การปนเปื้อนของโครเมียมในระบบนิเวศชายฝั่งทะเล บริเวณบางปู อำเภอเมือง จังหวัดสมุทรปราการ

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CHROMIUM CONTAMINATION IN MARINE ECOSYSTEM AT BANGPOO, MUANG DISTRICT, SAMUTPRAKARN PROVINCE

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การวิเคราะห์หาปริมาณโครเมียมในน้ำทะเล ตะกอน และสัตว์ทะเลในพื้นทำการศึกษา บริเวณบางปู อำเภอเมือง จังหวัดสมุทรปราการ พบว่าระดับความเข้มข้นของโครเมียมในตะกอนดินและสัตว์ทะเลผิวดินค่อนข้างสูง ความเข้มข้นของโครเมียมในตะกอนที่วัดได้คือ 102.46 - 527.23 ไมโครกรัม ต่อกรัม ส่วนในสัตว์ทะเลผิวดินพบการการสะสมของโครเมียมสูงในหอยกระปุก *Tapes turgidus* หอยขี้นก *Cerithidea* sp. เพรียงทราย *Perinereis* sp. และหนอนถั่ว *Sipunculida* sp. โดยวัดความเข้มข้นของโครเมียมได้ในระดับ 30-1,200 ไมโครกรัมต่อกรัม ความเข้มข้นของโครเมียมในปลาที่วัดได้โดยเฉลี่ยคือ 3 มิลิกรัมต่อกรัม นอกจากนี้ค่า Bioaccumulation factors (BAFs) ที่ได้จากการเปรียบเทียบความเข้มข้นของโครเมียมในสัตว์ทะเลกับความเข้มข้นในตะกอนและน้ำ พบว่าในปลาค่า BAF น้อยกว่าหนึ่ง แสดงว่าโครเมียมไม่สะสมในปลา แต่ในสัตว์หน้าดินพบค่า BAF ที่ได้มีค่าอยู่ระหว่าง 1-30 ซึ่งค่าที่ได้นี้บอกถึงระดับการสะสมของโครเมียมในสัตว์ทะเลหน้าดินที่ค่อนข้างสูง

ในการศึกษาระดับความเป็นพิษของโครเมียมในตะกอนต่อสัตว์ทดลอง *Perinereis nuntia* พบว่าระดับความเข้มข้นของโครเมียม 36-251 ไมโครกรัมต่อกรัมในดินตะกอนนั้นไม่มีผลให้เกิดการตาย แต่พบว่าทำให้การเติบโตช้าลงเมื่อเทียบกับสัตว์ทดลองในชุดควบคุม โดยที่อัตราการเจริญเติบโตคิดเป็น 60-80% ของชุดควบคุม

สาขาวิชา.....การจัดการสิ่งแวดล้อม..... ลายมือชื่อนิสิต.....
.....(สหสาขาวิชา)..... ลายมือชื่ออาจารย์ที่ปรึกษา.....
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KEY WORD: CHROMIUM/ BIOACCUMULATION/ SEDIMENT TOXICITY TEST

SUJAREE BUREEKUL: CHROMIUM CONTAMINATION IN MARINE ECOSYSTEM AT BANGPOO MUANG DISTRICT, SAMUTPRAKARN PROVINCE, THAILAND.
THESIS ADVISOR: SUCHANA CHAVANICH,Ph.D.
THESIS COADVISOR : ASST. PROF. EAKALAK KHAN, Ph.D.; 82 pp.
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Seawater, sediment, and marine organisms were collected from Bangpoo, Muang district, Samutprakarn Province and analyzed for chromium concentrations. The results showed relatively high chromium concentrations in sediments (102.46-527.23 mg/kg). For animals, the highest chromium concentrations were found in the bivalve *Tapes turgidus* (50-600 µg/g dry weight) and polychaete *Perinereis* sp. (30-1200 µg/g dry weight). In addition, chromium in fish tissues were an average of 3 µg/g dry weight. The accumulations of chromium among species were compared by Bioaccumulation factors (BAFs). In fish, BAFS ratios were less than 1, which meant that chromium tended not to accumulate in fish tissues. However, BAFs ratios were higher than 1 in bivalves, polychaetes, gastropods, and crabs, indicated that those species can accumulate chromium when in the exposure area and could cause adversely health impacts to marine organisms.

In addition, the 15 day experiment of chromium sediment toxicity test on *Perinereis nuntia* was conducted. The results showed chromium concentrations in the sediment between 36-231µg/g dry weight did not cause death to polychaetes. However, it reduced the growth rate of test species by 60-80 % of control set.

Field of study....Environmental Management... Student's signature.....
...Inter-Department..... Advisor's signature.....
Academic year....2005..... Co-advisor' signature.....

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LIST OF ABBREVIATIONS

BAFs	: Bioaccumulation Factors
ICP-AES	: Inductively Coupled Plasma Atomic Emission Spectroscopy
FAAS	: Flame Atomic Absorption Spectroscopy
DI	: Demineralized or Deionized water
CRM	: Certified Reference Material
BCS	: Basic Chromium Sulfate $[Cr(H_2O)_5(OH)SO_4]$

CHAPTER I

INTRODUCTION

1.1 Introduction

Bangpoo is a district located at the mouth of the Chaopraya River in Samutprakarn, Thailand. There are three industrial estates in the district: two tannery estates at kilometers 30 and 34 on Sukhumvit Road, and the Bangpoo industrial estate. A recent report on the characterization of hot pollution spots in Thailand in 2003 revealed that the concentrations of chromium in sediment near shoreline adjacent to the tannery estate at kilometer 30, Bangpoo industrial estate, and tannery estate at kilometer 34 were 370, 275, and 195 mg/kg, respectively (UNEP/GEF, 2003). These levels exceeded the sediment quality criteria for fishery water and normal industry uses (UNEP/GEF, 2003). According to the sediment quality criteria, the toxic effects threshold and USEPA Region VI proposed guidelines for sediment disposal is 100 mg/kg dry weight of total chromium (MacDonald, 1994; Johnson, 2000). Moreover, the ecotoxicological value of chromium concentration in sediment that is suitable for healthy living of animals was reported at 48.3 µg/g (MacDonald, 1994). Therefore, it is possible that living organisms in this area accumulate chromium in some significant amount. According to the United States Food and Drug Administration safety seafood guideline, chromium concentrations in crustacean and mollusk should not be more than 12 and 13 ppm (wet weight basis), respectively (Costa, 2004). Assessing sediment quality, chromium concentration, laboratory measurements of biological effects (toxicity test), and field identification of biological impacts, can give a clearer picture of the ecological quality in the study area that could initiative the policy makers and government to be more concern in managing contaminated marine ecosystem.

1.2 Objectives

The objective of this study was to determine chromium concentrations in marine ecosystem at the Bangpoo District. Seawater samples, sediment samples, and the representatives of marine organisms in each trophic level in this site were collected and analyzed for chromium concentrations. In addition, these data were used to determine the bioaccumulation factor in each trophic level and to determine sediment toxicity test.

1.3 Scope of the Study

The study was divided into two parts. The first part was the analysis of chromium concentrations in the samples. Chromium concentrations of seawater, sediments and living organisms in each trophic level were measured. Then, the results were used to calculate bioaccumulation factors (BAFs). The second part was the investigation of the toxicity of chromium in the sediment. The sediment toxicity test was conducted to determine the minimum chromium concentration suitable for living organisms**.**

1.4 Expected results

This study was expected to provide the following information:

1. Data on chromium concentrations in seawater, sediment and representative animals of the study site.
2. BAFs of chromium of representative animals.
3. Toxicity of the sediment.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Chromium

2.1.1 Introduction

Chromium occurs naturally in bound forms that constitute 0.1–0.3 mg/kg of the earth's crust (Zayed, 2003). In environment, chromium can present in different oxidation states. The most common forms are element, trivalent, and hexavalent. The trivalent and hexavalent states are the most stable in nature (Zayed, 2003).

Chromium is used widely in many industrial processes. The element chromium is used mainly for making steel and other alloys. Naturally, chromium is occurred as mineral chromites (trivalent species) that can be used as brick lining for high temperature industrial furnace and making alloys (Irwin, 1997). In addition, hexavalent and trivalent chromium are used for several industries such as for chrome plating, dyes and pigments manufacturing, leather tanning, and wood preserving. Smaller amounts are used in drilling mud, rust and corrosion inhibitors, textiles, and toner for copying machines (Irwin, 1997).

Trivalent chromium is an essential trace element. It is an active component of a molecule called the glucose tolerance factor (GTF) (ATSDR, 2000). It acts as a cofactor to bind insulin to receptor sites on membranes to improve the efficacy of insulin. In contrast, trivalent chromium deficiency reduces the ability of body to use sugars, proteins, and fat properly (ATSDR, 2000).

Hexavalent chromium is considered as human carcinogen. Hexavalent chromium is an extremely toxic carcinogen and may cause death to animals and humans if ingested in large doses (ATSDR, 2000). Routes of human exposure to chromium compounds include ingestion of food and water, inhalation of airborne particulates, and contact with numerous manufactured items containing chromium compounds (Irwin, 1997 and Wilbur, 2000).

2.1.2 Chromium Fates and Behaviors

The distribution of chromium in any environmental systems is controlled by three reactions, oxidation-reduction, precipitation-dissolution, and sorption-desorption (Zayed, 2003). In the open sea, the dominant species in surface seawater are hexavalent chromium species. Chromate ion (CrO_4^{-2}) is the most abundant followed by sodium chromate ion ($NaCrO_4^{-1}$). However, in near shore and estuarine water trivalent species is increasing as a result of human activities. The dominant species of trivalent chromium are hydroxide ions ($Cr(OH)^{+2}$,$Cr(OH)_2^{+1}$ and $Cr(OH)_3^0$) (Neff, 2002). Figure 2.1 showed the dominant chromium species at different pH.

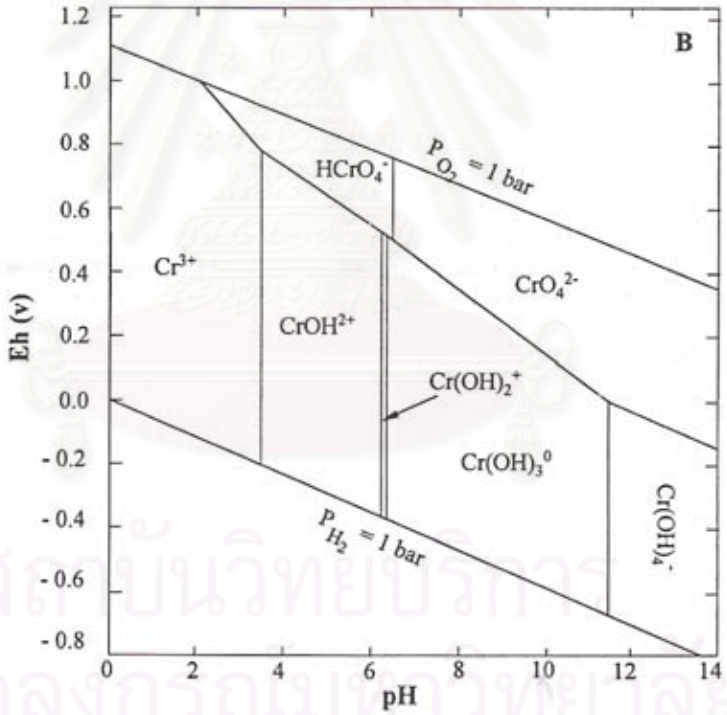

Figure 2.1 Predicted Eh-pH stability field for chromium species in aqueous systems (source: Adriano, 2000)

Trivalent chromium reacts with aqueous hydroxide ion to form precipitated insoluble chromium. It also reacts with oxides, hydroxides, and phosphate and forms highly insoluble compounds that can be rapidly absorbed by suspended particles (Zayed, 2003). Trivalent chromium can co-precipitate with $Fe(OH)_3$. In

addition, trivalent chromium can also form stable complexes with many dissolved or colloidal organic and inorganic ligands. This complex chromium does not absorb and precipitate. Thus, it remains in the water column (Irwin, 1997). The oxidation of trivalent to hexavalent chromium is very slow at pH of seawater and was influenced by ion in seawater such as borate. Figure 2.2 also show the distribution of trivalent chromium in aqueous systems at different pH. In addition, particulate manganese oxide is capable of oxidizing trivalent chromium (Neff, 2002).

