offer the data on the potential effects and actual integrated toxicities of pollutants, reflecting the corresponding deleterious degree in the environment (Zhou et al., 2008)

Biomonitoring of heavy metals and effect studies on natural populations of organisms must take into account the pollution induced community tolerance that is expected from communities that are exposed to particular pollutants for a long time (several generations). (Blanck et al., 1988; Chapman et al., 1998). By virtue of their wide spread geographic distribution, reasonable size and availability throughout the year, bivalves (mussels, clam and oysters) have been used as sentinel bioindicators of trace metal pollution monitoring in coastal regions of many countries.

In biological indication passive and active monitoring are accepted as a general approach, at different levels of organization. In passive monitoring degradation of the ecosystem, elimination of sensitive species and reduction of biodiversity can be revealed as adverse consequences of pollution at the level of populations, while at the level of individual's accumulation of toxic substances in specimen, in organs and tissues indicative of pollution in the environment can be traced. In active monitoring the response of artificial or modified populations, behavioral patterns of specimen, specific function of organs like movement, feeding, respiration, reproduction and the neural regulation as well as cellular and sub cellular events are studied under the effect of toxic substances (Solanki et al., 2003). The main purpose of biomonitoring heavy metal concentrations in biota has been to determine the toxicological threat posed to organisms and also for health risks to humans from the ingestion of edible species. (Valavanidis et al., 2000)

An important approach to assessment of risk from environmental and occupational exposures is biomonitoring which provides an estimate of the total dose absorbed and gives indirect access to determination of target site concentrations. To reveal the presence of pollutants and to measure their toxic effect biological indicators can be used. At the level of individual's accumulation of toxic substances in specimen, in organs and tissues indicative of pollution in the environment can be traced. (Sanjay et al., 2011). In biomonitoring surveys, the toxic elements arsenic, cadmium, chromium, cobalt, lead and

nickel etc are used as examples to illustrate the disturbing factors in the interpretation of biomonitoring results (Christensen J.M, 1995)

The use of aquatic organisms as bioindicators for trace metal pollution is very common these days. Molluscs are among the organisms most used for this purposes. It is widely observed that various species of molluscs are the effective sentinel organisms and can achieve high concentrations of metals and metalloids relative to concentrations gradient of these substances in the surrounding environment (Rainbow et al., 2000). In contrast, biomonitoring using specific organisms offers an appealing tool for the assessment of trace metal bioavailabilities over space and time in coastal water bodies by providing integrated measures of the ecotoxicologically significant fraction of trace metals partitioned in water, suspended particulates, and sediments (Rainbow 1995, 2006; Zhou et al., 2008).

Marine mollusks, including clams, mussels and oysters, can be employed as bioindicators to assess the marine environment, since they can accumulate (concentrate) trace elements and other substances (LaBrecque et al., 2004). In addition to using bivalve molluscs as bio-indicator organisms of coastal contamination, it is also recognized that they can be important links for contaminants between sediments and higher organisms, including man, that consume them. Hence, information on the contaminant concentrations in their tissues is potentially useful in considering toxicological and public health implications of estuarine contamination (Cheggour et al., 2005)

#### **1.4.2 Bioindicator**

Bioindicators are species that accumulate bioavailable chemical forms of contaminants. Among aquatic organisms, gastropods and bivalves mollusks have been recognized as a useful tool for monitoring of the environment they live in because of their ability to accumulate chemical elements and/or compounds in their tissues proportionally to their bioavailability and thus can be used as indicators of aquatic metallic pollution. They are filter feeders, herbivores or carnivores and have the potential to bioconcentrate contaminants, which would normally be present in the water or within sediments at concentrations too low for detection by routine monitoring techniques. They are also ideal species for environmental monitoring, because their sedentary nature does not require consideration of complex migratory factors in the interpretation of the bioaccumulation

data. They are sedentary organisms filtering large amounts of water allowing them to accumulate the substances from the environment. They also satisfy the other conditions to be bioindicators hence very appropriate for monitoring because of their abundance and wide geo-graphical distribution, relative longer life span, suitable dimensions, size, weight, easy identification and collection, abundance in an ecosystem and accumulate the elements to a degree suitable to measure for hazard and risk assessment (Chase et al., 2001).

Mollusks are benthic fauna in the food chain ecosystem, they live and move slowly to feed on the surface of sediments and thus accumulate higher concentrations of heavy metals than other organisms (Eisler, 1980). Many benthic organisms accumulate trace metals to the levels reflecting those in the environment. Tissue metal concentrations can reflect contamination, and molluscs in particular may therefore be sensitive bioindicators of anthropogenic metal inputs (Hendozko et al., 2010). To achieve a better estimate of bioavailable metal exposure, it is recommended that the tissues of the organisms be analysed for trace metals (Luoma S.N, 1983).

Benthic mollusks play an important role as bioindicators for trace metal pollution and appear more and more often in global monitoring programs (Rainbow et al., 2000). Among aquatic organisms suitable for biological monitoring mollusks occupy a prominent place and they are often used both for passive and active biomonitoring and in hazard and risk assessment (Salanki J, 1986). As even closely related species may exhibit different accumulation strategies for trace elements, there is a need to identify widespread cosmopolitan bioindicators to allow intra-specific comparison of accumulated metals concentrations over large geographical areas (Rainbow et al., 2000). Mollusks can accumulate and integrate concentrations of several metals in seawater for relatively long intervals. They also assimilate trace metals from their food and from the ingestion of inorganic particulate material (Philips D.J, 1977).

The potential ecological effects of rising levels of heavy metals concentrations in the environment are of great concern due to their highly bioaccumulative nature, persistent behavior and higher toxicity. These chemicals biomagnify in the food chain and impose various toxic effects in aquatic organisms. Mollusks reflect the higher degree of environmental contamination by heavy metals and are the most useful bioindicator tools. Several studies and research work have been cited to establish and evaluate the relationship

between metal contents of water column, sediment fractions, suspended matter and mollusk tissue concentrations.

Suggested characteristics for bioindicator organisms (ODEQ, 2007)

- Tolerance to a wide range of metal exposures, ability to accumulate metals without suffering mortality
- Sedentary habits, slow and limited range of movement
- Sufficient life span to allow for sampling of more than a one year class
- High presence, abundance and wide geographical distribution in the study area
- Sufficient size to allow chemical analysis of tissue samples
- Hardiness, ability to remain healthy during sampling and laboratory incubation
- Size, weight, relative ease of sampling and identification
- High metal accumulation rate
- Responsiveness to changes in metal exposure

The potential ecological effects of rising levels of heavy metals concentrations in the environment are of great concern due to their highly bioaccumulative nature, persistent behavior and higher toxicity. These chemicals biomagnify in the food chain and impose various toxic effects in aquatic organisms. Mollusks reflect the higher degree of environmental contamination by heavy metals and are the most useful bioindicator tools. (Cevik F et al., 2011)

#### **1.4.3 Marine molluscs as biomonitors for heavy metals**

Benthic mollusks are the organisms most often used for the biomonitoring of metal contamination. Bivalve mollusks have an ability to accumulate heavy metals to various orders of magnitude with respect to the levels found in their environment (Usero et al., 2005). The levels of heavy metals accumulated by marine mollusks are a function of water quality, but also of seasonal factors, temperature, salinity, diet, spawning and individual variation, among other factors.

Moreover, the levels of metals accumulated in some marine organisms may be many orders of magnitude above background concentrations, thus demonstrating the



potential of certain species as bioindicators of heavy metal pollution (Hamed et al., 2006). Mollusk, especially the species from bivalve class is frequently used as bioindicator in environmental monitoring. Bivalve is one of the organisms that have the criteria of potential bioindicator due to its ability to accumulate pollutants from its ambient. Usually, the level of pollutant accumulated in such an organisms tissue is used for assessing the level of pollution in its habitat (Al-Madfa et al., 1998; AbdAllah et al., 2002).

Blue mussels *Mytilus edulis* are commonly used to monitor heavy metal pollution (Riget and others, 1996). One study found the blue mussel to be a particularly effective sentinel organism because soft tissue residues closely paralleled the heavy metal concentration gradient of their surroundings (Julshamn et al., 1996). The mussels *Mytilus edulis* and *Mytilus californicus* are indigenous and abundant in bays and estuaries around much of the United States coastline. Mussels feed continuously throughout the year, making them a convenient species for physiological study. Furthermore, mussels are excellent for transplanting because healthy adult animals are plentiful and can be distributed easily into field cages at various sampling locales (EPA, 1989; Phelps et al., 1980).

American oysters *Crassostrea virginica* have shown an ability to concentrate cadmium (Cd) to a great degree in edible tissues (Eisler et al., 1972). Cadmium concentrations, exceeding levels considered dangerous for human consumption (13.0 mg/kg), were attained by oysters exposed to Cd concentrations in water considered safe for drinking (10.0µg/l).

In another study (EPA, 1976), butter clams *Saxidomus giganteus* contained the highest arsenic concentrations (2.2 mg/kg DW) and the second highest levels of copper (3.8 mg/kg DW) and nickel (7.8 mg/kg DW). The highest nickel concentration was exhibited by the native littleneck clams *Protothaca staminea* in Nesika Bay, Japan. Japanese littleneck clams *Venepuris japonica* collected at the same station exhibit nickel levels of only 2.7 mg/kg DW. Several of the metals levels measured in Japanese and native littleneck clams appeared to be correlated, to varying degrees, with companion surface sediment metal concentrations.

In general, marine shellfishes have a low bioaccumulation tendency for chromium, but a rather high tendency to accumulate arsenic, cadmium, copper, lead, mercury, silver

and zinc (Trocin et al., 1996; Saiz et al., 1996). Shellfishes, particularly oysters, passively accumulate many metals much more readily than fishes: this suggests a priority for monitoring in metal contaminated areas (EPA, 1978; Julshamn et al., 1996). Moreover, sediments are an important sink for most metals in aquatic environments. Further, information concerning the biological and physicochemical factors affecting metals mobilization in sediments, would be valuable.

Various metal accumulating bivalve and gastropod species show a high presence and abundance in marine ecosystems therefore they are suitable for different monitoring projects for example:

- *Perumytilus purpuratus*, *Semelle solida* and *Tagellus dombeii* (Gregori et al., 1996)
- *Bembicium nanum* (Gay D et al., 2003)
- *Pyganodon gandis* (Bonneris et al., 2005)
- *Crassostrea angulata*, *Scrobicularia plana*, *Palameon longirostris*, *Uca tangeri*, *Melicertus kerathurus* (Blasco J et al., 1999)
- *Crassostrea virginica* (Apeti et al., 2005)
- *Radix ovata*, *Vivitarus spp* (Gundacker C, 2000)
- *Rapana venosa*, *Neverita didyma* (Lee C et al., 2006)

Some mollusks species propose to use as bioindicator in specific regional as:

**Adriatic Sea, Black Sea:** *Mytilus galloprovincialis*, *Perna perna*, *Crassostrea angulata*, *Crassostrea virginica* (Blasco J et al., 1999; Apeti D.A et al., 2005)

**Mediterranean Area:** *Monodonta turbinata*, *Pattela caerulea*, *Mytilopsis sallei*, *Mytilus californicus* (EPA,1989)

**Chinese Bohai Sea:** *Mytilus edulis*, *Crassostrea talienwha nensis*, *Ruditapes philippinarum*, *Rapana venosa* (Chassard et al., 1989; Lee et al., 2006)

**Gulf of Aden, Yemen:** *Turbo coronatus*, *Acanthopleura haddoni*, *Ostrea cucullata*, *Pitar spp*

A preliminary investigation conducted to assess the pollution in the coastal part of Vietnam with focus on trace elements including heavy metals at significant site specific differences were reported in the Table 1.3 as bellow:

Table 1.3 Mollusk species are used as biomonitor in marine of Vietnam

N	Species	Region	References
1	<i>Saccostrea cucullata</i>	Nha Phu, Khanh Hoa	Vinh L.T, 2005
2	<i>Paphia undulata</i>	Son Tra, Da Nang	Mui L.T, 2008
3	<i>Anadara subcrenata</i>	Nam O, Xuan Trieu	Mui L.T, 2008
4	<i>Meritrix spp</i>	Central coastal zone	Tuyen B.C 2006
5	<i>Pletholophus Swinhoei</i>	North Viet Nam	Wagner A, 2004
6	<i>Conus textile</i>	Nha Trang	Nghi B.Q, 2011

The bay of Nha Trang is the protected beach in Viet Nam, yes its fauna with the world's richest diversity, which is very important in term of biodiversity conservation. A total of 694 species of molluscs in 2 classes Gastropoda (435 species) and Bivalvia (259 species) were recorded in Khanh Hoa coast (Nghi B.Q, 2001). Compared with some other areas, species composition of mollusks in Nha Trang Bay was more abundant than Cat Ba – Ha Long Bay (372 species) (Duc N.X, 2001) and Tonkin Gulf (470 species, Cai Yingya et al., 1988). Some of there dominated bivalve species in Nha Trang bay that are showed in Table 1.4:

Table 1.4 Dominated bivalve species in Nha Trang bay (Nghi B.Q, 2001)

N	Species	Family	Class
1	<i>Perna viridis</i> (Linnaeus 1758)	Mytilidae	Bivalvia
2	<i>Bufonaria rana</i> (Linnaeus, 1758)	Bursidae	Gastropoda
3	<i>Anomalocardia squamosus</i> (Linnaeus, 1758)	Veneridae	bivalvia
4	<i>Laternula anatine</i> (Linnaeus, 1758)	Laternulidae	Bivalvia
5	<i>Solen regularis</i> (Dunker, 1862)	Solenidae	Bivalvia
6	<i>Solen grandis</i> (Dunker, 1861)	Solenidae	Bivalvia
7	<i>Isognomon isognomum</i> (Linnaeus, 1758)	Isognomonidae	Bivalvia

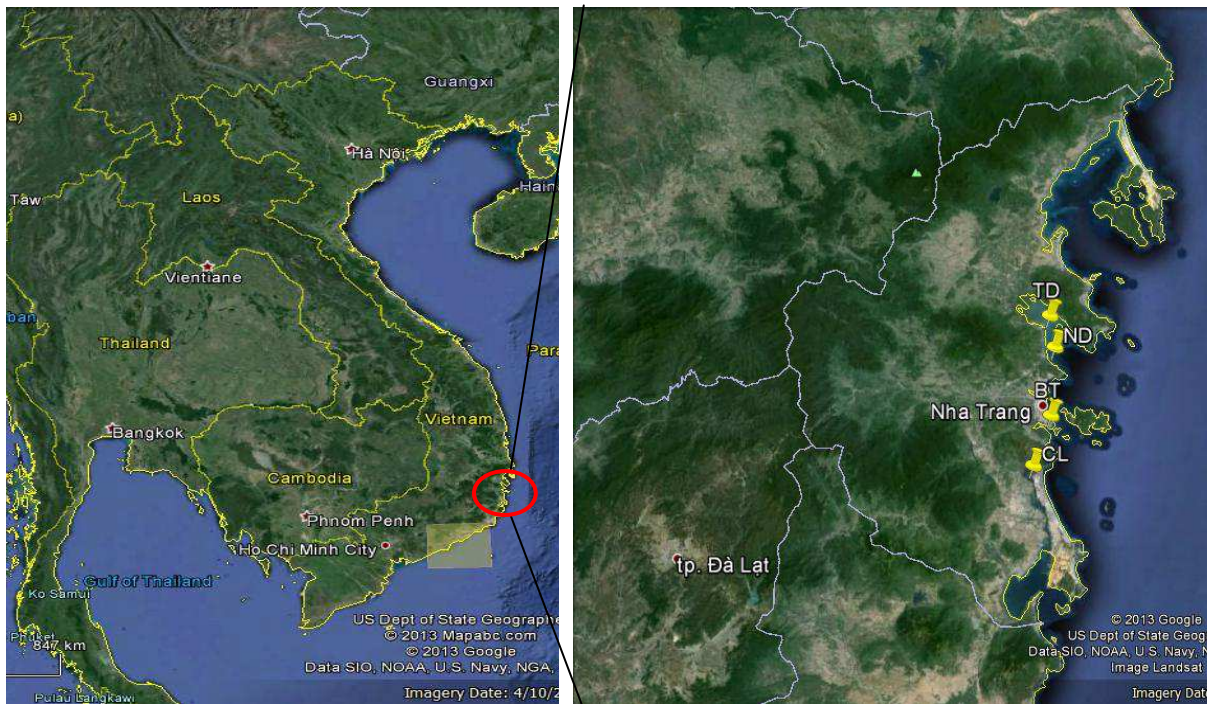
8	<i>Glaucanome virens</i> (Linnaeus, 1767)	Glauconomidae	Gastropode
9	<i>Anadara antiquate</i> (Linnaeus, 1758)	Arcidae	Bivalve
10	<i>Anadara granosa</i> (Linnaeus, 1758)	Mytilidae	Bivalve
11	<i>Circe scripra</i> (Linnaeus, 1758)	Veneridae	Bivalve
12	<i>Gafrarium dispar</i> (Holtel, 1802)	Veneridae	Bivalve
13	<i>Vasticardium elongatum</i> (Bruiguere, 1789)	Cardiidae	Bivalve
14	<i>Pinna atropurpurea</i> (Sowerby, 1825)	Pinnidae	Bivalve
15	<i>Atrina vecillum</i> (Born, 1778)	Pinnidae	Bivalve
16	<i>Modiolus elongatus</i>	Mytilidae	Bivalve
17	<i>Tapes literatus</i> (Linnaeus, 1758)	Veneridae	Bivalve
18	<i>Kataysia (Marcia) hiantina</i> (Lamarck, 1818)	Veneridae	Bivalve
19	<i>Crassostrea rivularis</i> (Gould, 1861)	Crassostrea	Bivalve
20	<i>Mactra maculate</i> (Gmelin, 1791)	Mactridae	Bivalve
21	<i>Lingula unguis</i> (Linnaeus, 1758)	Lingulidae	Lingulata
22	<i>Geloina coaxans</i> (Gmelin, 1791)	Cyreniidae	Bivalve

## **CHAPTER 2**

# **RESEARCH AREA**

### **2.1 INTRODUCTION OF RESEARCH AREA**

Nha Trang Bay is located in Khanh Hoa province - central-southern Viet Nam. between latitude: 12°09'–12°15'N and longitude: 109°13'–109°22'E (Picture 2.1). The Nha Trang bay is situated immediately adjacent to the coastal city of Nha Trang and its port at Cam Ranh. Nha trang city is a huge city with about 500.000 inhabitants, flanked by two rivers: the Cai river in the north and the Be river in the south, flow into Nha Trang bay, with the potential to influence water quality in the bay. The river catchments and riparian vegetation have been extensively modified for agriculture and aquaculture in recent decades. Apart from the inlands, which are located on the shelf in front of the bay, two large lagoons: Nha Phu lagoon is found just north of Nha Trang bay and a large lagoon Thuy Trieu is found just to the south with significant aquaculture and industrial activities. Nha Trang bay covers about 50.700 hectares and contains 19 islands, which are located about from 1km to 15 km offshore, with an average depth of 5- 20m. They provide the topographic basis for a wide range of coastal and marine habitat types in relation to prevailing oceanographic conditions and gradients in mainland - oceanic influences. The diverse array of tropical habitats includes coral reefs, soft bottom communities, and sea grass beds, small stands of mangroves, sandy beaches and rocky shores.



Picture 2.1 Map of Nha Trang Bay, Khanh Hoa province, Viet Nam

## 2.2 ENVIRONMENTAL STATE

The coastal province is under seasonal influence of the SW and NW monsoons. They have effect of tropical weather on the oceanography, particularly water temperatures, circulation pattern and monsoon winds. The climate is classed as humid tropical and is characterized by a relatively 6 months of dry season followed by a 6 months of rainy season with irregular rainfall. Rainfall generally occurs between November and April and the dry season is from May through October.

In Khanh Hoa province, according to Vietnam Meteorological Department, the monthly average temperature is 24°C low and 38°C high with all year temperature around from 27°C to 30°C, the rainfall ranges between 2000mm and 5268mm. The moisture level is also moderately high: 75% in dry season and 90% in rainy season.

In rainy season, the wind comes from the west or from the sea that can cause storms with duration of 3 to 7 days rendering travel by sea difficult. During strong winds and storms waves can reach 2-3.5m in height. They have effects large ocean swells (> 3m height) generated by the NW monsoon and typhoons in the South sea impact on the sublittoral communities most years. Wave energy during SW monsoon is usually more

moderate <1m high. Ocean currents of low/moderate velocity (<1knot) flow between the island driven by coastal winds, tides and regional oceanography. Sea surface temperatures usually peak is 30°C (SW) falling to 24°C during NW monsoon (An V.T et al., 2002). Upwelling off Vietnam occurs during SW monsoon when a cyclonic circulation cell in the northern and an anti-cyclonic give in the southern are initiated by spatial asymmetry in the wind field (Wu et al., 1998)

The bay of Nha Trang is the marine protected area (MPA) in Viet Nam; yes its fauna with the world's richest diversity, which is very important in term of biodiversity conservation. These mollusks are also indispensable for local communities because they provide livelihood and economy as fishery and tourism bases and protect land as a natural breakwater. However, they are at high risk of destruction due to coastal development accompanied with the rapid population growth in the neighboring coastal area. In this context this paper tries to evaluate the current state of knowledge trace metal impact on marine mollusks.

### **2.2.1 Human pressure**

There are a number of environmental issues in the coastal zone of Khanh Hoa province. Amongst these the most significant are the pollution problems (mainly along Nha Trang coast) and the occurrence of tropical cyclones. The pollution issues along Nha Trang coast that have arisen due mainly to the indiscriminate discharge of effluent from industrial and agricultural sources and disposal of untreated liquid and solid wastes generated from domestic sources into the coastal environment. The sewage waste generation in Nha Trang is some more than million cubic meters /day out of which 40% is domestic waste and 60% is industrial waste. This waste is dumped into the Nha Trang sea via Cai river, Be river, Nha Trang harbour area, and small waste drains. The other coastal areas having industrial pollution problems are ship breaking industries based in area. The heavy metals, persistent organic pollutants, air pollution and oil pollution are more significant.

### **Population Pressure**

The coastal city of Nha Trang has an estimated population of 500 thousands, and is the biggest tourist center of Viet Nam with more than 1 million tourists per year. In 1995, the total population of the coastal province was estimated to be 675,000 (6,5% of the total

national population). Population densities vary from 7.07 people/km in with an average density of 37 people/km<sup>2</sup>. Average growth rates in 1993 range from 2.7 to 4.6% in region.

### **Coastal Development**

The Government's goals for Khanh Hoa provinces and municipality originally planned for economic development in Viet Nam through the rebuilding of urban growth tourism port expansion and industry.

### **Urbanization**

Investment in coastal development will lead to increased urban growth as population from the rural areas move to tourism and associated services for increased economic opportunities. The Urban environment infrastructure is current insufficient to meet the requirement of even the current urban population. Without appropriate investments the environmental quality of this town will degrade.

### **Tourism development**

Coastal area is a matter of great in importance to the future of both in term of economical and environmental considerations. This development is expected to lead the way for Coast to develop as commercial centre which would substantially increase its population. The impact from this development may be affected to coastal inland resources coastal water resources. The number of tourists in this coastal province has been estimated at 1.206.000/year in 2010. An approximately 9% growth in tourism arrivals in Nha Trang is Russian and Chinese. This indicated that the positive growth in the potential tourism development sector in the country.

### **Port development**

Coastal ports expansions developments can make a contribution to the economy as a main hub for growth of maritime transport which should in turn attract manufacturing entries but may also have an adverse impact on the surrounding environment. These effects of new expansion port can be focused upon location port construction and port operation. These lead to impacts on water quality coastal hydrology bottom contamination marine ecology air noise waste management and visual quality.



### **Industrial development**

The industrial development zone was established in Khanh Hoa called Cam Ranh Industrial Zone. This industrial zone include petrochemical production to exploit recently confirmed oil and gas reserves in the seafood processing based on the local fisheries in the area timber processing and are manufacturing. However these industries pose potential damage to the environment outlets generally heavily contaminated with municipal sewage, industrial effluent and sediments contribute a significant quantity of pollution load to the coast. Regarding heavy metal presence in the biota, natural sources account for a background exposure. Among biota, zooplankton may contribute to the transfer of trace metals to higher trophic levels are the recommended groups for the base line studies of trace metals in the marine environment (Rejomon et al., 2008). Zooplankton accumulates metals directly from water by absorption, also by assimilation through food substances. The bioavailable fraction can be assessed only by determining the amount of metals incorporate into organisms which is the main goal in biomonitoring

### **Reversing environmental degradation trends in the Nha Trang bay**

In Nha Trang Bay, the Cai and Be rivers discharge into the sea, and the catchments of these two transboundary rivers cover parts of coastal region, and their water and sediment discharges greatly influence the coastal seas. The impact of human activities include changes in the quality of the coastal and marine environments due to the increased use and accumulation of pollutants and the loss of habitats. These impacts have resulted in increasing unpredictability and severity of coastal problems such as floods, erosion, sedimentation, and saltwater intrusion; environmental pollution; and the degradation of ecosystems, with accompanying decrease in biodiversity and fishery productivity (Thanh et al., 2004).

Environmental issues of Nha Trang coast have become more prominent recently mainly due to increasing population and intensifying industry after year of 1997. Among the various contaminants, trace metals are of particular concern due to their environmental persistence, available through biogeochemical recycling and encountering ecological risks. Hence, studies on trace metals influenced by seasonal variability and their concentrations in the coastal area are much needed.

## 2.2.2 Environmental quality

### 2.2.2.1 Water quality

According to the results of monitoring program of University of Fisheries Nha Trang in 2003, the salinity was ranged 25.2-34.0 mg/l, pH: 7.9-8.2, DO: 6.40-7.04 mg/l, BOD<sub>5</sub>: 0.64-2.56 mg/l and PO<sub>4</sub><sup>-3</sup>: 0.05-0.38mg/l except high concentration of total suspended solid TSS: 61.4-106.6mg/l (Tung H and others, 2003) Monitoring program in period 2005-2009 showed an overall excellent water quality of marine zone in Nha Trang bay: Concentration of COD were 11-16mg/l, TSS 5-6 mg/l, N-NH<sub>4</sub> 0.01-0.02mg/l and total oil 0.5-0.7mg/l (MOSTE, 2010). Water quality of Nha Trang Bay is showed in table 2.5 and 2.6.

*Table 2.5 Water quality of Nha Trang Bay*

<b>N</b>	<b>Components</b>	<b>Concentration</b>	<b>References</b>
1	pH	7.9-8.2	Tung H et al., 2003
2	Salinity mg/l	25.2-34.0	Tung H et al., 2003
3	DO mg/l	6.40-7.04	Tung H et al., 2003
4	BOD <sub>5</sub> mg/l	0.64-2.56	Tung H et al., 2003
5	PO <sub>4</sub> <sup>-3</sup> mg/l	0.05-0.38	Tung H et al., 2003
6	COD mg/l	11-16	MOSTE, 2010
7	N-NH <sub>4</sub> mg/l	0.01-0.02	MOSTE, 2010
8	Total oil mg/l	0.5-0.7	MOSTE, 2010

*Table 2.6 Water concentration of some heavy metal in Nha Trang Bay (Son P, 2007)*

<b>N</b>	<b>Metals</b>	<b>Concentration (µg/l)</b>
1	As	0.71 - 1.04
2	Zn	17.4 - 31.4
3	Cd	0.26 - 0.51
4	Cr	1.1 - 3.1

5	Cu	8.9 - 14.5
6	Fe	9.1 - 28.4
7	Hg	0.028 - 0.065
8	Mg	1402 - 1456

#### 2.2.2.2 Sediment quality

Across the fifteen sampling sites were analyzed by Faculty of Aquaculture, University of Fisheries Nha Trang, 2003, the sediment pH were 5.0-6.6 and Organic matter 0.51-2.45%. During the period 2000-2010, the concentrations of heavy metals rapidly increase in sediment samples collected in Van Phong coastal (Khanh Hoa province). The concentration of Cu was 4.2-5.4mg/kg DW in the year of 2001 (Vinh L.T, 2002) but it's was measured about 844.3mg/kg DW in 2010 (Du H.T, 2010). In coastal sediment, there was a considerable rise of the concentration of Pb from 5.7mg/kg DW in 2001 to 25.6mg/kg DW in 2010. In contract, the concentration of As increased sharply, more than twice from 3.9 mg/kg DW in 2001 to 8.6 mg/kg DW in 2010 (Du H.T, 2010). Chemical characteristics of sediment from Nha Trang Bay are showed in table 2.7, 2.8 and 2.9.

*Table 2.7 Chemical characteristics of sediment from Nha Trang Bay*

<b>N</b>	<b>Components</b>	<b>Concentration</b>	<b>References</b>
1	pH	5.0-6.6	Tung H et al., 2003
2	TOC %	0.51-2.45	Tung H et al., 2003
3	Al %	1.67-3.67	UNEP, 1987
4	Ca %	2.6-3.9	UNEP, 1987
5	Fe %	0.20-0.59	UNEP, 1987
6	Na %	0.85-1.28	UNEP, 1987

Table 2.8 Heavy metal concentrations of sediment from Nha Trang samples (Son P, 2007)

Number	Metals	Concentration (ppm)
1	As	5.4 - 8.1
2	Zn	101 – 147
3	Cd	1.45 - 3.04
4	Cr	18.7 - 36.1
5	Cu	13.5 - 23.2
6	Mn	387 – 621
7	Hg	1.59 - 2.78
8	Pb	22.8 - 35.9

Table 2.9 Concentration of metal ( $\mu\text{g/g DW}$ ) in surface sediment at period 1996-2011  
(MOSTE, 2010)

Times	Season	Zn ( $\mu\text{g/g}$ )	Cu ( $\mu\text{g/g}$ )	Pb ( $\mu\text{g/g}$ )	As ( $\mu\text{g/g}$ )	Cd ( $\mu\text{g/g}$ )
Jun1996	D	6,33	8,68	0,19	18,04*	ND
Oct1996	R	6,44	6,19	0,7	5,57	0,2
Sep1996	R	0,97	1,68	0,07	7,1	0,1
Mar1998	D	50,8	26	12,6	3,9	<0.05
Feb1999	D	61,73	12,26	28,11	6,26*	0,38
Jun1999	R	97,24	14,62	29,55	8,33*	0,04
Feb2000	D	99,5	18,3*	36,7*	8,5*	4,8*
Aug2000	R	57,53	10,23	26,09	2,32	Trace
Feb2001	D	52,3	10,6	33,4*	4,05	0,21
Aug2001	R	55,99	12,93	27,14	16,07*	0,104
Feb2002	D	24,8	5,37	15,63	1,53	0

Feb2003	D	56,38	11,19	14,62	4,02	0,07
Aug2003	R	57,4	13,3	4,7	3,19	0,5
Feb2004	D	56,9	12,7	26	3,2	0,07
Aug2004	R	58,64	11,21	36,1*	3,6	0,05
Feb2005	D	66,2	13,6	30,3	2,9	0,05
Aug2005	R	49,5	15,4	26	3,4	0,29
Feb2006	D	48,7	9,2	28,9	3,2	0,04
Aug2006	R	62,8	5,7	26,3	2,6	0,12
Feb2007	D	49,3	11,6	26,7	3	0,25
Aug2007	R	47,5	9,9	25	3,2	0,2
Feb2008	D	56,3	11,9	26,7	5,1	0,13
Aug2008	R	53	7,8	23,7	4,9	0,07
Feb2009	D	45,4	6,7	11,6	0,08	4,5*
Aug2009	R	21,9	4,3	9,2	0,06	2,6*
Mar2010	D	43	11,9	33,9*	3,8	0,7*
Sep2010	R	39,8	11,2	30,7	3,8	0,45
Mar2011	D	44,4	12,1	30,8	3,4	0,3
Aug2011	R	43,2	13,6	34,1*	2,7	0,33
LEL *		120	16.0	31.0	6.0	0.6
SEL*		270	110.0	110.0	33.0	9.0

Location of sampling: Sediment samples collected in Nha Trang port

Note: D: Dry season R: Rainy season ND: Not detected

\* Higher value than LEL: Lowest effect level (ppm)

\*\*Higher value than SEL: Severe effect level (ppm)

### 2.2.3 Biodiversity of bivalve molluscs in the bay of Nha Trang

A total of 490 species of molluscs in 164 genera from 62 families in 2 classes gastropoda (429 species) and bivalvia (68 species) were recorded in Nha Trang Bay. This represents 61% of the total species recorded in Khanh Hoa province (Nghì B.Q, 2011).

Saurin, 1959 published many species of *Pyramidellidae sp.* in Khanh Hoa province, of which 197 species were recorded in Nha Trang Bay. Many species in this family are ectoparasites, feeding on the body fluids of various invertebrates, mainly polychaete worms and other molluscs. The majority of pyramidellids live in subtidal to deep waters and prefer muddy or sandy substrates where annelid worms are abundant (Loi T.N, 1965). Compared with some other areas, species composition of molluscs in Nha Trang Bay was more abundant than Cat Ba – Ha Long Bay (372 species) (Duc N.X, 2001) and Tonkin Gulf (470 species, Cai Yingya et al., 1988).

Differences in geographical location and substrate types (mainland-oceanic gradient) are the major factors contributing to differences in distribution of molluscs. In the littoral, oysters were most numerous on rocks, developing into an oyster belt in the intertidal. Some species of families *Littorinidae*, *Patellidae* and *Turbinidae* were most strongly distributed in the lower littoral zone, particularly on soft substrates, with 27 species recorded in Cua Be estuary in 1999. In the deeper sea, species composition is more abundant and more diverse. In the coral reefs, molluscs have evolved many modes of life, free-living, boring in rocks or corals, in sandy bottom nearby reef. Some specific regional species listed as below:

#### ***Anadara antiquata* (Linnaeus, 1758)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Pteriomorphia*

Order: *Arcoida*

Superfamily: *Arcoidea*

Family: *Arcidae*

Genus: *Anadara*

Vietnam's name: Sò lông

The size structure:

Shell inequivalve, solid, inequilateral, obliquely ovate and elongate in outline, with an extended posteroventral part. Umbones much inflated, situated rather forwards, cardinal area narrow and elongate. About 40 radial ribs (35 to 44) at each valve; ribs usually with a narrow median groove on top, most visible towards the anterior ventral margin of valves in mature specimens. Periostracum coarse and velvety often eroded on umbones. Internal margins with strong crenulations corresponding with the external radial ribs. No byssal gape. Colour: outside of shell greyish white, often stained darker grey on umbonal and posterior areas; periostracum dark brown. Inner side is white, some times light yellow in the umbonal cavity.

Size / Weight / Age: Max length: 6.3 cm TL male/unsexed; common length: 4.0 cm TL male/unsexed;

Environment: Benthic; depth range 0 - 25 m

Climate / Range: Tropical

Distribution: *A. antiquata* is known from islands of the Pacific, Indo-Pacific: Eastern Africa to Japan, Australia, eastern Polynesia and Hawaii. (Poutiers, J.M. 1998)



Picture 1.2 *Anadara antiquata* (Linnaeus, 1758)

***Crassostrea rivularis* (Gould, 1861)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Pteriomorphia*

Order: *Ostreoida*

Superfamily: *Ostreoidea*

Family: *Ostreidae*

Subfamily: *Crassostreinae*

Genus: *Crassostrea*

Vietnam's name: Hàu sũa

The Japanese oyster, *Crassostrea rivularis*, seems to be uniform shell shape and attractive interior shell surface larger maximum size 10.5 cm, common size 7.0 cm, high spawning temperatures

Environment: Benthic, usually 0 - 5 m

Climate / Range: Tropical

Distribution: Western Pacific: Taiwan and China.(Wang.et al., 2004)



Picture 2.3 *Crassostrea rivularis* (Gould, 1861)



***Geloina coaxans* (Gmelin, 1791)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Heterodonta*

Infraclass: *Euheterodonta*

Order: *Veneroida*

Superfamily: *Cyrenoidea*

Family: *Cyreniidae*

Genus: *Geloina*

Vietnam's name: Vòp xanh

Size / Weight / Age: Max length: 10.5 cm SHL male/unsexed; common length 7.0 cm SHL male/unsexed.

Distribution: Indo-West Pacific: from India to Vanuatu; north to southern islands of Japan, and south to Queensland and New Caledonia. Sri Lanka, north Ahungala, SW Sri Lanka Taken on small estuaries (Poutiers, J.M. 1998)



Picture 2.2 *Geloina coaxans* (Gmelin, 1791)

***Glauconome virens* (Linnaeus, 1767)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Heterodonta*

Infraclass: *Euheterodonta*

Order: *Veneroida*

Superfamily: *Cyrenoidea*

Family: *Glauconomidae*

Genus: *Glauconome*

Local name: Phi Cai

Size / Weight / Age: Max length: 7.0 cm SHL male/unsexed; common length: 5.0 cm SHL male/unsexed;

Environment: Benthic; brackish

Climate / Range: Tropical

Distribution: Indo-West Pacific: from Thailand and the Philippines to northern Australia.  
(Poutiers, J.M. 1998)



Picture 2.3 *Glauconome virens* (Linnaeus, 1767)

***Katelysia hiantina* (Lamarck, 1818)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Heterodonta*

Infraclass: *Euheterodonta*

Order: *Veneroida*

Superfamily: *Vereroidea*

Family: *Veneridae*

Genus: *Katelysia* (*Marcia*)

Vietnam's name: Ngêu đen

Size / Weight / Age: Max length: 6.0 cm SHL male/unsexed; common length: 5.0 cm SHL male/unsexed;

Environment: Benthic; depth range 0 - 20 m. Climate / Range: Tropical

Distribution: Indo-West Pacific: from the Gulf of Aden to Papua New Guinea; north to southern Japan and south to Queensland. (Poutiers, J.M. 1998)



Picture 2.4 *Katelysia hiantina* (Lamarck, 1818)

***Lingula unguis* (Linnaeus, 1758)**

Classification:

Phylum: *Brachiopoda*

Subphylum: *Linguliformea*

Class: *Lingulata*

Order: *Lingulida*

Superfamily: *Linguiloidea*

Family: *Lingulidae*

Genus: *Lingula*

Vietnam's name: Duoi heo

Size / Weight / Age: Max length: 10.0 cm SHL male/unsexed; common length: 6.0 cm SHL male/unsexed;

Environment: Benthic; brackish

Climate / Range: Tropical

Distribution: Western Central Pacific: Singapore, Eastern Central Atlantic and Indo-West Pacific. (ABRS, 2000)



Picture 2.5 *Lingula unguis* (Linnaeus, 1758)

***Laternula anatina* (Linnaeus, 1758)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Heterodonta*

Infraclass: *Euheterodonta*

Order: *Anomalodesmata*

Superfamily: *Thracioidea*

Family: *Laternulidae*

Genus: *Laternula*

Vietnam's name: Phi đực

Size / Weight / Age: Max length: 9.0 cm SHL male/unsexed; common length: 6.0 cm SHL male/unsexed;

Environment: Benthic

Climate / Range: Tropical

Distribution: Indo-West Pacific: from India to eastern Indonesia; north to southern Japan and south to northwest Australia (Poutiers, J.M. 1998)



Picture 2.6 *Laternula anatine* (Linnaeus, 1758)

***Perna viridis* (Linnaeus 1758)**

Classification.

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Lamellibranchia*

Order: *Mytiloidea*

Family: *Mytilidae*

Species: *Viridis*

Genus: *Perna*

Common Name: Asian Green Mussel

Vietnam's name: Vẹm xanh



Picture 2.7 *Perna viridis* (Linnaeus, 1758)

Distribution:

The bivalve *Perna viridis* is an economically important mussel and a native to Asia-Pacific region where it is widely distributed. The *P.viridis* is found in the coastal waters of the Indo-pacific region (Benson and others, 2002). It is harvested for food but is also known to harbor toxins and cause damage to submerged structures such as drainage

pipes It is primarily found in estuarine habitat with salinities ranging from 18-33 ppt and temperatures from 11-32<sup>0</sup>C. *Perna viridis*

The size structure:

*Perna viridis* is a large mussel, 80-100 mm in length, occasionally reaching 165 mm (Rajagopal and others, 1998). The shell has a smooth exterior surface characterized by concentric growth lines and slightly concave ventral margin with a bright green colour in young and fading to brown with green edges as it matures.

Characteristic:

The sexes of the species are separate and fertilization is external. Spawning generally occurs twice in the year (Chatterji and others, 1984). Fertilized egg develops in to larvae and remains in the water column for two weeks. During this planktonic period, larvae will be widely dispersed by physical processes, but may aggregate periodically at certain depths through a variety of biological processes, most notably vertical migration (Hayes and others, 2005). The larvae completely metamorphose in eight to twelve days of growth. Sexual maturity typically occurs at 2-3 months of age with a length of about 15-30mm (Benson and others, 2001). *Perna viridis* has the greatest growth rate of the mussels studied, which have a life span of about 2-3 years (Shafee, 1979). Maximum growth of the green mussel occurs 2m below the surface because of the increased productivity of the water at that depth and a narrow area of temperature and salinity fluctuation (Sivalingam, 1977).

*Perna viridis* is commonly available, popular and most consumed seafood in Khanh Hoa region. It is available throughout the year and consumes by making various preparations.

### ***Solen regularis* (Dunker, 1862)**

Classification:

Phylum: *Mollusca*

Class: *Bivalvia*

Subclass: *Heterodonta*

Infraclass: *Euheterodonta*

Order: *Euheterodonta*

Superfamily: *Solenoidea*

Family: *Solenidae*

Genus: *Solen*

Vietnam's name: Ốc móng tay



Picture 2.8 *Solen regularis* (Dunker, 1862)

***Tapes literatus* (Linnaeus, 1758)**

Classification:

Phylum: Mollusca

Class: Bivalvia

Subclass: Heterodonta

Infraclass: Euheterodonta

Order: Veneroida

Superfamily: Vereroidea

Family: Veneridae

Genus: *Tapes*

Vietnam's name: Ngêu lũa



Size / Weight / Age: Max length: 10.8 cm SHL male/unsexed; common length: 8.5 cm SHL male/unsexed;

Environment: Benthic; depth range 0 - 20 m

Climate / Range: Tropical

Distribution: Indo-West Pacific: from East and Southeast Africa, to Melanesia; north to southern Japan and south to Queensland and New Caledonia. (Poutiers, J.M. 1998)



Picture 2.9 *Tapes literatus* (Linnaeus, 1758)

### 2.3 SELECTION OF SAMPLING SITES

The 4 sampling locations were selected based on differences in physicochemical conditions, contamination status, varied anthropogenic pressure and feasibility of sampling. These sites still sustain rich biodiversity and were selected to be far from direct discharges. The characteristics of the 4 studied locations were:

Location 1 (Tan Dao –TD): is characterized by dense mangroves, is located between Nha Trang and Nha Phu Bay. During the past 30 years, a considerable decline in mangrove stands occurred, threatening their survival in this area. This area far removed from obvious sources of metal pollution, with no appreciable human activities. Hence, this location was chosen as a reference site to provide information on natural metal levels in non contaminated macrobenthic.



Picture 2.10 Tan Dao - TD sampling site

Location 2 (Ngoc Diem – ND): Is located 15km from the North of Nha Trang bay, which receives agricultural effluents from the hinterlands predominantly which is an embayment influenced by strong tidal currents, a rare phenomenon in the Nha Trang bay.



Picture 2.11 Ngoc Diem – ND sampling site

Location 3 (Binh Tan - BT): The Be estuary is situated on the Southern coast of Nha Trang City (BT), which dump waste directly into the ocean. This is a estuary which is an almost enclosed ‘polluted’ area near Nha trang port receive disposal effluents of domestic sewage water as well as waste disposal through boats and ships and is affected by several activities of the Nha Trang City.



Picture 2.12 Binh Tan – BT sampling site

Location 4 (Cam Hai Tay – CL): This area is under direct anthropogenic influence urban sewage water as well as effluents from the agricultural activities, chemical industries, are affected by the over all industrialization and urbanization of the northern. The lagoon has less tidal influence with minimal marine influence. This is an area, which is also receiving the runoff of an influenced agricultural area and receives large amounts of agricultural effluents from the northern.



Picture 2.13 Cam Hai Tay – CL sampling site

## CHAPTER 3

# RESEARCH METHODOLOGY

### 3.1. SAMPLING TIMES AND SAMPLING SITES

One of the objectives of this study was to obtain a dataset of trace metal concentrations in bay of Nha Trang. Sampling density was adapted to assumed points of anthropogenic inputs and characteristic of the Nha Trang bay. In order to achieve the objective, surface sediment and mollusk samples were collected from 4 stations: Tan Dao (Ninh Loc, Ninh Hoa) – TD, Ngoc Diem (Ninh Ich, Ninh Hoa) – ND; Binh Tan (Nha Trang) – BT and Cam Hai Tay (Cam Lam) – CL.

Map of the sampling sites was showed in Figure 1. Their accurate positions were determined by GPS localities instrument with the accuracy of  $\pm 5\text{m}$  as is shown in Table 3.1.

Table 3.1 Localities of sampling sites

<b>NO</b>	<b>Samples</b>	<b>Northing</b>	<b>Easting</b>	<b>Remak : Sampling sites</b>
1	TD	12°26'17"N	109°08'14"E	Hoc Bo Dia, Ninh Loc, Ninh Hoa
2	ND	12°23'42"N	109°11'57"E	Ngoc Diem, Ninh Ich, Ninh Hoa
3	BT	12°12'22"N	109°11'17"E	Be estuarine, Binh Tan, Nha Trang
4	CL	12°04'45"N	109°10'42"E	Cam Hai Tay, Cam Lam

The bivalve mollusk and sediments were collected twice a year during the dry season and rainy season of 2 years 2012 - 2013 from 4 locations of the bay in low tide time of the day as shown in Table 3.2.

Table 3.2 Sampling dates

<b>Year</b>	<b>Season</b>	<b>Date</b>
2012	D	From 30 March to 14 Avril 2012
2012	R	From 15 to 24 September 2012
2013	D	From 22 to 28 March 2013
2013	R	From 20 to 27 September 2013

Common physicochemical parameters like pH, temperature, moisture, grain size, TOC; essential trace metals like zinc, copper, aluminum, iron, and non-essential arsenic, lead, cadmium, and chromium were estimated to know their levels present in the surface sediment and mollusk tissues.

### **3.2. ANALYZING METHODS**

The quality and quantity of sampling size, site selection and sample analysis methods for each of these parameters study is based on an International Standard Analytical Methods.

#### **3.2.1 Sediment samples**

Figure 3.1 shows the sampling techniques and processing of sediment samples for different analyses.