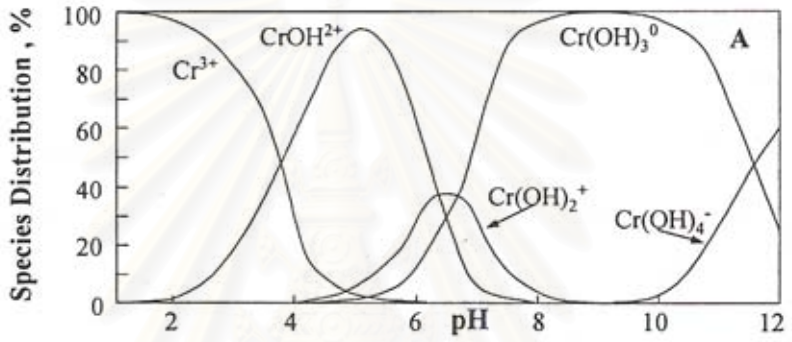

Figure 2.2 The distribution of trivalent chromium species at different pH in equilibrium with $Cr(OH)_{3(S)}$ (source: Adriano, 2000)

Chromium hexavalent species are insoluble species such as hydrochromate, chromate, and dichromate ionic species (Irwin, 1997) as shown in Figure 2.1. Dissolved hexavalent chromium can be reduced to trivalent chromium by reducing agents such as S^{2-}, Fe(II), fulvic acid, low molecular weight organic compounds, and proteins. Thus, chromium hexavalent can be removed from the water column, especially in deeper anaerobic waters. However, a small amount of hexavalent chromium is removed from water column due to the uptake of hexavalent chromium by planktons and release trivalent chromium at lower depths where oxygen is depleted (Irwin, 1997).

In aerobic sediments, trivalent chromium can be oxidized by manganese oxides and hydroxides present at the sediment-water interface. Thus, hexavalent chromium can be released to the overlying waters, especially during bioturbation processes (Irwin, 1997). The oxidation reaction is very slow and is limited by the amount of oxygen or manganese oxides. Even if at favorable pH (>5) with enough

manganese oxide, chromium oxidation is limited due to the immobile and insoluble properties of trivalent (Zayed, 2003). Recently, it has been suggested that unstable and dissolved trivalent chromium forms can be converted to hexavalent chromium relatively quick by strong oxidants such as H_2O_2 that are the production of photochemical reaction in aerobic surface waters (Zayed, 2003).

Under acidic or slightly alkaline condition, hexavalent chromium is adsorbed by mineral solids such as iron and ammonium oxides and kaolinite. Absorption of hexavalent chromium decreases with increasing of pH (Zayed, 2003). Hexavalent chromium adsorbs more tightly to oxide and clay particles than other anions such as chloride, nitrate, or sulfate (Adriano, 2000). However at high concentration of phosphate, desorption occurs due to the competition for the same adsorption site (Adriano, 2000; Zayed, 2003). In addition, trivalent chromium can adsorbed more strongly than hexavalent chromium to clay minerals. By increasing pH, the adsorption of trivalent chromium to clay minerals is increased (Zayed, 2003).

2.1.3 Chromium Toxicity

The toxicity of chromium to aquatic biota is influenced by abiotic and biotic factors. Abiotic factors are hardness, temperature, pH, and salinity. Biological factors are species, life stages, sizes, and variation of sensitivities of local populations. Toxicity of chromium varies widely, even among closely related species (Irwin, 1997).

It is suggested that hexavalent and trivalent chromium are likely to affect reproductive systems of animals by oral exposure (Irwin, 1997). The evidences showed that female mice fed with hexavalent chromium had a fewer offspring and had offspring with birth defects (ATSDR, 2000). Male mice fed with hexavalent and trivalent chromium had decreased numbers of sperms in the testes (ATSDR, 2000). In addition, chromium can pass through placenta and concentrate in fetus tissues and can be transferred from mothers to infants through breast milk (ATSDR, 2000). However, the dosages given to mice were greater than daily human intake by several orders of magnitude (ATSDR, 2000).

The reproductive effects of chromium (VI) to amphipods *Allorchestia compressa* and polychaetes *Neanthes arenaceodentata* were studied, and the results showed that there was no effect due to chronic exposure in their first generations. However, the effect occurred at third generations. It caused a cessation of reproduction in polychaetes (EC is 250µg/L) (Irwin, 1997; Neff, 2002).

Trivalent chromium normally has low toxicity due to its poor membrane permeability and non-corrosive capability while hexavalent form is highly toxic due to its strong oxidation characteristics and better membrane permeability (Irwin, 1997). In general, hexavalent chromium is 10 to 100 times more toxic than trivalent chromium. However, there are some species that are sensitive to trivalent chromium species. For example; salmonid fish reproductive cycles are particularly sensitive to trivalent chromium (Irwin, 1997). Moreover, trivalent chromium can induce exogastrulation in echinoids such as sea urchin and sea star embryos (Kobayashi, 2004).

In contrast, hexavalent chromium has high toxicity. Aquatic plants and marine polychaetes appear to be the most sensitive test groups (Irwin, 1997). The concentrations of hexavalent chromium that inhibited growth of algae and reproduction of worms were at 10.0 ppb and 12.5 ppb respectively (Irwin 1997). The median effective concentration (EC_{50}) of hexavalent chromium that reduced the rate of embryogenesis and larval attachment of *Ciona intestinakis* was 10,318 µg/L (Bellas, 2001). Fifty percents of oysters and mussels population exposed to hexavalent chromium concentration of 4,500 µg/L were developed abnormally (Neff, 2002).

Rainbow trout exposed to excessive hexavalent chromium developed severe gills damage and developed hypertrophy and hyperplasia (Irwin, 1997). Polychaetes worms, clams, crabs, oysters, and fish have also been shown to uptake significant amount of chromium. The excess chromium in their body leads to decreased weight gain, increased oxygen consumption, impaired reproduction, and increased hematocrits (Irwin, 1997; Neff, 2002). Hexavalent chromium is easily absorbed by

gut or body walls such as shells, gills, and mantle because of its higher solubility (Reish, 1976; Vranken, 1989; Hansan, 1995; Walsh, 1997).

The EPA determined the reference dose (RfD) for chromium that do not cause deleterious effects over a lifetime of exposure for human to be 1 mg/kg per day. The recommended estimated safe and adequate daily dietary intake (ESADDI) of chromium for infants up to 6 months old and for children up to seven years old and adult are 10–40 µg/day and 50–200 µg/day, respectively (Irwin 1997).

2.2 Bioavailability of Metals

Recently, more research on heavy metals is devoted to their bioavailability to establish the correlation between the concentration of metals in environment and in target species more clearly. The definition of bioavailability of metals in risk management is referred to the extent to which bioaccessible metals adsorb onto or absorb into and across biological membrane of organisms. It is expressed as a fraction of total amount of metal that the organisms is exposed to (at the sorption surface) during a given time and under defined conditions (USEPA, 2003).

Bioavailable fraction is referred to the portion of chemical in environment that is readily available for organisms to be taken up (Phillips, 1994). The fractions of metals in aquatic sediment can be divided according to the Tessier's sequential extraction procedure into acid soluble, reducible, oxidizable, and residual fraction (Langston, 1994; Sutherlanda *et al*., 2004). Single extraction with weak acid or salt solution was used to find the available fraction (Peijnenburg, 2003). The commonly extraction chemicals uses are HCl, $CaCl_2$ and $Ca(NO_3)_2$ (Peijnenburg 2003; Snape *et al*., 2004).

At equilibrium, the heavy metal partitioning in aquatic environment is present in three phases; free metal ions in solution, metal bound in organic and inorganic complexes, and metal bound in organic and inorganic particulates (Depledge, 1994). Free metal ion in solution and aqua ion (e.g. $M[OH]_2$) are the most readily available forms. Metals in the form of pure metal, precipitated and mineral are not available for uptake. In addition, the portion that is available for

animals to uptake is the portion that does not bind with sediments or particulates (Depledge, 1994). The most readily available metal forms in sediments are metals that adsorbed to amorphous iron and manganese hydroxide ion, carbonates, organic matter, and clay minerals. In anoxic environment, the bioavailability of metal depends on to the concentrations of sulfide (Neff, 2002).

The bioavailability of metals depends on the chemical properties, the characteristics of heavy metal, sediment, water, and organisms. In marine sediment, interaction of metal between sediment pore water and sediment is a source of metal available for benthic animals (Depledge, 1997). Metal speciation, pH, redox potential, particle size, concentration of organic ligands and major ions are the variables that control the bioavailability of metals (Depledge, 1994; Adriano, 2000). For example, metals bound with acid volatile sulfide form an unavailable and non-toxic portion. In contrast, in reducing environment, most metal are more soluble. At the same way, water quality characteristics, pH, dissolved oxygen, salinity and temperature determine the metal concentration in solution (Adriano, 2000). Moreover, the characteristics of organisms such as habitat and behaviors, types of food, source of water, size, and age can describe the ways that metals accumulate in body burdens (Phillips 1994; Adriano 2000)

Available heavy metals uptaken by organisms can pass through or bind with a surface membrane (Neff, 2002). The channels that metals can enter through the organisms are lipid permeation of charged species, complex permeation of metal ligands complex, carrier mediated transport, diffusion of hydrated ion through ion channels, ion exchanged pumps, endocysis of precipitated species, and solvent drag with water influx in diluted media (Neff, 2002). The routes of uptake are via ingestion, inhalation and dermal contact. In aquatic animals, uptake of metals via skin is assumed to be the major route since the better ability of dissolved metal to enter body via the respiratory surface, gills. In addition, food is a major source of metals entering organisms in the higher trophic level. For instant, mollusks, crustaceans, and annelids are uptaken metal via ingestion (Depledge, 1994).

Non-ionic and organic species can enter the organisms due to their high lipid solubility (Neff, 2002). Many metal ions bound to organic molecules are absorbed through the gut wall (Neff, 2002). Specific organometallic compounds such as cobalamine or arsenobetaine are more readily to absorb than their inorganic forms (Neff, 2002). Some non-essential metals for example, cadmium can pass through membrane via calcium pumps which are the channels for transporting the essential metals (Depledge, 1994). Insoluble metal species accumulate in granule and inert tissues and are not available to the other animals (Depledge, 1994).

Aquatic organisms are categorized into two groups according to the ability to handle metals: regulators and bioaccumulators. Regulators have ability to release and excrete metal to keep the metal at constant level in their body load. This ability can brake down when organisms are exposed to the rich metal condition at a long period of time (Adriano, 2000). The efficiencies of metal regulation depend on the route of uptake (Depledge, 1994). In addition, organisms handle metals by many mechanisms to prevent and reduce toxicity of metals to their body. The examples of the mechanisms occur in binding or transport stages are altering chemical speciation of metal to reduce bioavailability, complex metal at the surface of animals, decreasing permeability of membrane surface and reducing transport across lipid bilayer (Depledge, 1994). Bioaccumulators, on the contrary, tend to accumulate high chromium in their body.

2.2.1 Bioaccumulation

Bioaccumulation is the accumulation of contaminant via all routes available to organisms (Adriano, 2000). It is the net result of the uptake, distribution, and elimination of substances in organisms due to the exposure of contaminant in environmental media, air, water, food, and substrate (Neff, 2002). Bioaccumulation factor (BAF) is defined as the ratio of concentration of chemicals in tissues of organisms to concentration of chemical in ambient environment mainly sediment and water (Phillips, 1994). Considering the constant rate approach, BAF is referred to the ratio of the sum of uptake rate constant of chemical from all compartments to

the sum of the release rate constant (Neff, 2002). The mathematical expression of bioaccumulation factors are shown as:

$$\text{Bioaccumulation Factors:} \quad BAF_w = C_t/C_w$$
$$BAF_s = C_t/C_s$$

Where C_t, C_w, and C_s are the concentrations of contaminant in tissue, water and sediment, and BAF_w and BAF_s are the bioaccumulation factor of water and sediment, respectively (Lee, 1995)

Bioconcentration factor (BCF) is also a measurement of bioaccumulation. It defined as the ratio of concentration of concern contaminant in tissues to concentration of contaminant in water at equilibrium under the experimental condition. BCF also refer to the ratio of the uptake rate to the clearance rate (Chong, 1999). The mathematical expression is shown as:

$$BCF = \lim (C_t/C_w) \quad \text{when } t \rightarrow \infty$$
$$\text{Thus,} \quad BCF = k_1/k_2$$

Where C_t, C_w, are the concentrations of contaminant in tissue, and water and k_1 and k_2 are the rate constant of uptake and clearance, respectively (Nagal and Loskil, 1991)

Further study by Mountouris (2002), The BCF had calculated based on the concentration of metals in sediment and biota. The parameters that were used in this mathematical expression were focused on bioavailable metal in sediment which were iron, manganese and aluminum oxides, organic carbon, acid volatile sulfide (Mountouris, 2002). In addition, the expression of BCF for chromium was:

$$1/BCF = 7.64 + 0.9*10^{-8}[Al]^2 + 10.2 \pm 1.8*1/Cr \text{ biota (Mountouris, 2002)}$$

Hydrophobicity and biotransformation are the processes that influence ability of chemicals to accumulate in organisms. The transport system of marine organisms had developed to store essential trace metals without specific mechanisms (Neff, 2002). Along with essential trace metals, non-essential heavy metals can pass through and can be stored in organisms. In addition, lipid content, age, sex and size

influenced the bioaccumulation ability (Phillips, 1994). Trace metals can sometimes biotransform to organometallic compounds, which become more or less toxic to organisms. Moreover, pH, salinity, and temperature are the factors that can alter the bioaccumulation process (Depledge, 1994).

2.2.2 Chromium bioaccumulations

Chromium typically accumulates in the gill tissue of fish. Oldewage (2000) showed that gills were the primarily site for hexavalent chromium adsorption. Increasing pH value to 7.8, it resulted in higher accumulation of chromium in the internal organs rather than in the gills (Oldewage, 2000). However, higher bioaccumulation of chromium in gill samples was observed when pH of field study was well above 8.0 (Oldewage, 2000).

Reish (1976) studied the bioassay of two polychaetes; *Neanthes arenaceodentata* and *Ceratitis capitata*.. The results showed that adults of both species were more tolerant to chromium than juveniles (Reish, 1976). The study of chromium uptake on barnacles, *Balanus* sp., showed that hexavalent chromium accumulated in a soft tissue was 543 times of the level found in seawater and the bioconcentration factor was reported as 380 (Van Weerelt, 1984). In contrast, trivalent chromium did not concentrate in soft tissues of barnacles. Trivalent chromium precipitated quickly and was removed by water filtering through gills of barnacles (Van Weerelt, 1984).

The study of chromium bioaccumulation by the used seawater chrome lignosulphonate drilling mud on marine animals showed that chromium accumulated in the form of unassimilated mud components in gastrointestinal tract or in gill (Carr, 1982). Crustaceans and bivalves accumulated significant amount of chromium. However, most chromium was released within 24 hours when the animals were returned to clean seawater. Polychaetes accumulated significant amount of chromium and nearly all amount of chromium retained in polychaetes after 96 hours depuration period (Carr, 1982).

Walsh (1997) studied chromium accumulation in mussels *Mytilus edulis* from estuary receiving leather tannery effluent. The highest chromium concentrations were found in a gill and a digestive gland in both native and transplanted mussels (Walsh, 1997). In the contaminated area, chromium concentrations of mussel gill were between 400 to 1,000 µg/g dry weight, while the maximum chromium concentration at the reference site was 6 µg/g (Walsh, 1997).

Temperature and food also significantly influenced hexavalent chromium toxicity. In the study of Vranken (1989), J2-larvae of marine bacterivorous nematode, *Monhystera disjuncta*, were used to determine the influences of temperature and food on hexavalent chromium toxicity. Temperature influenced the mortality whereas both food and temperature can inhibit the development of J2-lavae (Vranken, 1989).

The study of Ayling (1974) on the heavy metals uptake by oyster *Crassostrea gigas* found that chromium concentration in oysters was limited by their size. American oyster *Crassostrea virginica* was investigated the route of chromium accumulation and found that chromium accumulated from both by direct absorption and by ingestion (Ayling, 1974). The assimilation efficiency (AEs) of chromium in green mussel *Perna viridis* and clam *Ruditapes philippinarum* have been studied (Chong, 1999). AEs is a physiological parameter to quantify metal bioavailability from ingested food (Chong, 1999). The AEs rates of chromium were 10 to 16% in the mussels and 11 to 24% in the clams (Chong, 1999).

Marine algae have ability to uptake heavy metal and are used as a bioindicator (Neff, 2002). In addition, sediment bacteria are able to accumulate trivalent chromium in the contaminated sites. Trivalent chromium bound to bacterial polysaccharide is bioavailable to infuanal invertebrates (Neff, 2002).

2.3 Sediment toxicity tests

Toxicity tests were commonly conducted to receive the reference doses (e.g. LC_{50}, EC_{50}) in order to determine the degree of risk (risk quotient). The study of toxicity test in marine ecosystems has been studied since 1970s (Bat, 1998). Most of

the studies have focused on the toxicity of chemicals associated with dissolved species (Bat, 1998).

The sediment toxicity test was first conducted in early 1990s since it can provide more effective information on the impact of environment due to the contaminated sediment (Bat, 1998). However, there is no standardized method due to high variation of sediment toxicity test (sensitivity of test species and variation of geographic distribution of test species) (Bat, 1998; Mudroch, 2000).

There are three components in the sediment quality assessment. These include the concentration of toxicants, laboratory measurements of biological effects (toxicity test), and field identification of biological impacts (Mudroch, 2000). Several types of research in risk assessment required the use of sediment toxicity test. Examples of the studies included measurement of the impact of dredging, measurement the efficiency of remedial program, and determination bioaccumulation of toxicants in tissues of aquatic animals (Mudroch, 2000).

Two proposed methods that are used to assess sediment toxicity are 1) aqueous phase bioassay, and 2) bulk sediment bioassay (Mudroch, 2000). Aqueous phase bioassays study the effect of tested animals towards the toxicity of toxicants that extract from sediment either; porewater or elutriate (water-soluble fraction) extraction (Mudroch, 2000). However, the stability of extraction and the lack of reality are drawbacks of this method. Bulk sediment bioassays (either the addition of toxicants into clean or artificial sediment) are more acceptable. However, temperature, dissolved oxygen, and settling period of sediment need to be controlled before carrying the experiment (Bat, 1998). The sediment used in toxicity test should also be stored at 2-4°C until the utilization. The storage time is between 2–6 weeks after the collection. If it is longer than 6 weeks, physicochemical changes will alter the bioavailability of nutrient and contaminants (Moore, 1994; Mudroch, 2000).

Two important issues of concern in the sediment toxicity test are; test species selection and sediment preparation (Bat, 1998). The cultured animals with short life

span are of interest due to it less variation. Typically, amphipod, polychaetes, oligichaetes, copepods and bivalves are animals using in the sediment toxicity tests (Bat, 1998; Mudroch, 2000; Gaffard, 2003; Bejarano, 2004). The sensitive life stages (e.g. juvenile) were also used in toxicity test (Gaffard, 2003). The endpoints of the study were determined by the percent of survival, development, reproduction and growth rate (Moore, 1994; Bejarano, 2004). The biomarker such as metallothioneins (MT) developed due to the metal exposure, can be used as an endpoint, too (Gaffard, 2003). Benthic microalgae are alternative test species that can be determined by the growth inhibition rate (Moreno-Garrido, 2001).

2.4 Tannery Industry

2.4.1 Chromium in Tannery Industry

Basic chromium sulfate (BCS) [$Cr(H_2O)_5(OH)SO_4$] is a major element use in tanning process. The amount of chromium that is necessary to produce the best leather is 3 g of Cr_2O_3 for 100 g of leather (Raju and Tandon, 1999). However, BCS is not totally taken up by the hide. The amount of chromium taken up is limited to 50-70% and the remaining is become wastes (Raju and Tandon, 1999). Workers can expose to chromium mainly in inorganic form or in protein bounded form via breathing, eating and direct contact (Carlos, 2002). Chromium had adverse effect on iron metabolism due to the similarities of Fe(III) and Cr(III) ions (Carlos, 2002). In the presence of chromium, the competition of these two ions leads to Fe deficiency, decreased catalyses activities and excessive excretion of irons (Carlos, 2002). Several recent studies were dedicated to chromium recovery and recycle to reduce chromium waste. For example, hydrogen peroxide can effectively oxidize $Cr(OH)_3$ to chromate(CrO_4^{2-}) in alkaline conditions (Awan, 2003).

2.4.2 Tannery Process

According to the Environmental Management Guideline for the Leather Tanning and Finishing Industry (1997), the tannery process can be divided into 3 sub-processes: beam house process, tanning process and finishing process (Figure 2.1).