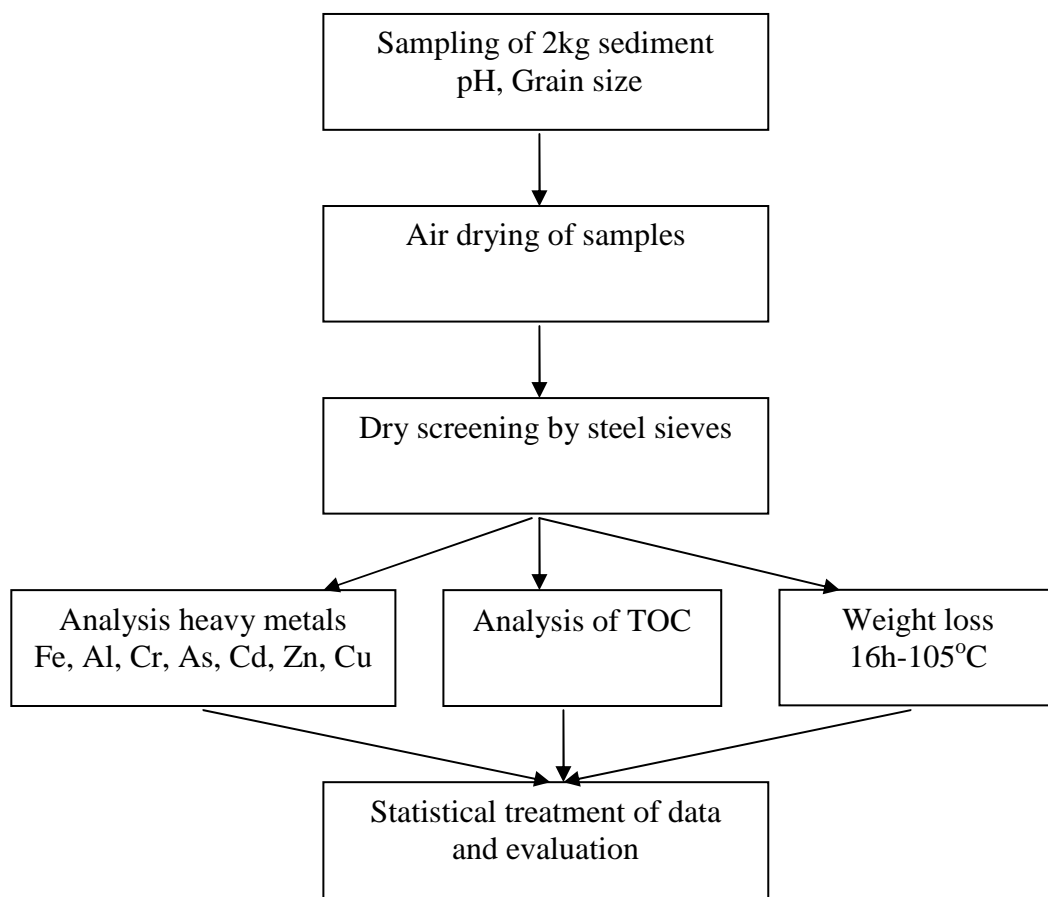


Figure 3.1 Sampling and evaluation scheme of sediment

### ***3.2.1.1 Method of collection sediment samples***

The uppermost 15cm of surface sediment was sampled using a grab sampler. Sub-samples were taken from the central part of the grab to avoid contamination. Further more, the state of the sediment surface was inspected to make sure it was relatively undisturbed (i.e., lack of channeling or sample wash out) and that the desired penetration depth was achieved. All sampling equipment (e.g., scoops, containers) was made of non contaminating material cleaned before and after each sample. If all the sampling criteria were met, the surface sediment was subsampled by a flat scoop. This device will allow a relatively large sub sample to be taken accurately to a depth of 2cm. This procedure avoided any sample pollution during collection. All objects coming in contact with the sample were made of glass or stainless steel. To avoid pollution, all sampling equipment had been cleaned in sequence with local seawater, chromatographically pure acetone and

methylene chloride prior to initial use and between uses at each station. Finally, the sediment samples were placed carefully into acid-rinsed glass vials via a stainless spatula and then stored at -4°C before analysis to insure undisturbed samples and eliminate cross contamination.

**3.2.1.2 Preparation and storage of sediment samples**

Using plastic container for storage sediment samples and frozen at -4°C to be transported to the laboratory of Environmental faculty of HCMUS. Here, the sediment samples were analyzed some physicochemical parameters as pH, Total organic carbon, moisture and grain size. There sediment samples were dried at room temperature then pulverized to fine powder using an agate mortar and pestle and then sieved through a 2mm mesh sieve to remove large particles. The samples were stored at -20°C until they were transported to the ECOMERS laboratory (UNS-France) for further analyses.

**3.2.1.3 Analyzing physico - chemical characteristic**

**Grain size analysis**

The grain size of the sediment samples was determined by standard sieving method for grain size >0.063mm and by pipetting for particle <0.063mm according to Casagrande method (Strmac, 1952). Fifty grams from dried sediment were prepared and then sieved to produce six sediment particle size fractions, starting with particles smaller than 2mm in diameter. The percentage of a given particle size in the sample, on a mass basis, was then calculated the size distribution of sediment and classification according to the Shepard classification (Shepard, 1954): fraction of sand, silt, clay or silt clay in table 3.3.

Table 3.3 Grain size classification

<b>N</b>	<b>Sediment main (%)</b>	<b>Grain size (mm)</b>
1	Large particular	2.0-1.0
2	Medium coarse sand	1.0-0.5
3	Fine medium sand	0.5-0.25
4	Very fine sand	0.25-0.125
5	Very fine sand and mud	0.125-0.1

6	Fine silt	0.1-0.0625
7	Silty clay	0.0039-0.0625
8	Clay	<0.002

### **pH**

The measuring the pH (H<sub>2</sub>O) of sediment following to ASTM D5464 – 11 Standard Test Method. Use a 1:5 dilution of sediment: water, and then take a pH measurement on the resulting solution with a laboratory meter. Use the 1:5 dilutions, but instead of water they use a dilute Kalium Chloride (KCl) solution and then take a pH (KCl) measurement on the resulting solution with a laboratory meter.

The pH measured in KCl is almost always lower than pH of the same soil measured in water due to the higher concentration of H<sub>+</sub>. The procedure gives a value similar to that for natural sediment solution because the sediment solution also contains dissolved K<sub>2+</sub> and other ions

### **Moisture content determination**

Standard procedures (Method ASTM 2974-87) were adopted for determination of moisture contents. Moisture was determined by two steps: One gram of the sediments was dried in air at room temperature then oven drying them at 110°C in 16h to constant weight. Moisture contents were measured by the weight loss as a percentage of the dry soil weight.

$$MC\% = \frac{\text{weigh wet soil} - \text{weigh of oven dry soil } 110^{\circ}\text{C}}{\text{weigh of wet soil}} \times 100\%$$

TOC Total organic carbon

### **Organic matter determination**

Organic matter was determined by ash dried sediments in the furnace to 750°C and hold until the samples completely ashed. (Method ASTM, 2974). The loss of weight after treatment was assumed to be due to the organic matter content

$$MC\% = \frac{\text{weigh dry soil of oven } 750^{\circ}\text{C}}{\text{Weigh of dry soil}} \times 100\%$$



### **Bulk density determination Db**

Soil bulk density is a measure of how dense and tightly packed a sample of soil is. It is determined by measuring the mass of dry soil per unit of volume (g/ml or g/cm<sup>3</sup>). Soils made of minerals will have a different bulk density than soils made of organic material. For dry density determination, 10 pots were filled with sediment samples and were to constant weight at 70°C. Dry bulk density was calculated as dry weight divided by wet volume. Soil Bulk Density is the dry mass of a soil divided by its volume. Bulk density value for soil is expressed as follows:

$$Db = (\text{Oven dry soilg/Total soil volume}) \text{ g/m}^3$$

Bulk density related to OC content (Y Avnimelech 2001) and show structure of mineral aquatic bottom sediment as mineral particles by organic micelles.

$$Db \text{ (g/m}^3\text{)} = 1.776 - 0.363\text{Log}_e\text{OC (R}^2\text{=0.70)}$$

$$Db=2.65 \text{ g/m}^3: \text{ inorganic sediment}$$

$$Db=1.25 \text{ g/m}^3: \text{ lighter organic matter}$$

$$Db=0.01-0.1 \text{ g/m}^3: \text{ hydrated microbial biofilm}$$

### **Carbonate content: CO<sub>3</sub>**

The carbonate content was determined as weight loss after the treatment with 4 M HCl (Loring et al, 1992).

#### ***3.2.1.4 Sediment digestion method***

Dried sediment samples were sieved through a nylon mesh to obtain particles smaller than 0.2mm in diameter for determination of heavy metal concentration. Schema of sediment digestion method show in figure 3.2.

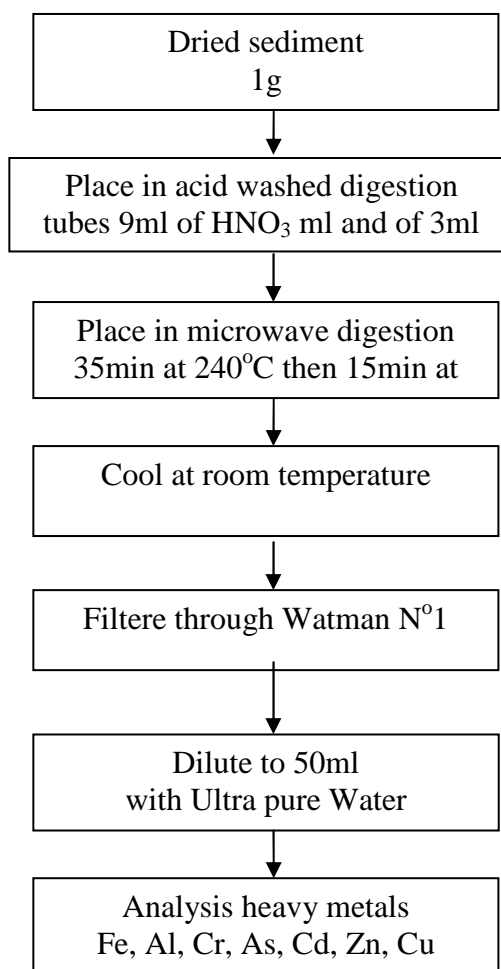


Figure 3.2 Schema of sediment digestion method

The samples were digested following the EPA guideline for siliceous sediments (EPA 3050b, 1996) for digestion of sediments using an Automatic Microwave Digestion System (Microwave 3000). A 0.1 gram of dried and sieved sediment samples were weighed out in a Teflon (R) reaction vessel then added with mixed of 9ml concentrated acid HNO<sub>3</sub> (69%) and 3ml of HCl (36,46%). Vessels then were placed in the rotor in the microwave. The vessels were heated to at least 240°C over 35 minutes and then held at 210°C for at least 15 minute. The samples were cooled and then filtered to remove insoluble materials, before dilution to volume to 50 mL with ultra-pure water (Figure 3.2). The solution in preparation of being analyzed the concentration of heavy metals measured using Perkin Elmer inductively coupled plasma optical mission spectrometer ICP-OES (Picture 3.1).



Picture 3.1 ICP – OE Spectrometer for digestion samples

Metals as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nikel (Ni), molipden (Mo), and zinc (Zn) as well as iron (Fe) and aluminum (Al) are all considered to be elements of concern to study, verified by literature reports.

The accuracy of the analytical procedures employed for the analysis of metals in sediment samples was checked using the marine sediment IAEA-433 as certified reference material (Appendix 4.1)

### **3.2.2 Mollusk samples**

All the mollusks examined in this study are commercial species which have sedentary life style, filter-feeder invertebrates and have the ability to accumulate metals from the surrounding environment. Mollusk samples were identified to species level by Biological department of HCMUS. Figure 3.3 shows the sampling techniques and processing of determination of traces of metal in organisms

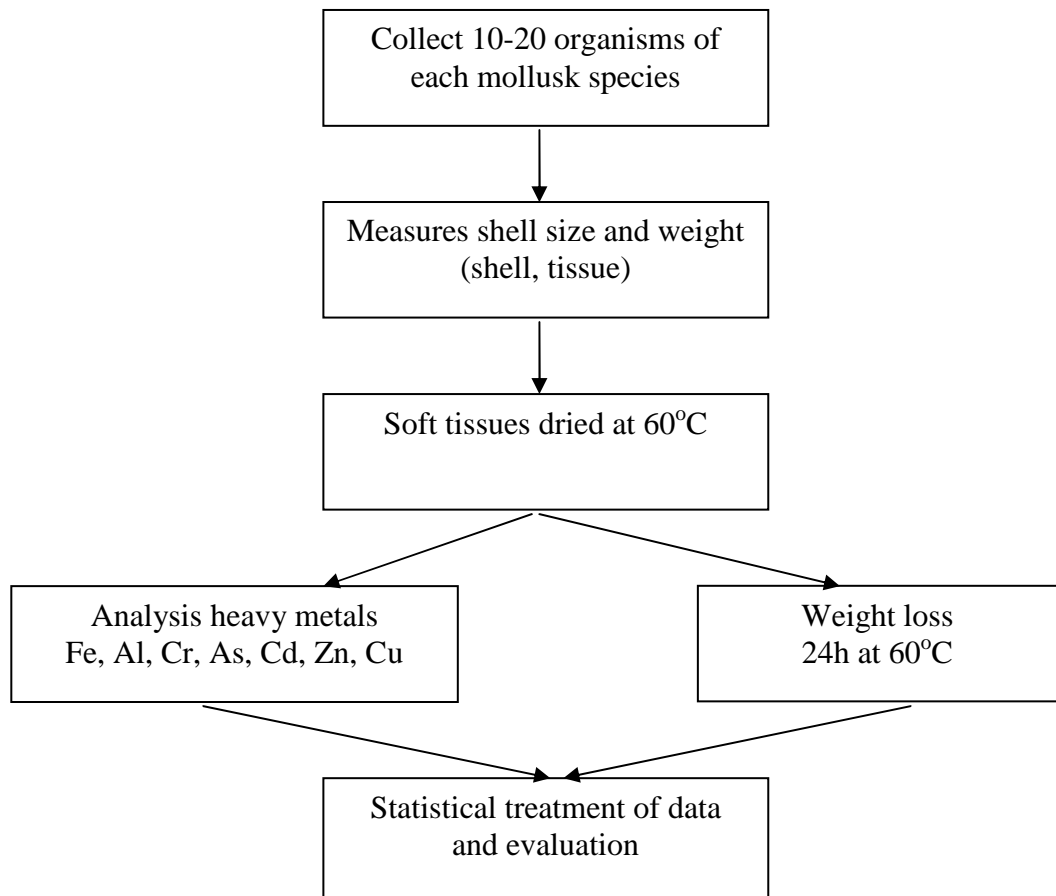


Figure 3.3 Sampling and evaluation scheme of mollusks

### 3.2.2.1 Collecting mollusk samples

About 10-20 number of big size organisms of each mollusk species were collected by hand with the help of local fishermen in low tide time of the day (Table 3.4). Samples were collected of the same size for mollusks through out the work to reduce possible variations in metal concentrations due to size and age and to assure sampling of mature specimens. The mollusks were rinsed with seawater from their sampling locations then put them in Ice box to transport in the laboratory of HCMUS. Some picture of collecting samples on site shown in Appendix 2.

Table 3.4 Mollusk species and collected numbers of them during 2012/2013

N	Species	TD		ND		BT		CL	
		D 2012/2013	R 2012/2013	D 2012/2013	R 2012/2013	D 2012/2013	R 2012/2013	D 2012/2013	R 2012/2013
1	<i>Anadara antiquate</i>	10/15	6/10	11/10	15/17			9/8	
2	<i>Crassostrea rivularis</i>	15/10	10/5			8/10	2/5		
3	<i>Glauconoma virens</i>			3/5	6/10	10/15	15/10	15/20	15/12
4	<i>Geloina coavans</i>	3/10	13/15	9/10	6/10			5/10	10/16
5	<i>Katelsia hiantina</i>	9/15	15/13	17/20	10/7	20/18	20/14	18/20	12/15
6	<i>Lingula unguis</i>	15/10	15/10			15/20	10/8	20/23	15/10
7	<i>Laternula anatine</i>					2/5	5/2	5/10	5/3
8	<i>Perna viridis</i>	20/15	10/5						
9	<i>Solen rivularis</i>					10/15	2/5	15/11	15/13
10	<i>Tapes literatus</i>			15/11	10/8				

### ***3.2.2.2 Preparation of tissue samples***

In the laboratory, all samples were removed from the plastic containers and washed with running distilled water. The whole soft tissues were removed from their shells and prepared for analysis. Mollusks were placed on a critically cleaned glass cutting board, and a scalpel was used to open the shell and remove the soft tissue from the shell (Picture 3.2). Tissue of mollusks were analyzed as a composite of 5-10 animals because tissue mass was small. Once all individuals that comprised a composite had been cleaned and weighed, they were placed in a stainless steel blender cup and dry at 60°C to constant weight. The dry sample was then placed in a labeled plastic bag, which was then capped and frozen until transferred to the Ecomers Laboratory UNS for analysis.



Picture 3.2 Measurement of mollusk samples

### ***3.2.2.3 Sample measurement***

#### **Moisture analysis**

Moisture analyses were conducted on all samples in duplicate at laboratory HCMUS. Three individuals from each mollusks species sample were placed into each of three aluminum weighing pans. Moisture was determined by measuring the difference between sample mass before and after drying in an oven at 60 degrees for more than 24 hours. Percentage moisture can be used to compute metal concentrations in the tissue on a dry weight basis.

#### **Body size measuring**

Each individual sample was classified, weighed and the weight was recorded on the sample composite form. Samples were measured using caliper 0.1mm for length, wide of shell and digital balance 0.01g for weight measurement

### ***3.2.2.4 Digestion method of mollusks***

Processing of the samples for analysis began in 1 month after collection. Determination of heavy metal concentration in mussels tissues according to Digestion method of organically based matrices (EPA 3052).

Before beginning the processing of the mollusks samples, in preparation for metals analysis, the equipment was cleaned using the method described in EPA 3052. The twelve individual mollusk samples from each sampling site were composited into a single sample by combining portions of each species into a common sample used for analysis of metals.

The dried tissues were powdered, and aliquots of 0,5g were digested for 30min at 95°C with 9ml HNO<sub>3</sub> (65%, Merck) in closed beaker. Additional nitric acid was added if the samples were evaporated to near dryness. After cooled, 3ml HCl (Merck) then 0.5ml of H<sub>2</sub>O<sub>2</sub> were added and heated for few minute to make the solution clear. The digests were cooled and diluted to 50ml with ultra pure water and metals were analyzed (Figure 3.4).

Digested tissues were analyzed for the same heavy metals as in sediment using the same procedure using an ICP-OES (Perkin Elmer). All metal concentrations in the tissues are reported in mg/kg dry weight. The precision and accuracy of analysis was checked by replicate measurements (n=5) of target metals in a standard reference material of marine biota sample MAB3



Picture 3.3 Digestion of mollusk samples by hot plate

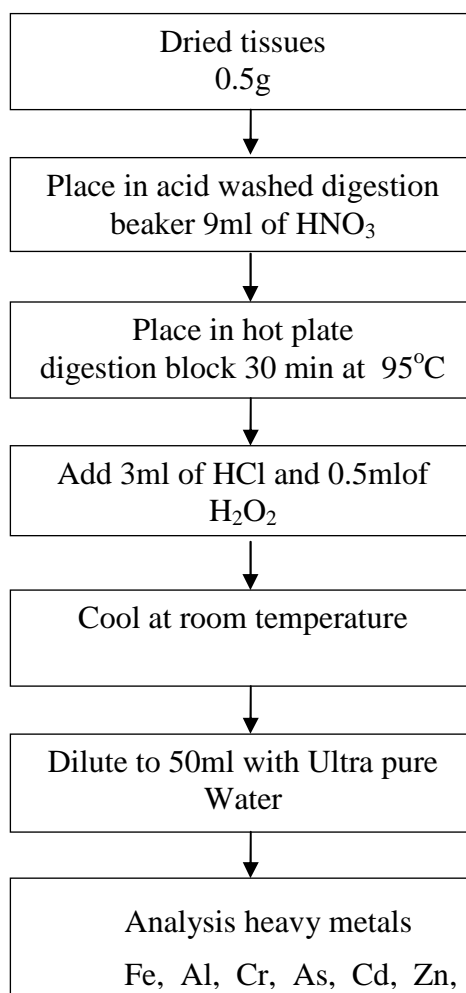


Figure 3.4 Schema of mollusks digestion method

### 3.2.3 Quality Assurance of analysis



### ***3.2.3.1 Standard solutions***

Standard solutions of known concentrations were prepared from purchased certified solutions. A standard curve was prepared each day of analysis using the standard solutions. Metal standards were made as sets of five concentrations plus a reagent blank with one set run at the beginning of the analysis and another full set analyzed with each set of forty samples. Three absorbance readings are taken for each sample by the instrument, with the reported concentration being an average of those readings to ensure accuracy of standards and calibration curve.

### ***3.2.3.2 Lab control***

Quality of analysis was monitored by several methods during this study. Analysis of reagent blanks determined if reagents contained appreciable quantities of metals or if contamination occurred during the sample preparation.



Picture 3.4 ECOMERS laboratory

Reagent blanks were processed with each digestion set by completing the digestion and analysis procedure on samples containing only reagents. This was done to determine if reagents contribute measurable quantities of the metals in question or if contamination is added during the digestion. Arsenic, cadmium, chromium, copper, lead and zinc were all below the Limit of Detection (LOD) or within the Limit of Quantitation (LOQ) in the

reagent blanks. LOQ is defined as 10/3 of the LOD. Both reagent blanks for iron and aluminum had values above the LOQ.

Analysis of blanks showed significant amounts of iron and aluminum compared to the reference material and would account for the high percentage agreements for these metals.

### ***3.2.3.3 Certified reference materials***

Accuracy is measured by analyzing certified reference standards IAEA – 433 for marine sediment and mussel tissue with MAB3 reference material (Appendix 4.1)

#### **Quality assurance for sediment analysis**

Reference material IAEA-433 was purchased to use as a reference standard for determining method accuracy. The marine sediment was prepared from the International Atomic Energy agency, Analytical Quality Control Services, A-1400 Vienna, Austria.

Metal concentrations measured in reference sediments following sample digestion procedures were in general agreement with expected values with a low mean agreement of 77.95% for magnesium and a high agreement of 319, 57% for manganese. The acceptable recoveries for sediment sample ranged from 88.52 % for arsenic to 114.19 % for lead (Appendix 4.1).

The Standard Reference Material IAEA- 433 certified concentrations of arsenic, cadmium, chromium, copper, zinc, and lead in sediment for method analysing. Certified values were not provided for nickel, molybdenum, magnesium and manganese which the method using.

#### **Quality assurance for mollusks analysis**

Mussel tissue was also purchased to use as a reference standard for determining method accuracy. The mussel tissue was purchased from the International Atomic Energy agency, AQCS, Laboratory Seibersdorf, A-1400 Vienna. The Standard Reference Material MA-B-3 was prepared from Lyophilised Tissue from the coast of Monaco. The values obtained for the reference material were in comparison with the certified values (Appendix 4.2).

The MA-B3 sample was analyzed as a quality assurance measure for metal in tissue. The sample has a recommended concentration of 2.11 (1.42-2.51)  $\mu\text{g As/g}$  of tissue. Agreements with the analyzed concentrations were  $2.43 \pm 0.78$  for 3 analyses. Again, measured values were in general agreement with certified values with a good agreement for Cu, Pb, Zn and Fe where in reference sample, recovery ranged from 95.87 % for zinc to 116.23 % for copper (Appendix 4.2).

In conclusion, the used method certified a good quality assurance for measure concentrations of arsenic, copper, iron, lead, zinc and aluminum in tissue. Mn and magnesium had recovery percentages ranging from 148.77 to 473.45 % which are both unreasonable and should be ignored. Certified values were not recommended for chrome, cadmium, nickel, molybdenum and aluminum.

#### ***3.2.3.4 Detection limit of the method***

Data are reported in one of three categories. Some samples sediment concentrations below the Detection Limit (DL) of the method. When this happens, the concentration for that sample is reported as a “less than” numerical value. Some data were measured above the detection limit (DL), but are less than ten-thirds of the detection limit and are marked as data between the DL and the Limit of Quantitation (LOQ). There is a lower confidence with values between the DL and LOQ than those above the LOQ. The third categories of data are values above the LOQ. Proposed general limit of heavy metals analysis method as show in Appendix 5

The sequence of metal concentration varies with the distance between stations and seasons. An overall view of the result indicated that the heavy metals Fe, Al, As, Zn, Ni, Pb, Cr, Cu, Cd and Mo concentrations were above the permissible limit. The highest metal levels estimated along the shoreline of the Be river, reflecting long-term exposure to various activities. Fe is the most abundant metal, whereas arsenic and chromium present in minimum concentration when compared to other metal's concentrations. The range of variation for each metal is unique; their concentration is not restricted only by anthropogenic input. The results suggest that complex combination of factors act on surface sediment thereby increase the metal concentration. If this valid point consider in future for bay of Nha Trang possible influences on metal accumulation in surface sediment may be worked out and follow up monitoring programmer may be initiated.

### 3.3 DATA ANALYSIS METHODS

#### 3.3.1 Method assessment of heavy metal contamination in sediment

##### 3.3.1.1 Metal assessment indices

##### Degree of contamination Cd

Contamination indices Cd, which compare the contaminants with clean and/or polluted stations, measured in the study area or simply aggregate the metal concentrations.

Contamination indices calculate as suggest by Hakason. L, 1980 as:

$$Cd = [\sum C_f]/n$$

Where: n: analysed element

i: element

Cj: contamination factor

Degree of contaminations is calculate as equation below:

$$C_f^i = \frac{C_{0-1}^i}{C_n^i}$$

Where Co – 1: mean content of metals from at least 5 sampling sites

Cn: concentration of element as a reference value

Gradation of Contmination indices Cd shows in table 3.5

Table 3.5 Gradation of Contmination indices Cd

N	Cd values	Classification of contamination
1	$1.5 \leq Cd < 2$	Low degree of contamination
2	$2 \leq Cd < 4$	Modarate degree of contamination
3	$4 \leq Cd < 8$	High degree of contamination
4	$8 \leq Cd < 16$	Very high degree of contamination
5	$16 \leq Cd < 32$	Extremely high degree of contamination
6	$Cd \geq 32$	Ultra high degree of contamination

##### Enrichment factor EF

Application of enrichment factor to the interpretations of result from the biomonitoring studies. EF is ratio of chemical concentration of an element in a soil or

sediment to that in its fresh parent material (Battelle 2003) (Hakanson, L 1980).  
 Calculating enrichment factor EF as

$$EF = \frac{([As]/[M]) \text{ sample (sediment)}}{([As]/[M]) \text{ background (crust)}}$$

[Hg]: Concentration of metal ex. As  
 [M]: Al or Fe concentration

Interpreting of EF as shows in Table 3.6. (Grousset et al, 1995)

Table 3.6 EF categories

	EF values	Categories of enrichment
1	<1.5	Crustal material, natural weathering
2	<2	Deficiency to minimal enrichment, considered to be lithogenic origin for a metal
3	2-5	Moderate enrichment, anthropogenic component input in the area or biogenical enrichment proces
4	5-20	Significant enrichment, anthropogenic component input in the area or biogenical enrichment proces
5	>40	Extremely high enrichment, anthropogenic component input in the area or biogenical enrichment proces

**Igeoaccumulation index**

Application of Igeoaccumulation index for classes metal enrichment propose by Muller, 1996.

$$I_{geo} = \log_2[C_n / 1,5 B_n]$$

Where: 1,5: Factor of enrichment due to lithological variation

C<sub>n</sub> Concentration of element in sediment

B<sub>n</sub> Concentration of element in background value

Table 3.7 shows classification of Igeo according to Muller, 1996)

Table 3.7 The 7 grades of classes Igeo

	<b>Igeo value</b>	<b>I geo class</b>	<b>Designation of sediment quality</b>
1	>5	6	Extremely contaminated
2	4-5	5	Strongly to extremely contaminated
3	3-4	4	Strongly contaminated
4	2-3	3	Moderaly to strongly contaminated
5	1-2	2	Moderaly contaminated
6	0-1	1	Uncontaminated to moderaly contaminated
7	0	0	Uncontaminated

If Igeo = 6: 100 fold enrichment about background value

### **3.3.1.2 Sediment Quality Guidelines (SQGs)**

As the mean to predict adverse effect derived from contaminated sediments numerous sediment quality guidelines (SQGs) were developed by overseas researchers. Basically, the purpose of SQGs development is to protect the aquatic and benthic organisms from the adverse effects of sediment contaminations. SQGs are also being used for prioritizing contaminated areas, evaluating contamination spatial patterns as well as for designing a monitoring programmer. Biological affects are not able to be predicted from metals concentrations in sediment. As an alternative, adverse ecological effects are identified and being used as the reference in the SQGs. The adverse ecological effects are attributable to sediment-borne metals concentrations. Among the most widely applied SQGs are as following:

- Effect-range guidelines (Long et al., 1995) - National Oceanic and Atmospheric Administration (NOAA), US
- Provincial Sediment Quality Guidelines for Metals and Nutrients (Persaud et. al., 1992)  
-Ontario Ministry of the Environment, Canada

- New York Sediment Screening criteria (1999) - Department of Environmental Conservation, New York
- Interim Sediment Quality Values (ISQVs) (Chapman et al., 1999). Hong Kong Special Administrative Region

These guidelines are summarized in Table 3.8 according to their terms and application.

Table 3.8 Sediment quality criteria

N	Institutions SQGs	Criteria and application
1	Hong Kong Special Administrative Region Interim Sediment Quality Values (ISQVs)	SQV low and SQV high (mg/kg) 1996. Seabed dredged sediment
2	Canada Ministry of Environment Ontario Provincial SQGs for Metals and Nutrients	LEL and SEL Freshwater sediment metal guidelines, 1996
3	US NOAA Effect-range guidelines NOAA	ER-M and ER-L, 1995
4	New York Dept. of Environmental Conservation New York Sediment Screening Criteria	LEL and SEL, 1999

### Effect-Range Guidelines

Effect-range guidelines is one of the most widely applied SQGs for estuarine and marine location. These SQGs was developed by National Oceanic and Atmospheric Administration (NOAA) in the United States of America (Longet al, 1995). This set of guidelines adopted the terms of effect-range-low (ER-L) and effect-range-median (ER-M). Effect-range-low (ER-L) illustrates 10th percentile of chemical concentrations corresponding to the adverse biological effect; while effect-range-median (ER-M) for 50<sup>th</sup> percentiles of chemical concentrations are corresponded. Three ranges in chemical concentration are evaluated using these terms:

Adverse effects were rarely observed < ER-L

Adverse effects were occasionally observed ≥ ER-L and < ER-M

Adverse effects were frequently observed  $\geq$  ER-M

The guidelines were derived for 25 chemicals (9 trace elements, 13 PAHs, 2 OCs and total PCB). Appendix 6.1 is a summary of ER-L, ER-M and Overall Apparent Effects Thresholds concentrations for selected chemicals in sediment (dry weight) (Long et al., 1990). These effect-range guidelines can be applied to both fresh and salt water sediments.

### **Provincial Sediment Quality Guidelines for Metals and Nutrients**

These guidelines were developed by Ontario Ministry of the Environment in Canada. They were derived by the Screening Level Concentration approach. This approach uses field data on co-occurrence of benthic animals and Contaminants (Persaud et al, 1992). Terms used to describe the sediment contamination conditions in these guidelines are Lowest Effect Level (LEL) and Severe Effect Level (SEL). Appendix 6.2 shows the Provincial Sediment Quality Guidelines for Metals and Nutrients. The values are in  $\mu\text{g/g}$  (ppm) dry weight unless otherwise noted by Persaud et al, 1992. These guidelines were meant for the application of metal concentration in fresh water sediment.

### **New York Sediment Screening Criteria**

Both Persaud's and Long and Morgan's SQGs were integrated to become the New York Sediment Screening Criteria. Two levels of protection were established following the Ministry of Ontario Guidelines. These levels are Lowest Effect Level (LEL) and Severe Effect Level (SEL). The LEL for each metal is the lowest of either the Persaud et al. (1992) LEL or the Long and Morgan (1990) ER-L. Similarly, the SEL for each metal is the lowest of either the Persaud et al. (1992) SEL or the Long and Morgan (1990) ER-M. Three ranges were defined for these levels:

- |                      |   |
|----------------------|---|
| $<$ LEL              | the effect in the sediment is considered to be acceptable |
| $\geq$ LEL & $<$ SEL | contaminated, moderate impacts to benthic life            |
| $\geq$ SEL           | contaminated and significant harm to benthic aquatic life |

Appendix 6.3 shows the New York Sediment Criteria for Metals proposed by Department of Environmental Conservation, New York, 1999. The units are  $\mu\text{g/g}$ , or ppm, except for iron, which is listed as a percentage. An "L" following a criterion means that it was taken from Long and Morgan, 1990; a "P" following a criterion indicates that it is from Persaud et al., 1992.



## **Interim Sediment Quality Values**

ISQVs from Hong Kong are meant for assisting in decision-making of dredged seabed sediment disposal by contamination level, they are adapted in this study as an extra reference. ISQVs displayed high similarity with other SQGs mentioned in above in terms of characteristics. However, there are differences in terms of contamination effect values. The contaminants range values in ISQVs are expressed as ISQV-low and ISQV-high. The contamination values below ISQV-low indicate adverse biological effects are unlikely, while values above ISQV-high indicate that severe adverse biological effect are very likely as a consequence of sediment contamination.

### **3.3.2 Evaluation of bioaccumulation**

The methods used in this study evaluate potential bioaccumulation by:

- Using the metal pollution index with mollusk tissues data
- Comparing the estimated or measured concentration of metals in tissue to acceptable tissue levels (ATLs) for humans and relevant classes of wildlife and/or to critical tissue levels (CTLs).
- Calculating bioaccumulation factor BSAF with site-specific benthic invertebrate tissue data.

#### ***3.3.2.1 Calculation of metal indices***

##### **Metal pollution index (MPI)**

MPI is a mathematical model which enables the presentation of all results from the trace metals concentrations in the muscle tissue of bivalve as one value to classify the biological variation from an unpolluted zone to a highly polluted zone. The overall metal content of bivalves at various sites investigated in this study was compared using the metal pollution index (MPI) calculated with the formula (Usero et al., 1996)

$$\text{MPI} = (\text{Al} \times \text{Fe} \times \text{Zn} \times \text{Cu} \times \text{Cr} \times \text{As} \times \text{Cd})^{1/7}$$

The MPI value is used for screening and estimating the spatial and temporal variation of trace metal pollution based on the metal content within muscle tissues of bivalves. The available data are of particular interest concerning filter-feeding bivalves

which are well known for their ability to reflect environmental levels of trace metal contaminants in estuarine ecosystems. According to Usero et al., 2005 reported that MPI values (based on Cu, Pb, Zn, Ni, Cr and Cd) in the bivalve *Donax trunculus* ranged from approximately 3.4 to 3.8 and from approximately 6.8 to 7.8 for unpolluted and polluted areas, respectively, from the southern Spanish Atlantic coast.

The MPI values for mollusks reported in this study broadly agree with the MPI value (based on metals Fe, Mn, Zn, Cr, Cu, Pb, Ni, Cd and Ag) range of 10.5–25.1 reported for the mussel *Mytilus edulis* from the coast

**Metal/shell weight indices (MSWI)**

The metal/shell weight index (micrograms of metal per gram dry shell weight or the metal content per unit of shell weight) was calculated according to Fischer, 1984. According to Sotoet al., 1995, the metal/shell weight index provides a reliable tool for the assessment of metal bioavailability to sentinel mollusks in coastal waters. It is useful in eliminating part of the variability due to the inherent biology of sentinel mussels (Bartoloméet al., 2010). Analysis tissues of cockle or mussels (popular seafood) to evaluate the bioaccumulation factor of toxic element by marine biota.

Metal bioavailability was calculated as metal concentrations in soft tissues (mg/kg dry weigh metal content per unit shell weight (mg/kg dry shell weight). Additionally, the MSWI was calculated according to Fischer, 1984 as below:

$$\text{Metal/shell weight index} = \frac{\text{Metal conc. in soft body } (\mu\text{g/g}) \times \text{Dry wt of soft tissues (g)}}{\text{Dry shell weight (g)}}$$

The metal/shell-weight index is independent of conditions such as nutritional stage, spawning, or tidal exposure (Fischer, 1983) and of environmental factors such as temperature, salinity and oxygen levels (Fischer, 1986). The metal/shell-weight index is also independent of changes in organism condition associated with the presence of pollutants, including metals (Marigomez et al., 1990) and has been significantly correlated with the biological effects of metals quantified at the cell and organism levels (Marigomez et al., 1990). According to Sotoet al., 1995 the metal/shell weight index does not exhibit seasonal fluctuations in non-polluted sites, as could be expected by the absence of local inputs. On the contrary, at a polluted site, the metal/shell weight index changed seasonally

in relation to the intensity of the industrial activity, indicating fluctuations in the metal bioavailability.

Consequently, the metal/shell weight indices provide a method to detect differences and changes in the over all availability of cumulative metals, as Fischer, 1988 concluded from laboratory studies and Regoli and Orlando, 1993 as applied to the particular case of metal exposed mussels.

### **Condition index (CI)**

According Romeo and Gnassia B., 1988, many factors, such as body size, could affect the concentration of trace metals in mollusks. Boyden, 1974 reported that the total trace metal content per individual ( $\mu\text{g/g}$ ) could be related to body weight (W) as the power function condition index CI.

A condition index (CI) was calculated (Crosby et al., 1990) for the majority of the cockles used for trace metal analysis:

$$\text{CI} = \frac{\text{Soft tissue dry weight (g)}}{\text{Shell cavity volume (litre)}}$$

The shell cavity volume is calculated by multiplying the length, width and depth measurements of the shell. However site had a greater affect on CI than season with seasonal differences being significant only a tone of the most coastal sites (MPI).

To investigate any relationship between trace metal concentrations and CI, the results from individual mollusks were combined for all sites and sampling times. Spearmanrank correlation coefficients comparing the adjusted mean trace metal concentrations for all sites and the mean condition index (CI)

#### ***3.3.2.2 Assessment bioaccumulation***

##### **Screening level value (SLVs)**

Compare the concentration of each metals in sediment at each location to its generic bioaccumulation screening level value (SLVs) or critical tissue levels (CTL). If the concentration is lower, no further action is required with respect to bioaccumulation for that metal. If the metal concentration is greater than its generic SLVs or CTLs, consider an area-wide statistical evaluation of the exposure point concentration taking into account the

appropriate range of relevant species. Using some guidance for assessing bioaccumulative potential with WDOE or DEQ's general screening approach for SLVs (mg/kg dry weigh) and WQC – USEPA for CTL (mg/kgdry weigh). The SLVs value of some potentially heavy metals in soil/sediment was developed by WDOE, 1994 and BCE, 1999 is seeing in appendix 6.4.

Compare the concentration of each heavy metal in mollusk at each location to its Maximum permissible levels MPL. If the concentration is lower, no further action is required with respect to bioaccumulation for that metal. If the metal concentration is greater than its MPL, consider an area-wide statistical evaluation of the exposure point concentration taking into account the appropriate range of relevant species. However, for benthic organisms that are stationary or range over small distances, a comparison with the maximum concentration is appropriate. Some MPL values depose by different institution is combined in the appendix 6.5.

### 3.3.2.3 Bioaccumulation factor

Biaccumulation or bioconcentration screening following constituent as likely to bioaccumulate or bioconcentrate for soil or sediment. The some of factors represent for accumulation of chemical in organisms are listed in Table 3.9 as below:

Table 3.9 The factors represent for accumulation of chemical in organisms

N	BF	Definition
1	BAF	Bioaccumulation factor: threshold for soil or sediment
2	BCF	Bioconcentration factor: for inorganic in surface water
3	BSAF	Biota to soil on sediment accumulation factor
4	BMF	Biomagnification factor
5	TMF	Trophic Magnification factor
6	R <sub>BAC</sub>	Bioaccumulation index for assessing contaminats of interest

#### **Biota sediment accumulation factor**

Acording to Szeferet al., 1999, Abdallahet al., 2008, BSAF is calculated as equation like:

$$BSAF = C_x/C_s$$

Where: C<sub>x</sub>: mean concentration in the organism

C<sub>s</sub>: Mean concentration in sediment

Classification of sites by BSAF according to Dalliger, 1993 shows in table 3.10

Table 3.10 Classification of sites by BSAF

N	BSAF values	Classification of contamination
1	>2	Macro concentrator
2	1 ≤ BSAF < 2	Micro concentrator
3	BSAF < 1	Deconcentrator

If BSAF > 2 suggested as suitable biomonitoring – organ/material

Using Biota sediment accumulation factor BSAF for organic COIs

(kg sediment organic carbon/kg organic lipid)

$$[C_{\text{tissue}}(\mu\text{g/kg wet weigh})/F_L]$$

$$BSAF = \frac{[C_{\text{tissue}}(\mu\text{g/kg wet weigh})/F_L]}{[C_{\text{sediment}}(\mu\text{g/kg dry weigh})/F_{OC}]}$$

$$[C_{\text{sediment}}(\mu\text{g/kg dry weigh})/F_{OC}]$$

F<sub>OC</sub> : fraction of total organic carbon in surface sediment (0-5cm for invertabletes) dry weigh. If TOC data are not available, assume that F<sub>OC</sub> is 1% (0.01)

F<sub>L</sub>: fraction of organic lipid content of whole body wet weigh. If organical lipid data are not available, assume that F<sub>L</sub> is 3% (0.03) for consumption fillets only and F<sub>L</sub> is 5% (0.05) for consumption whole organism.

Bioaccumulation index R<sub>BAC</sub> for assessing contaminats of interest (COI)

$$EPC \text{ (mg/kg)}$$

$$R_{BAC} = \frac{EPC \text{ (mg/kg)}}{SLV \text{ (mg/kg)}}$$

$$SLV \text{ (mg/kg)}$$

EPC: Exporsure point concentration of a given COI in sediment (mg/kg)

SLV: Screening level values for the COI and receptor class.(mg/kg)

Table 3.11 Classification of COPC by bioaccumulation index

N	Values $R_{BAC}$	Bioaccumulation screen
1	> 1	The individual bioaccumulation screen
2	>0.1	The cumulative bioaccumulation screen
3	<0.1	The chemical is not contaminant of potential concern

$R_{BAC} > 1$ : chemical is a contaminant of potential concern on the basis of the generic SLV. Chemical could be bioaccumulate threat to human that consume aquatic organism.

The most potentially bioaccumulative chemicals are: As, Cd, Pb, Hg,

### 3.3.3 Ecological risk analysis (ERA)

They are 3 guidance documents for interpret the ecological risk of metals:

- DEQ,2000: Guidance for assessing risk to human health
- DEQ,2001b: Guidance for ecological risk assessment
- DEQ, 2001a: Program quality assurance policy

#### 3.3.3.1 Potential ecological risk index (PERI)

The potential ecological risk index is used to assess the potential ecological risks from metals in the soil (Medici et al. 2011; Abdallah 2011). It is defined by where  $C_{sample}$  is the content of the metal measured in the studied soil sample,  $C_{reference}$  is the background value of the element (as mentioned above), and  $T_i$  is the response coefficient for the toxicity of the single element  $i$ . The values of  $T_i$  have been reported to be: As = 10, Cu = Pb = Ni = 5, Zn = 1, Cr = 2 and Cd = 30 (Hakanson, 1980; Madiseh and others, 2009). No  $T_i$  value appears to be given for Mn. Comparing its toxicity with that of Cu, Cr, and Zn, a  $T_i$  value of Mn = 1 was adopted.

$$PERI = \frac{T_i \times C_{sample}}{C_{reference}}$$

$$RI = \sum PERI$$

If the value PERI<40: the element poses a low ecological risk.  
 40 < PERI< 80 means a moderate ecological risk  
 80 < PERI<160 a higher ecological risk  
 160 < PERI<320 a high ecological risk  
 PERI>320 a severe risk to the ecology

Also, classification of risk index RI of all metals by Madiseh and others, 2009.

RI<150 means a low ecological risk,  
 150 < RI<300 a moderate ecological risk  
 300 < RI<600 a higher ecological risk  
 RI > 600 a severe ecological risk

**3.3.3.2 Determination of Estimated daily intake (EDI)**

Determination of EID for the metals in each shellfish species based on tissue concentration of metals and the average daily shellfish consumption rate (Santos and others, 2004). The EDI of metals for adults determine by the following equation

$$EDI = \frac{C \text{ metal} \times W \text{ food}}{BW}$$

Where:

C metal the concentration of metal in shellfish species (mg/kg on fresh weight basis)

W food: represent the daily average consumption of mollusk in region

BW: the body weight

Then the health risks from consumption of shellfish by local inhabitants assess based on the comparison with acceptable daily intake limit given by National research council of the National academy of sciences of the USA (for Cu 2000-3000µg), Environmental health criteria 221 (for Zn 5600-15000µg), Guidance document for Lead in shellfish, FAO/WHO 2003 for Cd: 60µg and USFDA, 1993 for Pb: 25-75µg (See Appendix 6.6)

### 3.3.3.3 *The target hazard quotient (THQ)*

The health risks from consumption of shellfish by local inhabitant also assess by THQ. The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed population is unlikely to experience obvious adverse effects. The method of estimating risk using THQ provides in the USEPA and it is described by the following equation:

$$\text{THQ} = \frac{\text{Efr} \times \text{ED} \times \text{FI} \times \text{MC}}{\text{RfD} \times \text{BW} \times \text{AT}} \times 0,001$$

Where EFr is exposure frequency (365 days/year)

ED is the exposure duration (70year)

FI is food ingestion (mollusks 9,8g/person/day FAO, 2005. NSF, 2006)

MC is metal concentration in shellfish (mg/kg fresh weight)

RfD is the oral reference dose (Cd: 0.001µg/g/day, Pb: 0.004, Ni: 0.006, Cr 0.003 USEPA, 1997 2000)

BW: is the average body weigh 60kg for VN

AT: is the average time for non-carcinogen in day (365 day/year x number of exposure years)

An important aspect in assessing risk to human health from potentially harmful chemicals in food is the knowledge of the dietary intake of such substances that must remain within determined safety margins.

### 3.3.3.4 *The target cancer risk (TR)*

The method for estimating TR values is provided by the US. We compared these levels to the recommended dietary intake levels published by the World Health Organization (Demirzen et al., 2006; WHO 1993).

The USEPA, 2006 has recommended the estimation method of the potential health risk for the intake of inorganic As from various seafood species, such as the target cancer risk TR. TR is the incremental individual lifetime cancer risk fromthe ingestion of



shellfish. The carcinogenic risk from inorganic As is expressed as the excess the probability of contracting cancer over a life time of 70years. The TR id difined as follows:

$$TR = \frac{E_{Fr} \times ED \times IR \times C_{inorg} \times CPSo}{BW \times ATc} \times 0,001$$

Where EFr is exposure frequency (365 days/year)

ED is the exposure duration (70year)

IR is the ingestion rate of shell fish (g/day)

Cinorg is As concentration in shellfish (mg/kg fresh weight)

CPSo is the oral carcinogenic potency slope obtained from the IRIS Integrated Risk Information system (1,5mg/kg/day USEPA, 2006)

BW: is the average body weigh 60kg for VN

AT: is the average time for non-carcinogen in day (365 day/year x number of exposure years)

0,001: conversion factor

### 3.3.3.5 Acceptable Tissue Levels for Humans

For human consumption, acceptable tissue levels for carcinogens (ATLhC) and noncarcinogens (ATLhN) are back-calculated from acceptable risk levels in accordance with federal guidance for establishing shellfish consumption limits and for conducting human health risk assessments (USEPA, 1989, 1997). Separate levels were not calculated for men and women, as differences in consumption rates relative to body weight are minor. Acceptable fish tissue levels for humans are calculated using the following equations.

For carcinogens:

$$ATL_{hC} = \frac{ARL_C \cdot BW \cdot AT}{SF_O \cdot IR_p \cdot ED}$$

and for noncarcinogens:

$$ATL_{hN} = \frac{RfD \cdot BW \cdot ARL_N}{IR_p}$$

Where:

ATL<sub>hC</sub> = Acceptable tissue level (carcinogen) in diet for human receptors (mg/kg);

ATL<sub>hN</sub> = Acceptable tissue level (noncarcinogen) in diet for human receptors (mg/kg);

ARL<sub>C</sub> = Acceptable risk level for carcinogens (unitless;  $1 \times 10^{-6}$ );

ARL<sub>N</sub> = Acceptable risk level for noncarcinogens (unitless; 1);

AT = Averaging time (years);

ED = Exposure duration (years);

SFo = Oral slope factor (mg/kg.day)<sup>-1</sup>;

RfD = Reference dose (mg/kg.day);

BW = Body weight (kg); and

IRP = Fish and/or shellfish ingestion rate for the exposed population (mean daily rate over a year in kg/day).

The ATL values represent the maximum concentration of a given chemical in shellfish tissue that will not generate a risk greater than the maximum acceptable risk level (ARL) used for carcinogens (See appendix 6.7)

Therefore, the ATL<sub>h</sub> values permit a specific population of humans to consume safely any combination of fish and/or shellfish for an extended period, provided that the combined daily consumption rate remains below the value of IR used to calculate ATL<sub>h</sub>.

CTL values are concentrations of bioaccumulative chemicals in tissue that will not cause significant adverse effects on the health of aquatic organisms containing those chemicals. Appendix 6.8 lists generic CTL values protective of populations and individuals of threatened or endangered species (WQC – USEPA, 2004)

## CHAPTER 4

# RESULTS AND DISCUSSIONS

### 4.1 PHYSICOCHEMICAL CHARACTERISTICS OF SEDIMENT

#### 4.1.1 Particles size of sediments

Grain size of particulate matter influences its adsorption capacity greatly. The specific surface area will determine the amount of pollutant adsorbed. Amount of adsorption is inversely proportional to the particle size. Concentrations of pollutants are generally highest at the finest particles as summarizes in Figure 4.1; 4.2; 4.3 and 4.4

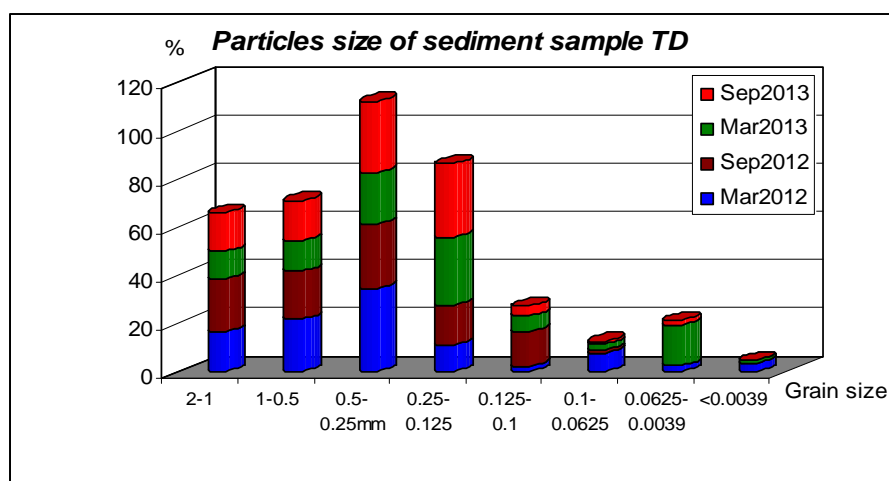


Figure 4.1 Percentage of particles size in Tan Dao during 2012-2013

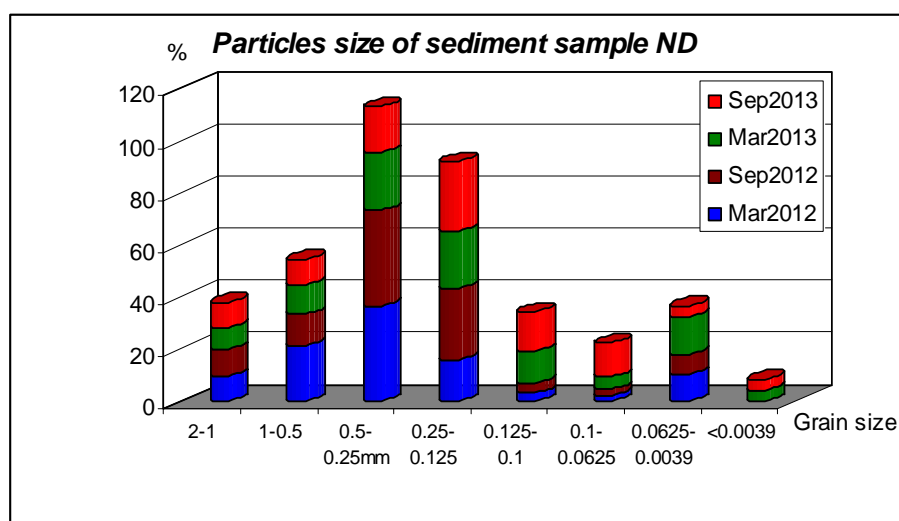


Figure 4.2 Percentage of particles size in Ngoc Diem during 2012-2013

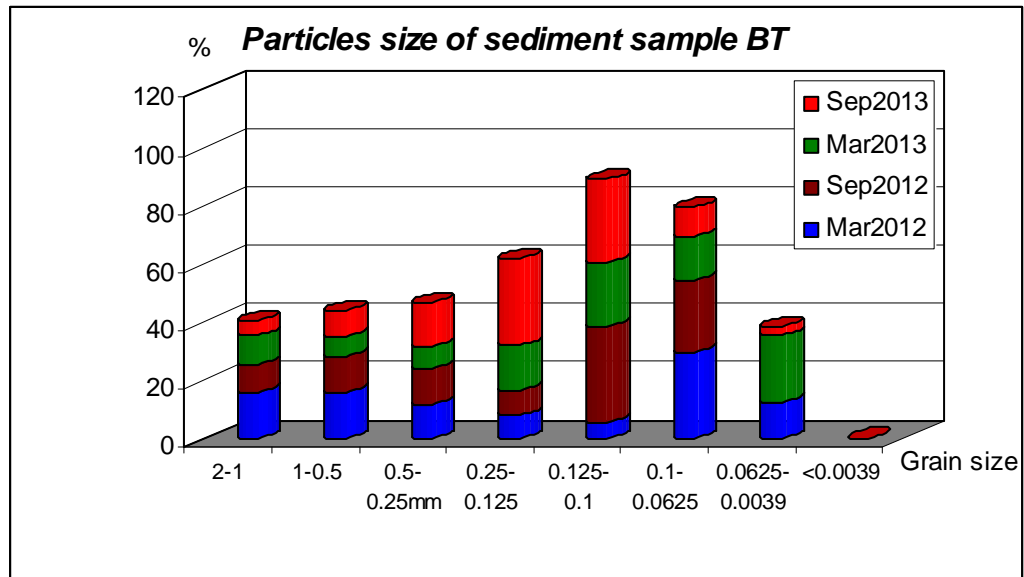


Figure 4.3 Percentage of particles size in Binh Tan during 2012-2013

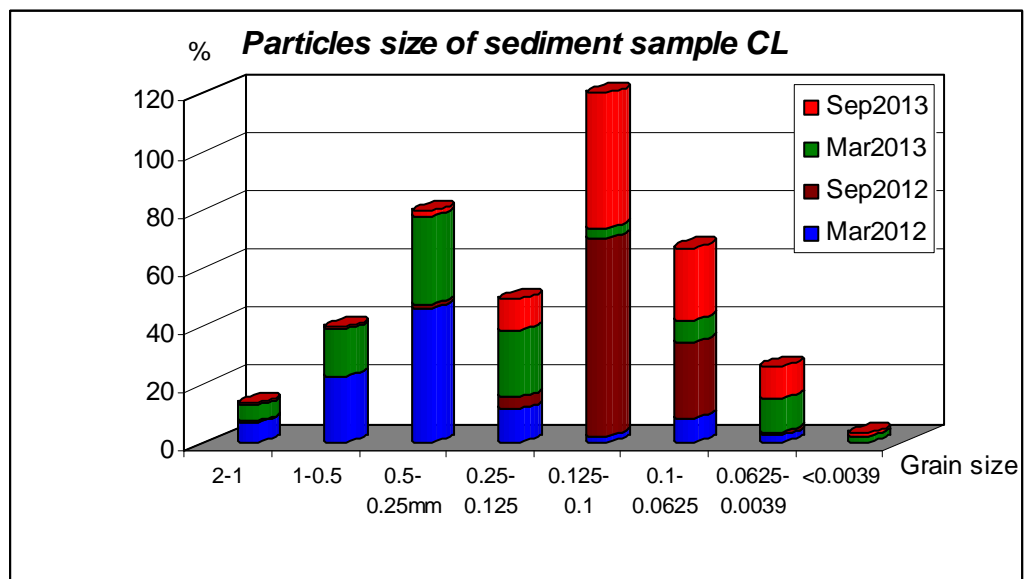


Figure 4.4 Percentage of particles size in Cam Lam during 2012-2013

At 4 stations, sediments were found to be highly sorted, indicating, therefore, a same origin of settled particles (terrigenous, anthropogenous, and biogenous). Grain size analysis and composition of sediment samples signified that they were mainly sand which range from 80.3-99.73% in TD, 76.24-90.76% in ND; 57.61-86.72% in BT; 62.79-89.28% in CL. Coarse clay fraction prevails in most of samples, after what follows silt, which is also obvious from Shepard's classification of material (Shepard,1954).

In TD and ND, the mean size of muddy particles is in the range from coarse clay to fine silt, while median of particles is in narrow range from very fine silt to fine silt were represented in BT and CL. Sediment type is determined according to Shepard (1954) and at BT and CL locations is classified as clayey silt. Other two samples TD and ND are classified as silty clay, due to some how increased percentage of clay. At all locations, percentage of gravel is very high, and only in the CL location, it is 0.54-6.8%, what could be due to close sea. Particles size of the sediments in rainy and dry season will compare and describe in the next part.

Study of change between seasons versus grain size fraction in the 4 sampled sites is an evident of this study. Figure 4.5; 4.6; 4.7 and 4.8 summarises the result of particles size of every sediment samples in two seasons.

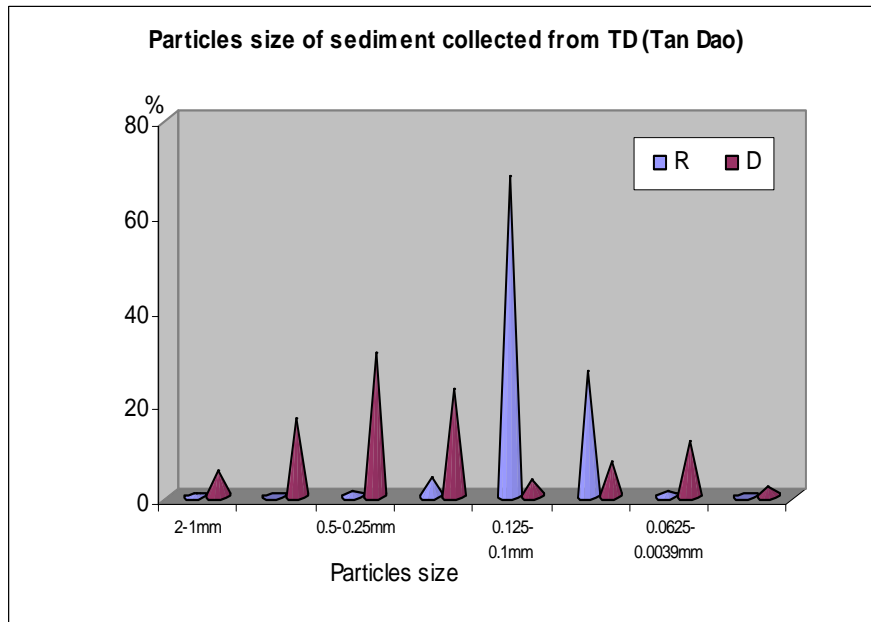


Figure 4.5 Particles size of sediment samples in Tan Dao at two seasons

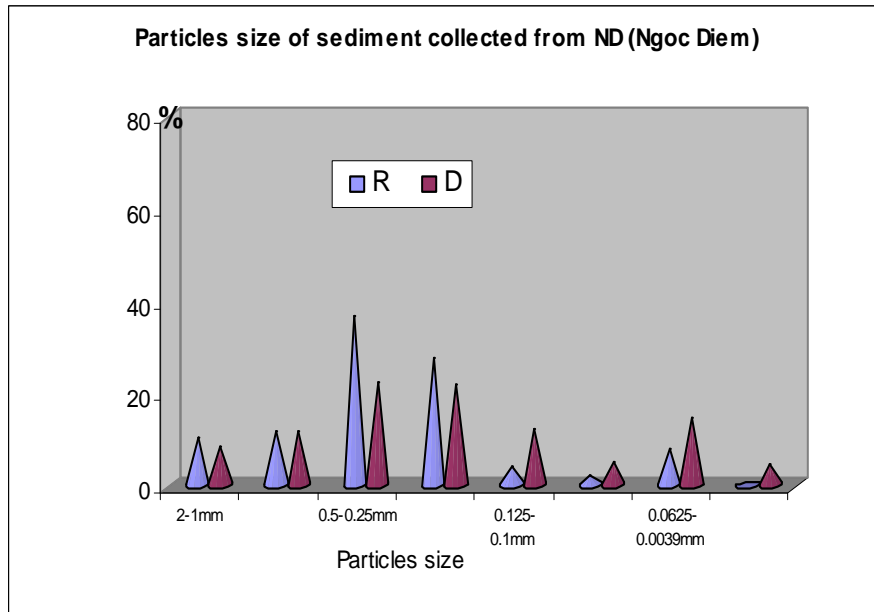


Figure 4.6 *Particles size of sediment samples in Ngoc Diem at two seasons*

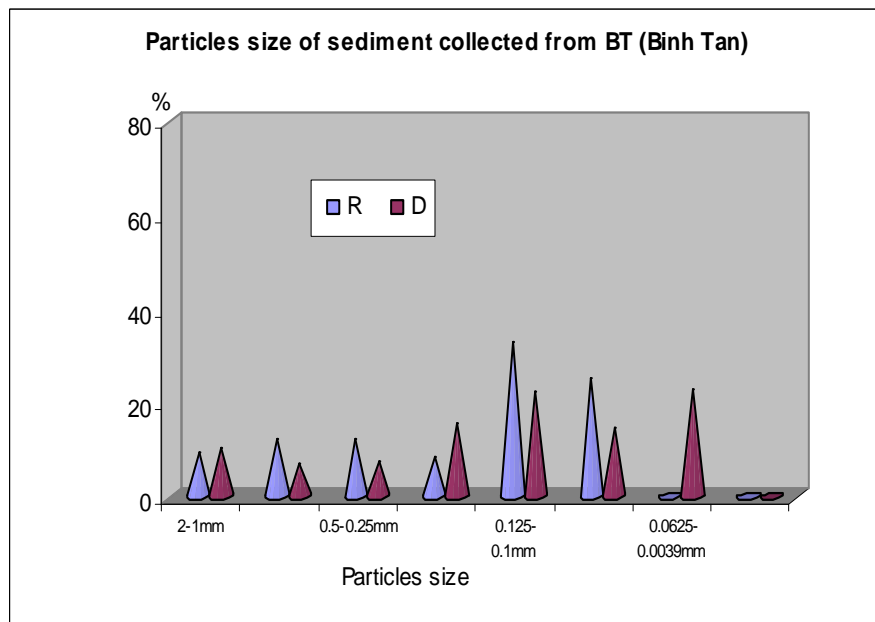


Figure 4.7 *Particles size of sediment samples in Binh Tan at two seasons*

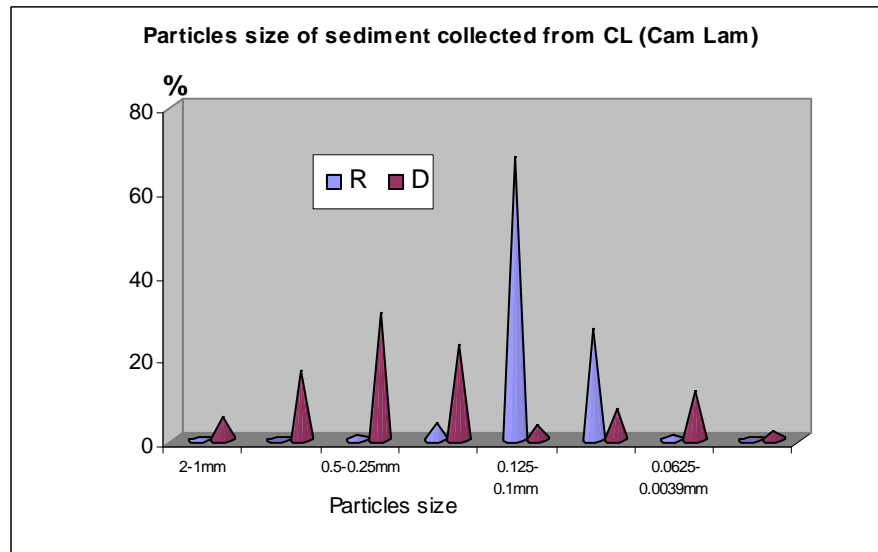


Figure 4.8 *Particles size of sediment samples in CL (Cam Lam) at two seasons*

**Note:** R - rainy season, D - dry season

Percentage of sand is rather high on all locations in dry season, which could be also due to anthropogenic influence. On rainy season, percentage of sand is some how decreased. Fine particles were found on the whole sediment samples in dry season but not in the rainy season. The fine-grained fractions (grain size <0.0063mm) content of more than 76% was found in quiet areas samples as CL and ND, mean while only 23% of very fine mud was found at estuaries zone sediment BT. In addition, the fraction of medium coarse sand (grain size 0.25-0.5mm) in sediments was strongly varies between sampling locations.

#### **4.1.2 pH of sediment**

Acidity is another main factor influencing the form of metal. Under low pH condition (<pH 5), metals such as Fe and Al can become soluble from most minerals. However, some elements may become insoluble under low pH and redox conditions. Acidic condition in the digestive tract of organisms can cause particulate pollutants become soluble as well as a consequence, metals are more available to the organism and lead to the occurrence of bioaccumulations.

Table 4.1 pH measurement of sediment samples

<b>pH KCl</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Mar 2012	7.43	7.43	7.43	7.4
Sep 2012	7.26	7.2	7.14	7.3
Mar 2013	7.47	7.44	7.58	7.53
Sep 2013	7.24	7.09	7.04	7.17

<b>pH H<sub>2</sub>O</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Mar 2012	7.56	7.55	7.48	7.52
Sep 2012	7.27	7.36	7.21	7.4
Mar 2013	7.63	7.58	7.68	7.54
Sep 2013	7.34	7.21	7.46	7.34

The pH values at the four sites ranged from 7.2-7.6 indicate that sediments were neutral medium, especially pH were higher in the dry season 7.5-7.6, which contrasted with those lower of the rainy season 7.2-7.4 (Figure 4.9)

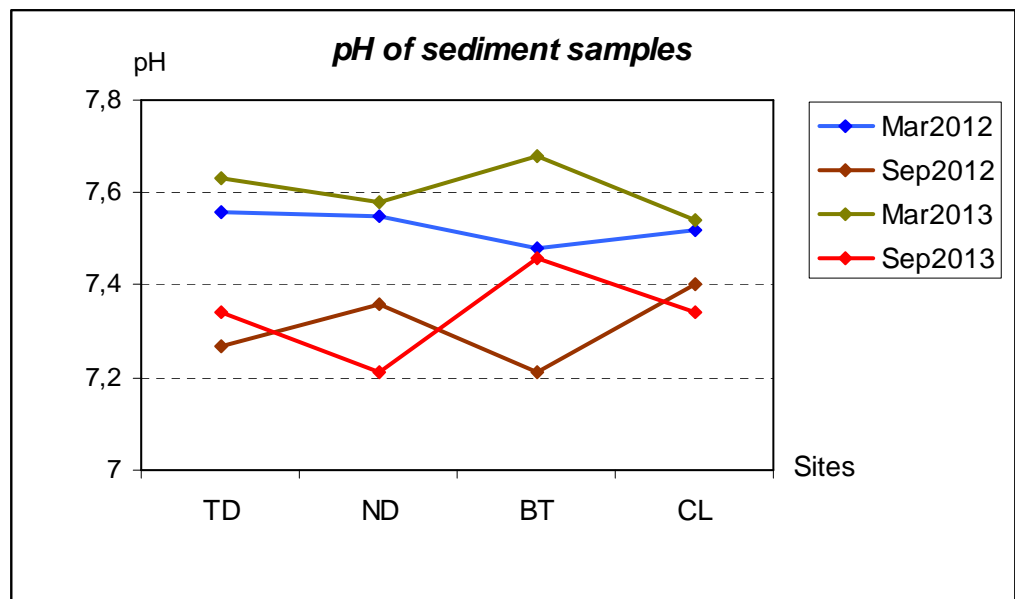


Figure 4.9 Trend of pH in 4 sampling locations from 2012 to 2013



### 4.1.3 Distribution of organic carbon in sediment

Adsorption is the most reactive form of metal. Metals are also tending to form bonding with the organic materials, has very high specific area. As a consequence, it has high adsorption capacity. Therefore, concentration of metal may also be proportional to the amount of organic particulates.

Table 4.2 Percentage of total organic carbon TOC (%) in sediment samples

<b>Locations</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Mar2012	9.98	18.47	3.08	3.03
Sep2012	9.06	27.06	10.4	3.14
Mar2013	6.84	10.01	3.16	1.71
Sep2013	6.45	19.64	8.26	3.17

Average values of sediment organic carbon (% dry weight) are showed in table 4.2, and the observed concentration of CL (1.71-3.17) was lowest when compared to that observed in other regions (TD, ND, and BT) that ranges from 3.08 to 27.06%. The values of organic carbon obtained in this study area are comparatively higher than those reported (2.78%) for various tidal and estuarine regions of southeast coast of India (Achyuthan and others, 2002). Relatively lowest concentration of organic carbon in sediments of CL is due to and mixing process in sediment–water interface and also due to high microbial activity in this region, which use the organic matter as nutrient source and removes it from the system as CO<sub>2</sub>. All the sediment collected showed relatively higher organic carbon values in the rainy season due to the nature of sedimentation in this season. The sediment collected from TD mangroves showed a decreasing trend of organic carbon due to restricted microbial activity in this anoxic or sub-oxic region and also due to partial decomposition of leaf litters, root exudates, and their preservation as burial in the sediments. Over all a high distribution of organic carbon was observed in both ND and BT due to solid waste coupled with boat activities and physical mixing processes. Trend of total organic carbon content in sediment samples during 2012-2013 is shown in figure 4.10.

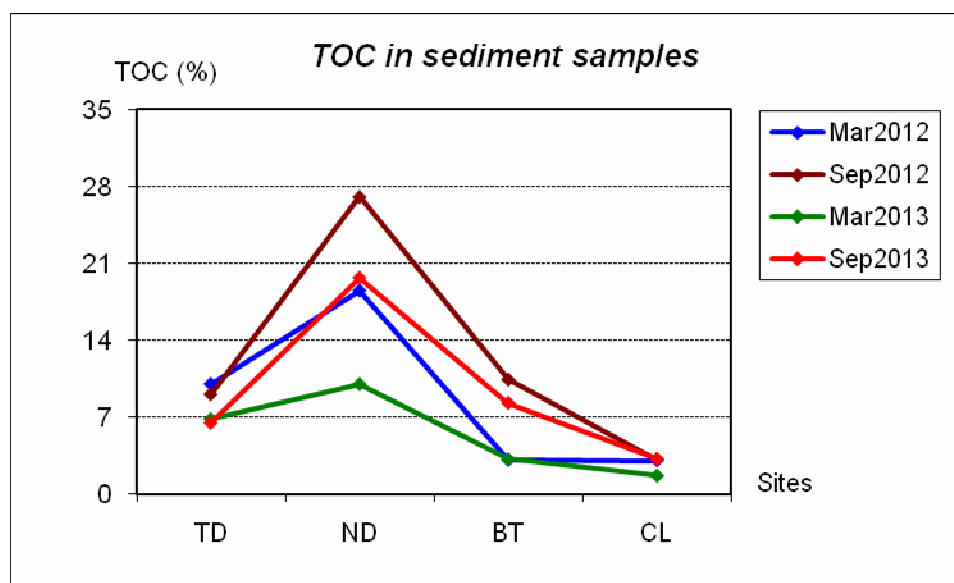


Figure 4.10 Trend of total organic carbon content in sediment samples

#### 4.1.4 Bulk density

Bulk density of sediments from Nha Trang bay and adjacent areas is shown in Table 4.3.

Table 4.3 Bulk density ( $\text{g}/\text{cm}^3$ ) of sediment samples

Locations	TD	ND	BT	CL
Mar2012	0.57	0.25	1.19	1.20
Sep2012	0.62	0.05	0.55	1.18
Mar2013	1.23	0.57	0.68	1.50
Sep2013	0.80	0.59	1.16	1.17

Generally there is inverse relationship between bulk density and organic carbon contents, bulk density increases as organic carbon content are decreased. The bulk density and organic carbon content of the sediment samples remained nearly constant with an average of 0.60-0.99 ( $\text{g}/\text{cm}^3$ ) with lowest bulk density was found 0.36 ( $\text{g}/\text{cm}^3$ ) in ND coast sediment and highest value was 1.26 ( $\text{g}/\text{cm}^3$ ) in CL lagoon area. The sediments were classified as lighter organic matter according to Avnimelech Y, 2001 classification. Figure 4.11 below shows a range of bulk density of sediment samples during 2012-2013:

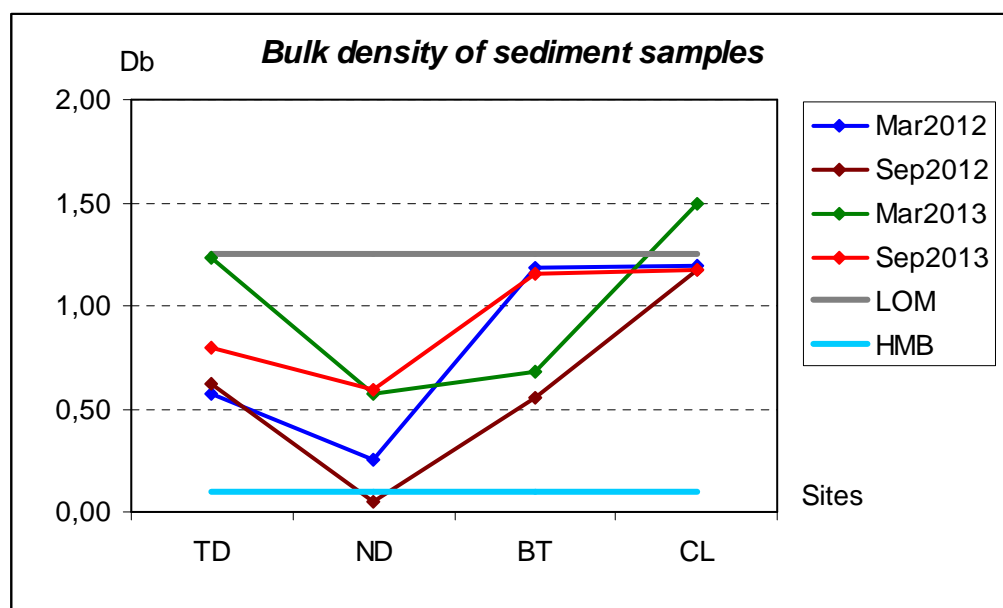


Figure 4.11 Range of bulk density of sediment samples during 2012-2013

Notes: LOM limit: lighter organic matter classification of sediment

HMB limit: hydrated microbial biofilm classification of sediment

#### 4.1.5 Moisture of sediment samples

Moisture concentrations were measured in the sediment samples. Percent moisture contents are showed in Tables 4.4

Table 4.4 Percent moisture contents in sediment samples

Locations	TD	ND	BT	CL
Mar2012	9.98	18.47	3.08	3.03
Sep2012	9.06	27.06	10.4	3.14
Mar2013	11.62	16.6	4.52	4.05
Sep2013	13.77	23.56	11.62	4.4

In general, rainy seasonal sediment contained the higher moisture of the dry sediment with a range of 9.06 to 13.77% in TD; 23.56-27.06% in ND; 10.4-11.62% in BT and 3.14-4.4% in CL in rainy season. A range of 3.08-4.52% in BT and CL with moisture measurements in dry season were very low moisture contents. Sediments in ND and BT had a grand difference of moisture between two seasons, but not for TD and CL sediments.

Moisture variation in sediment samples was noted as low for all of samples and most likely due to variations in location at the 4 times of sampling. Figure 4.12 is showed a trend of moisture content in sediment samples during 2012-2013.

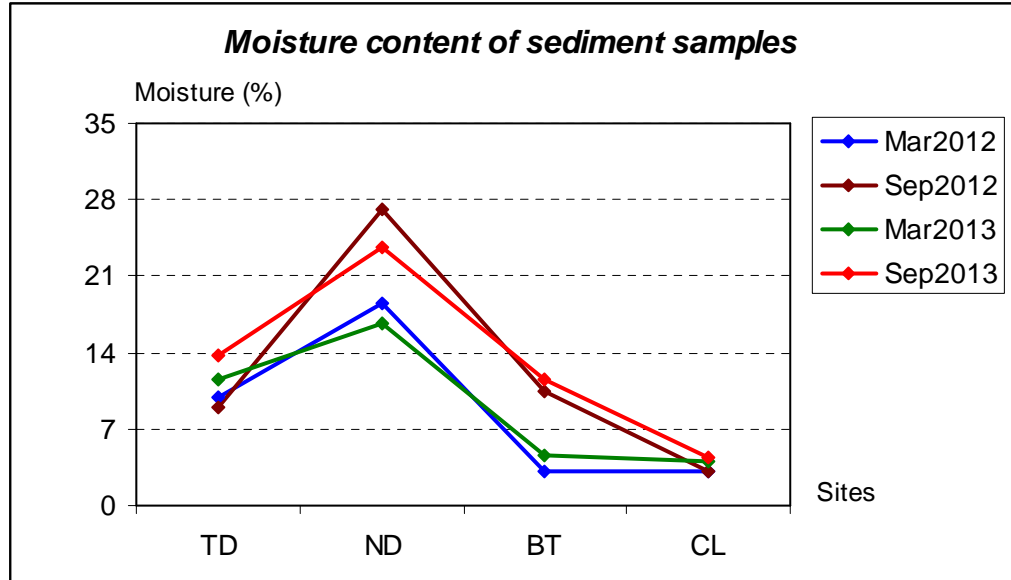


Figure 4.12 Trend of moisture content in sediment samples during 2012-2013

#### 4.1.6 Acid volatile sulfide (AVS)

Only one measurement was obtained for the AVS and Bicarbonat contents in September 2013. The acid volatile sulfide (AVS) concentrations (mg/kg) and Bicarbonat contents in the sampled sediments are listed in Table 4.5

Table 4.5 The AVS and Bi-carbonat content in the sampled sediments.

Parametres	TD	ND	BT	CL
AVS (mg/kg)	0,168	0,416	0,243	0,266
Bicarbonat (mgdl/100g)	0,446	0,786	0,488	0,457
Bicarbonat (%)	2,79	4,79	2,97	2,72

Significant variations in AVS in sediment samples were observed, with variation between 0,168 (TD) and 0,416 (ND), that justifies the low AVS levels in this coastal area according to the another region. The most significant AVS values were found in the ND sediments (0,416) and very distinct concentrations were obtained for the BT and CL (0,243 and 0,266). The highest bicarbonat content was obtained for the ND: 0,786mgdl/100g. The

percentage of bicarbonates varies from about 2,72 to 4,97% and is in all samples lower than percentage of organic matter.

Considering the physicochemical factor and accessibility, sampling locations were first being classified by referring to the structures. According to the physicochemical characteristics, the sediments type were classified as silt clay to clayely silt with pH neutral, lighter organic matter, low AVS and differences of moisture content from low to high, depends of location of sampling. Classification of sediment samples is shown in table 4.6.

Table 4.6 Sediment type classification

	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Sediment type	Silt clay	Silt clay	Clayely silt	Clayely silt
pH	Neutral medium	Neutral medium	Neutral medium	Neutral medium
OC	Lighter organic matter	Lighter organic matter	Lighter organic matter	Lighter organic matter
Moisture	Medium	High	Low	Low
AVS	Low	Low	Low	Low

## 4.2 METAL CONCENTRATIONS IN SEDIMENT SAMPLES

### 4.2.1 Ranges of heavy metal concentrations

For remaining heavy metals in sediments in both two seasons, metal concentrations in the sediments ranked in the following order: Aluminium > iron > zinc > copper > chromium > arsenic > cadmium. In particular, the average concentrations of heavy metals in sediments obtained from 4 locations were compared in sequential order as show in Table 4.7.

Table 4.7 Sequential orders of heavy metals in sampling sites

<b>Sites</b>	<b>Seasons</b>	<b>Metals in decreasing orders</b>
TD	Rainy	Zn > Cr > Cu > As > Cd
	Dry	Zn > As > Cr > Cu > Cd
ND	Rainy	Zn > Cr > Cu > As > Cd
	Dry	Zn > Cr > Cu > As > Cd
BT	Rainy	Zn > Cu > Cr > As > Cd
	Dry	Zn > Cu > Cr > As > Cd
CL	Rainy	Zn > As > Cu > Cr > Cd
	Dry	Zn > Cu > Cr > Cd > As

The concentrations of trace metals were showed a variation between the metals and between sites. The maximum average concentration values for Cu, Cr and Cd (mg/kg DW) were observed in BT estuary site. Site TD and CL showed similar concentrations of trace metals, where as mangrove site TD showed comparatively low concentrations due to poor sediment accumulation, and CL located in high energy system showed the least concentrations. In the present study, the elevated concentrations of Zn and Cu from in the all four sites could be attributed to the increase in natural weathering of soil materials and inputs from rapid industrial development and urbanization. In surface sediment, an increase in concentrations of chrome and copper at BT and CL sites showed exchange able fraction and gradual increase due to additional metal precipitation around there two boundaries.

It may be concluded that the increase in trace metals in the sediments mainly come from extensive fishing activity, public boat services, domestic sewage release from near by areas, and other industrial waste discharge. Concentrations of heavy metals in sediments from four collecting stations within the affected area are illustrated in Figures 4.13; 4.14; 4.15 and 4.16.

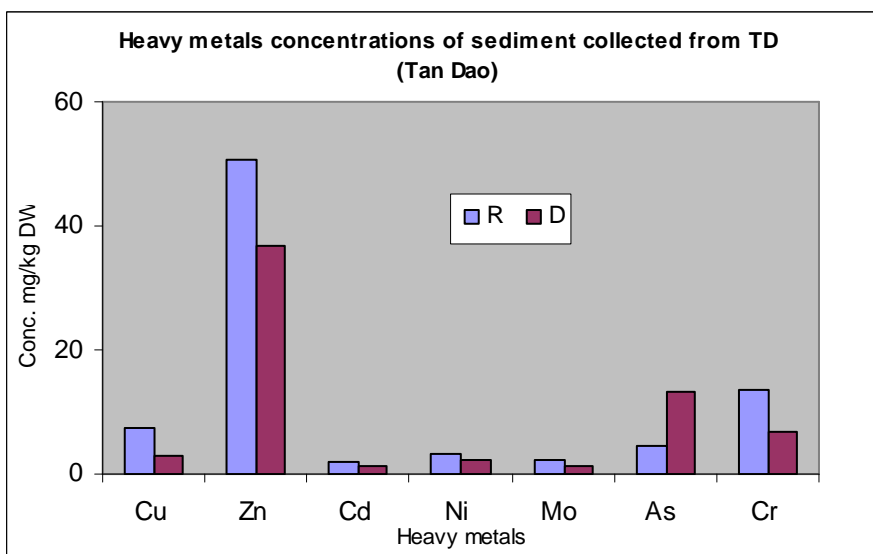


Figure 4.13 Heavy metals concentration of sediment collected from TD 2012-2013

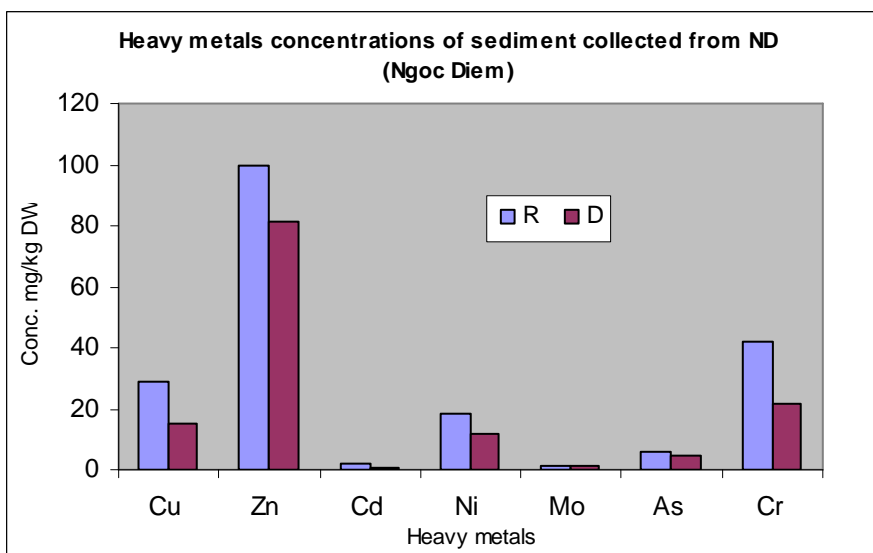


Figure 4.14 Heavy metals concentration of sediment collected from ND 2012-2013

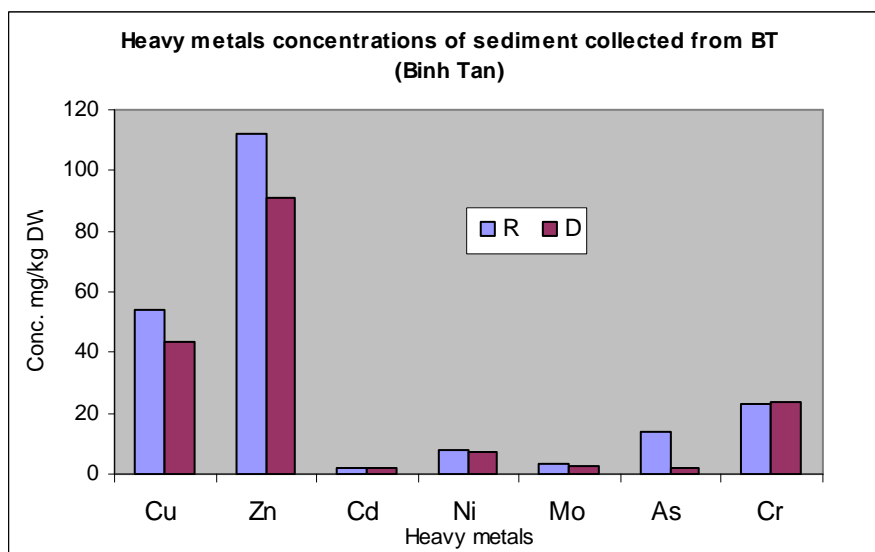


Figure 4.15 *Heavy metals concentration of sediment collected from BT 2012-2013*

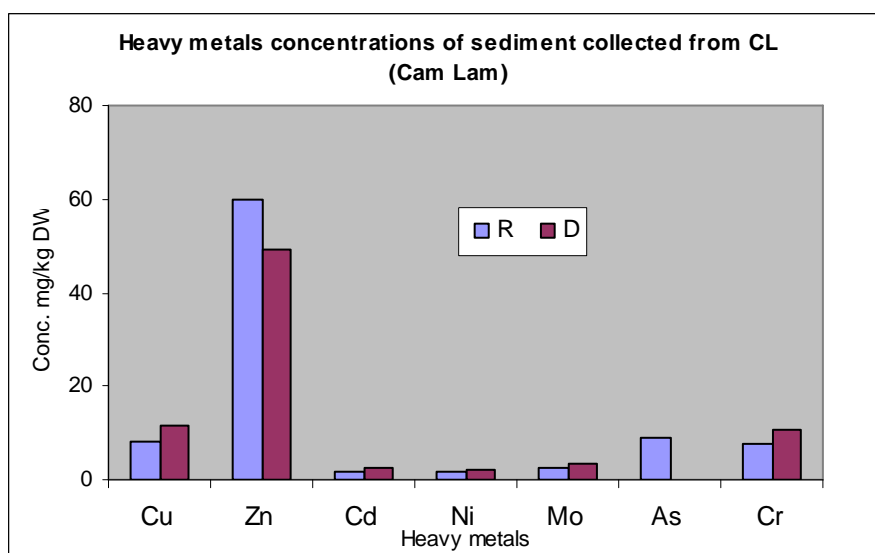


Figure 4.16 *Heavy metals concentration of sediment collected from CL 2012-2013*

When the analyses of a metal for seasonal, there rainy sediments contained higher concentrations than dry sediments of all most metal. Some significant that sediment in dry season contained higher concentrations of heavy metal as chromium at BT and CL sediment samples or arsenic in TD and copper in CL.

The average concentration of Zn was highest in both seasons compared with other metals. Furthermore, the highest concentrations of Zn were detected in rainy season (80.66mg/kg DW) and 64.67mg/kg DW in dry season. Nevertheless, the concentrations of



arsenic were not detected in dry seasons (arsenic concentration in CL = 0.15 mg/kg DW). The highest average concentration of Cu was in rainy compared by dry seasons (24.67 and 18.21 mg/kg DW, respectively). The concentrations of Cr were higher on rainy season than dry season, while concentrations of Cd were detected with an average of 1.85 mg/kg DW in rainy season but were lower of detected limit in dry season in ND sample. The elements that are essential for mollusk growth (copper, iron and zinc) are the most abundant elements measured in the sediment on the rainy basis.

In general, the area distribution of metals in the ND estuarine reflects the inputs from anthropogenic origins, with higher concentrations at locations close to the metal sources. So, Cr levels in the sediment were in the range of 21.81 to 41.96 mg/kg DW. The strong concentration of As and Cu were observed in BT estuary. When this local area is not considered, the increase of metal concentrations in the surface sediments are associated with the harbour areas: up to 53.94 mg/kg DW of Cu in rainy season to 43.24 mg/kg DW in dry season and to 14.1 mg/kg DW As in rainy season. Thus, the pattern of distribution of this metal shows the affect of the domestic activities and secondly the shipyard such as is observed in the middle of the Nha Trang habour. These results validate the industrial effects already pointed out for As.

The strong gradient in the As was observed around the CL, that shown a big difference which is in the TD because in this area the As concentration changes strongly from the rainy season to dry season although maximum values are found 14.10 mg/kg DW in dry season. The anthropogenic origin is clear in the sedimentary register, however the arsenic is dispersed throughout the dry season at TD and is not concentrated in the this area.

Zinc has a similar trend to copper with local differences: the highest concentrations in the surface sediment occur in the estuary zone of the BT (112 mg/kg DW) and the area of the ND (99.90 mg/kgDW). The influence of the Zn in TD and CL are low, however it is obvious in the TD (50.99 mg/kg DW) and in CL (59.60 mg/kg DW). Another difference is that the zinc levels in the CL show small signs of anthropogenic enrichment. The zinc contamination in the sediment of preindustrial (MOSTE, 1996) was very low found (6.6mg/kg DW).

Once the copper and zinc levels in the surficial sediments are established, the contamination state could be ascertained by comparison with typical concentrations of these two metals in the unpolluted sediments and/or their natural presence in the lithosphere. The measured values in the four areas are higher than average concentration in the preindustrial sediment and also exceed the range for unpolluted sediments. However, it is more appropriate to use local background equations to define the degree of contamination.

In the mouth of the estuary, copper and lead tend to precipitate, probably with iron oxides. Fe, Ni and Cr are found in varying concentrations along the bay of Nha trang. The chemical forms of these precipitations vary from one hydrological zone to the next. Nickel, most often associated with organic matter, can precipitate with manganese oxides and ferrihumic complexes in contact with salinated waters. These two metals are the most labile and ubiquitous. Concentrations of Zn, Pb, Cr and Cu reach their peak in the dry period, indicating the importance of the evaporation.

#### **4.2.3 Sediment Quality Guidelines (SQGs)**

As the mean to predict adverse effect derived from contaminated sediments, numerous sediment quality guidelines (SQGs) were developed by overseas researchers. Among the most widely applied SQGs are as following, both SQGs produced by NOAA and New York Sediment Screening Criteria were chosen as the most appropriate values, which is being used as main reference in this study.

Table 4.8 and 4.9 gives the values for elements tested in surface sediment collected in dry and rainy season for each parameter (where applicable). The element concentration was study with referred to the values of effect range (ERL and ERM) and effect levels (LEL and SEL). In the sediment, the recorded metal values did not reach biological effect levels (ERL), concentrations proposed by Long et al., 1995.

Table 4.8 Values for metals (mg/kg DW) in surface sediment collected in dry season

<b>Metals</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
As	2,49-13,09*	4,39-16,95*	2,09-5,82	0,15-5,54
Cd	0-1,15*	0-0,94*	0-2,20*	0-2,41*
Cr	2,33-6,83	5,94-21,81	4,92-23,43	10,84-10,97
Cu	2,95-7,95	15,31-21,93	0-43,24*	5,49-11,34
Zn	36,93-71,43	53,96-81,51	70,61-90,94	40,99-49,30

\*: Values above LEL (mg/kg DW)

The sediment samples that collected in dry season from TD had arsenic concentrations 13,09mg/kg DW in between the LEL-SEL values. Similar situations were with copper concentrations in sediments collected at BT (43,24mg/kg DW), that were more than two times higher when compared to LEL value. Only zinc concentration in sediment collected in dry season had all values below the LEL value with the mean value of 36,93-90,94mg/kg DW.