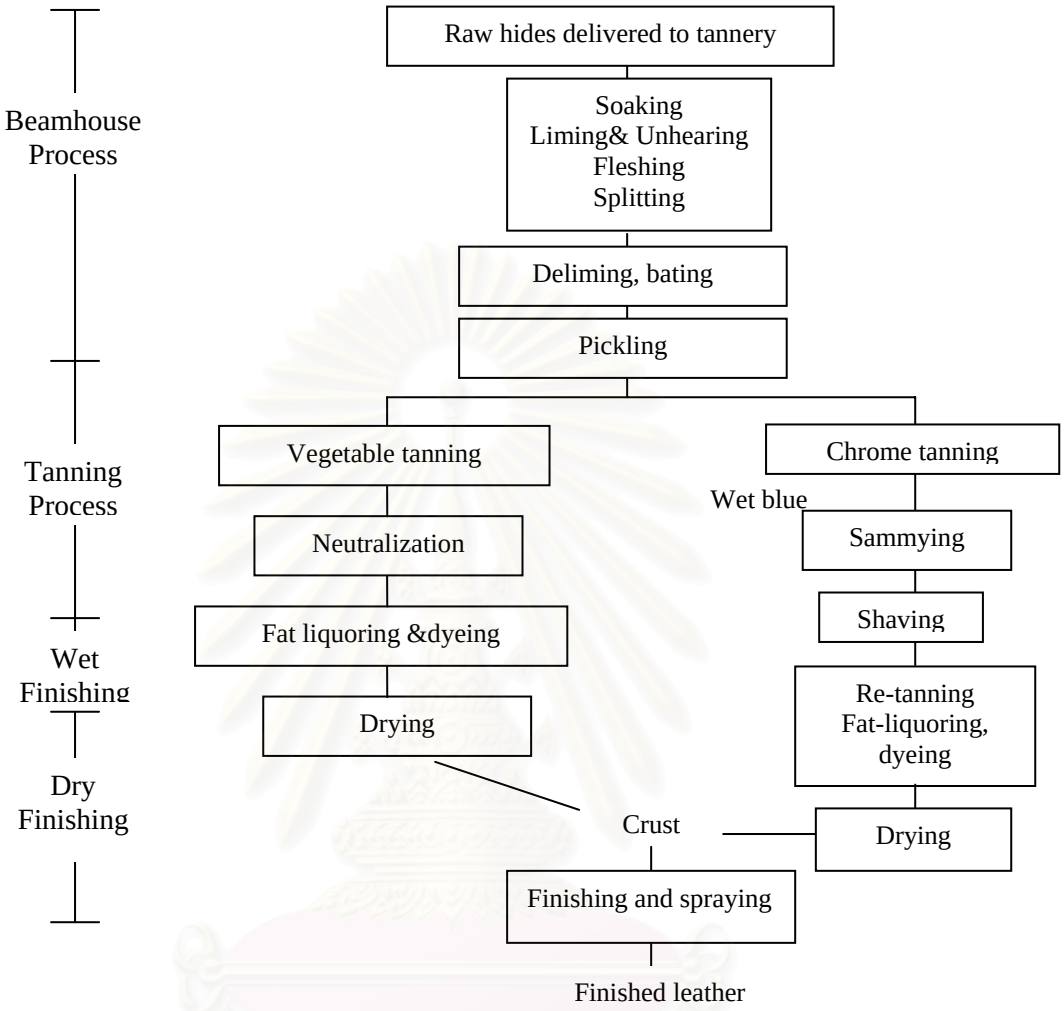

Figure 2.3 Tannery process **(**adapted from http://www.tto.or.th; The Tanning Organization Ministry of Defense, 2003)

Beam house process is the process to remove the unwanted part of raw hides and prepared the hide condition for the next process. Table 2.1 showed the sub-process and the materials and wastes produced. Salts usage in hide preservation is a concern issues when salt is disposed untreated to environment which posing negative effects to the growth of plants and aquatic animals.

Tanning process is the process that changes the decomposable raw hides to be more stable finished hides. The material and pollution contribution during tanning process was shown in Table 2.1. There are two types of tanning process namely vegetable tanning and chrome tanning.

Table 2.1 Materials and sources of pollution during the tanning process (Source: The Environmental Management Guideline for the Leather Tanning and Finishing Industry, 1997)

		Activities	Raw material and chemicals	Wastes
Beam house Process		Raw Hide preservation	Salt, bactericides and insecticides, water	Salts
		Washing and soaking	Surfactants, alkaline, bactericides, water	BOD, COD, insecticides, salts, flesh scraps, hair, skin and dirt
		Unhairing and liming	Sodium sulfide, lime, and water	High pH, BOD, COD, salt, sulfide, insecticides, bactericides, ammonia, alkaline, suspended solids, hair, lime and sludge
		Fleshing and splitting	Water	Hair scrap and wastewater
		Deliming of splitting (dog chew)	Ammonium chloride, hydrogen peroxide, sulfuric acid and water	BOD, COD, salt, sulfate, ammonium, alkaline, Suspended sludge and chloride.
		Deliming and bating	Sulfate, ammonium chloride or ammonium sulfate, bate and water	BOD, COD, salt, ammonium, alkaline, suspended solid, chloride
Tanning Process	**Vegetable**	Pickling and vegetable tanning	Sulfuric acid and formic acid, tannin	Water, acid and tannin
		Remove surplus vegetable tanning agent	Oxalic acid and water	Water, acid and tannin
		Drying	-	Wastewater without chromium
	Chrome	Pickling and chrome tanning	Sulfuric acid and formic acid, Basic chromium sulfate,	Chromium, BOD, suspended solids, fat residues, salt, acid
		Chrome fixation	Sodium bicarbonate	Chromium, BOD, suspended solids, fat residues, salt, acid
		Sammying and shaving	-	Wastewater with chromium
Finishing Process		neutralization	Sodium bicarbonate or ammonium salt and water	BOD,COD, suspended solid, chromium and ammonium
		Chrome re-tanning and fat liquoring	Syntans, formic acids and fat	acid, chromium, BOD, COD, syntans, fat
		Dyeing	Dyes	Water, dyes, fat
		Drying , finishing and spraying	-	-

Vegetable tanning Tannin or synthetic substance as a tanning is used as tanning agent.

Chrome tanning is a cheaper and faster process which requires the chromium sulfate as a tanning agent. Chrome tanning is more popular since its products are more resistant to humidity and hot water than vegetable tanning. The leather from the chrome tanning is called wet blue.

Finishing process is the last process to ensure that leather products meet the market requirement. The activities are shown with materials and wastes produced during tanning process in Table 2.1.

2.4.3 Tannery Industries in Thailand

The majority of tanneries in Thailand are located in clusters at Km. 30 and Km. 34 group on Sukhumvit Road., Bangpoo Mai, Samutprakarn (Figure 2.4). According to the Department of Industrial Work of Thailand, 148 factories were registered as tannery facilities in September 2001. One hundred thirty eight of them are located in the two tannery clusters. Eight of them use vegetable tanning while seventy factories of them manufacture raw hide and produce wet blue and crust (chrome tanning process). The rest of them are finishing industries. In each cluster, there is a central wastewater

A recent report on the characterization of hot pollution spots in Thailand in 2003 revealed that the concentrations of chromium in sediment near shoreline adjacent to the tannery estate at kilometer 30, Bangpoo industrial estate, and tannery estate at kilometer 34 were 370, 275, and 195 mg/kg, respectively. These levels exceed the sediment quality criteria for fishery water and normal industry uses (UNEP/GEF, 2003). Moreover, the ecotoxicological value of chromium concentration in sediment that is suitable for healthy living of animals was reported at 48.3 µg/g (MacDonald, 1994).

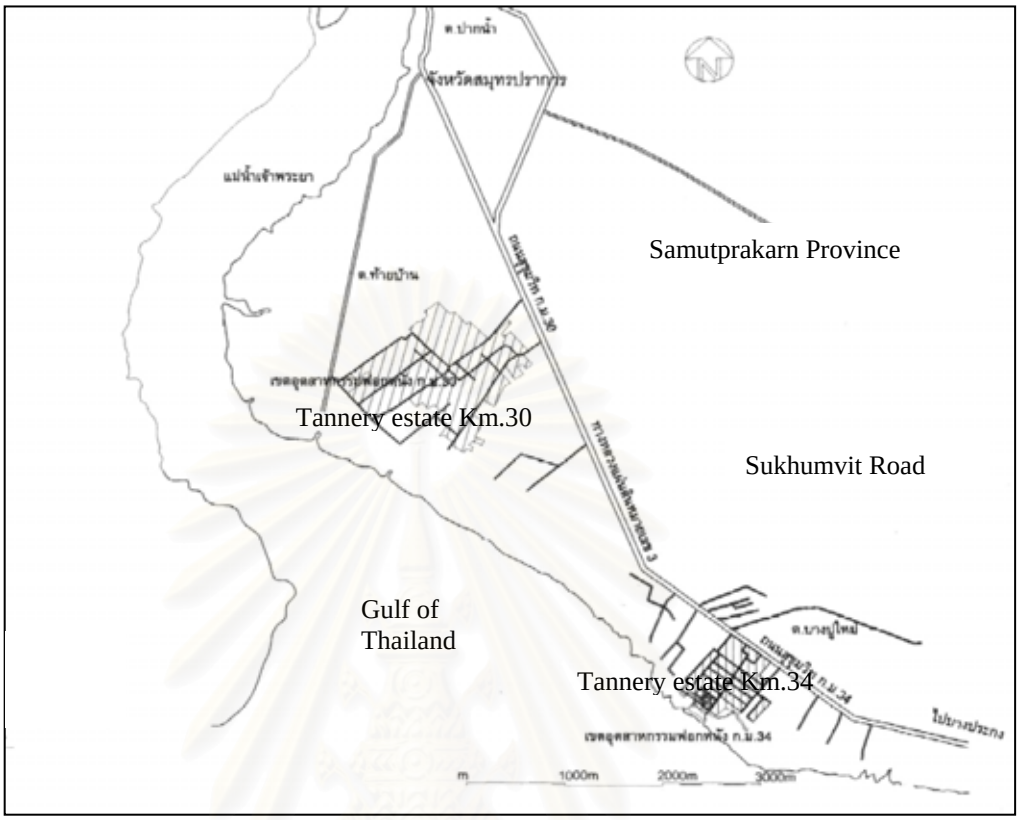

Figure 2.4 Location of tannery etates in Samutprakarn Province (Source: The Environmental Management Guideline for the Leather Tanning and Finishing Industry, 1997)

CHAPTER III

METHODOLOGY

The methodology of this study was divided into two parts: investigation on chromium concentrations in environmental media and organisms at the field site and experiment of sediment toxicity test. In the first part, seawater, sediment, and organism samples were collected from the study area, and were analyzed for chromium concentrations. Then, the results were used to calculate BAFs. In the second part, polychaetes *Perinereis nuntia*, were used as experimental animals for conducting the sediment toxicity test.

3.1 Investigation on Chromium Concentrations in Seawater, Sediment and Organisms at Field Site

3.1.1 The Study Area and Sampling Sites

The study area was located at Bangpoo, Muang district, Samutprakarn province covered 10 square kilometers along the shoreline. There were 12 sampling stations. Six stations were located near the shoreline. Each station was about 2 kilometers apart. The other six stations were approximately 500 meters away from the shoreline. The map showing the sampling stations is presented in Figure 3.1.

3.1.2 Sample Collection, Preservation, and Preparation

The field trip was conducted on October 24, 2004. Water, sediment, phytoplankton, zooplankton and benthic animals were collected form each station. Bivalves and fish samples were collected by local fishermen.