Table 4.9 Values for metals (mg/kg DW) in surface sediment collected in rainy season

<b>Metals</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
As	4,65-6,73	5,71-14,36	14,10-15,09*	9,09-14,87*
Cd	0-2,07*	1,14-1,93*	0-1,72*	9,02-1,69*
Cr	7,61-13,49	16,75-41,96*	5,81-23,14	7,73-19,83
Cu	0-7,36	28,70-91,22*	19,04-53,94*	8,00-99,21*
Zn	50,66-68,36	70,62-99,90	112,31-126,03	59,76-65,65

\*: Values above LEL

The sediment samples that collected in rainy season from BT and CL had arsenic concentrations in between the LEL-SEL values (6 - 33mg/kg DW), with mean concentration values were more than two times higher when compared to LEL value (14,10 and 9,09mg/kg DW respectively). Similar situations were with copper

concentrations in sediments collected at ND and BT (28,70 and 53,94mg/kg DW respectively).

Especially, cadmium concentration in sediment collected in both dry and rainy season had all values above the LEL value. Those concentrations are interpreted as a very high level which compare the values of cadmium were found in preindustrial sediments from near by port of Nha trang (Phuong et al., 2012) and in other northern coastal sediments. Additionally, that may conclude that Cd concentrations are anthropogenically influenced and that they are about 10 times higher than in unpolluted preindustrial sediments, but also that there are many spots in the Pacific Sea with much higher concentrations.

From the assessments in this study, most metals have acceptable (<LEL) or moderate ( $\geq$  LEL and < SEL) biological effects, except Cd. Percentage of samples amongst range of SQGs for dry and rainy sampling is showed in Figure 4.17 and Figure 4.18

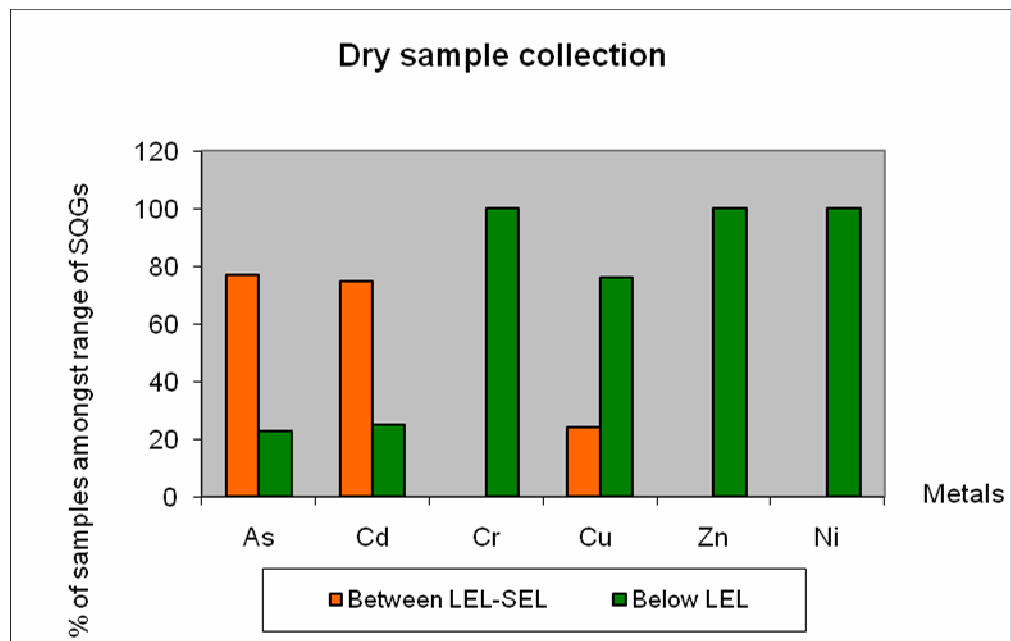


Figure 4.17 Percentage of samples collected in dry season amongst range of SQGs

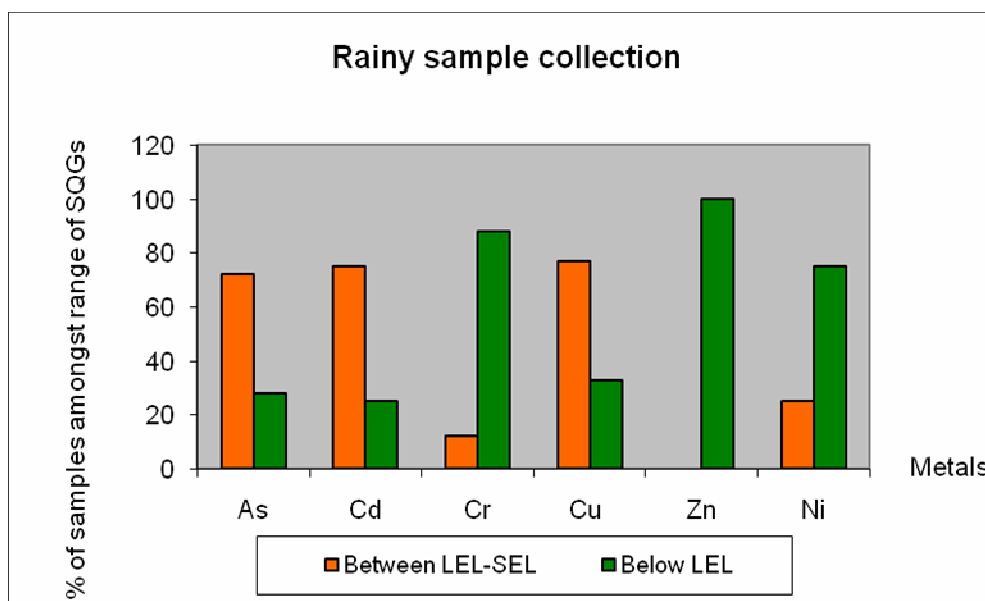


Figure 4.18 Percentage of samples collected in rainy season amongst range of SQGs

An interesting distribution pattern was found from this study. The metal contents are either highly distributed in the range below LEL or in between LEL and SEL. In rainy season, 5 metals as As, Cd, Cr, Cu and Ni dataset show equal distribution pattern among the SQGs' ranges. However, in the dry season, number of metal exceeding LEL reduced to 3 metals (As, Cd and Cu) in dataset. Following are the distributions of metal range in different effect level; Cd is the most critical contaminant in sediment as 100% of the collected samples exceeded effect level (LEL). On the other hand, As became the second critical contaminants in sediment samples. It shows the high numbers of sediment samples exceeding LEL (25-50%).

Mean concentrations of arsenic and zink in the sediments are in the same concentrations range like preindustrial sediments from the Nha Trang bay (Phuong T.T.M et al, 2012). Mean Cu concentrations in sediments from the ND and BT were 2 times higher in comparison with preindustrial sediments from the Nha Trang bay. Like copper, lead mainly originates from anthropogenic sources, but its concentrations were not very elevated, and they are comparable with other surface sediment. However, those concentrations were more than eight times lower than medium concentrations in the polluted sediments of the near by harbor.

#### 4.2.4 Interactions between metals

To study the inter-elemental associations, Pearson correlation coefficients of the trace metals were computed for both two seasons. One can observe a strong correlation between the amount of Fe and Al in both two season (Table 4.10 and 4.11). This can be mainly attributed to synergic co-precipitation in the form of hydrated aluminum oxide or hydrated ferric oxides. In sediment, a significant correlation exists between Cr and Al, Fe, Zn and Ni in both dry and rainy season and between Cu-Zn and Fe, Al, Ni in dry season. The same holds true for sediment concentrations of Fe has also shown that the high correlation of Al and Ni ( $r = 0.99$ ). Zn is high affected by a Cu concentration ( $r=0.94$ ). Negative correlations are obtained between As and Cd metal ( $r = -0.82$ ) and between Cd and Cu and Zn ( $r = -0.35$  and  $-0.38$ ) in rainy season. This can be attributed to the different longitudinal variations of these metals in the coastal. Major seasonal variations in metal concentrations occur in the four sites.

Table 4.10 Interactions between metals in rainy season

N	As	Cd	Cr	Cu	Zn	Fe	Al	Ni
As	1,0000							
Cd	-0,8223	1,0000						
Cr	-0,1312	0,2356	1,0000					
Cu	0,7467	-0,3853	0,5039	1,0000				
Zn	0,5915	-0,3541	0,7201	0,9443	1,0000			
Fe	-0,3489	0,3957	0,9746	0,3024	0,5464	1,0000		
Al	-0,1806	0,1776	0,9836	0,4060	0,6633	0,9739	1,0000	
Ni	-0,1552	0,2013	0,9951	0,4550	0,6944	0,9774	0,9966	1,0000

Table 4.11 Interactions between metals in dry season

	As	Cd	Cr	Cu	Zn	Fe	Al	Ni
As	1,0000							
Cd	-0,7048	1,0000						
Cr	-0,5329	0,0372	1,0000					
Cu	-0,5388	0,4726	0,8063	1,0000				
Zn	-0,5379	0,0836	0,9976	0,8452	1,0000			
Fe	-0,6656	0,1341	0,9839	0,7778	0,9783	1,0000		
Al	-0,8300	0,7642	0,6693	0,8941	0,7056	0,7205	1,0000	
Ni	-0,2678	-0,4031	0,8775	0,4361	0,8448	0,8507	0,2511	1,0000
Mo	-0,8418	0,9554	0,0823	0,3741	0,1114	0,2177	0,7403	-0,2850

To study the interactions between metals and physicochemical characteristics of sediments, Pearson correlation coefficients of the trace metals were computed for four locations separately (Table 4.12, 4.13, 4.14, 4.15 and 4.16). In all the sites studied, trace metals and their inter relationship showed strong correlation with Cr and Al, Fe and in significant correlation with organic matter and moisture content. Due to relatively low concentration of organic matter in sediments, it does not act as a carrier of metals, and therefore shows asynchronous distribution pattern with trace metals. Strong inter-element relationships suggest that one common process determines the sediment composition for all trace metals.

The matrix correlating the geophysical parameters (grain size, TOC, pH and moisture) and pollutant concentrations is presented in Table 4.12. A high correlation was observed between metal levels in the sediment and silt-clay content while a moderately negative correlation resulted between Cd in sediment and other metals in the sediment. Decreases in TOC and pH in the dry and rainy season appear to be linked to a accumulation of metals in the sediments.

In all the time sampling, trace metals and their inter relationship showed strong correlation with Al and in significant correlation with OC as well as moisture contents.

Due to relatively low concentration of OC in sediments, it does not act as a carrier of metals, and therefore shows asynchronous distribution pattern with trace metals. Strong inter-element relationships suggest that one common process determines the sediment composition for all trace metals. The strong positive correlation between Cr and other metals as Cu, Zn, Fe and Al ( $r = 0.80 - 0.96$ ) reveals that these metals are carried by pollution of Cr, which come from industrial discharges, agricultural activities into the near by environment. In moreover, the poor association of As with other metals suggests that As possibly due to different processes like biological effects and external inputs operating in the mangrove and adjacent estuarine sediments. Significant correlations of Cr, Cu, Zn with Fe, Al indicate the industrial contribution of these metals into the system.

Rank-order correlation coefficients for metal ion interconnections are showed in Table 4.12. The strong positive correlation between all the metals in sediment collected from TD that these metals are carried by industrial discharges, fishing activities, and constant movement of fishing and commercial boats into the near by environment. In TD, the poor association of As with other metals suggests that Fe oxide may only be a minor host phase for these metals in the mangrove environment. In TD, Cd showed no significant correlation with other metals possibly due to different processes like biological effects and external inputs operating in the mangrove and adjacent estuarine sediments. The differences in correlation among the above metals reflect differential behaviors, which require further examination. Significant correlations of Cr, Cu and Zn with Fe and Zn in TD indicate the industrial contribution of these metals into the system.



Table 4.12 The interactions between metals and physicochemical characteristics of sediments collected from Nha Trang bay and adjacent areas

	pH	Moisre	TOC	>0.063 mm	<0.063 mm	As	Cd	Cr	Cu	Zn	Fe	Al	Ni
pH H <sub>2</sub> O	1												
pH KCl	0,3023												
Moisture	0,1445	1											
TOC	0,0370	0,9814	1										
>0.063mm	-0,6894	0,0749	0,2266	1									
<0.063mm	0,5889	-0,0735	-0,2223	-0,9824	1								
As	-0,3075	-0,1367	-0,0414	0,0738	-0,0053	1							
Cd	-0,1199	-0,0364	0,0436	0,6853	-0,7644	-0,5395	1						
Cr	0,1897	0,9389	0,9460	0,1294	-0,1633	-0,1450	0,1122	1					
Cu	0,0799	0,3139	0,3957	0,2000	-0,2936	0,1399	0,2407	0,6037	1				
Zn	-0,0085	0,6096	0,6537	0,1478	-0,2020	0,1051	0,0481	0,8072	0,9004	1			
Fe	0,3258	0,9047	0,8642	-0,0175	-0,0369	-0,4250	0,1635	0,9481	0,4847	0,6999	1		
Al	0,2222	0,7324	0,7520	0,3396	-0,4225	-0,4026	0,5347	0,8665	0,6178	0,6945	0,8677	1	
Ni	0,2339	0,9564	0,9247	-0,0729	0,0658	-0,1115	-0,1275	0,9612	0,4628	0,7435	0,9291	0,7366	1
Mo	-0,3863	-0,5822	-0,5002	0,4597	-0,5030	-0,187	0,6250	-0,3435	0,3050	0,0500	-0,3309	0,0028	-0,5070

Table 4.13 Metals in correlation with the physico-chemical factors in Tan Dao station

	pH	Moisture	TOC	>0.063mm	<0.063mm	As	Cd	Cr	Cu	Zn	Fe
pH	1										
Moisture	-0,6699	1,0000									
TOC %	0,9930	-0,5772	1,0000								
>0.063mm	0,9993	-0,6976	0,9877	1,0000							
<0.063mm	-0,9993	0,6976	-0,9877	-1,0000	1,0000						
As	-0,3201	0,9178	-0,2056	-0,3559	0,3559	1,0000					
Cd	0,1148	-0,8145	-0,0037	0,1525	-0,1525	-0,977	1,0000				
Cr	0,5907	0,2034	0,6822	0,5596	-0,5596	0,5753	-0,733	1,0000			
Cu	0,7909	-0,0755	0,8578	0,7671	-0,7671	0,3266	-0,517	0,9610	1,0000		
Zn	0,8016	-0,0930	0,8668	0,7783	-0,7783	0,3099	-0,501	0,9559	0,9998	1,0000	
Fe	0,7878	-0,0704	0,8552	0,7638	-0,7638	0,3313	-0,521	0,9623	1,0000	0,9997	1,0000
Al	0,9940	-0,5844	1,0000	0,9891	-0,9891	-0,214	0,0052	0,6756	0,8532	0,8623	0,8506

Table 4.14 Metals in correlation with the physico-chemical factors in Ngoc Diem station

	pH	Moisture	TOC	>0.063 mm	<0.063 mm	As	Cd	Cr	Cu	Zn	Fe
pH	1,0000										
Moisture	0,7593	1,0000									
TOC	-0,0735	0,5931	1,0000								
>0.063mm	0,3380	0,8690	0,9138	1,0000							
<0.063mm	-0,3380	-0,8690	-0,9138	-1,0000	1,0000						
As	0,9802	0,8733	0,1256	0,5178	-0,5178	1,0000					
Cd	0,1086	0,7293	0,9834	0,9723	-0,9723	0,3035	1,0000				
Cr	0,1052	0,7270	0,9840	0,9715	-0,9715	0,3003	1,0000	1,0000			
Cu	0,4260	0,9122	0,8709	0,9955	-0,9955	0,5969	0,9456	0,9445	1,0000		
Zn	-0,8247	-0,2581	0,6247	0,2536	-0,2536	-0,6962	0,4728	0,4757	0,1604	1,0000	
Fe	0,2413	0,8147	0,9501	0,9949	-0,9949	0,4288	0,9909	0,9905	0,9808	0,3500	1,0000
Al	-0,6779	-0,0364	0,7830	0,4628	-0,4628	-0,5187	0,6572	0,6597	0,3763	0,9749	0,5499

Table 4.15 Metals in correlation with the physico-chemical factors in Binh Tan station

	pH	Moisture	TOC	>0.063mm	<0.063mm	As	Cd	Cr	Cu	Zn	Fe
pH	1,0000										
Moisture	0,7857	1,0000									
TOC	0,2186	0,7754	1,0000								
>0.063mm	-0,9524	-0,5596	0,0895	1,0000							
<0.063mm	0,8360	0,3175	-0,3527	-0,9635	1,0000						
As	-0,9515	-0,9379	-0,5081	0,8124	-0,6267	1,0000					
Cd	0,7573	0,9990	0,8028	-0,5221	0,2749	-0,9215	1,0000				
Cr	0,5937	0,9643	0,9150	-0,3200	0,0549	-0,8124	0,9751	1,0000			
Cu	0,1822	0,7514	0,9993	0,1263	-0,3871	-0,4758	0,7801	0,8994	1,0000		
Zn	0,1533	0,7317	0,9978	0,1554	-0,4140	-0,4498	0,7614	0,8862	0,9996	1,0000	
Fe	0,9600	0,9275	0,4831	-0,8289	0,6490	-0,9996	0,9099	0,7953	0,4503	0,4238	1,0000
Al	0,8192	0,9984	0,7387	-0,6052	0,3702	-0,9559	0,9949	0,9479	0,7132	0,6923	0,9470

Table 4.16 Metals in correlation with the physico-chemical factors in Cam Lam station

	pH	Moisture	TOC	>0.063mm	<0.063mm	As	Cd	Cr	Cu	Zn	Fe
pH	1,0000										
Moisture %	-0,7485	1,0000									
TOC %	-0,2641	-0,4419	1,0000								
>0.063mm	-0,9936	0,6688	0,3713	1,0000							
<0.063mm	0,9936	-0,6688	-0,3713	-1,0000	1,0000						
As	-0,9509	0,5066	0,5496	0,9798	-0,9798	1,0000					
Cd	0,7806	-0,9988	0,3967	-0,7050	0,7050	-0,5489	1,0000				
Cr	0,4772	0,2255	-0,9736	-0,5734	0,5734	-0,7257	-0,1768	1,0000			
Cu	0,5728	0,1148	-0,9419	-0,6617	0,6617	-0,7983	-0,0652	0,9937	1,0000		
Zn	-0,0655	-0,6126	0,9797	0,1778	-0,1778	0,3711	0,5725	-0,9082	-0,8555	1,0000	
Fe	0,9283	-0,4484	-0,6037	-0,9644	0,9644	-0,9978	0,4923	0,7697	0,8365	-0,4318	1,0000
Al	0,9052	-0,9594	0,1708	-0,8514	0,8514	-0,7293	0,9722	0,0585	0,1701	0,3647	0,6823

### 4.3 HEAVY METALS IN MOLLUSK TISSUES

#### 4.3.1 Concentrations of heavy metals between shell and tissue

Concentrations of metals in shell and tissues of these 7 species, which collected on the first time (March 2012) were showed in Figure 4.19 to Figure 4.25. These results showed that, for the metals, concentration of metals in shell and tissue were significantly correlated. These correlations may be partially attributed to metabolic rate of each species.

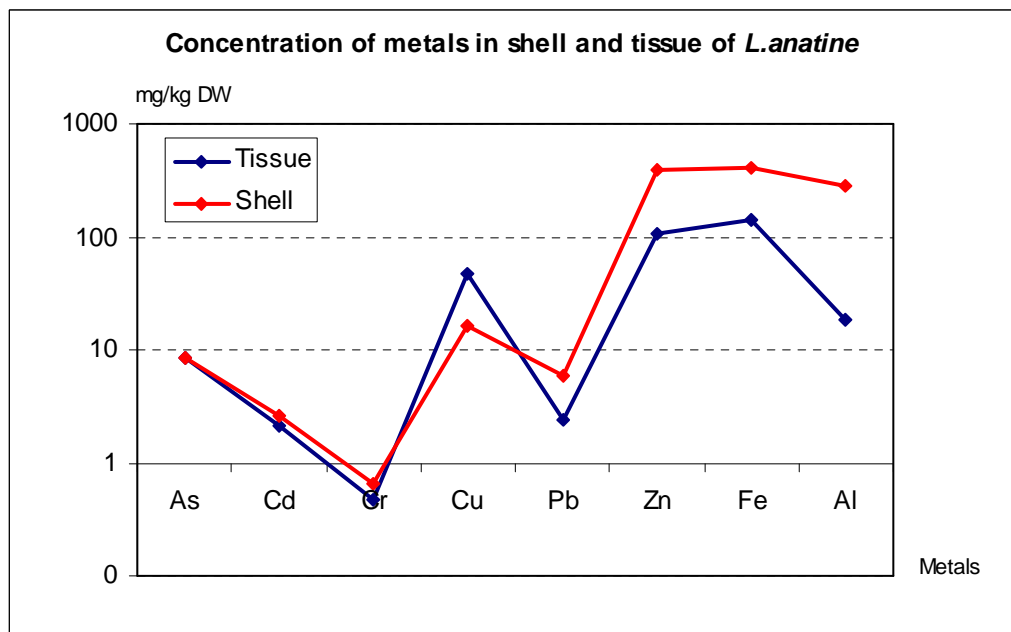


Figure 4.19 Concentrations of metals in shell and tissue of *L. anatine*

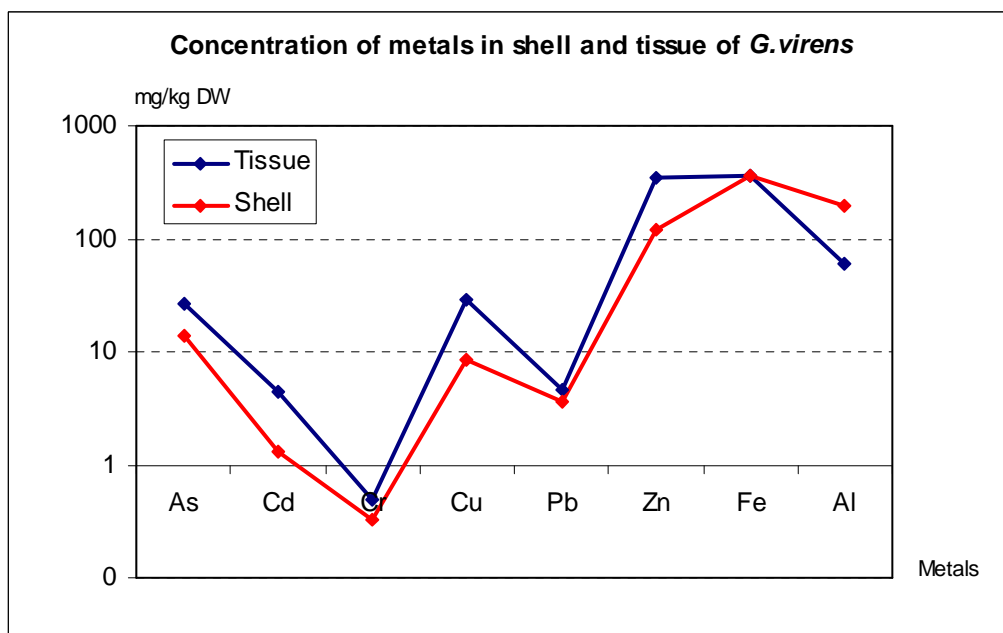


Figure 4.20 Concentrations of metals in shell and tissue of *G.virens*

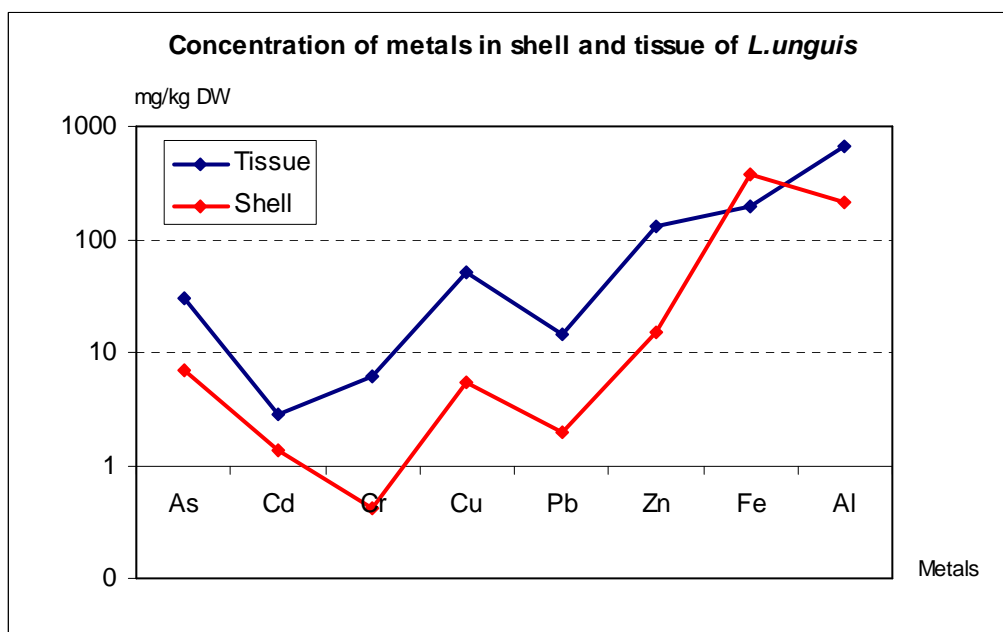


Figure 4.21 Concentrations of metals in shell and tissue of *L.unguis*

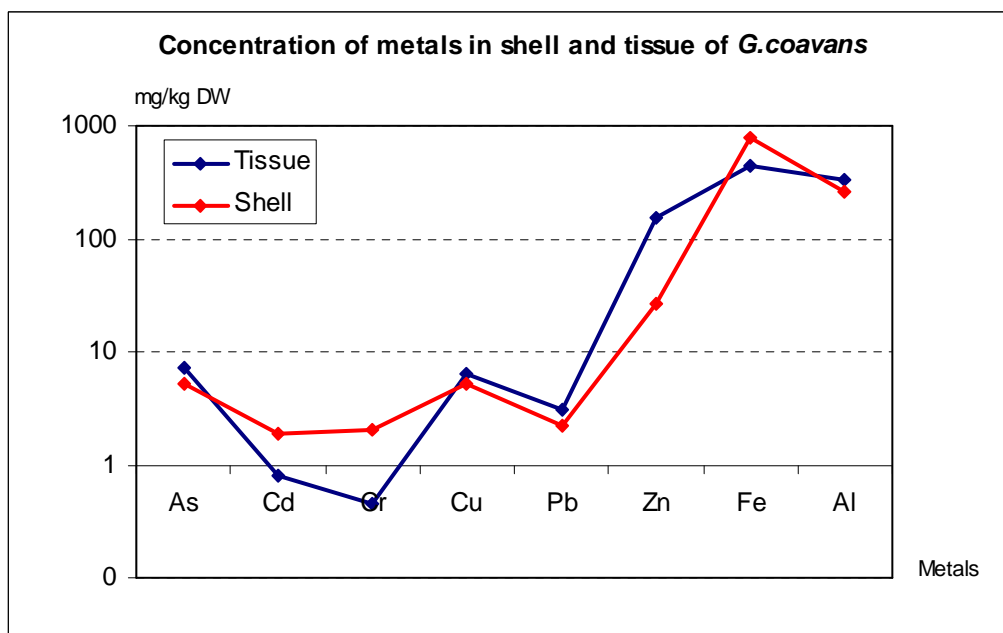


Figure 4.22 Concentrations of metals in shell and tissue of *G.coavans*

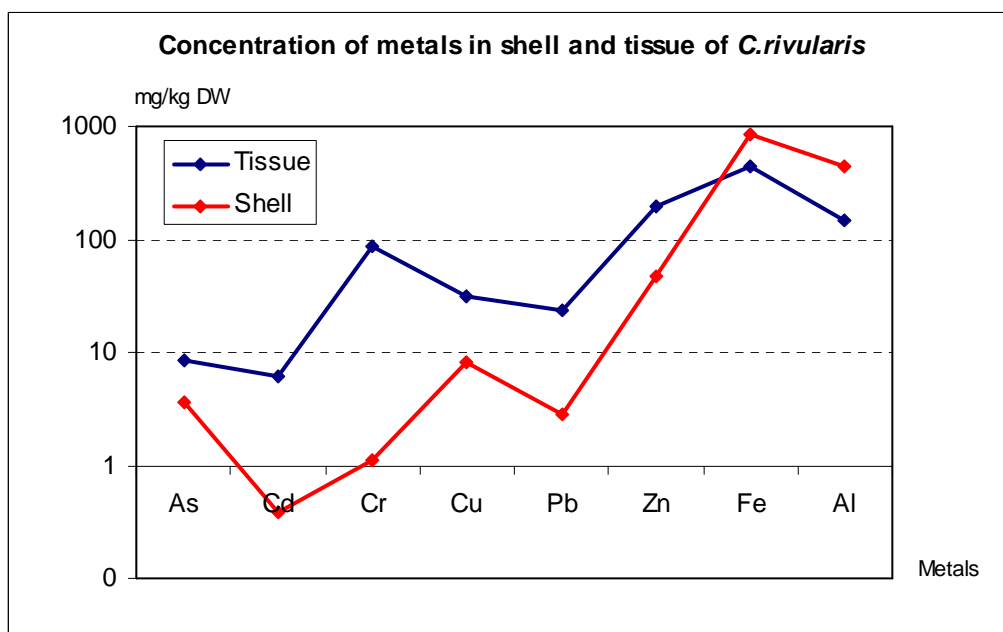


Figure 4.23 Concentrations of metals in shell and tissue of *C.rivlaris*



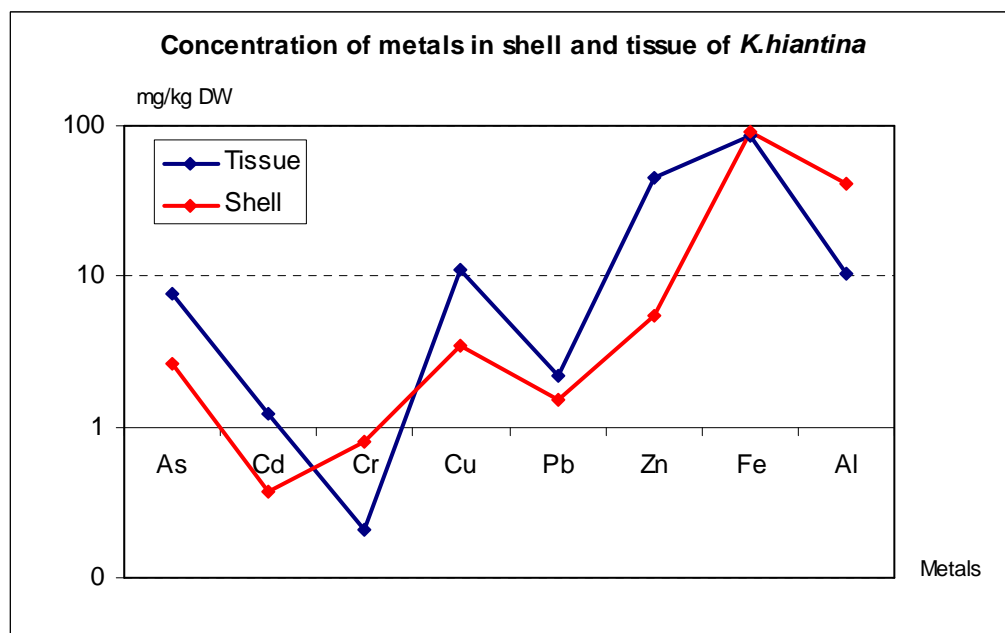


Figure 4.24 Concentrations of metals in shell and tissue of *K.hiantina*

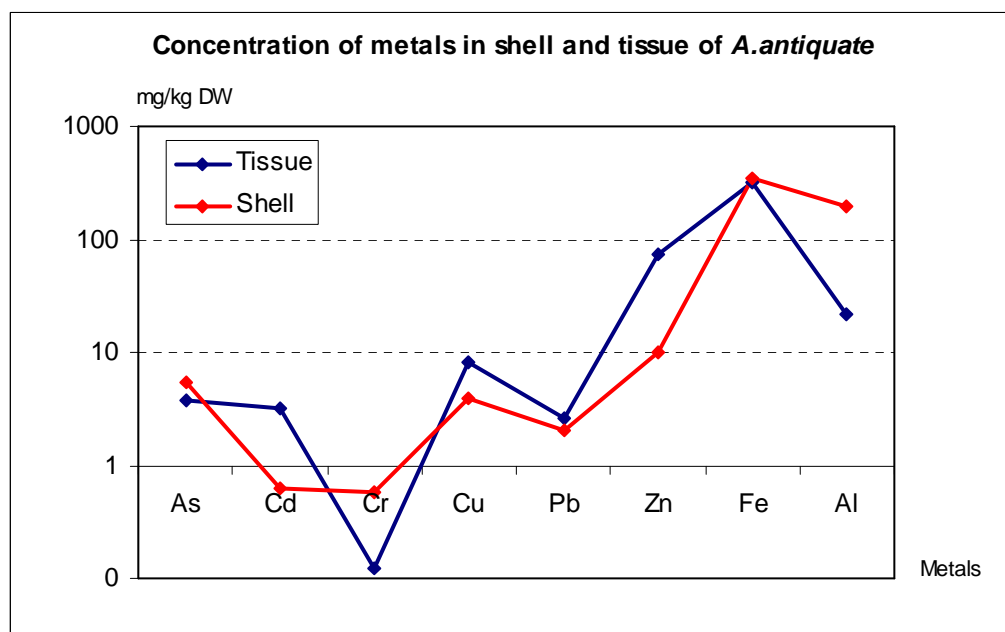


Figure 4.25 Concentrations of metals in shell and tissue of *A.antiquate*

In general, concentrations of Al and Fe in mollusk samples from Nha Trang bay were higher in shell than in soft tissue, but for other heavy metals, concentration of metals in shell and tissue were significantly correlated.

These results highlight that there not necessity to analyse metals in shell when comparing trace metal concentrations amongst mollusk samples.

### 4.3.3 Contents of heavy metals in soft tissues

Eleven species of mollusks collected from four locations were analyzed for metal contents. Results from the analyses of 5 metals As, Cd, Cr, Cu and Zn in these species are given in Figures 4.26 as below.

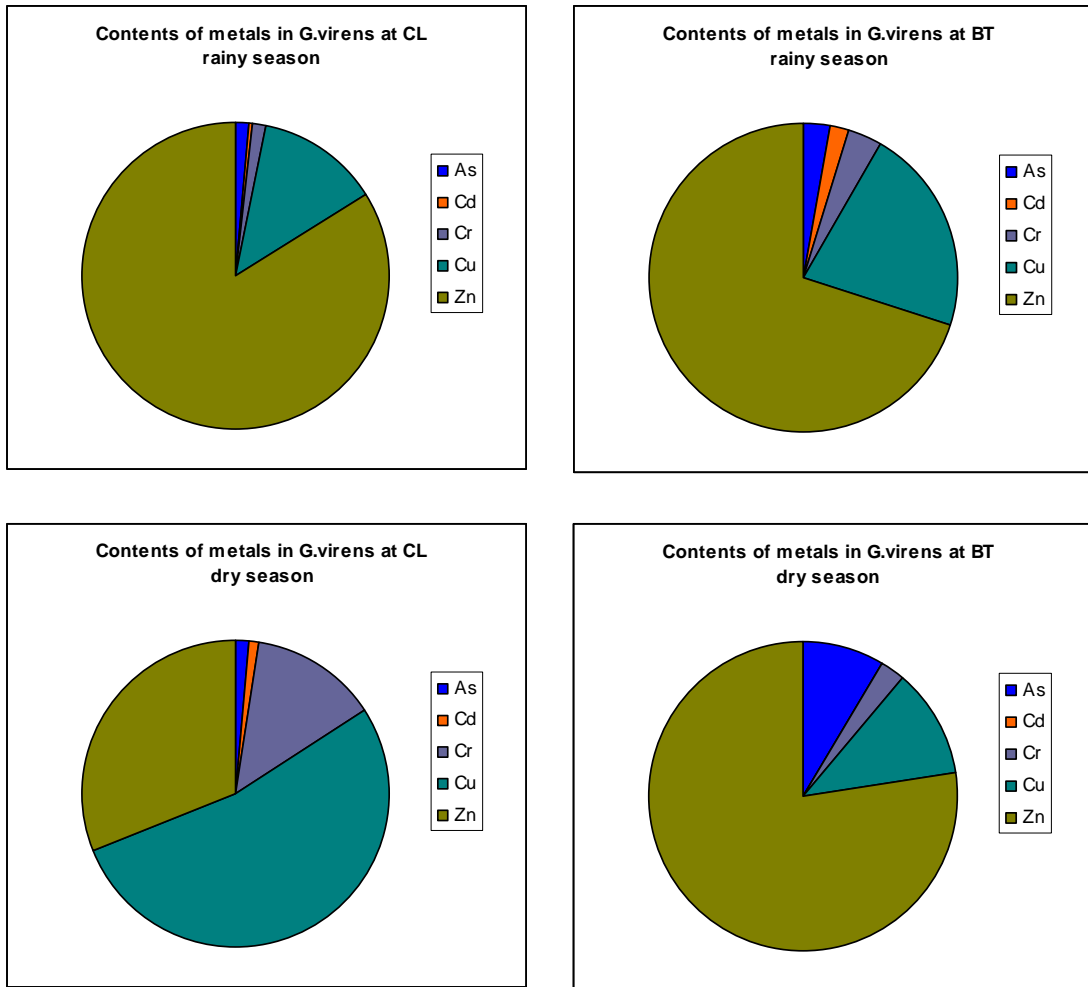


Figure 4.26 Contents of metal in soft tissue of *G. virens*

Generally, the highest values of heavy metals were observed during rainy season and the lowest during dry season, except Cu in CL. Mean concentrations of Zn found in mollusks were high in both seasons. Moreover, the concentrations of heavy metals in the *G. virens* of the ND could be arranged in the following sequence:  $As > Cd > Zn > Cr > Cu$ . From these results, the local distribution of metals in mollusk tissue of *G. virens* at different

stations also varied during dry and rainy seasons. The maximum value of As, Cd were found at ND and Cu, Zn at BT during rainy season, while the highest value of Cd, Cr, Cu, Zn were recorded at CL during dry season. The minimum values of Cd, Cr and Zn were found at station CL in rainy season, while Cu, Cd, Cr and Zn were found at BT during rainy season.

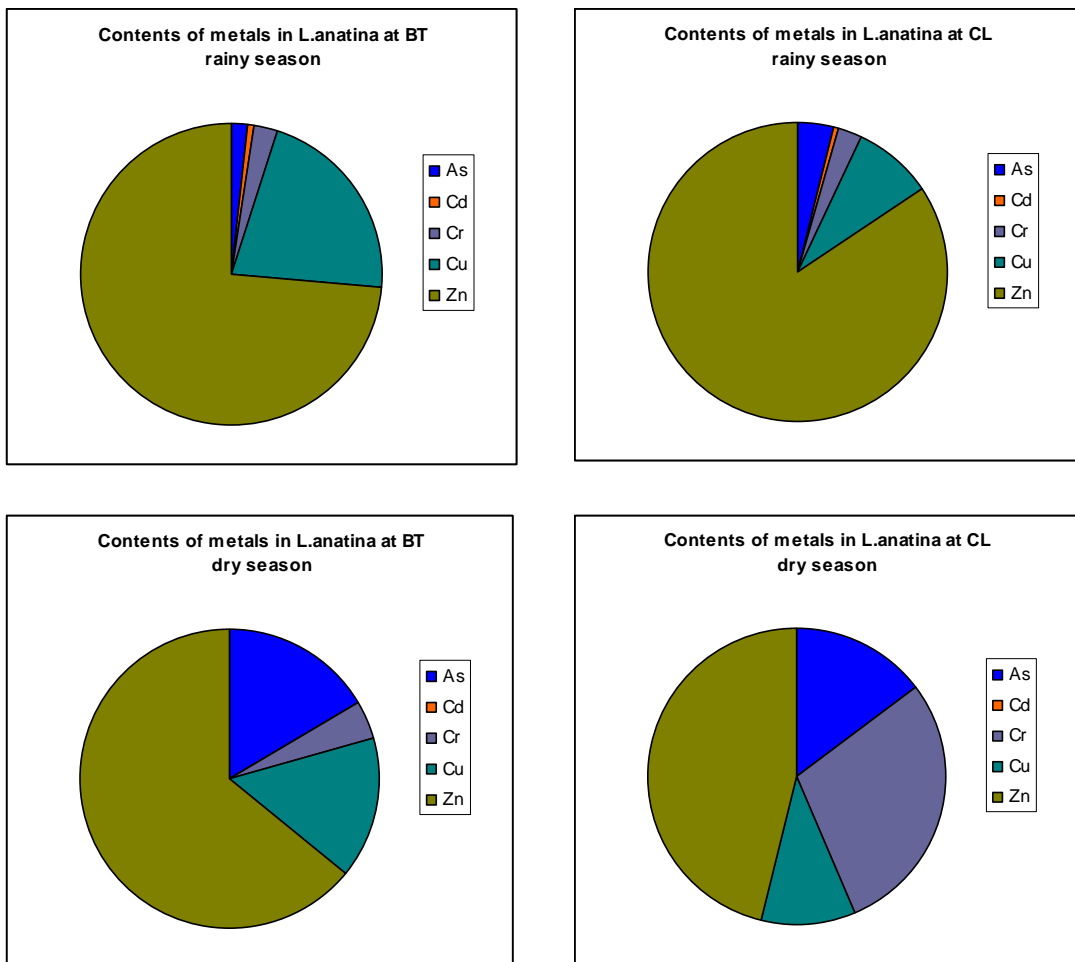


Figure 4.27 Contents of metal in soft tissue of *Laternula anatina*

On a seasonal scale, Zn and Cu showed their highest values during rainy season at both locations BT and CL (61.29 and 90.60mg/kgDW for Zn; 17.72 and 9.22mg/kg DW for Cu, respectively) where the highest values of As were exhibited during dry season. (6.58 and 6.84mg/kg DW ).The levels of Cr varied from 2.22 and 2.64 during rainy season to 1.62 to 13.42mg/kg DW during dry season, respectively. The maximum values of Cr recorded 13.42 during rainy season at CL location and their minimum values 1.62 during dry season at BT.

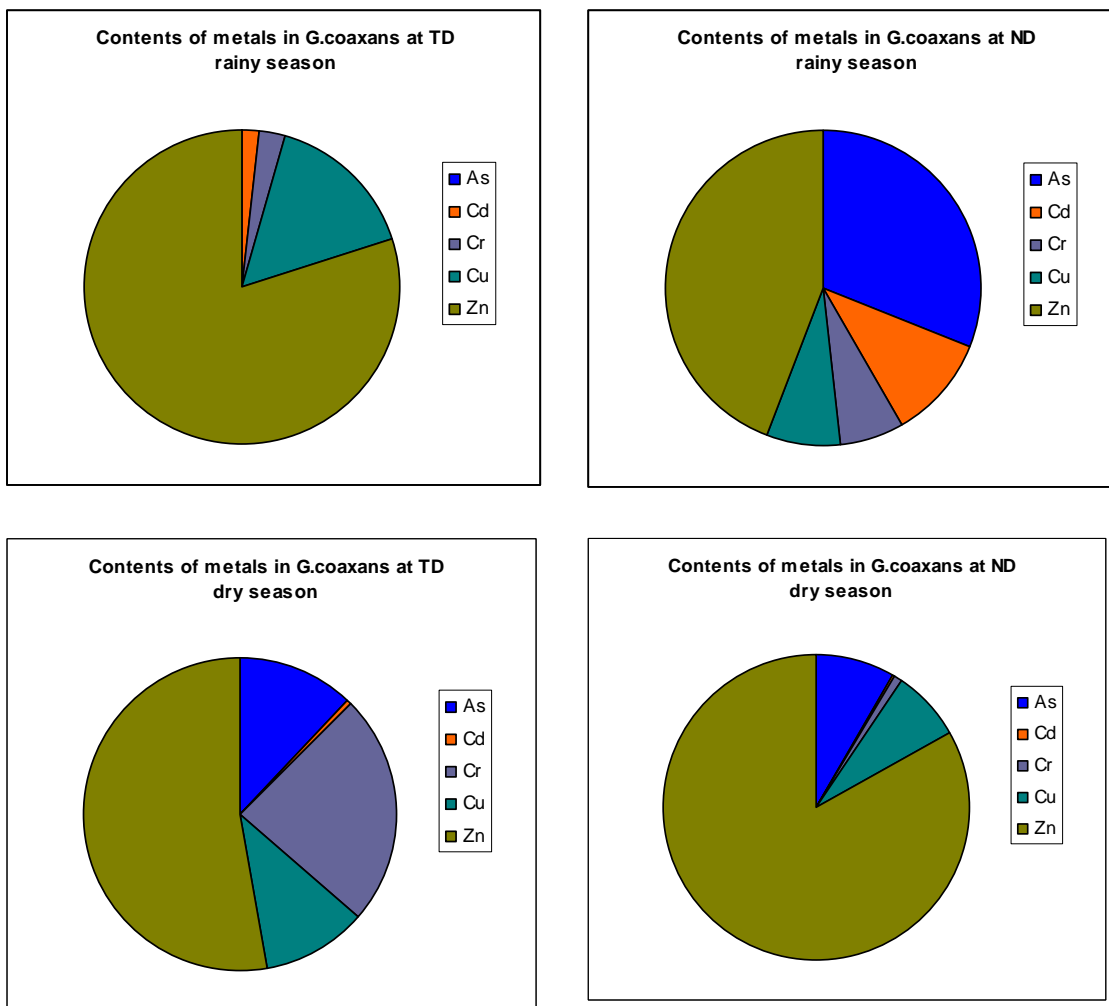


Figure 4.28 Contents of metal in soft tissue of *Geloina coxans*

For comparisons of data between two seasons the concentration of Cu and Zn in mollusks *G.coaxans* collected from TD during rainy season was much higher than that found in dry seasons. It was found that concentration of Zn was higher than Cu and Cr in mollusks in two seasons.

In addition, concentrations of As, Cd and Cr in *G.coaxans* collected in ND were higher than that in TD at rainy season (6.10, 2.10 and 1.28 mg/kg DW in ND and 0.01, 0.83 and 1.22mg/kg DW in TD, respectively) but concentration of Cu and Zn in *G.coaxans* collected from TD were higher than that found in ND.

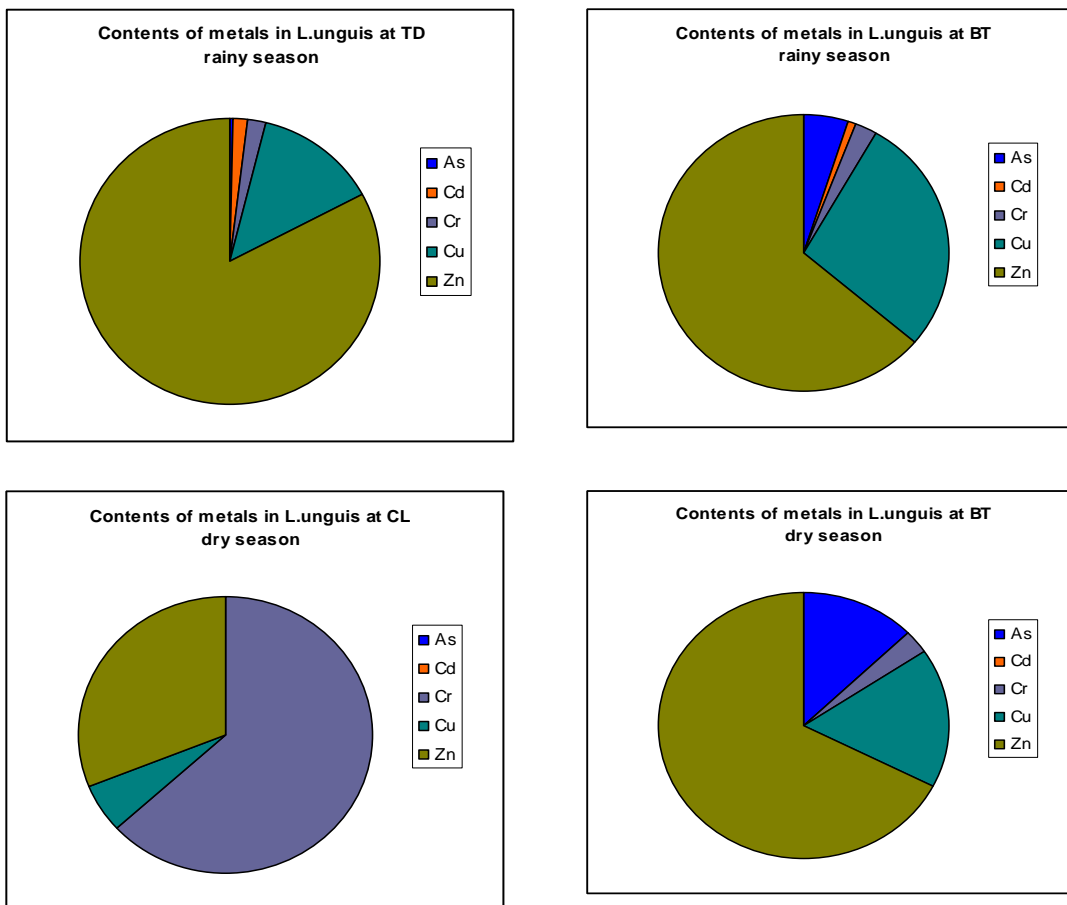


Figure 4.29 Contents of metal in soft tissue of *Lingula unguis*

In the present study, Cu content of tissue of *L. linguis* ranged from 5.41 to 9.35 and from 9.74 to 24.71 mg/kg DW at TD and BT during rainy season, respectively. The high accumulation of metal content may be due to the precipitation of decomposed organic matter of this location.

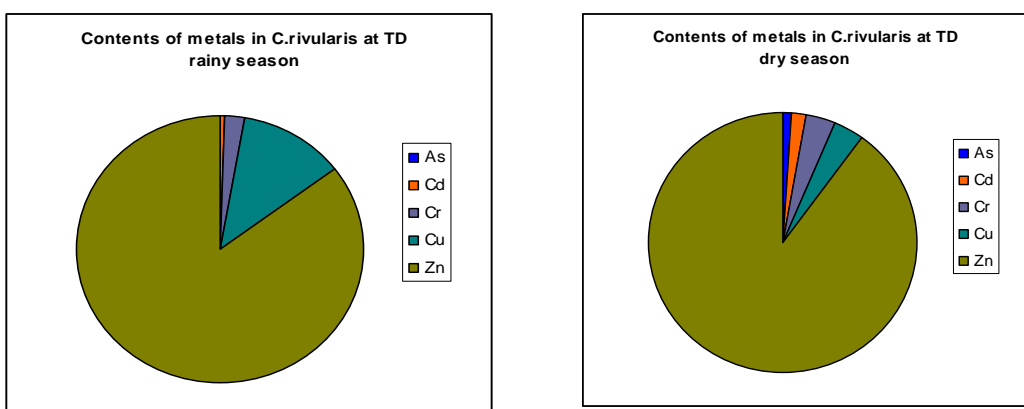


Figure 4.30 Contents of metal in soft tissue of *Crassostrea rivularis*

Mollusk tissues of *C.rivularis* were very rich of Zn contents. Maximum concentration of Zn in oyster was found in at BT in dry season about 562.01mg/kg DW.

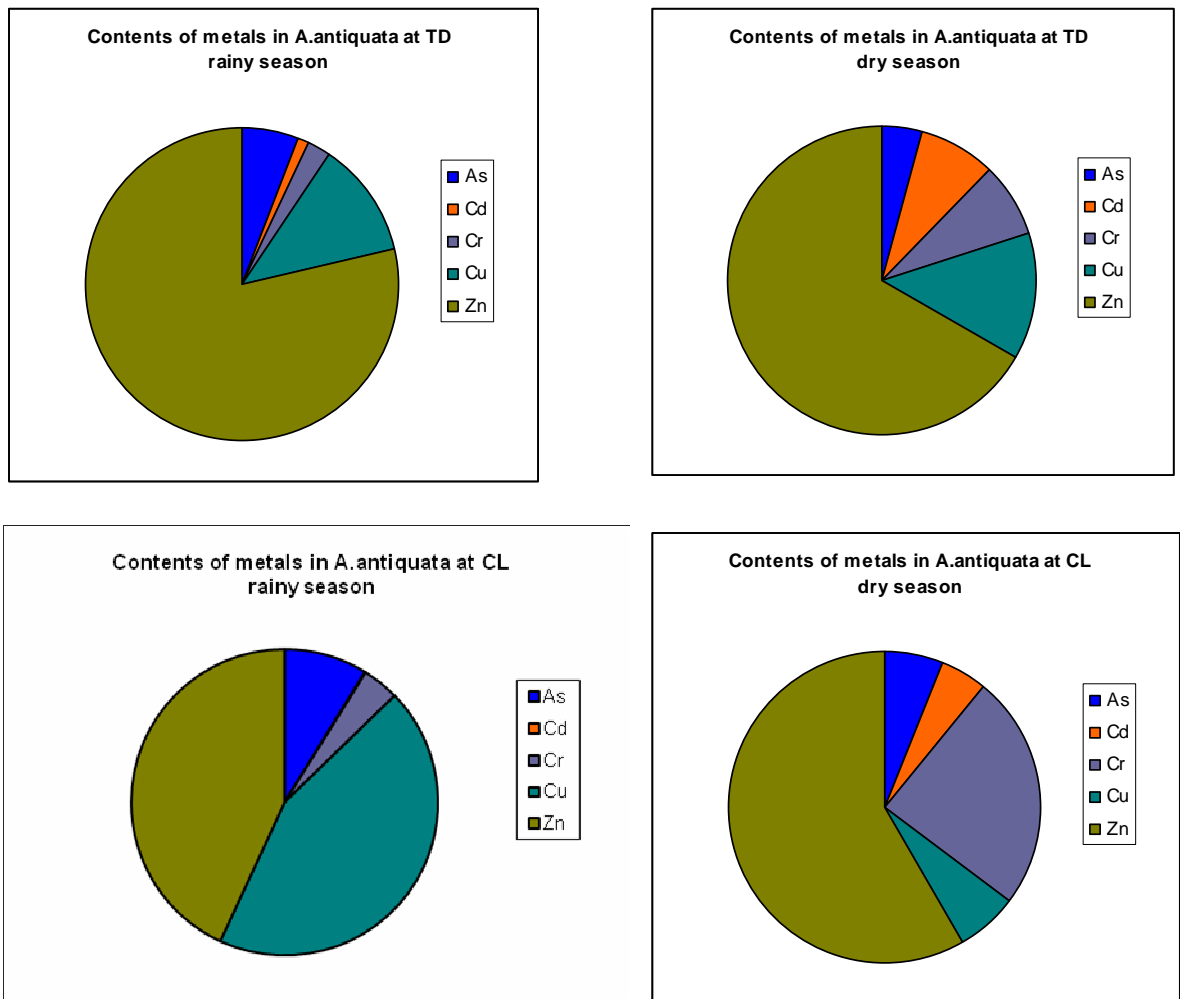


Figure 4.31 Contents of metal in soft tissue of *Anatina antiquate*

The local distribution of Cu in tissue of *A.antiquate* at TD showed higher concentration than the other stations. This may be due to the anti-fouling paints. The seasonal concentration of accumulate Zn showed higher level at TD (59.04 and 37.85mg/kgDW ) during rainy and dry season, respectively, while the level recorded 42.76mg/kg DW during dry season at station CL. This may be due to the high amount of suspended organic matter coming from different industrial wastes that precipitate to the bottom and also due to the decrease uptake of zinc by phytoplankton at lower temperature.

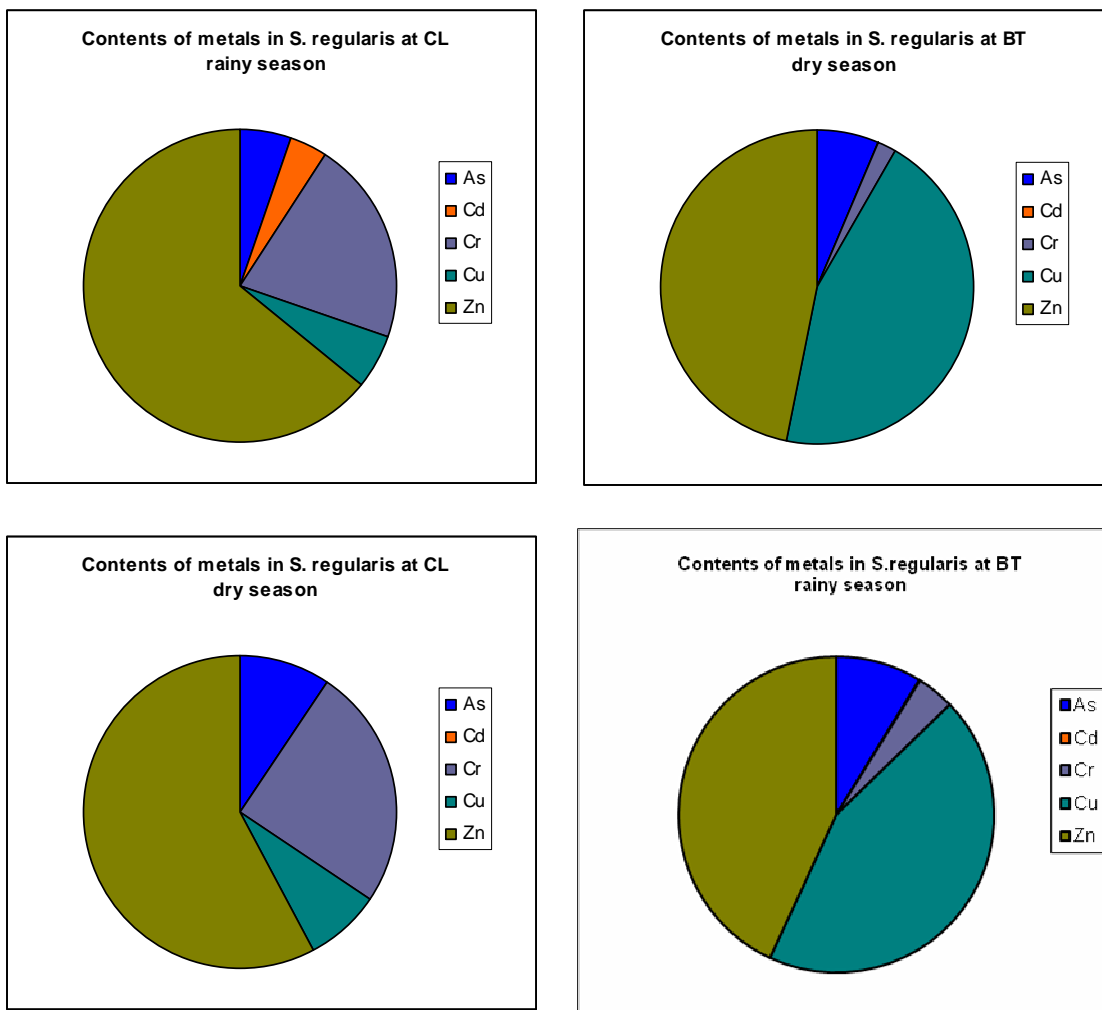


Figure 4.32 Contents of metal in soft tissue of *Solens regularis*

The present concentration of Cu in tissue of the *S. regularis* was higher than other sites. The maximum value of Cu (52.26mg/kg DW) was observed at BT in dry season probably due to sewage discharge and industrial effluents at the Nha trang city. This can be explained by the fact that their hydroxides are precipitated together in the bottom and the common geochemistry of both.

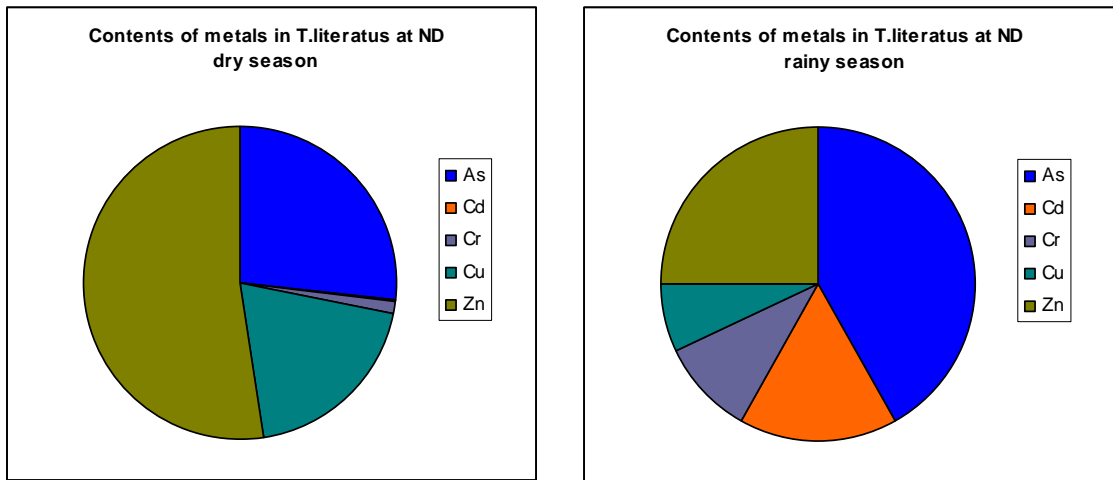


Figure 4.33 Contents of metal in soft tissue of *Tapes literatus*

In the present study, As content of tissue of *T. literatus* ranged from 17.98mg/kgDW in rainy season to 6.85mg/kg DW at ND and Zn concentration were decreased from 4.08.10 to 35.01mg/kg DW from rainy to dry season. The high concentration of Cd content in rainy season may be due to the precipitation of industrial leaching from north part of the bay.

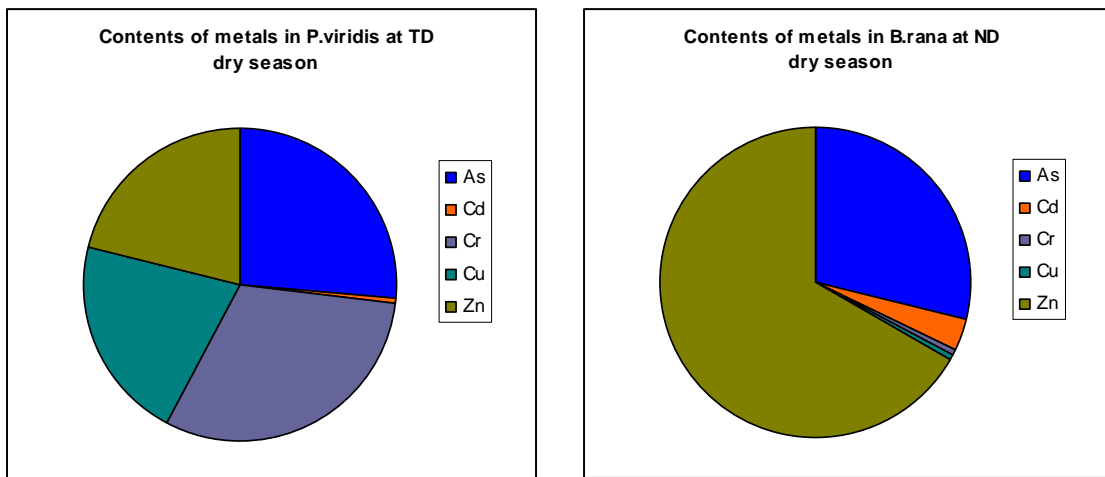
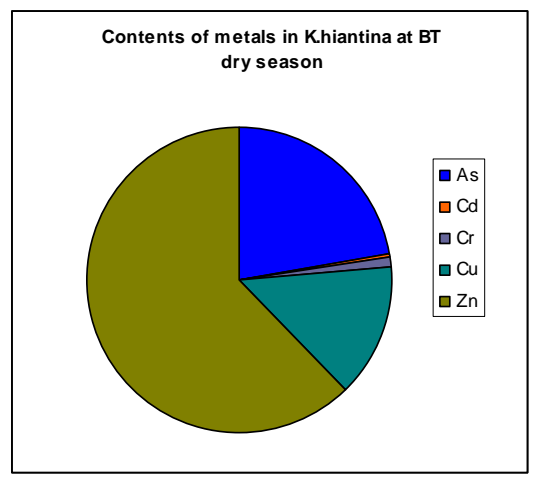
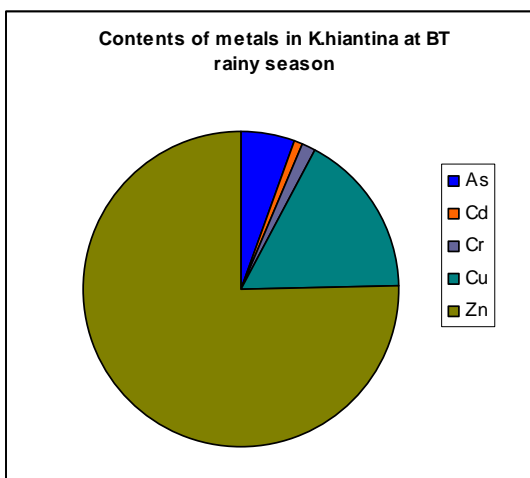
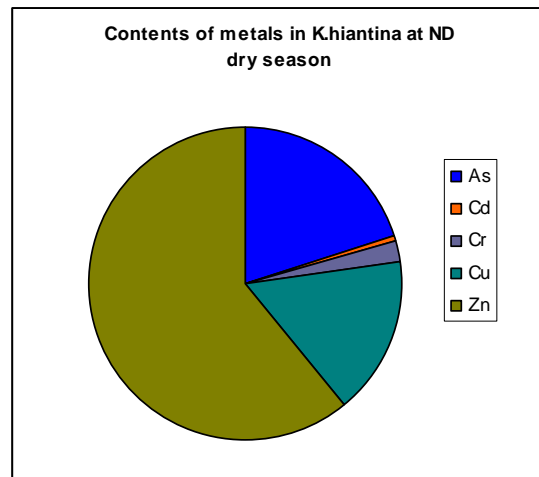
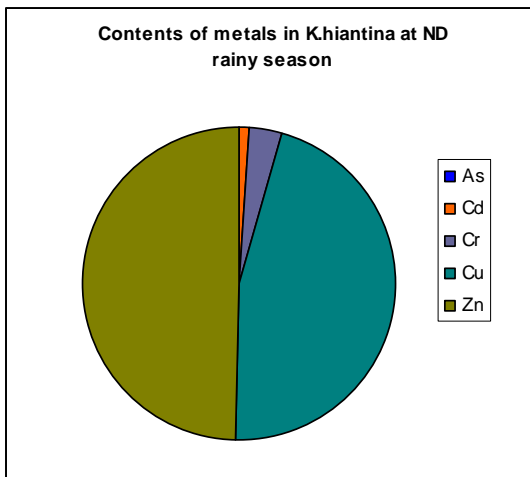
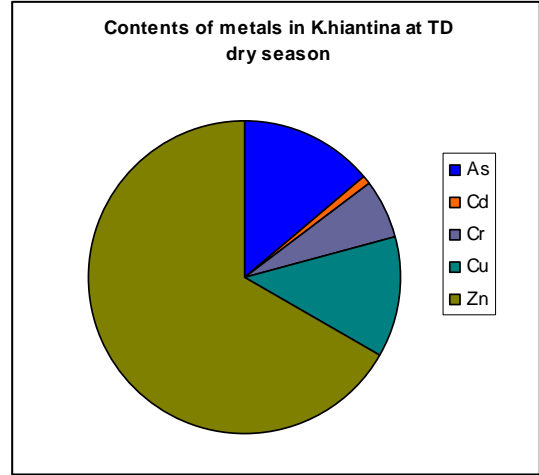
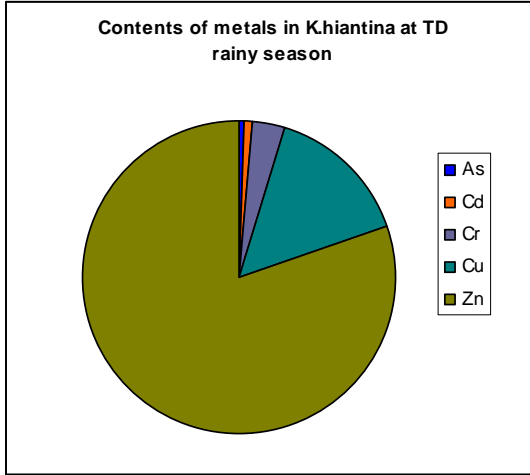


Figure 4.34 Contents of metal in soft tissue of *B.rana* and *P.viridis*

High differences of concentration of heavy metals in 2 species of Gastropode *B.rana* and Bivalve *P.viridis* were detected in rainy season for comparison. In gastropode *B.rana* tissues, concentrations of Zn and As were highest compared to Cu and others, but in bivalve *P.viridis*, these concentrations of As, Cr, Cu and Zn were similar.



Furthermore, the concentrations of Zn in *B.rana* were higher than in that in *P.viridis* during dry period. The comparative study showed that the accumulation of heavy metals in 2 mollusk tissues were significantly higher than in sediment samples.



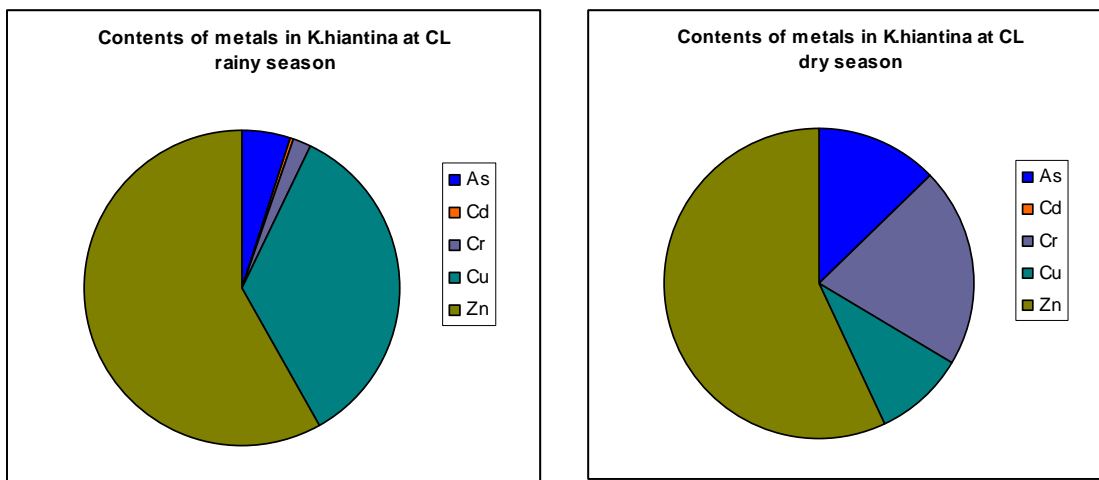


Figure 4.35 Contents of metal in soft tissue of *K. hiantina*

Samples contained arsenic concentrations that ranged from 0.01 to 6.7 mg/kg DW in rainy season and lower than concentrations of this metal in tissue at dry season 5.85-15.53 mg/kg DW. Among the five metals tested in all tissue of mollusk, zinc Zn concentration was the highest in all tissue of mollusks. This could be due to zinc is essential element which is important in the metabolic activities of mollusk. Copper concentration was the second elements found to be highest in the mollusk tissue. This could be explained due to the high concentration of this metal in the water body and the role of this metal as essential elements for aquatic organism.

#### 4.3.4 Metal pollution index

Metal pollution index MPI values provides a representative picture of contaminant impacts on bivalves due to their ability to concentrate and reflect the environmental levels of trace metal contaminants in coastal ecosystems. Table 4.17 and Table 4.18 show on the whole, the highest, moderate and lowest MPI ranges for 7 metals: As, Cd, Cr, Cu, Zn, Fe and Al. The averages occurred during the dry and rainy season for 4 locations are respectively.

Table 4.17 MPI during rainy season for 4 locations of sampling

	Rainy season	TD	ND	BT	CL
1	<i>Katelysia hiantina</i>	3,72	2,62	7,44	4,46
2	<i>Glaucanoma virens</i>		1,17	5,05	1,61

3	<i>Laternula anatine</i>			4,55	5,89
4	<i>Lingula unguis</i>	2,63		6,45	
5	<i>Geloina coaxans</i>	1,23	2,92		
6	<i>Crassostrea rivularis</i>	6,14			
7	<i>Anatina antiquate</i>	5,18			
8	<i>Tapes literatus</i>		2,69		
9	<i>Solens regularis</i>				5,17
	Mean	3,78	2,35	5,87	4,28
	STDV	1,96	0,80	1,14	1,88

The highest MPI values observed at BT is found to be coincided with the high overall percentage contribution of the rivers Be to the flow rate in rainy season (5,87), where as the lowest MPI valves observed at ND is found to be coincided with the low percentage contribution of the low flow rate.

Table 4.18 MPI during dry season for 4 locations of sampling

<b>N</b>	<b>Dry season</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
1	<i>Katelysia hiantina</i>	3,83	4,14	4,09	4,16
2	<i>Glauconoma virens</i>			3,57	8,52
3	<i>Laternula anatine</i>			1,76	2,49
4	<i>Lingula unguis</i>			2,75	0,74
5	<i>Geloina coaxans</i>	8,71	3,35		
6	<i>Crassostrea rivularis</i>	11,11		12,04	
7	<i>Anatina antiquate</i>	6,70			8,89
8	<i>Tapes literatus</i>		4,09		
9	<i>Solens regularis</i>			3,37	3,01

10	<i>Perna viridis</i>	3,67			
11	<i>Bufo rana</i>		12,54		
	Mean	6,80	4,82	4,60	4,63
	STDV	3,19	4,36	3,73	3,34

MPI values based on 7 metals: As, Cd, Cr, Cu, Zn, Al and Fe during the rainy season and dry season ranged from 2.35 to 5.87 (average  $4.07 \pm 1.44$ ) and 4.60 to 6.80 (average  $5.22 \pm 3.66$ ), respectively. According to classification of Usero et al, 2005 for MPI and in comparing these values from those in Table 4.17 and 4.18, it is clear that all of the sediment of Nha Trang bay are not contaminated by these trace metals of various magnitudes at 4 locations. During rainy season, BT is severely contaminated with highest MPI values (5.87), where as ND is lastly contaminated with moderate MPI values 2.35 and CL is moderately contaminated with low MPI values 3.78 for these metals. The highest MPI values found in BT were plausibly related to discharge of effluents from domestic. It's related to discharge of agricultural effluents from fields and industrial sectors, where as the moderate MPI values found in TD and ND. Similarly, the low MPI values found at CL is related to discharge of agricultural effluents from the basin. Thus, MPI values provides a representative picture of contaminant impacts on mollusks due to their ability to concentrate and reflect the environmental levels of trace metal contaminants in estuarine ecosystems. During dry season, the highest MPI values observed at TD with MPI value (6.80) is found to be coincided with the high overall percentage contribution of the rivers Be to the total annual flow rate, where as the lowest MPI valves observed at CL is found to be coincided with the low percentage contribution of the sea close to the total annual flow rate.

#### **4.3.5 Compare with the limit MPL**

Figures 4.36, 4.37, 4.38 and 4.39 present metal concentrations in seven mollusks collected in Nha Trang bay in comparison with FDA and WHO standards. On the contrary, higher than WHO standard concentrations of As, Cd, Cr and Cu were noted in mollusks collected in the 4 site. The high concentrations of these metals in mollusks from the site could be linked to the state of pollution in Nha Trang bay.

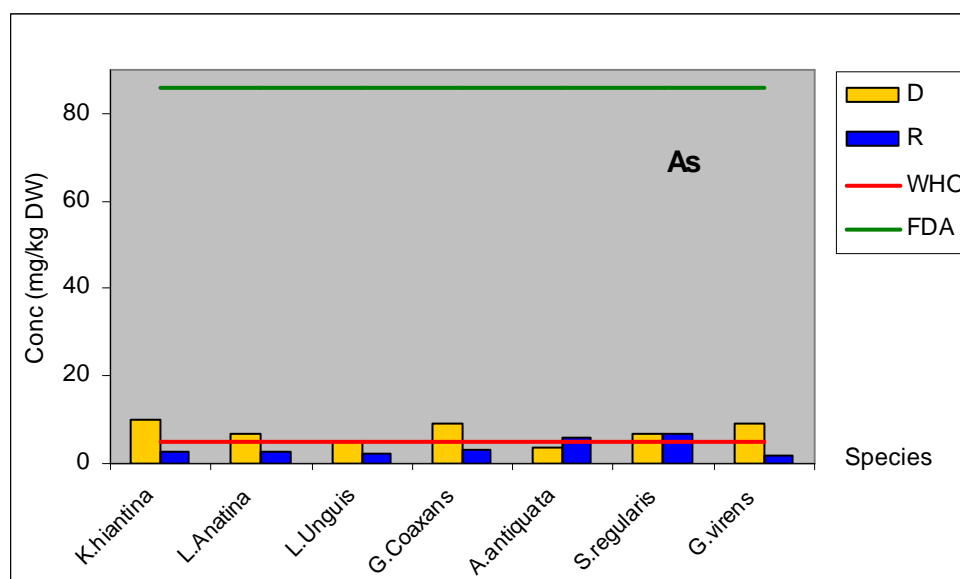


Figure 4.36 As contents in soft tissue of mollusks in comparing with standard values

Results from this study demonstrated that As were detected at high concentration for comparison with WHO standard. The mean range concentrations of As from 1.7 to 9.89 mg/kg DW. This is due to different species have different mechanism for metal binding and regulation in their body. Lower concentration of As in the *A.antiquate* tissue might be due to its toxicity and this metals is non essential metal to this clam, but As concentration in *K.hiantina* and *G.coaxans* were found high exceeded the permissible limit WHO (5 mg/kg DW).

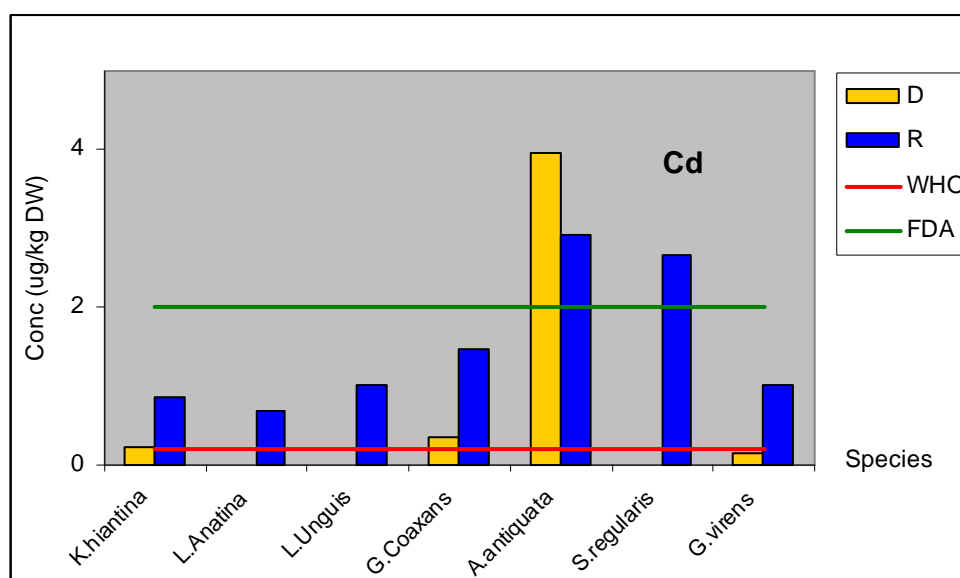


Figure 4.37 Cd contents (mg/kg DW) in soft tissue in comparing with standard values

Cd concentrations in the Nha Trang Bay mollusks in the general range 0.5–3.9mg/kg DW can be compared with those for other bivalves including coastal species. They are similar to concentrations reported for the bivalve *R.philippinarum* collected in Jiaozhou Bay (Lui et al, 1983). They are also similar to the Cd concentrations in the *S.plana* (Cheggouret al, 2005), where the average ranges was between 0.2 and 1.6 for irregular samples collected from the Moroccan estuarines. The Cd concentrations in the recent study were however lower than some other bivalves including *V.gigas* collected form Gulf of California (Ruelas I, 2003). The elevated Cd concentrations at two species *A.antiquate* and *S.regularis* in rainy season are very high compared with values reported for *P.viridis* from the Nha Phu bay, Khanh Hoa province where average Cd concentrations were between 0.03 and 0.31 µg/g (Ha D.et al., 2007).

Cadmium contents to different species have a big different for metal binding. Higher concentration of Cd was foud in the mollusk tissue of *A.antiquate* in both dry and rainy season. Their cadmium concentrations in were found exceeded the FDA permissible limit (3.95 and 4.84 mg/kg DW).

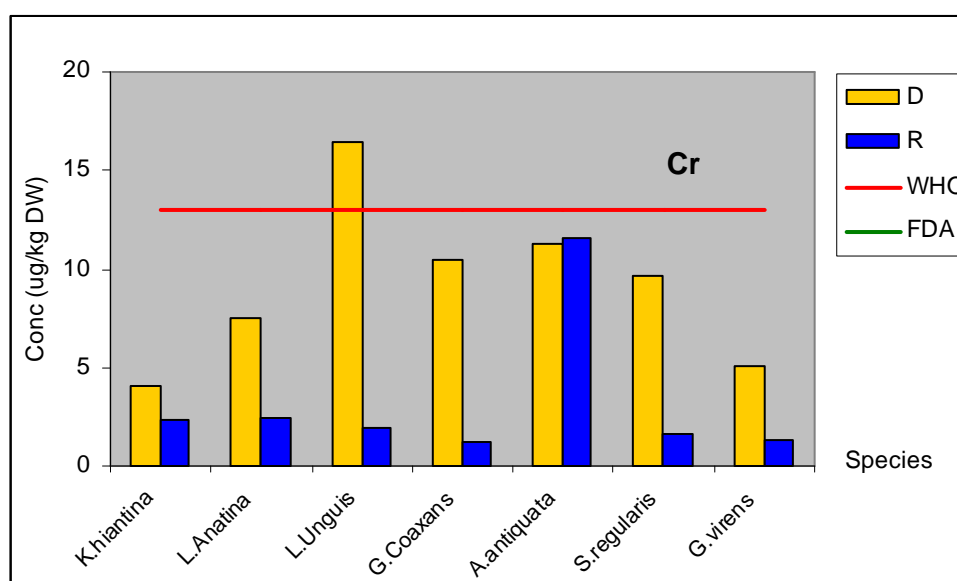


Figure 4.38 Cr contents in soft tissue of mollusks in comparing with standard values

However, concentrations of Chromium were still below the permissible limit when compared with FDA, 2001 (13mg/kg DW) exopt Cr concentration found in *L.unguis*, which concentration were exceed the permissible limit (16.46 mg/kg DW)

For chromium, which is considered a non-essential trace metal for bivalves, the tissue concentrations in the mollusks were lower values over rainy. These values are similar to those reported for mussels from Chile and pearl oysters from the Arabian Gulf where average values were below 3.8 mg/kg DW (Al-Sayed et al., 1994); Manley et al., 1996).

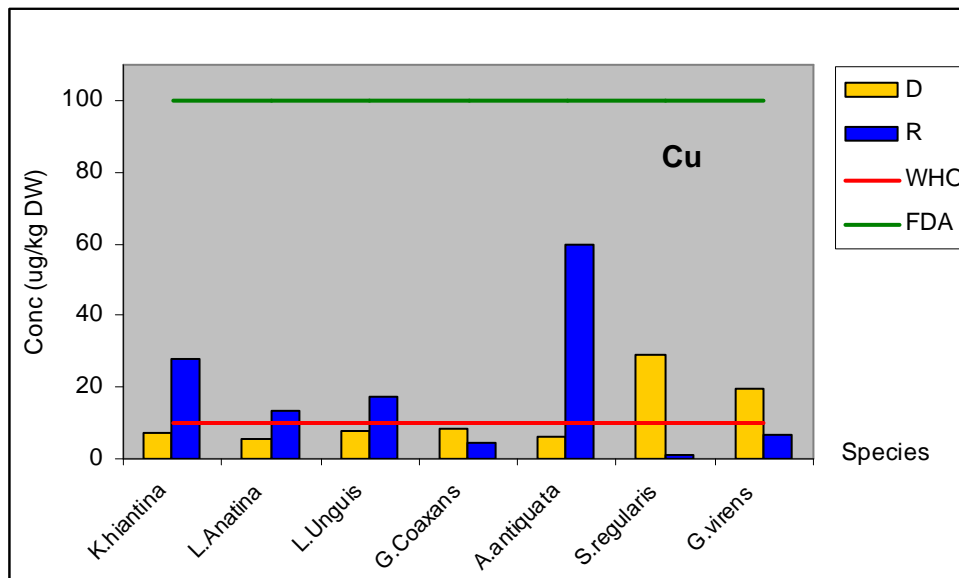


Figure 4.39 Cu contents (mg/kg DW) in soft tissue in comparing with standard values

From this study the concentration of copper was relatively lower when compared FDA (permissible limit 100 mg/kg DW) but Cu concentrations in almost mollusks are also most toxic metals when exceeded the certain limit of WHO (permissible limit 10mg/kgDW).

There have been fewer studies reporting copper concentrations in bivalve tissues (Table 1.2) but the average value of 15 mg/kg DW determined in the present study for the Nha Trang Bay mollusks is similar to the value reported for *S.plana* from Moroccan estuarines (Cheggour, 2005). Similarly, Zn concentrations in the Nha Trang bay mollusks were comparable to those reported for *T.mactroidea* collected from the Edo Miranda Venezuela (LaBrecque et al., 2004) from sites with low levels of trace metal contamination.

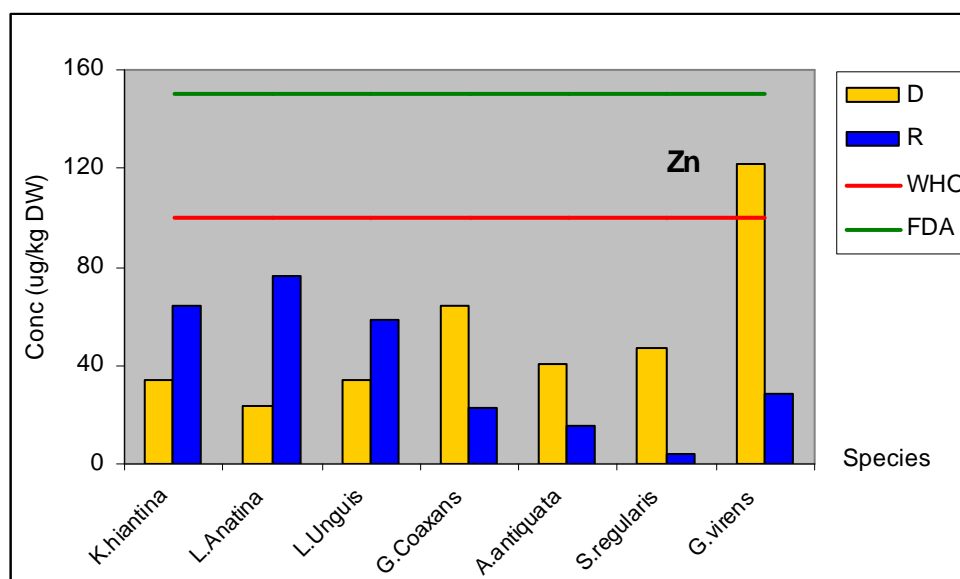


Figure 4.40 Zn contents (mg/kg DW) in soft tissue in comparing with standard values

This figure showed that Zn were concentrated at low concentration when compared WHO limit, then Zn concentration in *G.virens* was found exceeded the permissible limit WHO (100 mg/kg DW).

Results from this study demonstrated that Cr and Zn were detected at low concentration. This is due to different species have different mechanism for metal binding and regulation in their body. The low fluctuation level of Cr and Zn in the tissues of mollusks clearly showed that they need of same level of these metals to maintain the normal bodies function of marine organisms or might be due to its toxicity and those metals are non essential metal to mollusks.

On the other side, these observed concentrations of As, Cd and Cu in the samples were above permissible level WHO, but from this study the concentration of As and Cu were relatively lower when compared FDA (permissible limit 86 and 100 mg/kg DW respectively) but it's concentration in all mollusks was found exceeded the permissible limit WHO (5 and 10 mg/kg DW respectively). In conclusion, metals as As, Cu and Cd are also most toxic metals when exceeded the certain limit where they habited may pose hazard to human in terms of health risk.

Based on those results, it showed that the magnitude of the heavy metal accumulation in mollusks tissues depend on the type of the heavy metals, the species of the



mollusks, seasonal factors and also showed that mollusks living in the area accumulate heavy metals in their body tissues. Toxic heavy metals are dangerous since heavy metal tend to accumulate in the tissue of mollusk and could be transferred to human through the consumption of these mollusks.

#### 4.3.6 Heavy metal concentration and biological parameters

##### 4.3.6.1 Biometric parameters

##### Moisture of mollusks tissues

Tissue moisture was measured in all of the mollusks at the time of metal analysis as show in Table 4.19. There is a significant difference in moisture values between mollusks. The mean moisture concentration for mollusks was  $86.24 \pm 1.22$  % for 10 samples with a range of moisture concentrations of 81.99 to 90.49 %.

Table 4.19 Moisture contents in mollusk tissues

N	Species	Min	Max
1	<i>Katelysia hiantina</i>	92,59%	93,91%
2	<i>Glauconoma virens</i>	80,67%	84,99%
3	<i>Laternula anatine</i>	56,25%	75,47%
4	<i>Lingula unguis</i>	83,53%	91,63%
5	<i>Geloina coaxans</i>	89,59%	96,19%
6	<i>Anatina antiquate</i>	96,13%	98,58%
7	<i>Tapes literatus</i>	73,99%	94,55%
8	<i>Solens regularis</i>	62,23%	74,01%
9	<i>Perna viridis</i>	92,68%	97,97%
10	<i>Crassostrea rivularis</i>	92,21%	97,60%

##### Body size

In order to minimize the influence of the body weight of animals on metal concentrations, comparison was made between sites with animals where no significant differences in weight were registered. CI values obtained from seven mollusks of four sites

is showed in Table 4.20. Biometrics, which consisted of determining the length of the organisms and their fresh weight, were determined individually as Condition Index = 90 (normal); > 120 (high); < 70 - 80 (low) according to (Mann, 1978, Hetzel et al., 1983, Lucas et al., 1985). Table 4.20 shows the average values of condition index. The mean mollusk condition index ranged from 14.38 (min value) to 116.67 (max value) and was significant variation among the species.

Table 4.20 The calculated condition index for seven species

Species	CI min	CI max	Average
<i>K.hiantina</i>	27.18	54.52*	38.58±9.0
<i>G.virens</i>	44.94	105.52***	74.58±22.49
<i>G.coavans</i>	23.85	43.13*	33.06±8.90
<i>L.anatine</i>	78.12	116.67***	73.6±16.98
<i>L.unguis</i>	18.66	63.22*	44.64±19.87
<i>A.antiquate</i>	14.38	26.99*	22.64±1.75
<i>S.regularis</i>	93.49	99.26**	95.87±3.01

Note: \*Condition index low

\*\*\* Condition index high

The remaining biometric parameters were similar during all sample collections, and their weights and indexes were acceptable from the allometric point of view.

#### **4.3.6.2 Metals determination and condition index**

The matrix correlating the biometric parameters (length, weight, and condition index) and pollutant concentrations is presented in Table 4.21. Metal concentrations in tissues also depicted statistically significant correlations, from moderate to high, related to the condition index.

To investigate the relationship between trace metal concentrations and CI, the results from individual mollusks were combined for all sites and sampling times. The mean mollusk condition index (Table 4.21; 4.22; 4.23; 4.24) ranged from 22.64 (*A.antiquate*) to

95.67 (*S.regularis*) and was significant correlated with the metals in mollusk tissues. The matrix correlating the biometric parameters CI and pollutant concentrations is presented in tables bellow:

Table 4.21 Correlation coefficients between biometric and metal content in *K.hiantina*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1,000							
As	-0,274	1,000						
Cd	0,836	-0,526	1,000					
Cr	-0,142	-0,168	-0,268	1,000				
Cu	0,770	-0,563	0,668	-0,173	1,000			
Zn	0,700	-0,308	0,838	-0,315	0,372	1,000		
Fe	0,273	-0,426	0,376	-0,263	0,399	-0,048	1,000	
Al	0,008	-0,357	0,249	-0,335	0,086	-0,059	0,925	1,000

A high correlation was observed between Cd and Cu, Zn levels in the clam *K.hiantina* (0.668 and 0.838). While a moderately negative correlation resulted between As and Cd, Cu metals in the clam (-0.526 and -0.563). Metal Cd, Cu and Zn concentrations in tissues also depicted statistically significant high correlations related to the condition index ( $r = 0.836, 0.770$  and  $0.700$  respectively). Condition index was negative correlated with As and Cr concentrations in the clam ( $r = -0.274$  and  $-0.142$ ). Condition index correlated positively low with Fe and Al ( $r = 0.273$  and  $0.008$ , respectively). The clam may be a indicator for bioavailability for which the CI was independent of As and Cr concentration.