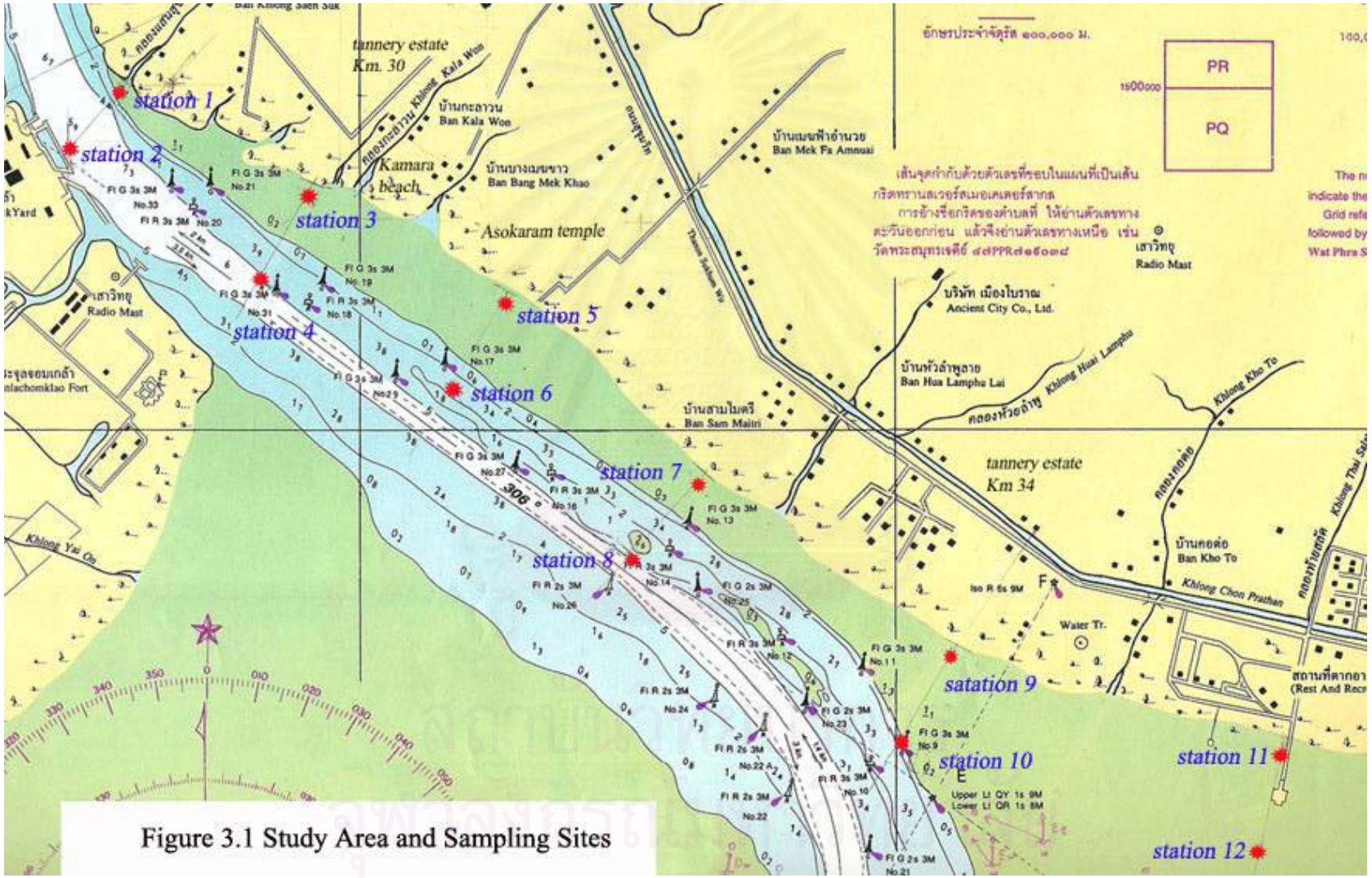

Figure 3.1 Study Area and Sampling Sites

3.1.2.1 Water Samples

Water Sample Collection and Preservation

Materials and Instruments

Water bucket, filter set, filter paper (0.45µm, polycarbonate Nucleopore), sample bottles, cool box, and vacuum pump

Reagents

Concentrated HNO_3 (suprapure, 65%)

Collection and Preservation Procedure

For each station pH, temperature, dissolved oxygen and transparency of the water were measured prior water sample collection. Then surface water samples were collected. The sampling bucket was rinsed with sample water. Water samples ware collected toward the upstream direction. The pre-cleaned bottles with 10% nitric acid were used for storing water samples. For each station, two bottles of seawater samples were collected.

Then, bottles were kept in double layer zip-lock plastic bags and stored in a box at 4°C for transportation. After the samples were transferred to the laboratory, the samples were filtered through a 0.45 µm pore size filter to separate dissolved and particulate matters. The water samples then were were preserved with concentrated nitric acid to pH < 2 and kept in refrigerator at 4°C until analysis of total chromium concentrations.

Water Sample Preparation: Total Chromium Concentration Analysis

Materials and Instruments

Microwave digester model ETHOS Sel, vessels, beakers, and pipette

Reagents

Concentrated HNO_3 (suprapure, 65%)

Digestion procedure

An aliquot of acidified water sample of 45 mL were digested with nitric acid according to the EPA Method 3015A. The aliquot was transferred

to a vessel with liner, added with 5 mL of concentrated HNO_3, swirled vigorously and capped. The vessel was then put in a microwave digester. The digested temperature were raised to 170 ± 5 °C within 10 minutes and maintained for 10 minutes. Then, the sample was cooled and transferred to plastic bottles before it was analyzed for total chromium.

3.1.2.2 Phytoplankton Samples

Phytoplankton Collection and Preservation

Materials and Instruments

Plankton net (mesh size 80 µm), centrifuge, 50 mL-centrifuged tubes and oven,

Collection and Preservation Procedure

Phytoplankton was collected by filtered 20 liters sample water through the plankton net with 80 µm mesh size and then concentrated phytoplankton were placed in plastic bottles (Figure 3.2). Plankton samples was centrifuged and oven dried at 60°C for 24 hour

Figure 3.2 Phytoplankton collection

Plankton sample preparation

Materials and Instruments

Oven, microwave digester model ETHOS Sel, vessels, beakers, pipette, volumetric flask, filter paper, filter funnel, and analytical balance

Reagents

Concentrated HNO_3 (65%), concentrated hydrogen peroxide (30%)

Digestion procedure

Dry sample was digested following the EPA Method 3052. The sample was weighed for 0.25 grams and transferred into a vessel liner. Then, 7 mL of concentrated nitric acid and 1 mL of hydrogen peroxide were added into the liner. After that, the vessel was capped and put in the microwave digester. Temperature of samples were raised to 200°C within 10 minutes and maintained for 10 minutes. Then, cooled samples were filtered and made to volume.

3.1.2.3 Sediment Samples

Sediment Sample Collection and Preservation

Materials and Instruments

Ekman grab, plastic container, scoop, plastic zip lock bag, cool box, plastic box, and freeze dryer model Heto LyoPro 6000

Collection and Preservation Procedure

Sediment samples were collected by the Ekman grab and placed in a container (Figure 3.3). The sediment was kept in plastic zip-lock bags. At the same time, pH of sediment was measured as the sediment parameter. Then, the samples were stored in a cool box at 4°C for transportation. Three replicates of samples were collected.

After the samples reached the laboratory, sediment samples were homogenized and placed in acid-cleaned plastic boxes and freeze-dried. Each sample was sieved through a 63 µm mesh-sized sieve.

Sediment Sample Preparation

Sediment that homogenized and freeze-dried were sieved to avoid grain size effect on metal concentration. Aliquot of sediment with pore size less than 63 µm was used to analyze for a) total chromium concentration and b) the bioavailable fraction.

Figure 3.3 Sediment collection

a) Total Chromium Concentration

Materials and Instruments

Microwave digester model ETHOS Sel, vessels, beakers, pipette, volumetric flask 100 mL, filter paper, filter funnel, and analytical balance

Reagents

Concentrated HNO_3 (suprapure, 65%), concentrated HCL (37%)

Digestion procedure

The sediment sample used for total chromium was digested with nitric acid and hydrochloric acid following the EPA Method 3051A. In a vessel liner, 0.5 gram of sample, 9 mL of concentrated HNO_3 and 3 mL of hydrochloric acid were mixed. Then, the vessel was capped and put in the microwave digester. The temperature of the sample was raised to 175 ± 5 °C within 5.5 minutes and maintained for 10 minutes. Then, the samples was cooled, filtered, and made to volume.

b) The Bioavailable Fraction

Materials and Instruments

Shaker, centrifuge and 50 mL-centrifuged tube, pipette, and balance,

Reagents

1M hydrochloric acid

Extraction procedure

The bioavailable fraction was analyzed by single extraction method (Peijnenburg 2003; Snape et al., 2004). One gram of dry sediment was placed in 50 mL-centrifuged tubes. Then, 20 mL of 1 M HCl were added. The sample tube were shaken for 4 hours and centrifuged at 1500 rpm for 20 minutes. The supernatants were filtered through a 0.45 µm pore size filter. The extractions were carried out in triplicate, including the analytical blanks.

3.1.2.4 Zooplankton, Benthic, Bivalves and Fish Samples

Zooplankton Sample Collection and Preservation

Materials and Instruments

Water bucket, bottles, oven, plankton net (Mesh Size 80 µm)

Collection and Preservation Procedure

Zooplankton samples were collected by boat hauling plankton net with mesh size of 315 µm near surface water for 5 minutes twice (Figure 3.4). Samples were kept in bottles and stored in a cool box. To obtain the dry mass volume, the samples were oven dried for 24 hour at 60°C.

Figure 3.4 Zooplankton collection

Benthic Sample Collection and Preservation

Materials and Instruments

Sieve with 0.5 mm mesh size, freeze dryer model Heto LyoPro 6000, and microscope

Collection and Preservation Procedure

Benthic organisms were collected by sieving the sediment samples through a 0.5 mm opening sieve. Animal samples were put in plastic zip-lock bags and placed in a cool box.

In the laboratory, benthic organisms were sorted with a microscope and eyes sighting. Each type of organisms was placed in a plastic box and then freeze-dried.

Fish and Bivalves Collection and Preservation

Materials and Instruments

Vernier, balance, plastic box and freeze dryer model Heto LyoPro 6000

Collection and Preservation Procedure

Mussels, cockles and fish were collected by local fishermen. Mussels and cockles were left alive one day to eliminate gut content. Then, the samples were grouped by size and weight. Later, the shells were removed. The soft parts were dissected and homogenized. Each pooled sample consisted of 10-12 bivalves. The samples were placed in plastic boxes and then freeze-dried.

Fish were separated by species and weight and length. For each sample, a composite making up with one to five fish were dissected and homogenized. Then, samples were placed into plastic boxes and freeze-dried.

Marine Organisms Samples Preparation

Materials and Instruments

Microwave digester model ETHOS Sel, vessels, beakers, pipette, volumetric flask, filter paper, filter funnel, and analytical balance

Reagents

Concentrated HNO_3 (65%), concentrated hydrogen peroxide (30%)

Digestion procedure

Samples were digested following the EPA Method 3052. 0.25 grams of samples were weighed in the vessel liner. Then, added 7 mL of nitric acid and 1 mL of hydrogen peroxide into the liner. Then, capped and put the vessel into the microwave digester. Temperature of samples were brought to 200°C within 10 minutes and maintained for 10 minutes. Cooled samples were filtered and made to volume.

3.1.3 Total Chromium Analysis

The digested samples were analyzed for total chromium using either an inductively coupled plasma atomic emission spectroscopy (ICP) or a flame atomic absorption spectroscopy (FAA), depending on sample concentration levels. The detection limits were 7µg/L and 0.05 mg/L for the ICP and FAA method, respectively.

Inductively Coupled Plasma Atomic Emission Spectroscopy

Sample was sprayed through a nubulizer and delivered to the plasma touch by an injector. ICP source consisted of a flowing stream of Argon gas ionized by radio frequency field. This field was coupled with coil that surrounding the torch. The high temperature of plasma (6000 to 8000 K) excited element-specific-atomic-line-emission spectra (Csuros M., 2002). The spectra were dispersed by a grating spectrometer and intensities of line were monitored. ICP was not subjected to self-absorption at low concentration (Csuros M., 2002). Thus, ICP was capable to detect concentration in microgram per liter (ppb).