Table 4.22 Correlation coefficients between biometric and metal contents in *G.virens*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1							
As	-0,181	1,000						
Cd	0,446	-0,479	1,000					
Cr	-0,817	0,534	-0,378	1,000				

Cu	-0,929	0,529	-0,540	0,926	1,000			
Zn	-0,777	0,702	-0,700	0,914	0,937	1,000		
Fe	-0,539	0,732	-0,750	0,469	0,707	0,738	1,000	
Al	-0,381	0,535	0,075	0,816	0,570	0,607	0,086	1,000

For Cr, Cu, Zn, Fe and Al, tissue concentration was dependent of As concentration. For Cd, the concentration appeared to decline with increasing As tissue concentration. Condition index was negative correlated with all metal concentrations in the clam, only correlated well with Cd concentrations in the *G.virens* ( $r=0.446$ ). The clam may become the best indicator for bioavailability than others mussel for which the CI was independent of all of metal concentrations (negative correlation).

Table 4.23 Correlation coefficients between biometric and metal contents in *L.anatine*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1,000							
As	-0,729	1,000						
Cd	0,269	-0,786	1,000					
Cr	0,093	0,520	-0,500	1,000				
Cu	0,795	-0,976	0,637	-0,513	1,000			
Zn	0,118	-0,690	0,988	-0,517	0,524	1,000		
Fe	0,624	-0,928	0,920	-0,351	0,831	0,850	1,000	
Al	0,551	0,019	-0,140	0,864	-0,019	-0,219	0,126	1,000

Table 4.23 shows metal relationships with other metals in soft tissue of *L.anatine* by using t-test and probability differences. All metals measured in *L.anatine* gave highly correlations as Cu, Cd and Zn with Fe ( $r = 0.831, 0.920$  and  $0.850$  respectively), Cr and Al ( $r=0.864$ ), while As with Cd, Cu, Zn and Fe were negative correlation. On the other hand, Cu and Fe metals in *L.anatina* showed highly correlations with condition index ( $r=0.795$  and  $0.624$ ). The clam may also be a better indicator for bioavailability than others mussel for As which the CI was independent of As concentration.

Table 4.24 Correlation coefficients between biometric and metal contents in *L.unguis*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1,000							
As	-0,728	1,000						
Cd	0,886	-0,436	1,000					
Cr	-0,167	-0,496	-0,563	1,000				
Cu	0,206	0,470	0,327	-0,699	1,000			
Zn	0,275	0,397	0,652	-0,994	0,690	1,000		
Fe	0,793	-0,498	0,947	-0,476	0,043	0,563	1,000	
Al	-0,107	-0,353	-0,556	0,892	-0,300	-0,890	-0,618	1,000

For the mussel *L.unguis*, there was a significant effect of CI on the trace metal concentrations for Cd and Fe. However in contrast, tissue arsenic and chrome concentrations were negative dependent of body size for mussels sampled in this study. The mussel may also be a indicator for bioavailability than others mussel for which the CI was independent of As and Cr concentration.

Table 4.25 Correlation coefficients between biometric and metal contents in *G.coavans*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1,000							
As	-0,657	1,000						
Cd	0,820	-0,236	1,000					
Cr	-0,677	0,560	-0,200	1,000				
Cu	-0,866	0,192	-0,918	0,497	1,000			
Zn	-0,731	0,348	-0,939	-0,007	0,732	1,000		
Fe	-0,681	0,165	-0,453	0,856	0,767	0,149	1,000	
Al	0,116	0,377	0,659	0,583	-0,415	-0,712	0,192	1,000

Mean tissue concentrations for Fe, Zn and Cu were positively correlated (Table 4.25) as were As and Cr concentrations. For these trace metals there was negative relationship between tissue concentration and the CI with contamination. Cd concentrations were negatively correlated with Cu and Zn. Condition index also correlated negatively with Cu, Zn and Fe ( $r=-0.866$ ,  $-0.731$  and  $-0.681$  respectively). The condition index also correlated low with Al concentrations in the bivalve ( $r=0.116$ ). The clam may also be a better indicator for bioavailability for which the CI was independent of all trace metal concentration. (Except Cd)

Table 4.26 Correlation coefficients between biometric and metal contents in *A.antiquate*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1,000							
As	0,996	1,000						
Cd	-0,775	-0,719	1,000					
Cr	0,333	0,411	0,339	1,000				
Cu	-0,128	-0,211	-0,528	-0,978	1,000			
Zn	0,702	0,640	-0,994	-0,438	0,616	1,000		
Fe	0,171	0,088	-0,756	-0,872	0,955	0,822	1,000	
Al	0,376	0,297	-0,878	-0,748	0,871	0,924	0,977	1,000

For the mussel *A.antiquate* collected from 4 sites, there was a significant effect of CI on the trace metal concentrations for As and Zn. However in contrast, tissue chromium concentrations were negative dependent of body size for mussels sampled at this site. Cu and Zn was high correlated with Fe and Al concentrations in the bivalve ( $r=0.822-0.955$ ). The clam may also be a better indicator for bioavailability for Cd and Cu for which the CI was independent of Cd and Cu concentration.

Table 4.27 Correlation coefficients between biometric and metal contents in *S.reguraris*

	CI	As	Cd	Cr	Cu	Zn	Fe	Al
CI	1,000							

As	-0,918	1,000						
Cd	0,974	-0,984	1,000					
Cr	-0,677	0,330	-0,494	1,000				
Cu	0,280	0,123	0,057	-0,896	1,000			
Zn	0,737	-0,409	0,567	-0,996	0,855	1,000		
Fe	0,088	0,314	-0,138	-0,793	0,981	0,738	1,000	
Al	0,995	-0,873	0,947	-0,748	0,376	0,802	0,189	1,000

A high correlation was observed between Cd, Zn and Al level and CI value in the *S.regulatis* tissue (0.974, 0.737 and 0.995). While a high negative correlation resulted between As and Cd in tissue clam (-0.984). The clam may also be a indicator for As and Cr pollutions for which the CI was independent of As and Cr concentration.

There was negative correlated with As for all mollusk species, but not in *A.antiquate* (Coefficient = 0.996) the condition index appeared to decline within creasing As tissue concentration. For Cd, tissue concentration was highly positive dependent of CI. For Cu, tissue concentration was not significant depend on the CI, except *A.antiquate* and *K.hiantian*. Coefficients of correlation were 0,795 and 0,775 respectively. For Cr, tissue concentration was independent of condition. For Al and Fe, where the range of tissue values examined was some what limited because of missing condition values for samples with highest Al and Fe concentrations. There was no clear significant effectt of any of these trace metal concentrations on mussel CI (regression analysis).

In order to establish this relation for 7 mollusks from the Nha Trang bay, we have considered all samples collected during the rainy and dry season.

Equation parameters for these 7 species were reported in Table 4.22 to 4.27. These results showed that, for the 7 metals, concentration and body weight were significantly correlated. The negative values of CI indicated that the higher metal concentrations were in small individuals. There negative correlations, more commonly observed than other correlations in mollusks, may be partially attributed to greater metabolic rate per biomass of smaller individuals or by a slight adsorption of metal by larger individuals (Blueweiss et al.,1978).

These results highlight the necessity to adjust for weight differences when comparing trace metal concentrations amongst mollusk samples.

#### 4.4 EVALUATION OF BIOACCUMULATION

The biota-sediment accumulation factor (BSAF) is an index of the ability of the species to accumulate a particular metal with respect to its concentration in the sediment. It was calculated as the ratio between the metal concentration in the organism and that in the sediment (Usero et al., 2005)

##### 4.4.1 Comparison of BSAF in soft tissue and shell

Metal concentrations in mollusks varied along the 7 species collected first time on dry season (March 2012) in Nha Trang were calculated for BSAF in shell (BSAF-S) and tissues (BSAF-T) is showed in Table 4.28

Table 4.28 Comparison BSAF in shell and tissue of different mollusk species

Species		As	Cd	Cr	Cu	Zn
<i>L.anatina</i>	BSAF-S	0,55	4,74	0,01	1,10	5,56
	BSAF-T	5,45	3,99	0,01	3,28	1,51
<i>L.unguis</i>	BSAF-S	0,72	1,70	0,02	0,38	2,26
	BSAF-T	13,41	5,89	0,02	1,29	65,46
<i>G.virens</i>	BSAF-S	0,54	2,54	0,02	0,27	0,22
	BSAF-T	2,36	5,25	0,34	2,49	1,83
<i>G.coaxans</i>	BSAF-S	0,83	4,12	0,19	0,47	0,64
	BSAF-T	1,17	1,76	0,04	0,57	3,81
<i>C. rivularis</i>	BSAF-S	0,46	0,25	0,10	0,40	0,69
	BSAF-T	10,57	4,15	7,51	14,71	46,68
<i>K. hiantina</i>	BSAF-S	0,19	0,23	0,02	0,12	0,08
	BSAF-T	0,56	0,76	0,01	0,39	0,64
<i>A. antiquate</i>	BSAF-S	0,42	0,38	0,02	0,07	0,08
	BSAF-T	0,29	1,95	0,01	0,15	0,58

The shell and tissue showed similar pattern in the accumulation of heavy metals analyzed and the tissue always had higher concentration than the shell. There was clear pattern of lowest bioaccumulation factor among the all metals and analyzed two body parts (shell and tissue) of the *K.hiantina*, *A.antiquate* and *L.anatine*. However, within the other species, Cd and Zn were recorded to have highest concentration in two body parts (shell and soft tissue).



The mollusks *L.anatine*, *L.unguis*, *G.virens*, *C.rivularis* and *G.coaxans* have high potential factor BSAF in tissue for As (BSAF > 2) but not for shell, exhibiting that this element possibly essential in its tissue. Comparatively, oyster *C.rivularis* recorded higher factor value for Cd, As, Cr, Cu and Zn in tissue and low factor value for these metals in shell. Such a condition indicated that all species are capable of accumulating that metal Cd in relatively larger quantity, which indeed shows that it is potential bioindicator for Cd pollution in sediement. On the other hand, it has low potential factor for the accumulation of Cr and Pb in both shell and tissue.

By comparing the accumulation of heavy metals in two body part of mollusks, this study found that the concentrations of metals were higher in tissue of mollusks and there was significant difference in metals accumulation between shell and tissue. Thus, the data from the study showed that mollusks living in the effluent from the study area high accumulate heavy metals in their body tissue.

#### 4.4.2 Bioaccumulation of heavy metal in the mollusk tissues

Metal concentrations of Al and Fe in the tissue of the four sites were similar and did not vary appreciably and presented very lower compared to concentrations of these metals in the sediments. Concentrations of Al and Fe in the sediments were 20-100 times higher than the concentration in the mollusks.

By comparing BSAF values, we can compare the ability of those mollusks in taking up metal from sediment. The highest or lowest BSAF values also varied for each different metals ans species during 2012-2013 was showed in Table 4.29

Table 4.29 Values of bioaccumulation factor of heavy metal in mollusk species

	<i>A.antiquate</i>	<i>C. rivularis</i>	<i>G. virens</i>	<i>G. coavans</i>	<i>K. hiantina</i>	<i>L. anatine</i>	<i>L. unguis</i>
As	0,57±0,55	<b>1,33±2,16</b>	<b>9,61±2,63</b>	<b>0,95±0,81</b>	<b>2,09±2,69</b>	<b>1,31±2,31</b>	<b>12,11±1,6</b>
Cd	<b>2,19±2,50</b>	<b>1,25±1,11</b>	<b>0,69±0,73</b>	0,53±0,42	0,34±0,24	0,30±0,31	0,24±0,27
Cr	<b>0,39±0,36</b>	<b>0,62±0,43</b>	<b>1,19±0,05</b>	<b>0,77±1,44</b>	0,32±0,35	<b>0,78±1,37</b>	0,44±0,55
Cu	<b>1,87±0,92</b>	<b>7,63±4,69</b>	<b>4,52±0,32</b>	<b>1,15±1,32</b>	<b>1,38±1,06</b>	<b>0,57±0,49</b>	0,51±0,44
Pb	0,27±0,21	ND	0,32±0,11	0,25±0,22	ND	0,60±0,26	0,54±0,16

Zn	<b>1,09±0,1</b>	<b>8,09±3,84</b>	<b>2,08±2,31</b>	0,21±0,36	<b>3,14±3,09</b>	<b>1,78±2,20</b>	<b>1,95±3,06</b>
Mo	0,16±0,23	ND	0,78±0,11	<b><i>1,11±0,46</i></b>	ND	0,22±0,31	ND
Ni	0,22±0,31	ND	ND	<b>1,77±2,17</b>	ND	0,36±0,41	ND
Fe	0,08±0,01	0,03±0,02	0,05±0,02	0,02±0,03	0,05±0,03	0,08±0,05	0,14±0,08
Al	0,05±0,03	0,03±0,03	0,06±0,11	0,06±0,09	0,04±0,05	0,01±0,01	0,03±0,01

**Note:** ND: not detected, **Red Bold:** High potential of accumulation, **Bold Italic:** Moderate potential of accumulation and Regular: Low potential of accumulation.

In general comparison the biocumulative factor of heavy metals from sediments within mollusks found in the Nha Trang bay, the BSAF indicated a low potential bioaccumulation for Pb, Mo and Ni (BSAF<1) and a high potential of accumulation for As, Cd, Cu, Cr and Zn. Arsenic had the high BSAF ratio for 5 species as: *Crassostrea rivularis*, *Glauconoma virens*, *Katelsysia hiantina*, *Lingula unguis* and *Laternula anatine*.

Copper had the highest BSAF ratio for 5 mollusk species *Anadara antiquate*, *Crassostrea rivularis*, *Glauconoma virens*, *Gelonia coaxans* and *Katelsysia hiantina*, while Cd had the highest ratio for the remaining two species *Anadara antiquata* and *Crassostrea rivularis*. The BSAF values for Pb obtained by all of the mollusks were below 1.0, indicating higher content of this metal were found in the sediments compared to the tissues of the mollusks. Such a condition possibly reflected that the mollusk were rather selective in accumulating Pb metal into their tissues. Comparatively, an interesting observation indicated that mollusk species from the study area showed highest BSAF for As, Cd and Zn.

The trends in the BSAF for metals in seven mollusks species were in the descending order of Cu > Zn > As > Cd > Cr. The trend in BSAF for metal in *G.coaxans* were in the decending order Zn > Cu > As > Cd > Cr. Finally, if we compare the seven species studied, we can conclude that *G.virens* by far had the highest BSAF for As followed by *K.hiantina*. It showed that the organisms living in the area were high accumulated heavy metals in their body tissues.

Based on the bioaccumulation factor (BSAF), all mollusks showed different degrees of the accumulation of heavy metal in their tissues which is depending upon the

type of heavy metals and the species of the mollusks. *G.coaxans* recorded high factor value of Cr, Cu and Ni. This indicates that *G.coaxans* is capable in accumulating Cr, Cu and Ni in relatively great quantity. Comparatively, *C.rivularis*, *G.virens* and *K.hiantina* have high potential factor for As, Cu and Zn, demonstrated that this element is probably essential element to they body. Therefore, these species also have the potential to be us as bioindicator for metals in aquatic environment. The study showed that the accumulation of heavy metals were different between species of mollusk. In general, higher metal concentration bioaccumulation was found in the samples from the vicinity of industrial complexes and urbanized area which lied between TD and CL.

#### **4.5 CHOISE K.HIANTINA AS BIOINDICATEUR**

The purpose of this study was to obtain information on the concentration of trace metals in mollusks from coastal area of Khanh Hoa Province and choising mollusk specie as a biomonitor for the area. The results reported here will provide valuable information and assessment the potential of using the *K.hiantina* as a biomonitors of heavy metal pollution. Another that the mineral composition of *K.hiantina* clam was to study their information on microstructure is one of the most significant relative datasets for clarification of evolutionary trends of shell structure. Thus, the findings may suggest the possibility of using the *K.hiantina* as an alternative biomaterial for bone substitute in managing bone defects.

##### **4.5.1 Seasonal and locational variation**

Although comparison between different stations is open to criticism, the mean concentrations of all studied metals in *K.hiantina* were of the same range with exception for the extreme values. For example, concentrations of heavy metals in *K.hiantina* from BT, in general, were significantly higher than that in samples from others locations as TD, ND and CL (as seen in Figure 4.41 to Figure 4.47). Only Cr and Cu concentrations in tissue samples from CL and ND were significantly higher than samples from BT.

For Al as well as Fe, although their concentrations were also higher in samples from BT, statistically they were not significantly different. Concentrations of metals in soft tissue of samples from BT were higher than that of samples from, but only Cd and Cu were statistically significant different. The highest mean value of Cr (11.58 mg/kg DW) in CL was higher 15.44 times than the lowest mean value (0.75 mg/kg DW) in BT, while other

values, which varied between 3-15 insignificant ranges. Moreover, Cd contents were higher about 2.08-5.47 times in rainy season than in dry season.

The high concentrations of Zn in BT and other metals in stations from Nha Trang bay were assumed to be related to the human activities along the coastal waters of these stations. Two large rivers are draining their load to these waters, one of which is passing the densely populated area in the Nha trang city center. However, the industrial and anthropogenic activities in Nha Trang are still much less when compared to those in the southern coast of Viet Nam

Higher concentrations of metals in samples from BT, in general, might be related to the more anthropogenic activities in this area. MOSTE, 2002 reported that the southern coast of Viet Nam is more developed than the north coast. Many activities such as petrochemical plants, land reclamation, urbanization, shipping and other industrial activities and some major ports are concentrated in this location.

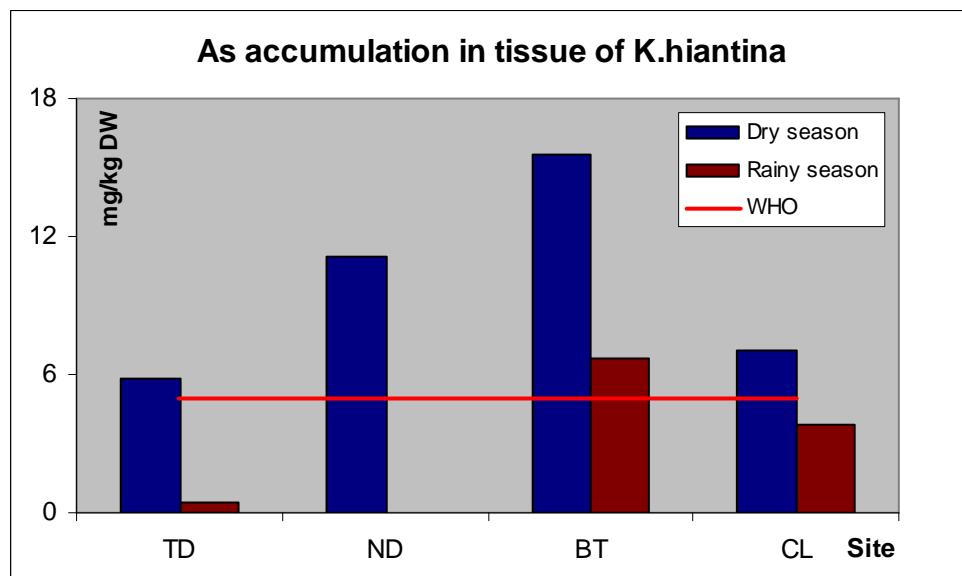


Figure 4.41 Arsenic accumulation in soft tissue of *K.hiantina*

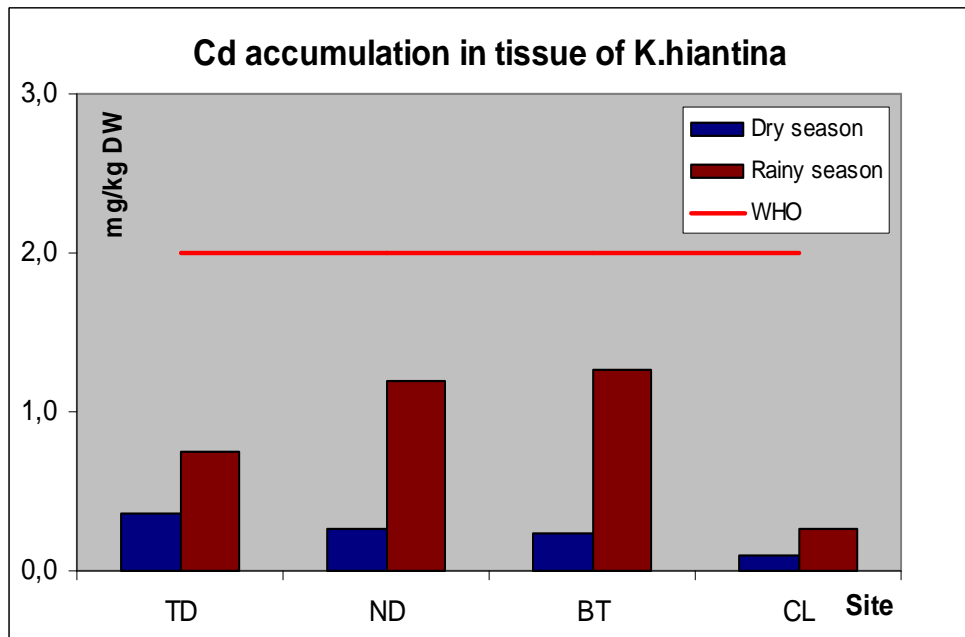


Figure 4.42 Cadmium accumulation in soft tissue of *K.hiantina*

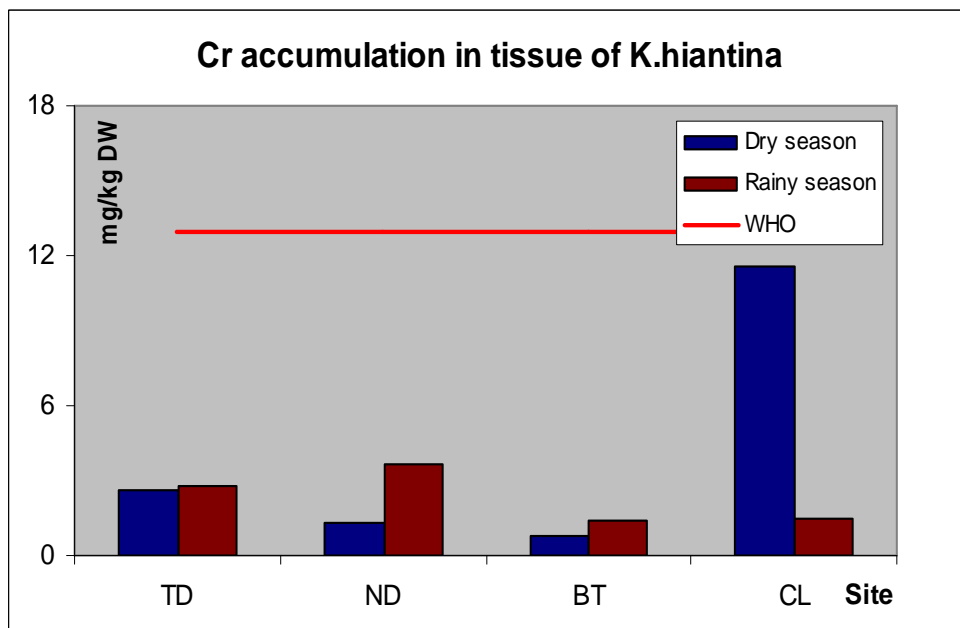


Figure 4.43 Chromium accumulation in soft tissue of *K.hiantina*

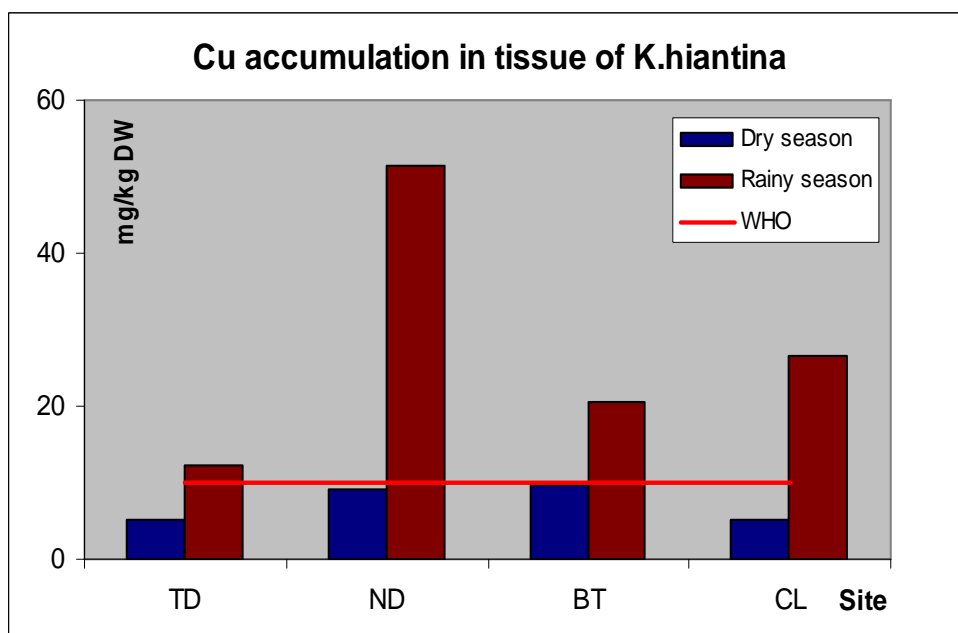


Figure 4.44 Copper accumulation in soft tissue of *K.hiantina*

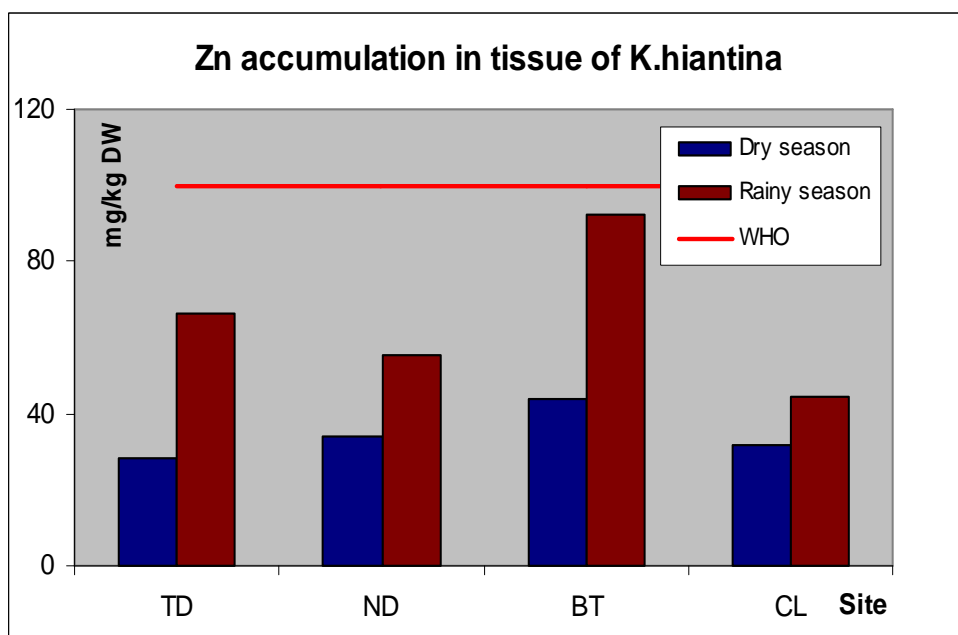


Figure 4.45 Zinc accumulation in soft tissue of *K.hiantina*

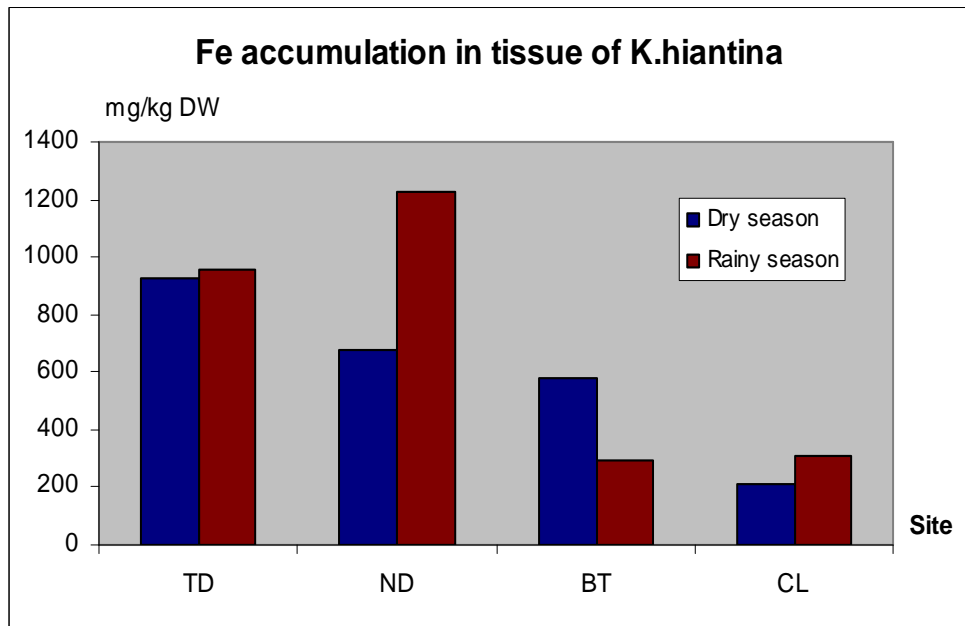


Figure 4.46 Iron accumulation in soft tissue of *K.hiantina*

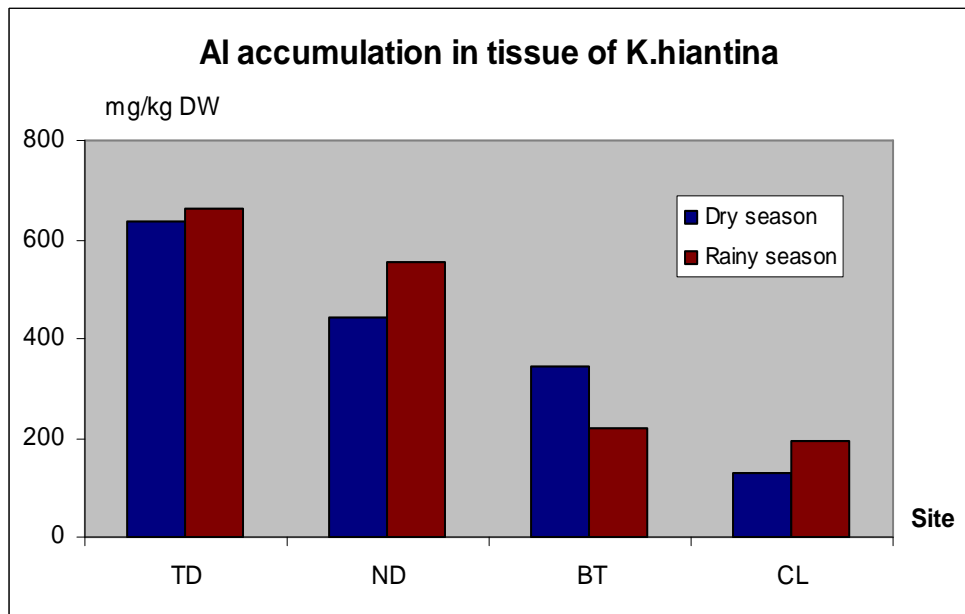


Figure 4.47 Aluminium accumulation in soft tissue of *K.hiantina*

Seasonal variations of 8 metal concentrations in *Katalysia hiantina* were also presented in Figure 4.40 to figure 4.47. Generally, in all sites, similar seasonal profile was showed for those metals: the higher levels were observed in rainy season. For example, in from the 4 site, the mean values were  $0.87 \pm 0.46$ ,  $27.72 \pm 16.78$  and  $64.53 \pm 20.41$  mg/kgDW respectively for Cd, Cu and Zn in the rainy season against  $0,24 \pm 0.11$ ,

7.33±2.41 and 34.38 ± 6.54 mg/kg DW in the dry season. Dry seasonal depending on As, Cr, Al pollutions as well as rainy season depending on Cd, Cu, Zn, Fe pollutions. The low concentrations in dry season could be explained by the metal dilution in the organism which caused by weight increase resulted and a metal reconcentration in clam.

Nevertheless higher concentrations of Al and Fe in the *K.hiantia* population can be found in areas of estuary BT at dry season; also a higher accumulation of As goes in the 4 station in dry has shown that physico-chemical factors and a stable metal concentration modify the concentration in species of the *K.hiantina*. Physiological processes linked to metabolic functions, especially reproduction - gametogenesis and emission of eggs, described by Cheggour, 1988 seem to play a key role in determining seasonal variations in the level of metals present in the tissue of the organisms. This laying period is followed by rainfall mating characterized by high metal levels. At the beginning of dry period metal concentrations clearly peak.

#### 4.5.2 Metal/shell weight indices (MSWI)

Metal bioavailability was first calculated as metal concentrations in soft tissues (mg/kg DW) and shell weigh of organisms. Biometric data of samples for analysis was presented in Table 3.22. Mean shell height of clams *K.hiantina* varied from 5.5 to 6.2 cm, total weight from 64.0 to 81.6 g/clam and body mass from 5.3–9.9 g/clam. Calculation of MSWI for *K.hiantina* is showed in table 4.30

Table 4.30 Calculation of MSWI for *K.hiantina*

Sites	Dry season				Rainy season			
	TD	ND	BT	CL	TD	ND	BT	CL
As	0,50	0,95	1,34	0,60	0,03	0,00	0,51	0,29
Cd	0,03	0,02	0,02	0,01	0,06	0,09	0,10	0,02
Cr	0,23	0,11	0,06	0,99	0,21	0,27	0,11	0,11
Cu	0,45	0,77	0,84	0,45	0,93	3,84	1,56	2,01
Zn	2,42	2,88	3,76	2,71	5,00	4,17	6,95	3,35
Fe	78,93	57,61	49,73	18,09	72,06	91,69	22,34	23,20
Al	54,27	37,66	29,78	11,14	50,07	41,64	16,48	14,78
MSWI	136,83	100	85,53	33,99	128,36	141,7	48,05	43,76

Metal/shell weight indices were used to characterize each site and season depending on metal bioavailability. It is evident that the bioaccumulated Zn in *K.hiantina*



from the TD and BT and Cu from the ND, during both dry and rainy, demonstrated the highest values among the total samples analyzed.

Data of the study showed higher mean Cd, Cu and Zn shell/weight indices in mollusks in rainy season in comparison to dry season, excluding As and Cr. Ratios of MSWI rainy season/dry of As and Cr were 0.83/3.39 and 0.7/1.4 respectively. In clams, higher mean Pb shell/weight indices were found in rainy season in all the examined areas ( $p < 0.001$ ), where as no significant seasonal variation was observed regarding Pb in dry season. Concerning Fe and Al, no specific seasonal trends were recorded. The metal/shell weight indices of the mollusks were found to correlate with the metal concentrations in the sediment. Regarding the clam *K.hiantina*, its Zn/shell weight indices are associated with Zn concentrations in the fraction of sediment.

The intense correlation of Zn and Cu shell/weight indices in *K.hiantina* species with their corresponding concentrations in the weakly bound fraction of sediment confirms the impact of sediment to the specific organism living within the sediment, under these a bottom surface, since in its filter-feeding mode it is exposed to the dissolved metals of its burrow water, which is a mixture of hypoxic/anoxic pore water and overlying (oxygenated) water (Griscom et al., 2002).

Metal/shell-weight indices were used to characterize each site and season depending on the metal bioavailability. In general terms, metal shell-weight indices also indicate that metal bioavailability was very low in the CL sea close between dry and rainy season. MSWI values were 33.19 and 43.76, respectively. As an exception, chrome Cr bioavailability was high in CL in dry season. Occasionally, highest Fe, Cd and Cu bioavailability was found in ND at rainy season and Al in TD in dry season. Low Cd, Cu, Fe and Al bioavailability were usually found in CL in dry season and Cu and Zn in TD. Metal bioavailability was highest in TD in both seasons than at any other site.

In dry season, TD showed higher metal bioavailability than other sites with values over those recorded in the rainy season. In rainy period, a general decline in metal bioavailability was observed, the highest bioavailability being restricted to the ND location. In this season, the metal bioavailability found in TD was close to that exhibited by ND. Afterwards, metal bioavailability decreased at all sampling points, mostly in BT

then CL. As, Cr, Al bioavailability were higher in dry than in rainy season and Cd, Cu, Zn, Fe bioavailability occasionally increased in rainy season.

In the present field investigation, shell dry weights did not change significantly over the year for clams *K.hiantina* measuring 2.5 to 3.5 cm in shell length. At a polluted site, such as CL, the Cd concentration, for instance, changed seasonally but, conversely, the Cd/shell-weight index did not exhibit seasonal fluctuations in relation to the intensity of the industrial activity, indicating fluctuations in metal bioavailability. At a non polluted site, such as TD, both Cd concentration and Cd/shell-weight index changed seasonally as could be expected by absence of local inputs. Consequently, the present field study supports the proposition that metal/shell-weight indices provide a method to detect differences and changes in the bioavailability of cumulative metals (Fischer, 1988). Since the term metal/shell-weight index (Fischer, 1983, 1984, 1986, 1988) might be misinterpreted as a measure of shell metal content, we suggest using Fischer's bioavailability index as a routine name for the metal burden in flesh related to shell weight in sentinel mussels. Such an index was applied in this study to investigate the metal bioavailability in the Nha trang bay.

Different metal bioavailabilities have been found, however, at different sites in relation to the influence of the waste waters out coming from the highly industrialized Nha Phu. Thus, metal bioavailability was generally higher on the north part of the bay than on the southern part and the outer sampling sites. The north receives the direct influence of the industrialized Nha Phu whereas the south is mainly influenced by water coming from the close sea, as a result of wind and water currents. Interestingly, according to the metal concentration values a high metal bioavailability was envisaged in TD where flesh weight was heavily depressed due to the continuous stress provoked by very strong wave turbulence. When metal/shell-weight indices were applied, however, TD resulted to be a non polluted site, as expected from the scarce influence of industrial inputs. Finally, although the metal/shell-weight index appears to be kept unchanged by seasonal factors other than metal bioavailability, the present results indicate the existence of seasonal trends in metal/shell-weight indices. Thus, these fluctuations might be attributed to different metal inputs at different times of the year.

### 4.5.3 Bioaccumulation of heavy metal in clam *K.hiantina*

Metal concentrations of Al and Fe in the tissue of the four sites were similar and did not vary appreciably and presented very lower compared to concentrations of these metals in the sediments. Concentrations of Al and Fe in the sediments were 20-100 times higher than the concentration in the mollusks. By comparing BSAF, we can compare the ability of clam *K.hiantina* in taking up metal from sediment. Table 4.31 shows the calculated BSAF values in the different sampling sites.

Table 4.31 Bioaccumulation of heavy metal in clam *K.hiantina*

Metals	Dry season				Rainy season			
	TD	ND	BT	CL	TD	ND	BT	CL
As	0,45	<b>2,53</b>	<b>7,42</b>	<b>46,16</b>	0,09	0,00	0,48	0,42
Cd	0,31	0,28	0,10	0,04	0,36	0,62	0,73	0,16
Cr	0,39	0,06	0,03	<b>1,07</b>	0,21	0,09	0,06	0,19
Cu	<b>1,78</b>	0,59	0,23	0,46	<b>1,67</b>	<b>1,79</b>	0,38	<b>3,32</b>
Zn	0,77	0,42	0,48	0,64	<b>18,29</b>	<b>6,52</b>	<b>4,03</b>	<b>7,51</b>
Mo	0,40	0,93	0,39	0,13	ND	ND	ND	ND
Ni	0,31	0,06	0,12	0,03	ND	ND	ND	ND
Fe	0,11	0,03	0,03	0,01	0,06	0,04	0,02	0,03
Al	0,15	0,02	0,01	0,00	0,03	0,01	0,01	0,01

**Note:** ND: not detected, **Bold:** High potential of accumulation, **Bold Italic:** Moderate potential of accumulation and Regular: Low potential of accumulation.

BSAF is defined as the ratio of metal concentration in the mollusk to that in sediment. In this study, it was found that *K.hiantina* from ND, BT and CL sites showed higher BSAF values for As. The BSAF values were 2.53, 7.42 and 46.16 respectively in dry season indicating that the mollusk contained much more metals than the sediment. It should also be noted that the concentration of As metals in the sediment and *K.hiantina* from there sites were lower in rainy season than dry season, as shown in Figures 4.41. Similar pattern was also found for Zn in all 4 sites, although the BSAF values were higher

than 2.0 in rainy season. This investigation also indicated that the *K.hiantina* were able to accumulate higher quantity of Cu and Cr than Cd, Mo and Ni. The BSAF values for Cd, Mo and Ni were lower than 1.0, which means limited ability of these heavy metals to be accumulated by the mollusk.

The BSAF indicated a low potential bioaccumulation for Cd, Mo and Ni (BSAF<1) and a high potential of accumulation for As in dry season and for Zn in rainy season. Based on those results, it show that the magnitude of the heavy metal accumulations in tissues depend on the type of the heavy metals and seasonal.

In conclusion, the clam *K.hiantina* has characteristics as sampling efforts, locational pollution classification according to MSWI, seasonal depending on metal pollution and could choice as biomonitor in Nha Trang bay, Khanh Hoa coastal.

## **4.6 RISK ASSESSMENT**

### **4.6.1 Daily trace metals intake EDI**

Environmental health risk in this study was assessed by a comparison between the concentrations of contaminants in bivalves based on wet weights and “threshold” values recommended by the WHO/FAO and the US Food and Drug Administration which may cause adverse effects in human consumers. For the purpose of this calculation, it was assumed that total exposure to a specific metal was derived solely from shellfish. The average daily mussel consumption rate in Nha Trang assumed to vary between 50 and 100g/person. In this study, the low available consumption rate of 50 g/person/day was used to calculate the level of concern for the average shellfish consumption group. Daily trace metal intake EDI through bivalve consumption (Table 4.32 – Table 4.39) was calculated based on the minimum and maximum concentration range of metal concentration (wet weight) of each mollusk species at 4 stations during the dry and rainy seasons.

Table 4.32 Daily trace metals intake EDI through *K.hiantina* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	1,062	0,001	0,431
<b>Cd</b>	0,086	0,006	0,038
<b>Cr</b>	0,792	0,051	0,219
<b>Cu</b>	3,507	0,359	1,198
<b>Zn</b>	6,293	1,936	3,380

Table 4.33 Daily trace metals intake EDI through *G.virens* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	1,468	0,084	0,589
<b>Cd</b>	0,230	0,001	0,086
<b>Cr</b>	0,890	0,089	0,362
<b>Cu</b>	3,093	0,066	1,546
<b>Zn</b>	17,978	0,130	8,465

Table 4.34 Daily trace metals intake EDI through *L.anatina* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	1,168	0,240	0,805
<b>Cd</b>	0,137	0,002	0,059
<b>Cr</b>	2,293	0,277	0,850
<b>Cu</b>	3,027	0,821	1,618
<b>Zn</b>	15,477	3,672	8,502

Table 4.35 Daily trace metals intake EDI through *L.unguis* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,500	0,001	0,182
<b>Cd</b>	0,063	0,000	0,025
<b>Cr</b>	1,535	0,075	0,459
<b>Cu</b>	1,236	0,136	0,628
<b>Zn</b>	2,984	0,758	2,308

Table 4.36 Daily trace metals intake EDI through *G.coaxans* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,992	0,001	0,616
<b>Cd</b>	0,210	0,008	0,091
<b>Cr</b>	1,997	0,091	0,585
<b>Cu</b>	0,898	0,148	0,642
<b>Zn</b>	8,473	0,870	4,376

Table 4.37 Daily trace metals intake EDI through *A.antiquata* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,647	0,000	0,263
<b>Cd</b>	0,053	0,004	0,023
<b>Cr</b>	0,483	0,031	0,133
<b>Cu</b>	2,138	0,219	0,730
<b>Zn</b>	3,836	1,180	2,060

Table 4.38 Daily trace metals intake EDI through *S.regularis* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,876	0,296	0,646
<b>Cd</b>	0,045	0,000	0,015
<b>Cr</b>	2,014	0,238	0,834
<b>Cu</b>	6,097	0,634	3,456
<b>Zn</b>	6,517	4,674	5,849

Table 4.39 Daily trace metals intake EDI through *C.revularis* consumption

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	2,588	0,002	1,052
<b>Cd</b>	0,211	0,015	0,092
<b>Cr</b>	1,930	0,125	0,533
<b>Cu</b>	8,552	0,875	2,921
<b>Zn</b>	15,343	4,722	8,242

Thus, moderate trace level consumption of bivalve from the Nha Trang bay is not harmful to humans for As, Cr, Cd, Cu and Zn, but due to high-level accumulations of metals in bivalve muscle, heavy consumption of bivalves from the Nha Trang bay may pose a health risk to consumers (human beings). Assuming a tolerable daily intake of metals per kg of body weight (USFDA,1993), it can be calculated that a 60kg human would reach there commended level of intake with a daily consumption of about 1kg of mussel flesh from this area. Additional sources of metals are thus required to cause adverse effects on human health. In addition, other factors should be considered.

The need for regular monitoring of trace metal pollution is emphasized to acquire data for environmental protection and conservation measures. Because shellfish consumption rates can vary greatly between specific sections of acommunity, the values reported here should only be used for screening purposes. Clearly, further assessment is necessary before more definitive conclusions can be drawn.

#### 4.6.2 The target hazard quotient THQ

The target hazard quotient THQ has been recognized as a useful parameter for evaluation of risk associated with the consumption of metal contaminated food. Table 4.40 to Table 4.47 are showed the provisional THQ of As, Cr, Cd, Cu, and Zn recommended by the WHO/FAO and the USA for a reference weight of 60kg. It is evident from the table that target hazard quotient As, Cr, Cd, Cu and Zn from mollusks did not exceed the provisional THQ recommended by the WHO/FAO and the USA. However, the estimated Fe and Al THQ from the 5 species exceed the provisional THQ recommended by the WHO/FAO and the USA.

Table 4.40 The target hazard quotient for *K.hiantina*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,02	0,00	0,01
<b>Cd</b>	0,00	0,00	0,00
<b>Cr</b>	0,01	0,00	0,00
<b>Cu</b>	0,06	0,01	0,02
<b>Zn</b>	0,11	0,04	0,06
<b>Fe</b>	1,52	0,26	0,80
<b>Al</b>	0,82	0,16	0,50

Table 4.41 The target hazard quotient for *G.virens*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,033	0,002	0,013
<b>Cd</b>	0,005	0,000	0,002
<b>Cr</b>	0,020	0,002	0,008
<b>Cu</b>	0,070	0,001	0,035
<b>Zn</b>	0,406	0,003	0,191
<b>Fe</b>	2,892	1,335	2,151
<b>Al</b>	2,624	0,522	1,548

Table 4.42 The target hazard quotient for *L.anatina*



	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,031	0,006	0,021
<b>Cd</b>	0,004	0,000	0,002
<b>Cr</b>	0,060	0,007	0,022
<b>Cu</b>	0,080	0,022	0,043
<b>Zn</b>	0,407	0,096	0,223
<b>Fe</b>	8,795	3,920	6,450
<b>Al</b>	5,530	2,135	3,724

Table 4.43 The target hazard quotient for *L.unguis*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,017	0,000	0,006
<b>Cd</b>	0,002	0,000	0,001
<b>Cr</b>	0,053	0,003	0,016
<b>Cu</b>	0,043	0,005	0,022
<b>Zn</b>	0,103	0,026	0,080
<b>Fe</b>	3,936	1,291	2,160
<b>Al</b>	1,097	0,395	0,687

Table 4.44 The target hazard quotient for *G.coaxans*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,017	0,000	0,011
<b>Cd</b>	0,004	0,000	0,002
<b>Cr</b>	0,034	0,002	0,010
<b>Cu</b>	0,016	0,003	0,011
<b>Zn</b>	0,146	0,015	0,076
<b>Fe</b>	1,961	0,759	1,290
<b>Al</b>	1,363	0,345	0,897

Table 4.45 The target hazard quotient for *A.antiquata*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,003	0,002	0,003
<b>Cd</b>	0,003	0,001	0,002
<b>Cr</b>	0,012	0,001	0,006
<b>Cu</b>	0,006	0,003	0,005
<b>Zn</b>	0,041	0,026	0,032
<b>Fe</b>	0,746	0,299	0,505
<b>Al</b>	0,337	0,176	0,239

Table 4.46 The target hazard quotient for *S.regularis*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,054	0,000	0,022
<b>Cd</b>	0,004	0,000	0,002
<b>Cr</b>	0,040	0,003	0,011
<b>Cu</b>	0,177	0,018	0,061
<b>Zn</b>	0,318	0,098	0,171
<b>Fe</b>	4,229	0,729	2,232
<b>Al</b>	2,289	0,449	1,375

Table 4.47 The target hazard quotient for *C.rivularis*

	<b>Max</b>	<b>Min</b>	<b>Average</b>
<b>As</b>	0,011	0,003	0,007
<b>Cd</b>	0,004	0,000	0,002
<b>Cr</b>	0,008	0,004	0,006
<b>Cu</b>	0,162	0,049	0,106
<b>Zn</b>	0,777	0,206	0,492
<b>Fe</b>	0,595	0,395	0,495
<b>Al</b>	0,406	0,310	0,358

As shown in Table 4.40 to 4.47, there were no THQ values for all metals greater than 1 for any metal, suggesting that the local inhabitants will not be exposed to a potential health risk from dietary As, Cd, Cr, Cu and Zn. However, the TTHQ values for Al and Fe

caused by consuming mollusks were quite high, and likely would have been even higher had other metals and metalloids been included in the study. These species may pose a potential risk to the local inhabitants through their consumption in the diet. Consequently, some effective measures may be necessary to reduce Al and Fe metal contamination in sediments in this region. Further in depth studies are urgently needed, and should be identified to help safeguard human health.

## CHAPTER 5

# CONCLUSION AND SUGGESTION

### 5.1 CONCLUSIONS

The trace metal concentrations determined in the present study for 4 sediments and soft tissues of the 11 mollusk species collected in period 2012-2013 in Nha Trang bay, Khanh Hoa, province, Viet Nam are reported for each of ones. During each sampling event, both organisms and sediment were collected from the 4 areas and analyzed for the metals of concern. The variation in trace metal concentrations between individual mollusks was predictable to some extent based on environmental factors, their body weight/shell size, patterns of essential trace metals depending on the group and appears to be affected by both seasonal and locational variations. Results from this study demonstrated that:

#### **5.1.1 Heavy metal contaminations in sediment samples**

##### **The sediments type**

According to data analysis of physicochemical characteristics, the sediments collected in Nha Trang bay were classified as silt clay to clayey silt with pH neutral, lighter organic matter, low AVS and differences of moisture content from low to high, depends of location of sampling

##### **Metal contents in sediments**

For remaining heavy metals in sediments in both two seasons, metal concentrations in the sediments ranked in the following order: Aluminium > iron > zinc > copper > chromium > arsenic > cadmium. The most of metals concentrations were acceptable (<LEL) or moderate ( $\geq$  LEL and < SEL) biological effects, except Cd.

##### **Correlation of metal concentration and environmental factors**

A high correlation was observed between metal levels in the sediment and silt-clay content while a moderately negative correlation resulted between Cd in sediment and other metals in the sediment. Decreases in TOC and pH in the dry and rainy season appear to be linked to an accumulation of metals in the sediments.

One can observe a strong correlation between the amount of Fe and Al in both two seasons. On the other side, a significant correlation exists between Cr and Al, Fe, Zn and Ni in both dry and rainy season and between Cu-Zn and Fe, Al, Ni in dry season.

### **5.1.2 Bioaccumulation of heavy metals in mollusks**

#### **Metal contents in mollusks**

In general, concentrations of Al and Fe in mollusk samples from Nha Trang bay were higher in shell than in soft tissue, but for other heavy metals, concentration of metals in shell and tissue were significantly correlated.

Among the five heavy metals tested in all tissue of mollusk, Zn concentration was the highest in all tissue of mollusks and Cu concentration was the second elements found to be highest in the mollusk tissue. But, these observed contents of Cu and Zn were not exceeded permissible level WHO. These concentrations of As, Cd and Cr were detected at low concentration, but As, Cd and Cr in the samples were above permissible level WHO, where they habited may pose hazard to human in terms of health risk.

#### **Bioaccumulation of heavy metals**

The study also showed that the magnitude of the heavy metal accumulation in mollusks tissues depend on the type of the heavy metals, the species of the mollusks, seasonal factors.

There was well obvious overall pattern in the seasonal dependence of the metal concentrations measured for the mollusks in this study although the concentrations of some metals were generally high in rainy season, which is the period of somatic growth and gametogenesis. The majority of this research supports accelerated accumulation during the rainy season.

Physicochemical factors such as pH, TOC, together with the inorganic components of the sediments, can affect the uptake of metals and the subsequent accumulation of metals in the tissues. While in most mollusks the increased CI reflects directly the accumulation of metals, in some clams increases can occur without apparent weight gain. This was also found in the present study where the variation in mollusks CI amongst species was greater than it was between sampling locations. These results highlight the

necessity to adjust for weight differences when comparing trace metal concentrations amongst mollusk samples.

Mollusk species have served as good bioindicator organisms. Thus, the data showed that mollusks living in the effluent from the study area high accumulate heavy metals in their body tissue. The mollusks *L.anatine*, *L.unguis*, *G.virens*, *K.hiantina* and *G.coaxans* have high potential factor in term of metal BSAF in their tissues. They have served as good bioindicator organisms, in which *K.hiantina* might be the best indicator of metal pollution. Trace metal concentrations in this clam may be low compared with some other species, but it is high presence, abundance and wide geographical distribution also in potentially contaminated sites.

### **5.1.3 Ecological risks**

A moderate trace level consumption of bivalve from the Nha Trang bay is not harmful to humans for Cd, Cu and Zn, but due to high level accumulations of metals in soft tissues, heavy consumption of mollusks from the Nha Trang bay may pose a health risk to consumers.

## **5.2 LIMITATIONS AND SUGGESTIONS**

However this study also consider for a local programs. There was disturbance of the monitoring area (i.e., dredging, harvesting, recreational activities), as this was suspend contaminated material and possibly desorbs the sediment bound metals, as well as compromise the diversity of the benthic

Sediment analysis should include salinity, DO, pyrite  $\text{FeSO}_4$ , LOI, total sulfite volatil, as well as determination of  $\text{CaCO}_3$  and Mn in sediment. These physicochemical parameters are important for explain of bioavailability and uptake.

The number or organisms should be sufficient so that a level of statistical significance can be assigned to the data. At least 10 surviving mollusks or better still, 20–30 bivalves per monitoring site should be sufficient.

These results highlight that there not necessity to analyse metals in shell when comparing trace metal concentrations amongst mollusk samples. The differences in correlation among the above metals reflect differential behaviors, which require further

study. Although seasonal fluctuations in metals accumulation by mollusks has been reported, tissue sampling and analysis should occur four times per year.

Because shellfish consumption rates can vary greatly between specific sections of a community, the value ecological risks reported here should only be used for screening purposes. Clearly, further assessment is necessary before more definitive conclusions can be drawn.

### **5.3 FURTHER RESEARCH**

When undertaking monitoring studies using mollusks, it is important to account for differences in biomonitoring considerations. The need for regular monitoring of bioaccumulation of trace metal pollution is emphasized to acquire data for environmental protection and conservation measures in Nha Trang bay in particular or .coastal of Vietnam in general.

Clam *K.hiantina* as good bioindicators, that found that clams had an ability to survive very well under natural conditions. A field experiment of the clam will be transplant, either suspended in seawater or in sand over the 6 month duration of the study.

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# **APPENDIX**

## **APPENDIX 1**

### **PUBLICATION**

Appendix 1.1: Heavy metal contamination in sediments of Nha Trang bay, Khanh Hoa province. Tran Thi Mai Phuong, Nguyen Ky Phung, Nicolas Marmier. Journal of Science and Technology. Vol 50, N° 1C, ISSN 0866 708X. (159-165). 2012

Appendix 1.2: Bioaccumulation and concentrations of heavy metal in the different mollusks collected from Khanh Hoa coastal, Viet nam. Tran Thi Mai Phuong, Nicolas Marmier, Charlotte Hurel, Nguyen Ky Phung. Journal of Science and Technology. Vol 50, N° 4A, ISSN 0866 708X. (234-241). 2012

Appendix 1.3: Bioaccumulation of heavy metals in some mollusks species at the Khanh Hoa province, Viet Nam. Tran Thi Mai Phuong, Nicolas Marmier, Charlotte Hurel, Nguyen Ky Phung. Proceeding of the International conference on BIENDONG, 2012. Volume 1 :(225-234). 2013





# HEAVY METAL CONTAMINATION IN SEDIMENTS OF NHA TRANG BAY, KHANH HOA PROVINCE

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## ABSTRACT

More than ten years ago, Khanh Hoa province has developed a progress in monitoring the coastal environment. This monitoring program takes place in period from 1996 to 2011, during which the sediment sampling in the Nha Trang Bay and the set of heavy metal were established: Pb, Cd, Cu, As, Hg and Zn. With the aim to review the contamination evaluation, distributions of metals in 1996 were required as pre-industrial reference, which concentrations of heavy metals can be compared with those measured in the next ten years 1998-2011. To assess the pollution of heavy metals, a method for calculating contamination indices was used, which can evaluate the impact of anthropogenic pollution by the ratio of Geoaccumulation (Igeo index) for each metal in a given station. The degree of contamination in marine sediments, based on the calculation of the Igeo index can be defined as a moderate to considerable contamination level in the sediments of Khanh Hoa province.

**Key words:** Heavy metal, contamination, enrichment factor, sediment, Nha Trang bay.

## I. INTRODUCTION

Heavy metals like Pb, Cd, Cu, As, Hg and Zn, coming from natural and/or anthropogenic sources, are significant input components of the sediment in marine zones, which are being important areas for concentration of contamination. The most significant sources of pollution include oil from ships, sewage and other domestic wastes, and industrial effluents [4]. Since 1996, in Viet Nam, the increase of metal concentration in the sediment had been observed in Quang Ninh [1], Hai Phong [7], Da Nang [3] and Khanh Hoa [3], and receives attention from local managers.

The objective of this study was to evaluate the ranges and state of contamination by 6 metals in the period of time going from 1996 to 2011 in Nha Trang bay, Khanh Hoa province by making comparison with standard criteria for sediment. Concentrations of these heavy metals for two different seasons have been monitored: the dry and the rainy seasons, and calculations of geoaccumulation using the Igeo index, which compare the metal contaminants concentrations for the pre-industrial period (1996) with values

measured for sediments collected from 1998 to 2011, supposed to be affected by human activities.

## II. MATERIAL AND METHODS

### 2.1 Survey area

The station of monitoring with latitude  $12^{\circ}12'45''\text{N}$  and longitude  $109^{\circ}13'12''\text{E}$ , is located in Nha Trang bay – Central southern Vietnam, which encompasses some  $507\text{km}^2$  of offshore waters and island coasts. The Nha Trang bay is situated immediately adjacent to the coastal city of Nha Trang and its port at Cau Da. Two rivers, the Cai River in the north and the Be River in the south, flow into Nha Trang Bay, with the potential to influence water quality in the Bay. There are 19 islands included in the Nha Trang Bay, situated from ~ 1km to ~ 15 km offshore, with an average depth of 5 - 20m, providing a wide range of coastal and marine habitat types in relation to prevailing oceanographic conditions and gradients in mainland - oceanic influences. The river catchments and riparian vegetation have been extensively modified for agriculture and aquaculture in recent decades.

The surface sediment samples were collected every year in the Nha Trang bay from 1996 to 2011 during both dry and rainy seasons, by Institute of Oceanography of Nha Trang.

### 2.2 Field sampling

These sediments were taken using an Ekman grab from a boat. Samples of the top surface centimeter were collected with a polyethylene spool and placed in a plastic bag, which were stored in an ice box at  $4^{\circ}\text{C}$ , to be transported in the laboratory of Faculty of Environment (HCMUS) Ho Chi Minh City. In the laboratory, some of sediment characteristics were determined as pH, moisture content, organic matter and particles-size.

Sediment samples were dried at room temperature, and then were sieved through a nylon mesh to obtain 4 different particle-size fractions, starting with particles smaller than 0,2mm in diameter for determination of heavy metal concentration. For each particle-size fraction, 1.0 gram was taken for heavy metal analysis.

### 2.3 Analysis

#### *Determination of sediment characteristics*

Moisture was determined in two steps: sediments were dried in air at room temperature then oven drying at  $105^{\circ}\text{C}$  in 16h (Method ASTM 2974-87). Moisture contents were measured by weight loss.

Organic matter was determined by ash dried sediments in the furnace to  $750^{\circ}\text{C}$  and hold until the samples were completely ashed. (Method ASTM, 2974)

For the bulk density determination, 10ml of post were added in sediment samples and allowed to dry to a constant weight at  $70^{\circ}\text{C}$ . Bulk density was calculated as dry weight divided by wet volume.

For grain size analysis, a standard sieving method has been used for particles bigger than 0.1mm, and by pipetting method for particles smaller than 0.1mm.

### Heavy metal analysis

The samples were digested, following the guideline EPA 3050 for digestion sediments using a hot plate. About 1,0 gram of dried and sieved shells were transferred in 250 ml pyrex beaker and added with 4 ml of acid (1+1) HNO<sub>3</sub> and 10 ml of (1+4) HCl for decomposition of organic matter. Then the beakers were put on a hot place for reflux extraction at 95°C, for 30min. Then, the samples were cooled and the extract quantitatively transferred in a 100 ml volumetric flask, then final solutions diluted and mixed to volume to 100 mL with ultra-poor water. The obtained solution was then centrifuged and the concentration of heavy metals measured using AAS - Atomic Absorption Spectrophotometry.

## III. RESULTS AND DISCUSSION

### 3.1 Sediment characteristics

The results have showed that the pH value in the station corresponding to the samples varies from 7.15 to 7.32. The sediment sample was then in a neutral environment. The moisture content of sediment sample was about 7.4%. The total organic matter of sediments is equal to 17.97%, and the bulk density gave an average value of 0.26g/cm<sup>3</sup>.

Fine particles were found on the sediment sample. Meanwhile the average fraction of very fine sand and mud (grain size 0.01-0.125mm) in sediment was 26.77%, compared with an amount of about 73.02-74.71% of silty clay. The distribution of the coastal sand was not found in this zone.

The grain size distributions of sample showed a highest muddy fractions, while there is only 0,66% medium coarse sand (fraction whose practice-size >0.1).

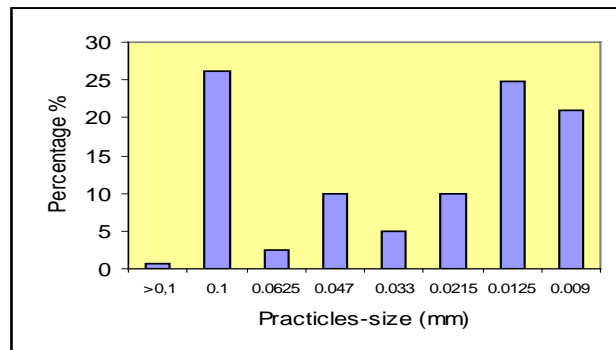


Figure 1: The grain size distributions of sample

### 3.2 Heavy metal concentrations

The concentrations of contaminated heavy metal in period 1996-2011 are summarized and compared with the New York guidelines criteria [6] for the effect of sediment on benthic organisms. Assessment of quality of sediment was focused on the LEL (Lowest effect level) and SEL (Severe effect level) as proposed by the Department of Environmental Conservation of New York, 1999. Definition of LEL and SEL [6] is:

- Metal values < LEL: the effect in the sediment is considered to be acceptable
- LEL < Metal values < SEL: contaminated, moderate impact to benthic life
- Metal value > SEL: contaminated and significant harm to benthic aquatic life

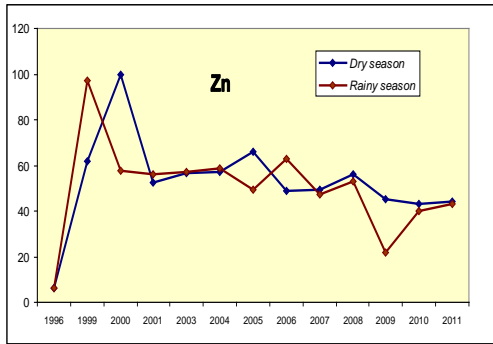
Table 1 shows the based concentration of metal in sediment samples from 1996 to 2011 and gives the LEL and SEL for each metal.

Table 1: Based concentration of metal in surface sediment at period 1996-2011

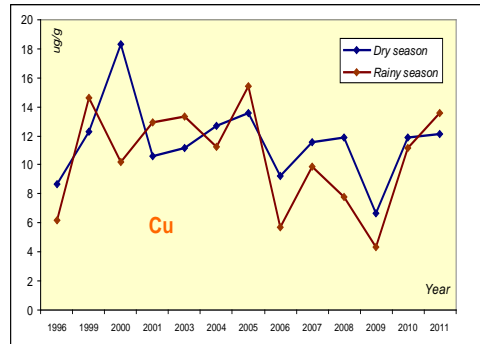
Time	Season	Zn (µg/g)	Cu (µg/g)	Pb (µg/g)	As (µg/g)	Cd (µg/g)	Hg (µg/g)
Jun1996	D	6,33	8,68	0,19	18,04	ND	ND
Oct1996	R	6,44	6,19	0,7	5,57	0,2	<0.1
Sep1996	R	0,97	1,68	0,07	7,1	0,1	0,09
Mar1998	D	50,8	26	12,6	3,9	<0.05	<0.02
Feb1999	D	61,73	12,26	28,11	6,26	0,38	0,62
Jun1999	R	97,24	14,62	29,55	8,33	0,04	0,78
Feb2000	D	99,5	18,3	36,7	8,5	4,8	1,6
Aug2000	R	57,53	10,23	26,09	2,32	Trace	2,76
Feb 2001	D	52,3	10,6	33,4	4,05	0,21	1,99
Aug2001	R	55,99	12,93	27,14	16,07	0,104	0,684
Feb2002	D	24,8	5,37	15,63	1,53	0	0,28
Feb2003	D	56,38	11,19	14,62	4,02	0,07	0,74
Aug2003	R	57,4	13,3	4,7	3,19	0,5	0,53
Feb2004	D	56,9	12,7	26	3,2	0,07	0,31
Aug2004	R	58,64	11,21	36,1	3,6	0,05	0,33
Feb2005	D	66,2	13,6	30,3	2,9	0,05	0,35
Aug2005	R	49,5	15,4	26	3,4	0,29	0,28
Feb2006	D	48,7	9,2	28,9	3,2	0,04	0,22
Aug2006	R	62,8	5,7	26,3	2,6	0,12	0,26
Feb2007	D	49,3	11,6	26,7	3	0,25	0,26
Aug2007	R	47,5	9,9	25	3,2	0,2	0,39
Feb2008	D	56,3	11,9	26,7	5,1	0,13	0,72
Aug2008	R	53	7,8	23,7	4,9	0,07	0,8
Feb2009	D	45,4	6,7	11,6	0,08	4,5	0,5
Aug2009	R	21,9	4,3	9,2	0,06	2,6	0,25
Mar2010	D	43	11,9	33,9	3,8	0,7	0,33
Sep2010	R	39,8	11,2	30,7	3,8	0,45	0,41
Mar2011	D	44,4	12,1	30,8	3,4	0,3	0,31
Aug2011	R	43,2	13,6	34,1	2,7	0,33	0,43
LEL*		120	16.0	31.0	6.0	0.6	0,15
SEL*		270	110.0	110.0	33.0	9.0	1,3

Note: D: Dry season R: Rainy season ND: Not detected  
LEL\*: Lowest effect level (ppm) SEL\*: Severe effect level (ppm)

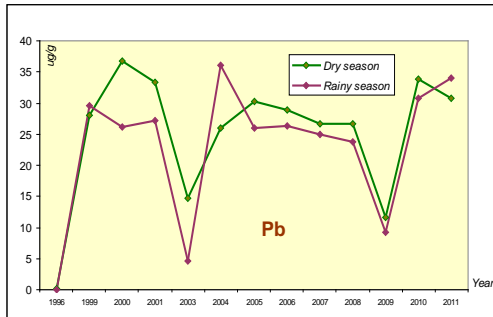
In general, the results demonstrated that concentrations of heavy metal in Nha Trang were lower than LEL\*. That means that most of the elements have no biological effect, except Hg. Hg is the most critical element that can have an effect on the organism living in sediment, as 100% of the collected sediments from 1998 to 2011 were contaminated with values exceeded LEL.



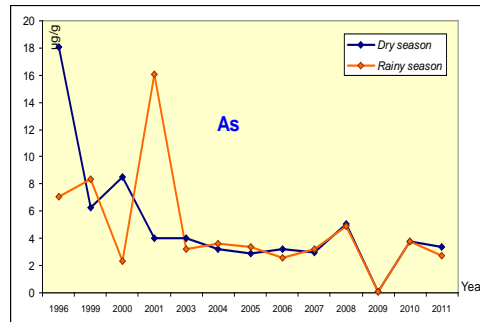
Zn concentration in period 1996-2011



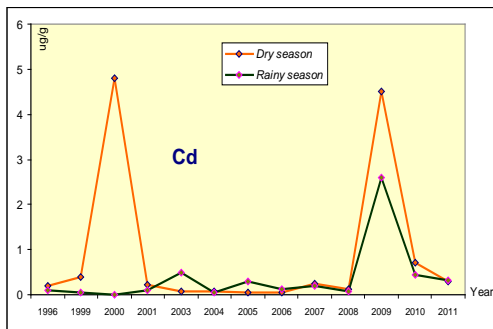
Cu concentration in period 1996-2011



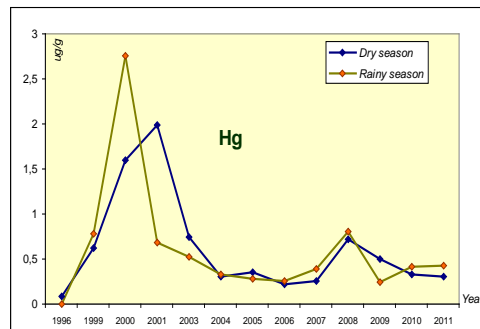
Pb concentration in period 1996-2011



As concentration in period 1996-2011



Cd concentration in period 1996-2011



Hg concentration in period 1996-2011

Figure 2: Heavy metal concentrations of Nha Trang Bay in period 1996-2011

In general, the concentrations of heavy metal in dry season were higher than in rainy season for the ten years.

### 3.3 Classification of contamination level

The geoaccumulation Igeo index [2] was used to calculate the metal contamination level for the period 1996-2011. Table 5 describes classes for increasing Igeo values. The geoaccumulation index is calculated as follow

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

Where: Cn: concentrations of elements in samples (from 1998-2011)

Bn: concentration values of the element in pre-industrial (1996)

Table 2: Descriptive classes for increasing Igeo values [2]

N	Igeo values	Igeo class	Designation of sediment quality
1	0	0	Uncontaminated
2	0-1	1	Uncontaminated to moderately contaminated
3	1-2	2	Moderately contaminated
4	2-3	3	Moderately contaminated to strongly contaminated
5	3-4	4	Strongly contaminated
6	4-5	5	Strongly contaminated to extremely contaminated
7	>5	6	Extremely contaminated

Detail of the results of calculating Igeo values for individual element is showed in table 3. The negative Igeo presented in the table are the results of relatively low concentration for metal in sample.

Table 3: Igeo values and classing of level contamination of each metal

Time Year	Igeo Zn	Igeo Cu	Igeo As	Igeo Pb	Total Igeo
Mar 1998	1,02	0,85	0,19	2,45	0-3
Feb 1999	1,07	0,66	0,26	3,22	0-4
Jun 1999	1,19	0,70	0,30	3,27	0-4
Feb 2000	1,19	0,76	0,30	3,48	0-4
Aug 2000	1,05	0,61	0,12	3,15	0-4
Feb 2001	1,03	0,62	0,20	3,39	0-4
Aug 2001	1,04	0,67	0,39	3,19	0-4
Feb 2002	0,83	0,44	0,06	2,65	0-3
Feb 2003	1,05	0,63	0,20	2,59	0-3
Aug 2003	1,05	0,68	0,16	1,49	0-2
Feb 2004	1,05	0,67	0,16	3,14	0-4
Aug 2004	1,06	0,63	0,18	3,46	0-4
Feb 2005	1,09	0,68	0,15	3,29	0-4
Aug 2005	1,01	0,72	0,17	3,14	0-4
Feb 2006	1,01	0,58	0,16	3,25	0-4
Aug 2006	1,07	0,46	0,13	3,16	0-4
Feb 2007	1,01	0,64	0,15	3,17	0-4

Aug 2007	1,00	0,60	0,16	3,11	0-4
Feb 2008	1,05	0,65	0,23	3,17	0-4
Aug 2008	1,03	0,54	0,22	3,05	0-4
Feb 2009	0,99	0,50	-0,36	2,37	0-3
Aug 2009	0,80	0,38	-0,40	2,14	0-3
Mar 2010	0,98	0,65	0,19	3,40	0-4
Sep 2010	0,96	0,63	0,19	3,30	0-4
Mar 2011	0,98	0,65	0,17	3,31	0-4
Aug 2011	0,98	0,68	0,14	3,41	0-4

Based on the classification of metal pollution proposed by the calculated Igeo factor, both Cu and As were in the range below uncontaminated or moderate contaminated level, when Zn was classified as moderately contaminated level. Only Pb shows a moderately to strongly contaminated level in this zone.

For Cd and Hg, low concentrations in the samples do not allow calculations.

#### IV. CONCLUSION

With regard to an overall review of contamination of heavy metal in Nha Trang bay, the heavy metal concentration was lower than LEL, meaning that most of the measured elements have no biological effect, except Hg. The concentrations of heavy metal in the dry season were higher than in the rainy season during the all ten years period of time.

The impact of contamination by heavy metal in Nha Trang bay in period from 1996-2011 was evaluated using Geoaccumulation Igeo indices. The Igeo suggests that fine fraction of sediment in Nha Trang bay goes from moderately to strongly contamination with respect to the analysis of 4 heavy metals (Zn, As, Cu and Pb). The Igeo index was not able to applied to the Hg and Cd concentrations for the assessment of their pollution impact.

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## BIOACCUMULATION AND CONCENTRATIONS OF HEAVY METAL IN THE DIFFERENT MOLLUSKS COLLECTED FROM KHANH HOA COASTAL, VIET NAM

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### ABSTRACT

Eight species of gastropod and bivalve (*Bufonaria rana*, *Isonomon perna*, *Modiolus vaginus*, *Crasostrea rivularis*, *Katelsysia hiantin*, *Anadara antiquata*, *Glauconome virens* and *Geloina coaxans*) were collected from 8 different stations in the coastal of Khanh Hoa Viet Nam in April 2012 and analyzed for heavy metals such as Arsenic (As), Copper (Cu), Cadmium (Cd), Chrome (Cr), Lead (Pb) and Zinc (Zn) in their soft tissues. The capability to accumulate metals from sediment was measured by calculating values BSAF (Biota – Sediment Accumulation Factor). From this study, it was found that the concentration levels of heavy metal in tissues were observed higher than in shells and ranged between 33.82-262.91; 0.80-6.24; 0.12-87.67; 8.28-23.72; 4.31-3525.63 ( $\mu\text{g/g}$  dry wt) for As, Cd, Cr, Cu and Zn respectively. On the other hand, highest BSAF values were obtained in tissues of *Isonomon Perna* for As (13,41), Cd (5,89) and Zn (65,46), while highest BSAF were obtained in tissues of *Crassostrea rivularis* for Cr (7,51) and for Cu (14,71). The highest bioaccumulated metals were Zn, As and Cu, while the lowest one was Cd.

*Key word:* Heavy metal, mollusk, bioaccumulation, Khanh Hoa coastal.

### 1. INTRODUCTION

In the recent year, contamination of heavy metals has been negative impact to the natural environment, especially to marine ecosystems in coastal areas. The effects of heavy metal contamination can be severe and are a significant concern in the Arctic [1]. Once heavy metals are in the sediment, they can be easily be uptaken by organisms and accumulate in living tissues of bivalves as well as mussels community. The bioaccumulation of heavy metals by marine mollusks may reach many orders of magnitude above background concentration of certain

locality [2]. Thus, collecting and analyzing the concentrations of heavy metals may help us to understand the bioaccumulation capacity for heavy metals by different species of mollusks, which habitats locates along the coastal of Khanh Hoa province. This research conducted the heavy metal analysis in the sediment and in the bivalves from the same location could be used to estimate bioaccumulation capacity in marine ecosystem and will help to propose the best bivalve indicators for an environmental biomonitoring.

## 2. MATERIAL AND METHODS

### 2.1 Research area

Nha Trang bay locates in Khanh Hoa province – central-southern Viet Nam, which is an important ecological zone and providing a wide range of coastal and marine ecosystems in relation to prevailing oceanographic conditions and gradients in mainland – oceanic influence. The sites of collection samples from the different geographic locations and substrate types (mainland-oceanic gradient). Sediments and mollusks samples were collected from eight sites in March and April 2012. The locations of sampling sites are showing in Table 1.

Table 1. Sampling location

Samples	Sites	Longitude	Latitude
NT1	Nha Trang port	12°12'45"N	109°13'12"E
NT2	Be estuary	12°12'22"N	109°11'17"E
NT3	Lon estuary	12°15'44"N	109°11'58"E
NT4	Vinh Luong	12°20'10"N	109°12'15"E
NT5	Ninh Loc	12°26'17"N	109°8'14"E
NT6	Ninh Ich	12° 23'42"N	109°11'57"E
NT7	Dinh estuary	12°27'00"N	109°09'38"E
NT8	Cam Lam	12°04'45"N	109°10'42"E

### 2.2 Sample collection and preparation

#### 2.2.1 Mollusks collection

About 5-10 numbers of different sizes organisms from each sampled site were picked by hand and then put in Ice box to be transported in the laboratory. Different tissues are separated by knif and dried at 60°C during two days to constant weight. These dried tissues were then stored in polyethylene bag and sent to Ecomer's laboratory (UNS-France) for the determination of heavy metals concentrations.

#### 2.2.2 Sediment collection

These sediments were taken using an Ekman grab from a boat. Samples of the top surface centimeter were collected with a polyethylene spool and placed in a plastic bag, which were stored in an ice box at 4°C and sent to the laboratory. Sediment samples were dried at room temperature, and then were sieved through a nylon mesh to obtain particles smaller than 0,2mm

in diameter for determination of heavy metal concentration. For each sediment sample, 1.0 gram was taken for heavy metal analysis.

### **2.3 Sample digestion and analysis**

#### *2.3.1 Mollusks digestion*

Digestion method of organically based matrices was based on the guideline EPA 3052. Each biological sample (tissues or shells) was dried, pulverized and sieved for digestion and determination of metals concentrations. A weight of about 0, 5 gram of shell or 0,1gram of tissue was then transferred in a 100 ml Pyrex beaker and added with 4 ml of acid HNO<sub>3</sub> and 10 ml of HCl. Then the beakers were placed on a hot place for reflux extraction at 95°C, for 30min. The digested samples were diluted and filled up with de-ionized water to a total volume of 50ml before metal analyzing by ICP-OES (Perkin Elmer). The accuracy of the analytical procedures employed for the analysis of metals in organic samples was checked using the Fish tissue reference IAEA MA-B-3/TM as certified reference material, and showed good agreement with the certified values.

#### *2.3.2 Sediment digestion*

The samples were digested, according to the guideline from EPA 3050 for digestion of sediments using a hot plate. About 1,0 gram of dried and sieved sediment were transferred in 100 ml Pyrex beaker and added with mixed of concentrated acid HNO<sub>3</sub> and HCl. Then the samples were put on a hot place for reflux extraction at 2 etapes: 95°C, for 30min then 2h at 140°C. The samples were cooled and mixed to volume to 100 mL with ultra-pure water. The obtained solution was then centrifuged and the concentrations of heavy metals were measured using ICP-OES (Perkin Elmer). The accuracy of the analytical procedures employed for the analysis of metals in sediment samples was checked using the marine sediment IAEA-433 as certified reference material.

### **2.4 Method of analyzing data**

A mollusks capacity to accumulate metals from living medium into its tissue can be estimated using BSAF factor. BSAF were calculated for the selected metal in the mollusks studied according to formula  $BSAF = C_x/C_s$  where  $C_x$  and  $C_s$  were the metal concentrations in the organisms and in associated sediments [3].

By comparing BSAF, we can compare the ability of those mollusks in taking up metal from sediment. The categories of BSAF are presented as: High potential of accumulation if  $BSAF > 2$ , Moderate potential if  $1 < BSAF < 2$  and Low potential if  $BSAF < 1$ .

## **3. RESULTS AND DISCUSSION**

### **3.1 Heavy metal distribution in mollusk tissues**

The overall heavy metal concentrations in the 7 analyzed species are included in Figure 1 and presented in µg/gram of dry weight. For most of the metals, when mean values were calculated, values were close to the maximum permissible level FDA (USA, 2001), except for

Pb. It indicated that the coastal has been polluted by heavy metal which eventually leads to bioaccumulation of that pollutant in food chain of the marine environment.



Figure 1. Heavy metal concentrations in mollusk tissues

On the other side, the observed concentration of As, Cd, Cr and Cu in the samples were within permissible level, where the bivalves are safe for human consumption and where the ecosystems where they habited do not pose any hazard to human in terms of health risk. The low fluctuation level of Cd, Cu and Cr in the tissues of mollusks clearly showed that they need of same level of these metals to maintain the normal bodies function of marine organisms.

Based on there results, it showed that the magnitude of the heavy metal accumulation in mollusks tissues depends on the type of the heavy metal and the specie of the mollusks and also showed that mollusks living in the area accumulate heavy metals in their body tissue.

### 3.2 Heavy metal concentration in sediments

The concentrations of heavy metal in eight collected sediments are summarized in Figure 2 and compared with the New York guidelines criteria [4] for the effect of sediment on benthic organisms. Assessment of quality of sediment was focused on the LEL (Lowest effect level) and SEL (Severe effect level) as proposed by the Department of Environmental Conservation of New York, 1999.

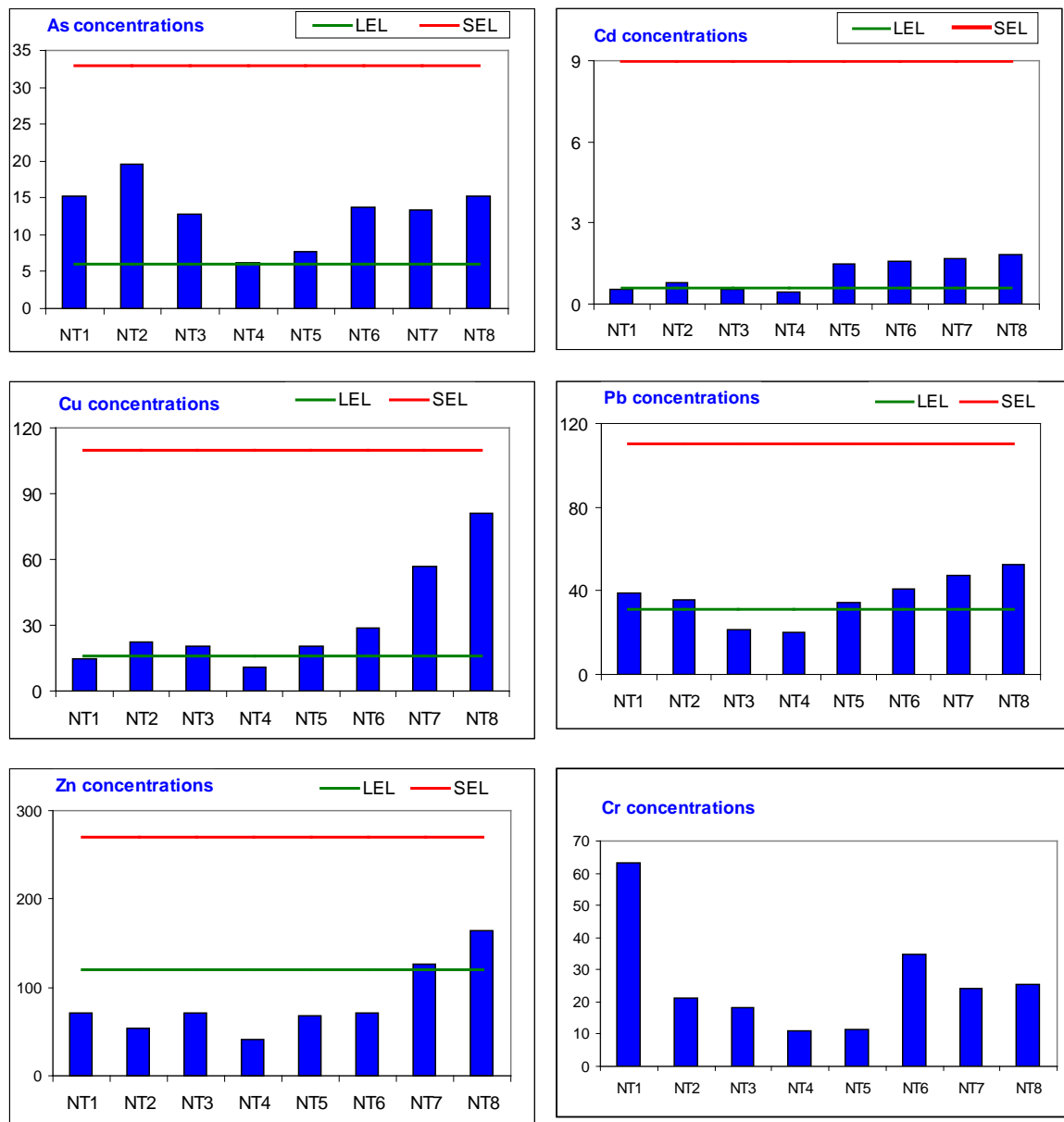


Figure 2. Heavy metal concentrations in sediment samples ( $\mu\text{g}/\text{gram}$  dry weight)

These variations between 8 sites may be due to their different geographic locations. The concentrations of heavy metal in sediments collected were ranged between 6.26-19,61; 0,45-1,82; 10,94-63,15; 11,03-381,30; 20,23-52,65, 40,99-465,65 ( $\mu\text{g/g}$  dry wt) for As, Cd, Cr, Cu, Pb and Zn respectively. The highest concentrations of Cu, Pb and Zn were in sediments sampled at NT8 and the value measured were 381, 30; 52, 65 and 465, 65  $\mu\text{g/g}$  respectively. The highest concentration of As was observed in the samples from NT2, whereas the highest concentration of Cr was observed in those from NT1. However, the lowest value of Pb was measured in NT4. The mean concentrations of the heavy metals As, Cd and Pb in eight collected sediments were higher than the lowest effect level (LEL) but were lower than the severe effect level (SEL). In classification, these sediments are considered as contaminated, with a moderate impact on benthic life, except sample NT8. Indeed, the Cu and Zn concentrations in sample NT8 was exceeded the SEL, that means the NT8 was contaminated and can cause significant harm to benthic aquatic life.

In conclusion, the results showed that concentrations of heavy metal in Nha Trang are between LEL and SEL values. That means that most of the elements have a moderate impact to benthic life. At NT8 site, some heavy metals can cause harm to benthic life. In terms of geographical location the highest values appeared to be associated with river estuary areas and this may be due to the discharge of untreated water wastes.

### 3.3 Bioaccumulation of heavy metals

The BSAF were calculated for the selected metal in the mollusks studied and showed in the table 2.

Table 2. BSAF values for each mollusks for heavy metals

Mollusk names	As	Cd	Cr	Cu	Pb	Zn
<i>Bufo rana</i>	5,45	3,99	0,01	3,28	0,06	1,51
<i>Isonomom perna</i>	13,41	5,89	0,02	1,29	0,13	65,46
<i>Modiolus vaginus</i>	2,36	5,25	0,34	2,49	0,68	1,83
<i>Geloina coxans</i>	1,17	1,76	0,04	0,57	0,15	3,81
<i>Crasostrea rivularis</i>	10,57	4,15	7,51	14,71	0,69	46,68
<i>Katelysia hiantina</i>	0,56	0,76	0,01	0,39	0,05	0,64
<i>Anadara antiquata</i>	0,29	1,95	0,01	0,15	0,06	0,58
<i>Glauconome virens</i>	0,78	0,44	0,02	0,03	0,04	0,38

The data from table 2 also suggested that there are differences in accumulation of heavy metals among the different mollusk species. As indicated in the table 4, *Bufo rana*, *Isonomom perna*, *Modiolus vaginus* and *Crasostrea rivularis* were found to have a large capacity for As and Cd intake. That could be explained by the role of those metals as essential elements for them. Comparatively, the lower potentials of accumulation of metals as Pb and Cr in the tissue are possibly due to non essential metals for these mollusks.

It was also found a very high heavy metals level in the oyster *Crasostrea rivularis* collected in NT6, but this site of coast is an unpolluted area in terms of industrial impact. In the other hand, the heavy metal concentration in harbor of Nha trang NT1 were also found very low level in organisms, where this site has sources of anthropogenic inputs.

The living condition has a major impact on the accumulation rate of heavy metals in those mollusks. *Anadara antiquata* and *Geloina coxans* are the species of mollusk that live on the bottom, where they burrow in the sand or mud. They are very much exposed to the heavy metal accumulated in the sediment. The BSAF values of these organisms were lower than 2, thus show that they have a low potential of accumulation of heavy metal from sediments. On the other hand, *Crasostrea rivularis*, *Bufo rana* and *Modiolus vaginus* which have a high potential of accumulation (BSAF values were higher than 2), are mollusks that live by sticking to any solid material such as rocks in the sea. As a result, they are more exposed to water and accumulate heavy metal by trapping food from water.

In conclusion, the accumulations of heavy metals were different between species and may exhibit different accumulation capacity for heavy metals. The protection of coastal pollution from damage due to these metals and other chemical requires an understanding of the sensitivity of mollusks to these substances and their ecological requirement. Monitoring programs for coastal pollutions may help to obtain relevant information.

#### 4. CONCLUSION

As a conclusion, the bivalve mollusks can be useful as bioindicator for a coastal contaminant, especially soft tissues of mollusks which are suitable to be used as a toll for heavy metal contamination, due to its natural capacity to regulate and accumulate concentration of various metals. It is clear that the biomonitoring plan is not only site specific, but that it considers the use of species whenever possible. This research provides the evidences a general review of several bivalves and mollusks in Nha Trang Bay impacted by heavy metals, and give suggestion that the mollusks can be an established and essential biomonitor of heavy metal contamination particularly in the coastal Khanh Hoa area.

**Acknowledgement:** The authors wish to acknowledge and thank staff of Institute of Oceanography Nha Trang, especially to Mr.Vu Tuan Anh and Ms.Nguyen Thi Thanh Thuy for their helps and helpful comments in this study. In addition, we wish to thank to Mr. Hua Thai Tuyen for help and assistance classification of mollusks.

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## SỰ TÍCH LŨY KIM LOẠI NẶNG Ở MỘT SỐ LOÀI NHUYỄN THỂ TẠI VÙNG BIỂN KHÁNH HÒA, VIỆT NAM

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**Tóm tắt:** Nghiên cứu bao gồm việc xác định thành phần một số kim loại không ảnh hưởng đến sinh vật như sắt (Fe), nhôm (Al) cũng như hàm lượng các kim loại nặng độc hại khác như arsen (As), đồng (Cu), cadmium (Cd), crôm (Cr), chì (Pb) và kẽm (Zn) trong các mẫu trầm tích cũng như thịt của một số loài nhuyễn thể. Mẫu các loài nhuyễn thể cũng như mẫu trầm tích được lấy tại 4 điểm thuộc vùng ven biển từ đầm Nha Phu (thuộc huyện Ninh Hòa) tới đầm Thủy Triều (thuộc huyện Cam Lâm) vào tháng 9/2012. Mẫu 4 loài nhuyễn thể phi cái (*Glauconome virens* (Linnaeus, 1767), phi đực *Laternula anatina* (Linnaeus, 1758), ngao đen *Katelsysia hiantina* (Lamarck, 1818) và sò lông *Anadara antiquata* (Linnaeus, 1758) được mang về phòng thí nghiệm, sấy khô trong 24 giờ ở 60°C, nhiệt phân hủy với hỗn hợp axit HNO<sub>3</sub> (69%) và HCL (36,46%) và phân tích các kim loại nặng bằng máy khối phổ plasma cảm ứng ICP-OES. Hàm lượng nhôm (Al) và sắt (Fe) không có sự khác biệt nhiều trong thịt các loài nhuyễn thể khác nhau nhưng rất thấp so với hàm lượng kim loại trong trầm tích. Hàm lượng kim loại nặng As, Cu, Cr, Cd trong các mẫu sinh vật nằm dưới ngưỡng cho phép (MPL) theo tiêu chuẩn của Cục an toàn thực phẩm Mỹ FDA nhưng hàm lượng chì (Pb) và kẽm (Zn) cao hơn 4 lần ngưỡng cho phép của FDA.