To determine chromium concentration, suggested wavelengths were 357.9 and 267.7 nm. The detection limit was at 7 µg/L (Csuros M., 2002). Calibration curve consisted with at least three standards and a blank.

Flame Atomic Absorption Spectroscopy

Samples were atomized and then excited at high temperature in flame. Atoms of analyzed element absorbed light at specific wavelength emitted from hollow cathode lamp (HCL). This lamp emitted the energy passing through the flame. The amount of energy absorbed by these atoms was measured by detector. Since the amount of energy was proportional to the number of atoms, concentration of samples can be measured at specific wavelength. Detected concentration region was in a milligram per liter (mg/L). Air-acetylene flame was used for most elements with produced flame with temperature ranged from 2000-2800°C. According to Csuros M., 2002, detection limit was 0.05 mg/L whereas the optimum concentration ranged from 0.2-10 mg/L.

To determine chromium concentration, chromium hollow cathode lamp was used with air-acetylene flame. The wavelength that was used in determining chromium concentration was 357.9 nm. Calibration curve was consisted with at least three standards and a blank.

The concentration obtained from the instruments was in mg/L and could be converted to µg/g of dry weight for the solid samples according to the following equation.

$$\text{Sample concentration (µg/g)} = \frac{C^* V^* D}{W}$$

Where C = Concentration from instrument (mg/L)
V = Volume of sample after digestion (mL)
W = Dry weight of sample (g)
D = Dilution factor

In the case of water sample, conversion was

$$\text{Sample concentration (µg/L)} = \frac{C^* V2}{V1}$$

Where C = Concentration from instrument (µg/L)
V1= Volume of sample before digestion (45 mL)
V2= Total volume after digestion (50 mL)

3.1.4 Laboratory Quality Control

Reagent blanks or method blanks were used to determine contamination that may occur due to the use of reagent. Reagent blank was processed in the same way as samples but only reagent free water was used. The analyses of duplicate and triplicate samples were separated and considered to be different samples.

CRM certified reference materials were used to verify the method used in this study. CRM was treated and analyzed in the same way as samples

3.2 The Experiment of Sediment Toxicity Test

3.2.1 Test Species Characteristics

Polychaete *Perinereis nuntia*, was used in this test. Nereid polychaetes are an important fresh feed for black tiger shrimps (Poltana *et al*, 2005). The worms are essentially omnivorous which feed on both small animals and plants. Polychaetes can be found in most habitats ranging from estuaries and inshore waters especially on sandy or muddy beaches (Lim, 2000). The uses of polychaetes in bioassay studies are due to their short live cycles, their abundant, and ease to maintain under laboratory conditions (Lim, 2000). In addition, polychaetes are considered as bioindicator species. The presence and absence of specific polychaetes in sediments provides information of environmental health and conditions (Lim, 2000).

3.2.2 Sediment Toxicity Test Procedure

In this experiment, the procedure had been adapted from Bat and Raffaelli (1998) and Borgman(2001). Control and tested sediment samples were placed in 10 cm deep containers. Seawater was then added to a level of 4 cm. After settling for 24 hours, water above sediment was replaced with clean seawater. Dissolved oxygen in the containers was maintained constant (above 60% of saturation level) for 24 hours prior adding polychaetes by the aeration without disturb sediment surface. Seven polychaetes washed with clean seawater were placed in each container. The experiments were conducted in four replicates at each chromium concentration. The tanks were checked everyday for the number of survival,

emergence and death. After 15 days period, polychaetes, sediment, and water were analyzed for total chromium following the method described above.

3.2.3 Polychaete Preparing for Sediment Toxicity Test

To prevent the variation, commercial polychaetes were used in this study. Polychaetes were transferred into a darken tank filled with seawater for 24 hours prior the experiment for acclimation and gut content release purpose. Two-month old, *Perinereis nuntia,* with approximately 0.3 gram of dry weight were acquired from Sand Worm Aquaculture Research Group, Aquatic Research center of Chulalongkorn University. Each experimental set was conducted for four replicates. Every tank was consisted of 7 worms. After 15 days of experiments, polychaetes form each set was left for 1 day to get gut content to release. Then, they were weighed for final dry weight which was compared with the initial dry weight to obtain the growth rate.

3.2.4 Sediment Preparing for Sediment Toxicity Test

Prior the test start, sediment samples collected from an area that was free from pollutant and from the study site were sieved through 0.5 mm mesh and transferred to laboratory in a cold box. The sediments were washed and stirred with clean-filtered seawater three times, and settled for 24 hours. Next, water was decanted. Then, sediment was placed in plastic containers that had chromium solution at different concentration. The mixture was shaken continuously for 3-4 hours. Control sediments were treated using the same procedure but chromium solution was not added. After mixing, the supernatant was poured off.

The sediments used in this toxicity test were come from two sources. First, the field sediment was collected near station 9. The second source of sediment was collected from the area near the study site that was free form contaminant. These sediments in toxicity test were collected prior the experimental started to prevent the altering of sediment properties (Moore, 1994). There were 8 test sets in this study. Sediment in the first test set was field sediment designated as Field Sed., whereas, the second test set was the half mixture of field and clean sediment here after Field-Cont. The third to seventh test set were the clean sediment spiked with chromium

(Cr_2O_3) solution here after Control and ConC 1 to 4. Sediment in the eight test set, however, was artificial sand. This test set was here after experimental control set that was used as a compared set for the endpoint of toxicity test (Moore, 1994). The end point of this study was the growth rates that can be calculate as the following equation:

$$G = WTt_2\text{-}WTt_0/(t_2\text{-}t_0)$$

Where G is the growth rate (mg/day)

WTt_2 is an individual dry weight of polychaete at test termination

WTt_0 is an individual dry weight at test initiation

$t_2\text{-}t_0$ is a duration of exposure

The percentage of growth rate was calculated by comparing the growth rate of experimental control set with the other test set as shown in the following equation:

$$\% \text{ growth rate} = \frac{(G_{control} - G_{test})}{G_{control}} * 100$$

Where $G_{control}$ is the growth rate of the experimental control

G_{test} is the growth rate of the test set

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Investigation on Chromium Concentrations in Seawater, Sediment and Organisms at Field Site

4.1.1 Seawater and Sediment Properties

Field study was conducted on October 24, 2004 at Bangpoo, Muang District Samutprakarn Province. Table 4.1 showed the locations of sampling stations and properties of seawater and sediment. There was a trend of the salinity increase from station 1 to station 12 or as the distances was farther from the mouth of the Chaopraya River. For the near shore stations, station 1, 3, 5, 7, 9, and 11, transparency was not more than 50 centimeters because of high particulate matters in the water column. Temperature and pH of seawater and sediment for each station were in the normal range. Dissolved oxygen levels were above the acceptable level for aquatic lives (>4 mg/L). For station 2 and 4, sediments could not be collected since to the stations were located in inaccessible deep channel. As a result, benthic organisms from these stations were not available for chromium analysis. Sediment temperature and pH were also not measured.

Table 4.1 Seawater and sediment quality parameters

Station	Latitudes	Longitudes	Transparency (cm)	DO mg/L	Salinity (‰)	Seawater		Sediment	
						Temp °C	pH	Temp °C	pH
1	13° 33' 32"	100° 34' 55"	10	5.84	6.5	29.8	6.98	28.7	7.13
2	13° 32' 42"	100° 35' 6"	25	5.96	5.5	30.3	7.13	N.A	N.A.
3	13° 32' 55"	100° 35' 34"	10	6.18	12.5	29.6	7.24	28.5	7.43
4	13° 32' 35"	100° 35' 31"	70	6.57	5.8	32.0	7.16	N.A	N.A.
5	13° 32' 29"	100° 36' 23"	35	6.70	14.1	29.8	7.28	28.5	7.32
6	13° 32' 16"	100° 36' 21"	60	5.97	12.5	30.2	7.16	27.5	7.59
7	13° 31' 49"	100° 37' 15"	10	5.90	19.0	30.2	7.36	27.4	7.35
8	13° 31' 38"	100° 37' 3"	90	6.70	14.4	30.3	7.18	27.9	7.33
9	13° 31' 7"	100° 37' 59"	50	6.60	23.0	29.4	8.05	27.9	6.88
10	13° 30' 52"	100° 38' 6"	90	6.30	23.5	30.2	8.00	29.2	7.06
11	13° 30' 46"	100° 39' 23"	35	6.20	30.5	29.8	7.68	29.5	7.53
12	13° 30' 29"	100° 39' 15"	85	6.02	31.7	29.9	8.11	28.6	7.58

4.1.2 Chromium Concentrations in Seawater

Concentrations of total dissolved chromium in seawater were in between 1.44-4.55 µg/L (Table 4.2), and were below the industrial effluent standard of water quality of Thailand (total chromium of 0.75 mg/L) and water quality standard of total chromium recommended for marine life (50 µg/L) (Zayed,2003 and PCD, 2005). The highest concentration was at station 7 (Figure 4.1) whereas, the lowest chromium concentration was found at station 12. The stations near coastline, which were designated as the near shore stations in Table 4.2 tended to have higher chromium concentration levels than the off shore stations.

Table 4.2 Chromium concentrations in sediment, seawater, and phytoplankton and zooplankton

Station	Sediment			Seawater (µg/L)	Phytoplank (µg/g DW)	Zooplank (µg/g DW)
	Total Cr (µg/g DW)	Bioavailable (µg/g DW)	%available			
1	265.01	162.28	60.65	1.56	26.99	15.16
2	N.A.	N.A.	N.A.	2.46	N.A.	N.A.
3	335.85	240.95	71.98	2.99	29.47	20.59
4	N.A.	N.A.	N.A.	1.80	9.74	11.88
5	416.56	279.73	67.22	3.04	28.28	13.47
6	131.27	75.48	58.11	1.60	20.61	14.61
7	297.12	214.84	72.17	4.55	29.09	77.05
8	102.46	47.78	46.88	2.30	15.16	87.79
9	527.23	385.94	73.82	1.86	38.08	69.82
10	138.37	84.16	59.76	2.87	20.79	28.72
11	28.30	15.40	54.50	1.72	24.13	N.A.
12	88.93	49.15	55.17	1.44	14.86	26.13

The distribution of chromium in environment is controlled by oxidation-reduction, precipitation-dissolution, and sorption-desorption (Zayed, 2003). Chromium reacts with aqueous hydroxide, hydroxide or phosphate ions and precipitated (Zayed, 2003). Since chromium concentrations among the stations were not much different. It is likely that chromium reacted with ions in the water column and formed insoluble compounds that can be absorbed on to particulate matter (Neff, 2002). Then, chromium was removed from water column by precipitation (Zayed, 2003).

In the study area, point sources of chromium were expected to come from the outfall near tannery estates (Figure 3.1). Chromium seemed not to disperse into the large area because it likely to precipitate to the sea bottom. Thus, less dissolved chromium remained in water column.

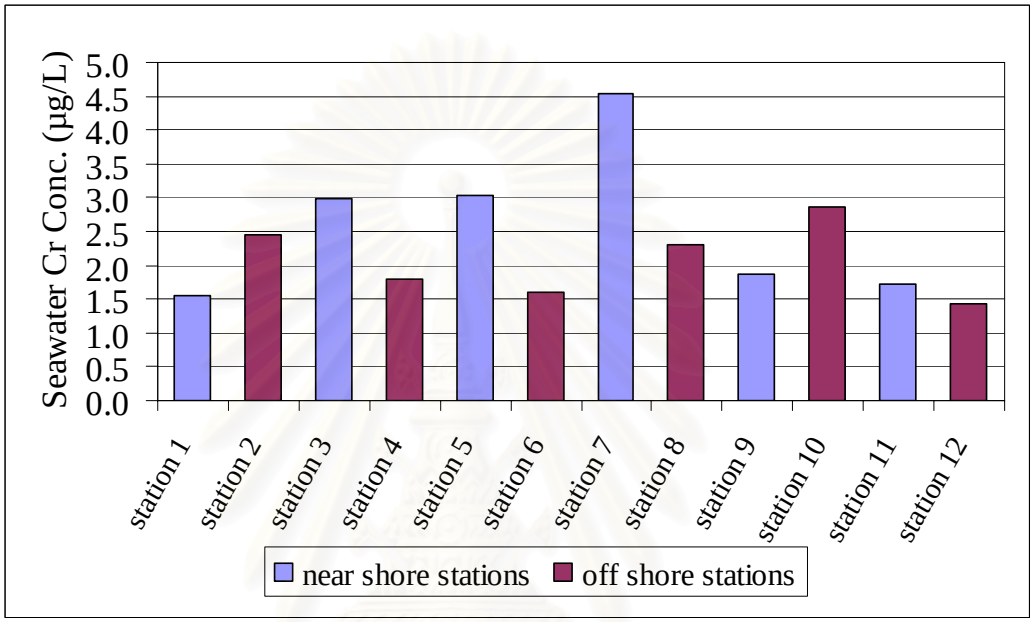

Figure 4.1 Total chromium concentrations in seawater at 12 sampling stations (µg/l)

4.1.3 Chromium Concentrations in Sediment

Total chromium concentrations in sediments ranged between 28-527 µg/g dry weight (Table 4.2). Figure 4.2 suggested that chromium was relatively high in sediment near shoreline along the study site, especially station 9. When compare with mean chromium concentration of world soil, chromium concentrations in sediment of this field study were above this level, 40µg/g (Zayed, 2003). Moreover, chromium concentrations in sediment were also higher than toxic effects to sediment dwelling-organisms (52.3 µg/g) according to Canadian interim marine sediment quality guidelines (UK marine sacs, 2005). However, at station 11 which had the lowest chromium concentration in sediment, chromium concentration did not exceed this guideline.

The highest chromium concentrations were found at station 5 and 9 (Figure 4.2). This was corresponded to the location of tannery estates. Station 5 and 9 were in the downstream direction and located near water channels (Figure 3.1). Chromium from these stations was distributed to the nearby stations. The distribution of chromium in sediment is controlled by chemical and physical properties. Chromium is likely to bind with particles and settled to the sea bottom (Zayed, 2003). Thus, chromium did not disperse in to the large area. Wave, tidal, and current also influenced the chromium distribution.

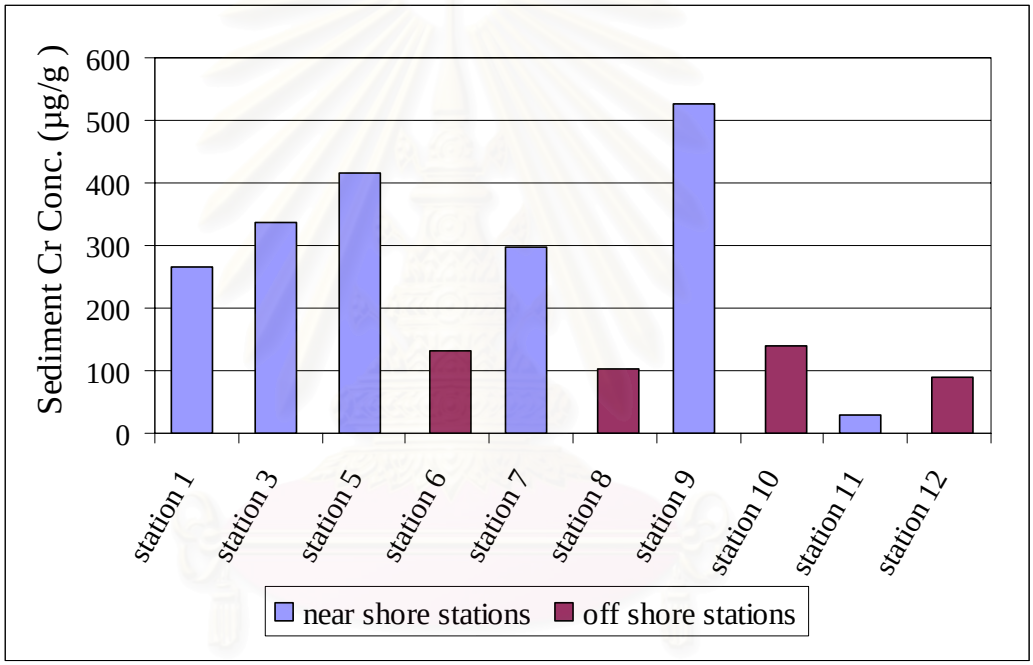

Figure 4.2 Total chromium concentrations in sediment at 10 sampling stations (µg/g dry weight)

The bioavailable fraction results indicated that more than half of the total chromium in the sediments. The percentages of bioavailable fraction were shown in Table 4.2. The highest percentage of bioavailable form (74%) was found in sediment at station 9. It indicated that more than half of chromium in this sediment was available for organisms to uptake.

4.1.4 Chromium Concentrations in Phytoplankton and Zooplankton

Chromium concentrations in phytoplankton and zooplankton were ranged between 10-90 µg/g dry weight. Much higher chromium concentrations in zooplankton were observed at station 7, 8, and 9. at the near shore stations, especially at station 9 while those in phytoplankton were more comparable (Figure 4.3).

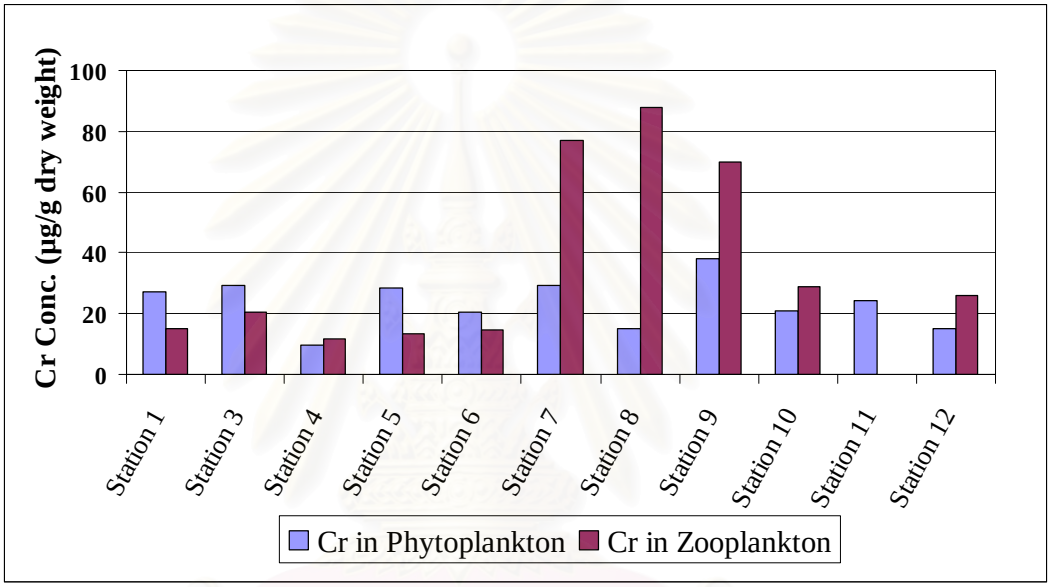

Figure 4.3 Chromium concentrations in phytoplankton and zooplankton (µg/g dry weight)

4.1.5 Chromium Concentrations in Benthic Organisms

Nine species of benthic organisms were found in studying sediment samples. They were peanut worm *Sipunculida* sp., polychaete *Perinereis* spp., ridged venus clam *Tapes turgidus*, razer clam *Solen regularis*, cockle *Arca granulosa*, gastropod *Cerithidea* sp., crab *Dotilla wichmani*, crab *Leucosia haswelli*, and shrimp *Acetes* sp. As shown in Table 4.3, nereid worm *Perinereis* sp, was found in every station.

According to Lim (2000), Family Nereide was an indicator species of an environmental discovery after pollution has been abated. In addition, most species found in the studying sediment were detritus feeders, which preferred to live in heavy load of organic contents. Although, only nine species were found in the study

area, the abundance of each species were high, especially, peanut worm *Sipunculida* sp., polychaete *Perinereis* spp., ridged venus clam *Tapes turgidus*, gastropod *Cerithidea* sp., and crab *Dotilla wichmani*.

Table 4.3 Total numbers of species and names of each species found in each station

Station	Total Numbers of Species	Names of Each Species
1	2	*Perinereis* spp., *Tapes turgidus*
3	3	*Perinereis* spp., *Tapes turgidus, Cerithidea* sp.
5	1	*Perinereis* spp.
6	5	*Perinereis* spp., *Tapes turgidus, Cerithidea* sp., *Dotilla wichmani, Acetes* sp.
7	2	*Perinereis* spp., *Tapes turgidus*
8	6	*Perinereis* spp., *Tapes turgidus, Cerithidea* sp., *Dotilla wichmani, Sipunculida* sp., *Solen regularis*
9	1	*Perinereis* spp.
10	6	*Perinereis* spp., *Tapes turgidus, Cerithidea* sp., *Dotilla wichmani, Sipunculida* sp., *Arca granulosa*
11	3	*Perinereis* spp., *Tapes turgidus, Leucosia haswelli*
12	6	*Perinereis* spp., *Tapes turgidus, Cerithidea* sp., *Dotilla wichmani, Sipunculida* sp.

Chromium concentrations in *Perinereis* spp., were demonstrated in Figure 4.4. The highest chromium concentrations in the polychaetes were observed at station 9 (Appendix A-4).

Figure 4.4 displayed chromium concentrations in polychaetes and sediments. Polychaetes at station 1, 6 to 10 and 12 had two times of chromium concentrations higher than in the sediment. Chromium concentrations in sediments and polychaetes at station 3 and 5 were nearly the same levels. Thus, this species can accumulate chromium in their bodies more than twice of chromium found in sediments.

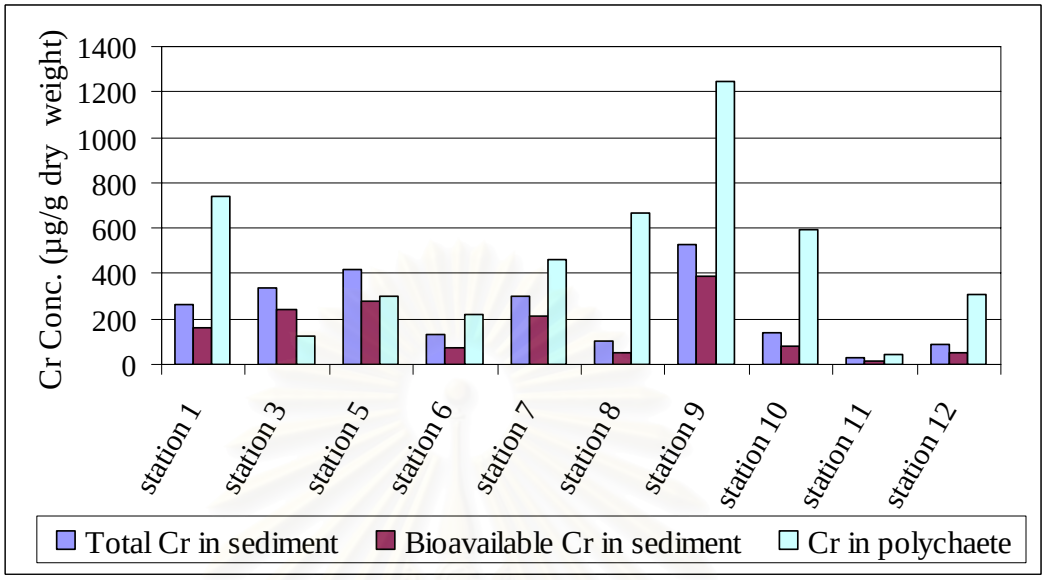

Figure 4.4 Chromium concentrations in sediment and polychaetes, *Perinereis* spp.