**Từ khóa:** Tích tụ sinh học Kim loại nặng, Nhuyễn thể, Khánh Hòa.

## BIOACCUMULATION OF HEAVY METAL IN SOME MOLLUSK SPECIES AT THE COAST OF KHANH HOA PROVINCE, VIET NAM

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**Abstract:** Levels of non-critical metals, Iron (Fe) and/or Aluminum (Al), as well as toxic and relatively accessible elements including Arsenic (As), Copper (Cu), Cadmium (Cd), Chrome (Cr), Lead (Pb) and Zinc (Zn) in soft tissues of seven gastropod and bivalve species (*Bufo rana*, *Isonomon perna*, *Modiolus vaginus*, *Crassostrea rivularis*, *Katelsysia hiantin*, *Anadara antiquata* and *Geloina coaxans*) collected from 8 different stations in the Nha Trang Bay, Khanh Hoa province, Viet Nam were analysed. Tissues samples were dried 24 hours at 60°C, digested in a mixed HNO<sub>3</sub> (69%) and HCL (36%) solution, and then analysed for heavy metals using ICP-OES. The concentrations of heavy metal in

the tissues ranged between 33.8-262.9, 0.80-6.24, 0.12-87.6, 8.28-23.7, and 4.31-3525 ( $\mu\text{g/g}$  dry wt) for As, Cd, Cr, Cu and Zn, respectively. Mean concentrations of As, Cu, Cr, and Cd in the selected mollusk species were below the maximum permissible level (MPL) when compared with the FDA guidance document.

**Key words:** *Bioaccumulation, Heavy metal, Mollusk species, Khanh Hoa.*

## I. INTRODUCTION

The pollution of aquatic ecosystems by trace metals is a significant problem, as trace metals constitute some of the most hazardous substances that can bioaccumulate Nguyen Van Khanh & Pham Van Hiep (2009). The bioaccumulation of heavy metals by marine mollusc and other marine organisms may reach many orders of magnitude above background concentrations of certain locality. This phenomenon may demonstrate the potential of these species as a biomonitor of heavy metal pollution Chan (1989), Hamed & Emara (2006). Many studies have shown that intertidal molluscs can be good biomonitoring organisms Ismail (2006). Due to heavy metals contamination are very localised and closed to discharge point sources (Ismail et al. 1993) and molluscs inhabit in different microhabitat of intertidal areas, a detail studies on different species and tissues are important (Berandah et al. 2010).

A lot of studies on mollusks associated with heavy metal pollution have been done by many researchers (Abdullah et al. 2007), but studies of this kind have been rare in Vietnam due to local difficulties in organizing major studies and legal restrictions regarding the export to fin digenous biologic material (Wagner & Johan 2004) For example, the concentrations of some heavy metals in the tissues of bivalve molluscs have been observed in the samples collected along the coastal of Viet Nam (Le Thi Mui, 2008; Nguyen Van Khanh & Pham Van Hiep, 2009; Tu et al. 2010). However, none of those studies specifically investigated the bioacumulative factor of such a species.

The objective of this study is to provide important information of the metal distribution in the sediments and different mollusk tissues. Thus, helping in proposing the potential bioaccumulation by determining its Biota-Sediment Accumulation Factor (BSAF) (Dallinger et al. 1993) and suggesting bio-monitor for heavy metals in Khanh Hoa coastal.

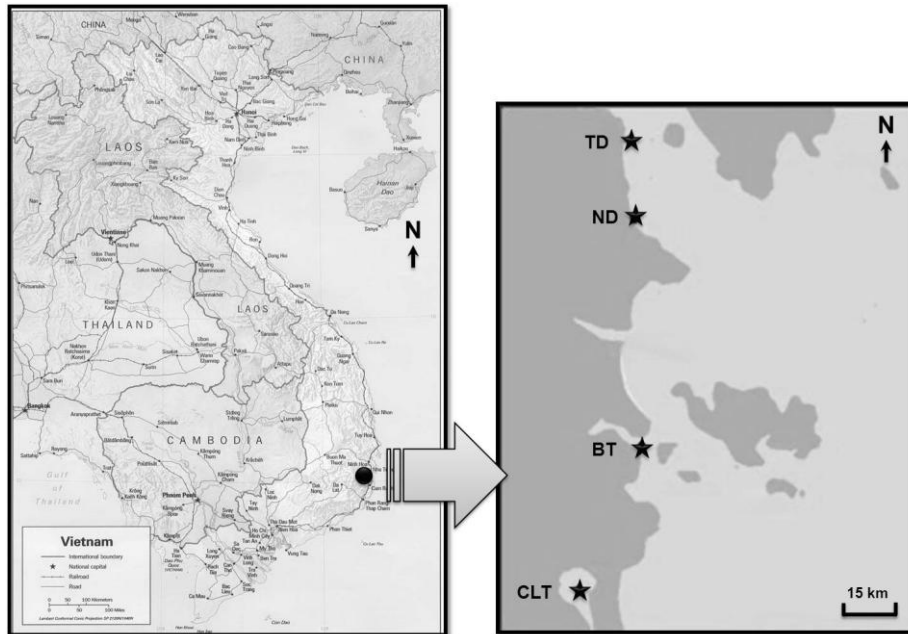
The work is based on study of samples of sediments and 4 mollusks species (*Glaucanome virens* (Linnaeus, 1767), *Laternula anatina* (Linaeus, 1758), *Katelysia hiantina* (Lamarck, 1818) and *Anadara antiquata* (Linaeus, 1758) taken in September 2012 in 4 locations within Nha Phu lagoon (Ninh Hoa) - Nha Trang bay and Thuy Trieu lagoon (Cam Lam). There locations provided wide intertidal zone across the Khanh Hoa coastal in terms of the sandy-muddy areas, represented high community of the bivalve mollusks.

## II. MATERIAL AND METHOD

### 1. Study sites

Khanh Hoa province – central-southern Viet Nam, which is an important ecological zone and providing a wide range of coastal and marine ecosystems in relation to prevailing oceanographic conditions and gradients in mainland – oceanic influence. Compared with some other areas, species composition of molluscs in Nha Trang Bay (490 species) was more abundant than Cat Ba – Ha Long Bay (372 species) and Tonkin Gulf (470 species) Vo Si Tuat et al. (2002).

Sediments and mollusks samples were collected from four sites: Tan Dao (Ninh Loc, Ninh Hoa) – TD ( $12^{\circ}02'17''\text{N}$ ;  $109^{\circ}08'14''\text{E}$ ), Ngoc Diem (Ninh Ich, Ninh Hoa) – ND ( $12^{\circ}23'42''\text{N}$ ;  $109^{\circ}11'57''\text{E}$ ); Binh Tan (Nha Trang) – BT ( $12^{\circ}12'22''$ ;  $109^{\circ}11'17''\text{E}$ ) and Cam Hai Tay (Cam Lam) – CL ( $12^{\circ}04'45''\text{N}$ ;  $109^{\circ}10'42''\text{E}$ ). The locations of sampling sites are showing in Figure 1.



**Figure 1:** Map and locations of sampling site

### 2. Sample collection and preparation

#### 2.1 Organisms collection

About 10-20 numbers of the same sizes of each mollusk species (*Glaucanome virens* (Linnaeus, 1767), *Laternula anatina* (Linnaeus, 1758), *Katelysia hiantina* (Lamarck, 1818) and *Anadara antiquata* (Linnaeus, 1758) were picked by hand from four locations and then put in ice box at  $4^{\circ}\text{C}$  to be transported to the laboratory. The tissues were separated by a plastic knife and dried at  $60^{\circ}\text{C}$  during two days to constant dry weight. These dried tissues were then stored in a polyethylene bag to be sent in ECOMERS laboratory (UNS-France) for the determination of heavy metals concentrations.

## 2.2 Sediment collection

The sediment samples of the top 20cm surface centimeter were collected with a polyethylene spool or Ekman grab, then placed in a plastic bag, which were stored in an ice box at 4<sup>0</sup>C, to be transported to the laboratory. There sediment samples were dried at room temperature, and then were sieved through a nylon mesh to obtain particles smaller than 0.2mm in diameter for determination of heavy metal concentration. For each sediment sample, 0.1gram was taken for heavy metal analysis.

## 2.3 Sample digestion and analysis

### Sediment digestion

The samples were digested following the guideline from EPA 3050b US EPA (1996) for digestion of sediments using a Microwave 3000. A 0.1 gram of dried and sieved sediment samples were weighed out in the reaction vessel then added with mixed of 9ml concentrated acid HNO<sub>3</sub> (69%) and 3ml of HCl (36,46%). Vessels then were placed in the rotor in the microwave. The vessels were heated to at least 240°C over 35 minutes and then held at 210°C for at least 15 minute. The samples were cooled and mixed to volume to 50 mL with ultra-pure water. The obtained solution was then centrifuged and the concentration of heavy metals measured using ICP-OES (Perkin Elmer). The accuracy of the analytical procedures employed for the analysis of metals in sediment samples was checked using the marine sediment IAEA SP-M1 as certified reference material.

### Tissues digestion

The digestion method of organically based matrices used is the one described in the guideline EPA 3052 US EPA (2008). The tissue sample was dried, pulverized and sieved for digestion and determination of metals concentrations. A weight of about 0.5 gram of dry tissue was then transferred in a 100 ml Pyrex beaker and added with 9 ml of acid HNO<sub>3</sub> (69%) and 1ml of HCl (36,46%). Then the beakers were placed on a hot place for reflux extraction at 95°C, for 30min. The digested samples were diluted and filled up with de-ionized water to a total volume of 50ml before metal analyzing. Heavy metals were analyzed by ICP-OES (Perkin Elmer). The accuracy of the analytical procedures employed for the analysis of metals in organic samples was checked using the Fish tissue reference IAEA MA-B-3/TM as certified reference material, and showed good agreement with the certified values.

## 2.4 Data analysis

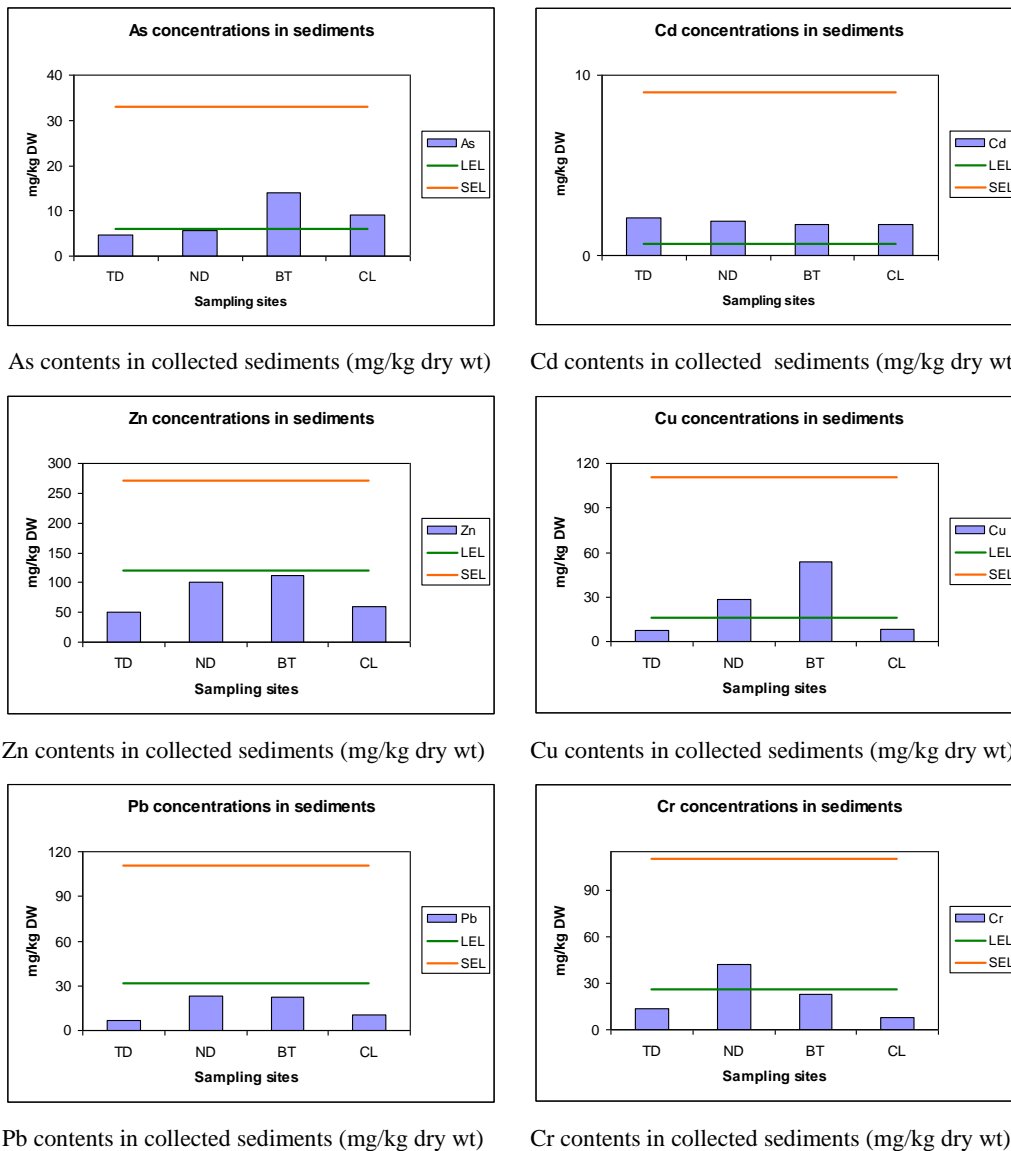
A mollusk's capacity to accumulate metals from living medium into its tissue can be estimated using Biota-Sediment Accumulation Factor (BSAF). BSAF were calculated for the selected metal in the studied mollusks according to the formula  $BSAF = C_x/C_s$  where  $C_x$  and  $C_s$  were the metal concentrations in the organisms and in associated sediments, respectively (Phillips 1997). By comparing BSAF values, we can compare the ability of those mollusks in taking up metal from sediment. The categories of BSAF are presented as: High potential of

accumulation if  $BSAF > 2$ , Moderate potential if  $1 < BSAF < 2$  and Low potential of accumulation if  $BSAF < 1$  (Wich & Colvin 1993).

### III. RESULTS AND DISCUSSION

#### 1. Sediments quality

The sediment qualities from the four sites are given in Figure 2.



**Figure 2:** Concentrations of heavy metals in collected sediments.

Concentrations of As, Cd, Cr and Cu in collected sediments were between LEL (Lowest Effect Level) and SEL (Severe Effect Level) values even though the concentrations of Zn and Pb were lower than LEL. It indicated that the coast has been polluted by most of the heavy metal. Indeed, the effect of these sediments is considered to be contaminated, moderate impact to benthic life Fletcher et al. (2008).

## 2. Heavy metal concentrations in the tissues of 4 studied species

The overall 8 heavy metal concentrations in the 4 analyzed species *Glaucanome virens* (Linnaeus, 1767), *Laternula anatina* (Linnaeus, 1758), *Katelsysia hiantina* (Lamarck, 1818) and *Anadara antiquata* (Linnaeus, 1758), which were collected at 4 locations are included in Table 1 and presented in mg/kg of dry weight.

**Table 1.** Metal concentrations (mg/kg DW) in tissues of four collected organisms.

Names	As mg.kg <sup>-1</sup>	Cd mg.kg <sup>-1</sup>	Cr mg.kg <sup>-1</sup>	Cu mg.kg <sup>-1</sup>	Pb mg.kg <sup>-1</sup>	Zn mg.kg <sup>-1</sup>	Fe mg.kg <sup>-1</sup>	Al mg.kg <sup>-1</sup>
<i>A.antiquata</i>	3,4±1,48	2,72±2,6	3,14±1,9	8,22±1,1	2,53±0,0	48,4±15	879±2 85	392±134
<i>G.virens</i>	1,7±0,98	1,02±0,9	1,32±0,9	6,96±6,9	6,43±3,9	49,8±13	642±1 64	398±214
<i>K.hiantina</i>	9,89±4,39	0,24±0,1	4,08±5,1	7,33±2,4	3,52±1,4	34,4±6,5	597±2 96	388±201
<i>L.anatina</i>	2,72±1,85	0,69±0,2	2,43±0,3	13,5±6,0	8,25±2,0	225±232	1880± 112	805±131
<i>FDA</i>	86	4	13	100	1,7	150	-	-

Metal concentrations of Al and Fe in the tissue of the four sites were similar and did not vary significantly and presented very lower compared to concentrations of these metals in the sediments. Concentrations of Al and Fe in the sediments were 20-100 times higher than the concentration in the mollusks. For most of the heavy metals, when mean values were calculated, values were close to FDA maximum permissible level (Rainbow & Phillips, 1993; Rainbow et al., 2000), except for Pb. The Pb concentration in all of samples was more accumulated and was higher than the FDA maximum permissible level (Pb<1.7µg/g dry wt), which eventually may pose hazard to human in term of health risk. The observed concentration of heavy metals in the mollusk samples showed that the magnitude of the heavy metal accumulation in the tissues depend on the type of the heavy metals and the species. There values of BSAF in the different mullusk species are shown in Table 2.

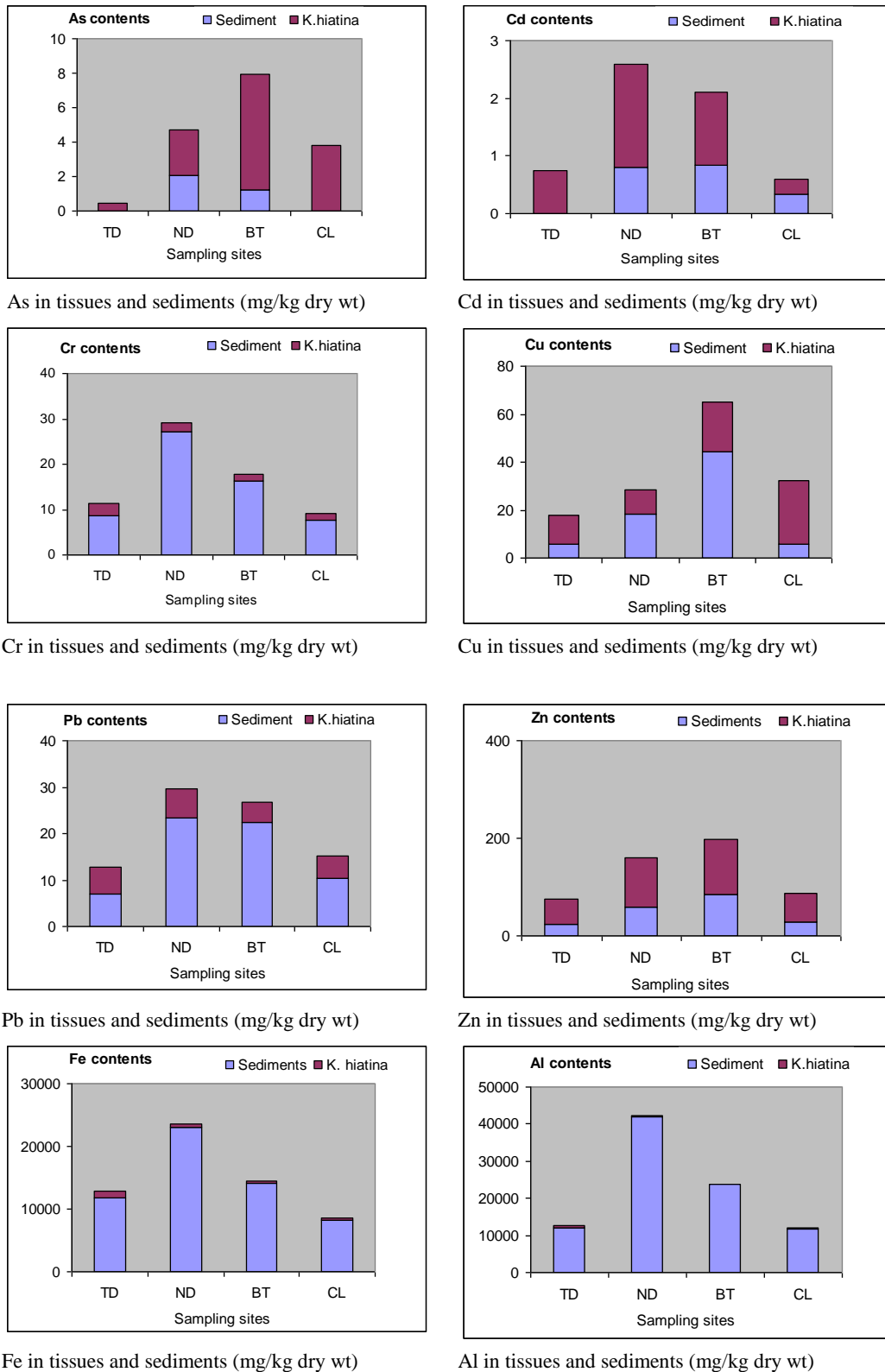
**Table 2.** The BSAF values in the different mollusk species.

Names mollusks	As	Cd	Cr	Cu	Pb	Zn
<i>Anadara antiquata</i>	0,57±0,55	2,19±2,50	0,39±0,36	1,87±0,92	0,27±0,21	1,09±0,1
<i>Glaucanoma virens</i>	9,61±2,63	0,69±0,73	1,19±0,05	4,52±0,32	0,32±0,11	2,08±2,31
<i>Katelsysia hiantina</i>	5,01±3,6	4,87±6,76	0,32±0,35	1,38±1,06	0,44±0,29	3,14±3,09
<i>Laternula anatina</i>	12,11±1,67	0,24±0,27	0,44±0,55	0,51±0,44	0,54±0,16	1,95±3,06

Notes:

	High bioaccumulation
	Medium bioaccumulation
	Low bioaccumulation

On the other side, when BSAF were calculated, it indicated that different species showed different bioaccumulation of metals levels depending on the geographical location. In general, for all of the studied metals, the BSAF values for As were highest than those for Pb were lowest.



**Figure 3:** The contents of metals in soft tissue of the clam and sediments

The BSAF indicated a low potential bioaccumulation for Pb and Cr (BSAF<1) and a very high potential of accumulation for As, Cd, Cu and Zn.

From the results, it was found that clam *Katelsia hiantina* (Lamarck, 1818) highly accumulate As, Cd, Cu and Zn (BSAF>2), and then could be chosen as sensitive indicators for monitoring heavy metal pollutions in Khanh Hoa coastal, Viet Nam.

### **3. The heavy metal contents in the tissues of *Katelsia hiantina* and sediments**

The clam *Katelsia hiantina* (J.B.Lamarck, 1818) also known as other names *Marcia hiantina* and local names “ngao den” or “ngao gia”, is an economical important bivalve and can be also represented in Khanh Hoa coast - Nha Trang bay as one of the local abundant mollusca. This clam belongs to the class Bivalve, order Veneroidea in which shell is medium, oval and thick, that live on the loose bottom of water bodies. They are filter feeder and thus very potential to accumulate toxic substances from water and sediment.

The concentrations of metals (mg/kg dry wt) in whole soft tissue of the clam *K.hiantina* and surface sediments from the four sites are given in Figure 3.

It was observed that clam *K.hiantina* accumulate 8 metals at different concentrations and at different potential of bioaccumulation. The distribution of metals in the tissue of *K.hiantina*, which inhabit in different habitat of intertidal areas, shows that metals contamination are very localized and closed to the anthropogenic influences. The study has concluded that clam *K.hiantina* could be a good bio-indicator of the particular environment through bio-monitoring process.

The higher bioavailability of heavy metals to TD and CL could be explained by the significantly lower organic matter contents, which could potentially affect metal availability, or affect several physiological processes that influence the accumulation of heavy metals by *K.hiantina*.

## **IV. CONCLUSION**

The coast has been polluted by most of the heavy metal as As, Cd, Cu, Cr. Indeed, the effect of these sediments is considered to be moderate impact to benthic life.

Concentrations of almost heavy metals in 4 species mollusk collected were closed to FDA maximum permissible level except for Pb.

The BSAF indicated a low potential accumulation for Pb and Cr and a high bioaccumulation for As, Cd, Cu and Zn.

Clam *K.hiantina* is represented in study zone as the most local abundant mollusca. This species high accumulate heavy metals and then could be chosen as sensitive indicators for monitoring heavy metal pollutions in Khanh Hoa coastal, Viet Nam.

This preliminary sampling should merit further studies using the BSAF values for assess contamination in coastal areas by demonstrate the potential of bioaccumulation of mollusk species



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## APPENDIX 2

### SOME PICTURE OF COLLECTING SAMPLES



Picture A2.1 Collecting mollusk samples at Binh Tan



Picture A2.2 Collecting mollusk samples at Tan Dao site



Picture A2.3 Collecting samples at Cam Lam site



Picture A2.4 Collected mollusk samples

**APPENDIX 4**  
**CERTIFIED REFERENCE MATERIALS**

Appendix 4.1 The values obtained for the reference material IAEA-433 in comparison with the certified values.

Metals	N	Average conc (g.kg <sup>-1</sup> )	Std Deviation (g.kg <sup>-1</sup> )	IAEA 433 (g.kg <sup>-1</sup> )	95% confidence interval	% recovery
As 188.979	3	16,73	3,75	18,9	18,4-19,4	88,52
As 193.696	3	10,72	1,32	18,9		56,72
Cd 228.802	3	0,164	1,15	0,153	0,145-0,161	107,19
Cd 214.440	3	0,297	0,59	0,153		194,12
Cr 267.716	3	141,57	4,76	136	134-138	104,10
Cr 205.560	3	138,99	1,7	136		102,20
Cu 327.393	3	31,58	3,1	30,8	30,2-31,4	102,53
Cu 324.752	3	29,57	3,21	30,8		96,01
Ni 231.604	3	0,22	0,03	39,4		0,56
Ni 221.648	3	2,97	0,54	39,4		7,54
Mo 202.031	3	0,10		NR		ND
Mo 203.845	3	0,00		NR		ND
Mn 257.610	3	34,66	3,42	11,5	11,1-12,0	301,39
Mn 259.372	3	36,75	4,22	11,5		319,57
Mg 285.213	3	246,32	21,45	316	312-320	77,95
Mg 279.077	3	267,19	20,51	316		84,55
Pb 220.353	3	29,69	2,76	26	25,4-26,6	114,19
Pb 217.000	3	34,14	3,57	26		131,31
Zn 206.200	3	98,62	4,02	101	99-103	97,64
Zn 213.857	3	97,82	12,32	101		96,85
Fe 238.204	3	138,72	16,45	40,8	40,3-41,3	340,00
Fe 239.562	3	141,74	15,38	40,8		347,40
Al 396.153	3	167,23	11,35	78,2	76,8-79,6	213,85
Al 308.215	3	182,52	13,46	78,2		233,40

Appendix 4.2 The values obtained for the reference material MAB3 with the certified values.

Metals	N	Average conc (g.kg <sup>-1</sup> )	Std Deviation (g.kg <sup>-1</sup> )	IAEA 433 (g.kg <sup>-1</sup> )	95% confidence interval	% recovery
As 188.979	3	2,43	0,78	2,11	1,42-2,51	115,17
As 193.696	3	3,48	1,02			164,93
Cd 228.802	3	1,38	0,03	NR		ND
Cd 214.440	3	1,05	0,15			
Cr 267.716	3	1,11	0,13	NR		ND
Cr 205.560	3	0,38	0,28			
Cu 327.393	3	3,58	2,16	3,08	9-13	116,23
Cu 324.752	3	5,02	1,95			162,99
Ni 231.604	3	0		NR		ND
Ni 221.648	3	0				
Mo 202.031	3	0,07	0,01	NR		ND
Mo 203.845	3	0,11	0,03			
Mn 257.610	3	4,24	2,71	2,85	2,62-3,57	148,77
Mn 259.372	3	4,18	2,34			146,67
Mg 285.213	3	5,35	1,65	1,13	1,04-1,2	473,45
Mg 279.077	3	5,15	2,54			455,75
Pb 220.353	3	4,57	2,52	4,62	3,58-5,13	98,92
Pb 217.000	3	3,04	2,35			65,80
Zn 206.200	3	104,69	10,04	109,2	106,4-111,9	95,87
Zn 213.857	3	114,46	12,81			104,82
Fe 238.204	3	93,35	13,25	95,4	87,3-107,2	97,85
Fe 239.562	3	56,1	16,28			58,81
Al 396.153	3	11,59	0,55	NR		ND
Al 308.215	3	8,1	2,62			

## APPENDIX 5

### QUALITY ASSURANCE OF ANALYSIS

#### Appendix 5.1 Check of DL for sediment samples

Analyte Name	DL (mg/kg)	RSD (Conc)	SD (Samp)
As 188, 979	1-100	0,214	0,497
Al 396, 153	1-10000	2,156	520,373
Fe 238, 204	1-10000	1,776	561,221
Mg 279, 077	1-10000	1,809	224,028
Cd 228, 802	1-100	0,656	0,206
Cr 267, 716	1-100	0,246	0,549
Cu 327, 393	1-100	1,085	0,78
Zn 206, 200	1-500	0,584	1,312

#### Appendix 5.2 Determination of standard deviation and limit of qualification of analyzing method for heavy metals

Metals	SD (µg/L)	LD (µg/L)	LQ (µg/L)
Ag 328.068	0,07	0,20	0,66
Ag 338.289	0,26	0,78	2,59
Al 396.153	6,55	19,64	65,48
Al 308.215	8,12	24,36	81,20
As 188.979	4,52	13,57	45,22
As 193.696	2,74	8,22	27,41
B 249.677	6,36	19,08	63,60
B 249.772	6,19	18,57	61,89
Ba 233.527	0,07	0,22	0,73
Ba 455.403	0,12	0,35	1,15
Be 313.107	0,06	0,18	0,59
Be 313.042	0,13	0,38	1,25
Ca 317.933	27,94	83,81	279,36
Ca 315.887	28,73	86,19	287,30
Cd 228.802	0,17	0,51	1,72
Cd 214.440	0,12	0,35	1,16
Co 228.616	0,25	0,74	2,46
Co 238.892	0,26	0,78	2,59
Cr 267.716	0,21	0,62	2,08
Cr 205.560	0,20	0,60	2,00
Cu 327.393	0,09	0,27	0,91

Cu 324.752	0,20	0,59	1,96
Fe 238.204	0,71	2,12	7,06
Fe 239.562	0,76	2,29	7,65
K 766.490	0,25	0,76	2,53
K 404.721	78,67	236,00	786,67
Mg 285.213	2,48	7,44	24,79
Mg 279.077	3,12	9,37	31,22
Mn 257.610	0,07	0,20	0,65
Mn 259.372	0,07	0,22	0,73
Mo 202.031	0,37	1,12	3,74
Mo 203.845	0,72	2,16	7,19
Na 589.592	13,30	39,90	132,99
Ni 231.604	0,36	1,07	3,57
Ni 221.648	0,36	1,08	3,61
Pb 220.353	1,54	4,62	15,39
Pb 217.000	4,64	13,92	46,39
Sb 206.836	1,93	5,78	19,28
Sb 217.582	1,69	5,07	16,91
Se 196.026	4,09	12,28	40,93
Se 203.985	6,57	19,71	65,71
Si 251.611	1,51	4,54	15,14
Si 212.412	1,54	4,63	15,42
Ti 334.940	0,06	0,18	0,59
Ti 336.121	0,04	0,13	0,42
Tl 190.801	1,89	5,66	18,88
Tl 276.787	4,36	13,08	43,58
V 290.880	0,36	1,07	3,58
V 310.230	4,07	12,22	40,72
Zn 206.200	1,19	3,58	11,92
Zn 213.857	1,14	3,41	11,36



## APPENDIX 6

### QUALITY GUIDELINES FOR METALS

Appendix 6.1 ERL, ERM and Overall Apparent Effects Thresholds values for sediment

Metals	Effect range low ER-L (ppm)	Effect range median ER-M (ppm)	Overall Apparent Effects Thresholds
As	33	85	50
Cd	5	9	5
Cr	80	145	No
Cu	70	390	300
Pb	35	110	300
Zn	120	270	260
Ni	30	50	NSD

Appendix 6.2 Provincial sediment quality guidelines for metals and nutrients

Metals	Lowest Effect level ERL ( $\mu\text{g/g DW}$ )	Severe Effect level ERM ( $\mu\text{g/g DW}$ )
As	6	33
Cd	0.6	10
Cr	26	110
Cu	16	110
Pb	31	250
Zn	120	820
Ni	16	75

Appendix 6.3 New York Sediment Criteria for metals

Metals	Lowest Effect Level $\mu\text{g/g (ppm)}$	Severe Effect Level $\mu\text{g/g (ppm)}$
As	6.0 (P)	33.0 (P)
Cd	0.6 (P)	9.0 (L)
Cr	26.0 (P)	110.0 (P)
Cu	16.0 (P)	110.0 (P)
Zn	120.0 (P)	270.0 (L)
Pb	31.0 (P)	110.0 (L)
Ni	16.0 (P)	50.0 (L)

Appendix 6.4 Sediment bioaccumulation screening level values (SLVs)

	Metal	CASRN	SLV(mg/kg DW)	References	Reference dose (mg/kg.day)
1	As	7440-38-2	7	WDOE,1994	0.0003
2	Cd	7440-43-9	1	WDOE,1994	0.001

3	Pb	7439-92-1	17	WDOE,1994	(d)
4	Hg	7439-97-6	0.07	BCE 1999	0.00001

Maximum permissible levels MPL

#### Appendix 6.5 Criteria of the limit MPL

Institutions Criteria	As	Cd	Cr	Cu	Pb	Zn
International standard						
WHO, 1982	5	2		10	5	100
FDA, 2001	86	0.2	13	100	1.5	150
MFR, 1985		1		30	2	100
FAO		0.5	13	60	5	40
DEQ, 2007	7	1			17	
National standards						
USA	86	4	11		1.7	
MFA Malaysian Food Act						100
CAC standard		1			1	
	1	0.1			0.5	
Australia standard	1.5	5.5				
Canada standard	3.5					
Hongkong standard	10	2			6	

#### Appendix 6.6 Toxicity reference values (TRVs) for mammal (mg/kg/day)

Metal	Individual (mg/kg/day)	Population (mg/kg/day)
As	1.04	5.2
Cd	0.77	3.85
Pb	4.7	23.5

(USEPA, 2006)

ATLs: Acceptable tissue level for chemical in fish, shell fish and other aquatic organism (mg/kg wet weigh)

#### Appendix 6.7 Acceptable Tissue Levels (ATLs) for chemicals in Fish/Shellfish consumed by wildlife and humans

Metal	Carcinogens (mg/kg Wet wt)		Non-carcinogens (mg/kg Wet wt)	
	General	Substance	General	Substance
As	0.0062	0.00075	1.2	0.15
Cd	NA	NA	4.0	0.49
Pb	NA	Na	0.5	0.5

Appendix 6.8 Critical tissue level for chemical in fish, shell fish and other aquatic organism

Metal	Fresh water			Marine	
	Recomended BCF (l/kg)	National recomended WQC ( $\mu\text{g/l}$ )	CTL (mg/kg)	National recomended WQC ( $\mu\text{g/l}$ )	CTL (mg/kg)
As	44	150	6,6	3,6	1,6
Cd	64	0,25	0,15	8,8	0,15
Pb	49	2,5	0,12	8,1	0,40
Hg	7,342	0,012	0,088	0,025	0,18

Note: hardness = 100mg/l

National Recommended Water Quality Criteria (WQC) (USEPA, 2004).

## APPENDIX 7

### RESULT OF ANALYSIS

Appendix 7.1 Particle size of collected sediment samples during 2012-2013

<b>Tan Dao</b>	<b>Mar2012</b>	<b>Sep2012</b>	<b>Mar2013</b>	<b>Sep2013</b>
2-1mm %	6,79	0,54	5,50	0,71
1-0.5mm %	22,55	0,46	16,42	0,66
0.5-0.25mm %	46,20	1,24	30,40	1,74
0.25-0.125mm %	11,39	4,12	22,94	10,79
0.125-0.1mm %	2,32	67,65	3,76	49,07
0.1-0.0625mm %	8,18	26,38	7,50	24,92
0.0625-0.0039mm %	2,54	1,00	11,52	10,96
<0.0039mm	0	0,00	2,00	1,22
	10,72	27,38	21,02	37,1
	89,28	73,55	79,02	62,97
<b>Ngoc Diem</b>	<b>Mar2012</b>	<b>Sep2012</b>	<b>Mar2013</b>	<b>Sep2013</b>
2-1mm %	9,52	10,34	8,52	9,71
1-0.5mm %	21,58	11,83	11,58	9,42
0.5-0.25mm %	36,52	36,79	22,12	17,78
0.25-0.125mm %	15,95	27,33	21,98	26,77
0.125-0.1mm %	3,26	3,9	12,04	15,04
0.1-0.0625mm %	2,32	2,13	4,86	13,13
0.0625-0.0039mm %	10,28	7,65	14,6	4,02
<0.0039mm	0	0	4,3	4,17
	12,6	9,78	23,76	21,32
	86,83	90,19	76,24	78,72
<b>Binh Tan</b>	<b>Mar2012</b>	<b>Sep2012</b>	<b>Mar2013</b>	<b>Sep2013</b>
2-1mm %	16,05	9,34	10,05	5,15
1-0.5mm %	16,10	12,14	7,12	8,57
0.5-0.25mm %	11,70	12,38	7,56	14,98
0.25-0.125mm %	8,07	8,54	15,45	29,7
0.125-0.1mm %	5,68	32,88	22,22	28,32
0.1-0.0625mm %	29,65	24,96	14,8	10,1
0.0625-0.0039mm %	12,64	0	22,86	3,02
<0.0039mm	0,00	0	0	0,16
	42,29	24,96	37,66	13,28
	57,61	75,28	62,40	86,72
<b>Cam Lam</b>	<b>Mar2012</b>	<b>Sep2012</b>	<b>Mar2013</b>	<b>Sep2013</b>
2-1mm %	6,80	0,54	5,50	0,71
1-0.5mm %	22,56	0,46	16,42	0,66

0.5-0.25mm %	46,20	1,24	30,40	1,74
0.25-0.125mm %	11,39	4,12	22,94	10,79
0.125-0.1mm %	2,33	67,65	3,76	49,07
0.1-0.0625mm %	8,18	26,38	7,50	24,92
0.0625-0.0039mm %	2,54	1,00	11,52	10,96
<0.0039mm %	0,00	0,00	2,00	1,22
	10,72	27,38	21,02	37,10
	89,28	73,55	79,02	62,97

Appendix 7.2 Heavy metals contents in collected sediment samples during 2012-2013

<b>Tan Dao</b>	<b>Mar2012</b>	<b>Sep2012</b>	<b>Mar2013</b>	<b>Sep2013</b>
As	15,30	4,65	13,09	4,61
Cd	0,54	2,07	1,15	0,06
Cr	63,15	13,49	6,83	4,97
Cu	14,46	7,36	2,95	3,97
Zn	71,43	50,66	36,93	<LQ
Fe	2536,64	1490,46	8550,79	>LQ
Al	2367,77	2134,98	4198,46	ND
<b>Ngoc Diem</b>				
As	13,78	5,71	4,39	15,65
Cd	1,59	1,93	0,94	0,57
Cr	34,97	41,96	21,81	11,34
Cu	28,48	28,70	15,31	56,57
Zn	70,62	99,90	81,51	<LQ
Fe	2844,86	3031,49	2178,29	>LQ
Al	1326,90	7459,74	2323,22	ND
<b>Binh Tan</b>				
As	19,61	14,10	2,09	10,46
Cd	0,77	1,72	2,20	0,00
Cr	21,07	23,14	23,43	5,37
Cu	22,41	53,94	43,24	9,52
Zn	53,86	112,31	90,94	<LQ
Fe	1329,17	1592,62	2243,08	>LQ
Al	8620,48	3618,47	5650,40	ND
<b>Cam Hai Tay</b>				
As	6,26	9,09	0,15	10,21
Cd	0,45	1,69	2,41	0,46
Cr	10,94	7,73	10,84	15,40
Cu	11,03	8,00	11,34	52,35
Zn	40,99	59,76	49,30	<LQ
Fe	1176,95	1011,62	1450,50	>LQ
Al	7870,52	2008,27	3651,24	ND

Appendix 7.3 Bioparametrics of mollusks collected in September 2012

<b>Katelysia hiantina</b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Long (mm)	4,10	4,33	3,84	3,81
Wide (mm)	3,31	3,57	2,88	3,08
Weight total fresh (gr)	12,05	16,63	10,68	11,49
Weight tissue dry (gr)	0,52	0,84	0,53	1,39
Weight shell dry (gr)	7,56	13,12	7,94	6,38
Weight total dry (gr)	8,08	13,96	8,47	7,77
<b><i>Glaucanoma virens</i></b>		<b>ND</b>	<b>BT</b>	<b>CL</b>
Long (mm)		5,03	5,07	5,18
Wide (mm)		2,78	2,61	2,88
Weight total fresh (gr)		18,41	13,74	9,03
Weight tissue dry (gr)		5,48	0,81	1,22
Weight shell dry (gr)		9,85	4,19	4,33
Weight total dry (gr)		15,32	5,00	5,54
<b><i>Laternula Anatina</i></b>				
Long (mm)			4,80	5,35
Wide (mm)			2,50	2,60
Weight total fresh (gr)			8,27	16,80
Weight tissue dry (gr)			3,40	2,17
Weight shell dry (gr)			3,20	4,77
Weight total dry (gr)			6,60	6,94
<b><i>Lingula Unguis</i></b>	<b>TD</b>		<b>BT</b>	
Long (mm)	2,97		3,34	
Wide (mm)	1,54		1,60	
Weight total fresh (gr)	4,34		5,09	
Weight tissue dry (gr)	0,29		0,30	
Weight shell dry (gr)	0,77		1,29	
Weight total dry (gr)	1,06		1,59	
<b><i>Geloina Coaxans</i></b>	<b>TD</b>	<b>ND</b>		
Long (mm)	4,95	5,18		
Wide (mm)	3,98	4,67		
Weight total fresh (gr)	19,22	20,37		
Weight tissue dry (gr)	0,74	3,04		
Weight shell dry (gr)	10,52	10,02		
Weight total dry (gr)	11,26	13,07		
<b><i>Crassostrea rivularis</i></b>	<b>TD</b>			
Long (mm)	5,88			
Wide (mm)	4,12			
Weight total fresh (gr)	15,72			
Weight tissue dry (gr)	0,85			
Weight shell dry (gr)	8,16			
Weight total dry (gr)	9,01			
<b><i>Anatina antiquata</i></b>				
Long (mm)	4,20			
Wide (mm)	2,92			

Weight total fresh (gr)	13,24			
Weight tissue dry (gr)	1,33			
Weight shell dry (gr)	8,55			
Weight total dry (gr)	9,88			
<b><i>Tapes literatus</i></b>		ND		
Long (mm)		7,01		
Wide (mm)		3,69		
Weight total fresh (gr)		17,36		
Weight tissue dry (gr)		2,52		
Weight shell dry (gr)		9,67		
Weight total dry (gr)		12,19		
<b><i>Solens regularis</i></b>				CL
Long (mm)				5,44
Wide (mm)				1,01
Weight total fresh (gr)				5,61
Weight tissue dry (gr)				0,54
Weight shell dry (gr)				1,44
Weight total dry (gr)				1,98

Appendix 7.4 Bioparametrics of mollusks collected in March 2013

<b><i>Katelysia hiantina</i></b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Long (mm)	4,18	3,61	3,48	3,43
Wide (mm)	3,54	3,11	2,76	2,61
Weight total fresh (gr)	12,89	13,12	15,91	10,30
Weight tissue dry (gr)	0,40	0,37	0,39	0,30
Weight shell dry	5,60	10,12	0,23	6,30
Weight total dry (gr)	6,00	10,49	5,68	6,60
Condition index (CI)	27,18	32,98	40,51	33,57
<b><i>Glaucanoma virens</i></b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Long (mm)			4,04	4,60
Wide (mm)			2,24	2,51
Weight total fresh (gr)			7,95	7,58
Weight tissue dry (gr)			0,72	0,52
Weight shell dry (gr)			3,95	3,50
Weight total dry (gr)			4,67	4,02
Condition index (CI)			79,56	44,94
<b><i>Laternula Anatina</i></b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Long (mm)			5,10	6,10
Wide (mm)			2,55	2,90
Weight total fresh (gr)			5,81	9,62
Weight tissue dry (gr)			1,02	0,69
Weight shell dry			2,21	4,60
Weight total dry (gr)			3,23	5,29
<b><i>Lingula Unguis</i></b>	<b>TD</b>	<b>ND</b>	<b>BT</b>	<b>CL</b>
Long (mm)			3,70	2,73
Wide (mm)			1,58	1,46
Weight total fresh (gr)			3,86	4,34

Weight tissue dry (gr)			0,11	0,06
Weight shell dry			1,30	0,34
Weight total dry (gr)			1,41	0,40
<b><i>Geloina Coaxans</i></b>	TD	ND	BT	CL
Long (mm)	5,20	5,31		
Wide (mm)	4,60	4,73		
Weight total fresh (gr)	19,87	21,20		
Weight tissue dry (gr)	0,17	0,69		
Weight shell dry	15,87	18,20		
Weight total dry (gr)	16,04	18,89		
<b><i>Crassostrea rivularis</i></b>	TD	ND	BT	CL
Long (mm)	4,35		5,89	
Wide (mm)	3,21		4,48	
Weight total fresh (gr)	18,15		26,86	
Weight tissue dry (gr)	1,02		0,57	
Weight shell dry	13,10		23,86	
Weight total dry (gr)	14,12		24,43	
<b><i>Anatina antiquate</i></b>	TD	ND	BT	CL
Long (mm)	3,71			3,34
Wide (mm)	2,52			2,21
Weight total fresh (gr)	13,45			10,67
Weight tissue dry (gr)	0,13			0,20
Weight shell dry	9,45			5,67
Weight total dry (gr)	9,58			5,87
<b><i>Tapes literatus</i></b>	TD	ND	BT	CL
Long (mm)		3,21		
Wide (mm)		3,99		
Weight total fresh (gr)		7,73		
Weight tissue dry (gr)		0,26		
Weight shell dry		4,73		
Weight total dry (gr)		4,99		
<b><i>Solens regularis</i></b>	TD	ND	BT	CL
Long (mm)			3,82	6,69
Wide (mm)			0,69	1,06
Weight total fresh (gr)			2,52	6,56
Weight tissue dry (gr)			0,25	0,67
Weight shell dry			0,55	2,56
Weight total dry (gr)			0,80	3,23
<b><i>Perna viridis</i></b>	TD	ND	BT	CL
Long (mm)	3,90			
Wide (mm)	2,00			
Weight total fresh (gr)	5,55			
Weight tissue dry (gr)	0,19			
Weight shell dry	2,55			
Weight total dry (gr)	2,74			
<b><i>Bufonaria rana</i></b>	TD	ND	BT	CL
Long (mm)		6,13		



Wide (mm)		2,70		
Weight total fresh (gr)		17,56		
Weight tissue dry (gr)		0,30		
Weight shell dry		14,56		
Weight total dry (gr)		14,86		