Beside the ability to accumulate chromium of this organism, high chromium contents in nereid worms may be due to the collection method. After, collecting benthic animals, samples were placed immediately in cool box and transferred to the lab. Thus, there was no period for releasing gut content of the organisms.

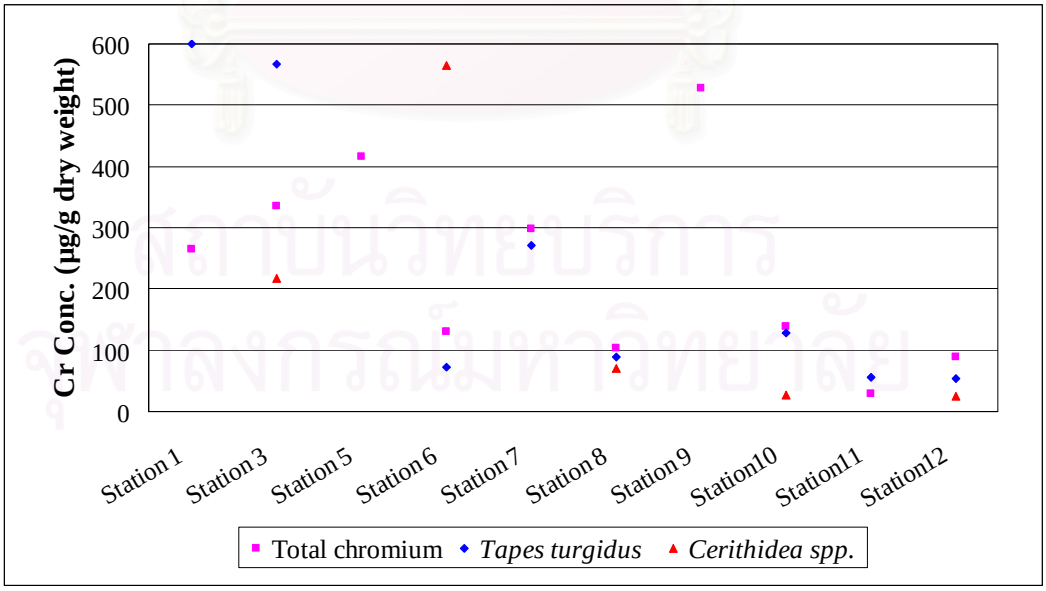

Figure 4.5 Chromium concentrations in benthic organisms: *Tapes turgidus* and *Cerithidea* sp.

Chromium concentrations in *Tapes turgidus,* and *Cerithidea* sp. were present in Figure 4.5. *Tapes turgidus* had chromium concentrations between 50-600 µg/g dry weight while chromium levels in *Cerithidea* sp were in the wider range (from 30-600µg/g). However, chromium concentrations in sediments, *Tapes turgidus,* and *Cerithidea* sp. were not much different. Only *Tapes turgidus* in station 1 and 3 and *Cerithidea* sp. in station 6 had chromium in their bodies higher than the chromium levels in the sediment (Figure 4.5).

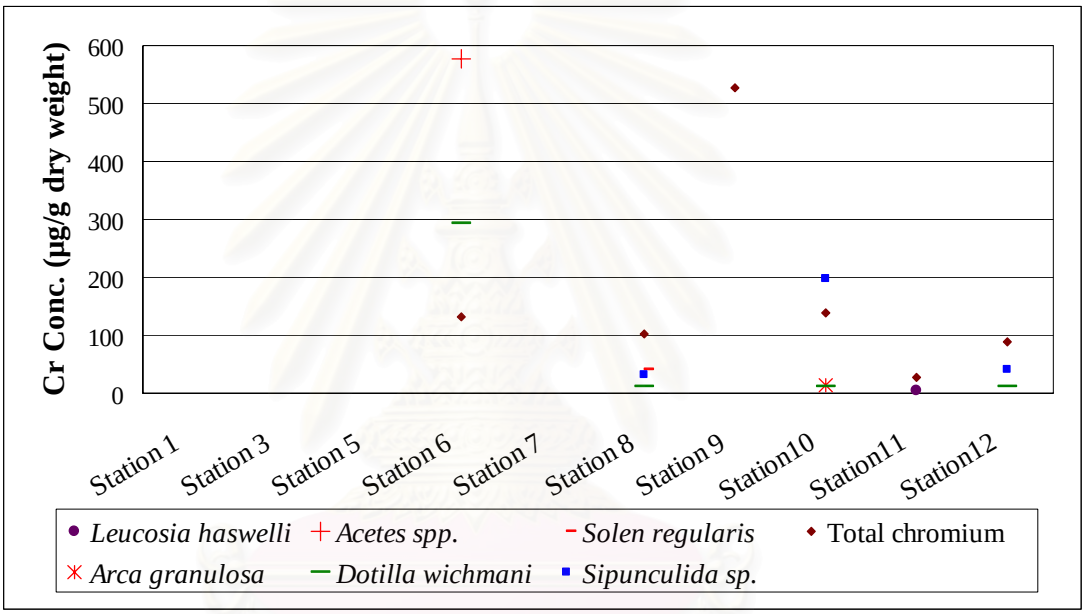

Figure 4.6 Chromium concentrations in benthic organisms: *Dotilla wichmani, Acetes* sp., *Sipunculida* sp., *Solen regularis, Arca granulosa,* and *Leucosia haswelli*

Chromium concentrations in other benthic animals; *Dotilla wichmani, Acetes* sp., *Sipunculida* sp., *Solen regularis., Arca granulosa,* and *Leucosia haswelli* were illustrated in Figure 4.6. These organisms were found at station 6, 8 and 10 to 12. Chromium in these organisms was at the same levels as those in sediment. *Dotilla wichmani* and *Acetes* sp from station 6 and *Sipunculida* sp from station 10 had chromium concentrations higher than 200 µg/g dry weight while the others contained chromium less than 40 µg/g dry weight (Appendix A-5)**.**

4.1.6 Chromium Concentrations in Mussels, Oysters and Fish

Chromium concentrations in seven fish, mussel, and cockle tissue samples were between 2.17 -3.48 µg/g dry weight as shown in Table 4.4. Chromium concentrations in the tissues of most organisms were below 4.2 µg /g dry weight which is a suggested value converted form wet weight value (at 85 % water content) by the Food and Drug Administration safety food guideline (SFEI, 1999 and Costa, 2004). Only cockles found at station 10 had higher chromium concentrations in their tissues (13.63 µg /g dry weight).

Table 4.4 Chromium concentrations in fish, mussels and cockles

Type	Common Name	Scientific Name	Cr Conc. (µg/g DW)
F1G1	Java Tilapia	*Oreochromis mossambicus*	3.02
F1G2	Java Tilapia	*Oreochromis mossambicus*	2.98
F1G3	Java Tilapia	*Oreochromis mossambicus*	2.98
F2G1	Java Tilapia	*Oreochromis mossambicus*	2.99
F2G2	Java Tilapia	*Oreochromis mossambicus*	3.06
F2G3	Java Tilapia	*Oreochromis mossambicus*	3.13
F3G1(S)	Threadfin	*Eleutheronema tetradactylum*	3.28
F3G2(L)	Threadfin	*Eleutheronema tetradactylum*	2.93
F4	Sand Whiting	*Sillago sihama*	3.18
F5	Sea Catfish	*Plotosus lineatus*	3.21
F6	Soldier Crocker	*Nibea soldado*	3.27
F7G1	Greenback Mullet	*Liza subviridis*	2.78
F7G2	Greenback Mullet	*Liza subviridis*	2.17
F7G3	Greenback Mullet	*Liza subviridis*	3.03
Mussels	Green Mussel	*Perna viridis*	3.48
Cockles	Bloody Cockle	*Arca granulosa*	2.58

4.1.7 Bioaccumulation Factors (BAFs)

Two food webs found in the study area. There were the classic food web and the benthic food web. These were classified by the organisms found in field study. The classic food web was the phytoplankton-zooplankton-fish pathway (Chamberlin, 2005), whereas, the benthic food web was the pathway transfered energy form detritus to benthic animals and fish.

BAFs ratios show the abilities of organisms to accumulate chromium in their bodies. Organisms that have BAFs higher than 1 can accumulate chromium in their bodies. Thus, BAF of each organism in the food web can give more details about chromium bioaccumulation via food web.

Table 4.5 Bioaccumulation factors (BAFs) from the study site

Bioaccumulation Factor				
Loop 1 Classic Food Web			BAF	min	max
Seawater					
Phytoplankton				5.42	20.44
Zooplankton				6.61	38.23
Fish Species					
	Oreochromis mossambicus (F)			0.30	0.93
	Oreochromis mossambicus (M)			0.32	1.01
	Liza subviridis			0.14	0.45
Loop 2 Benthic Food Web			BAF	min	max
Sediment					
Benthic Species					
	Peanut worms	*Sipunculida* sp.		0.35	1.51
	Polychaetes	*Perinereis* spp.		0.31	7.26
	Bivalves	*Tapes turgidus*		0.53	2.07
	Cockles	*Arca granulosa*			0.1
	Gastropods	*Cerithidea* sp.		0.21	7.09
	Razor clams	*Solen regularis*			0.46
	Crabs	*Leucosia haswelli*			0.14
	Crabs	*Dotilla wichmani*		0.13	2.16
Fish Species					
	Java Tilapia	*O. mossambicus(F)*		0.003	0.05
	Java Tilapia	*O. mossambicus(M)*		0.003	0.05
	Threadfin	*E. tetradactylum*		0.001	0.02
	Sand Whiting	*Sillago sihama*		0.002	0.029
	Catfish	*Plotosus lineatus*		0.002	0.033
	Soldier Crocker	*Nibea soldado*		0.002	0.030
	Greenback Mullet	*L. subviridis*		0.001	0.024

For the first loop or the classical foob web, BAF ratios in phytoplanktons, and zooplanktons were higher than 1 indicating the chromium accumulation of these organisms (Table 4.5). However, BAF of 3 fish fed on planktons were less than 1. Thus, fish in this food web did not accumulate chromium in their tissues.

For the benthic food web, BAFs of benthic organisms were both higher and lower than 1 (Table 4.5). Thus, benthic animals can be categorized into 3 groups according to their BAFs. First, high BAF ratios were found in polychaetes *Perineris* spp. and gastropods *Cerithidea* sp. These organisms can accumulate chromium in their body as high as 7 times of the ambient sediment. Second, moderate BAF ratios were presented in peanut worm *Sipunculida* sp., crab *Dotilla wichmani* and bivalve *Tapes turgidus*. These organisms contained double of chromium concentrations found in the sediment. Last, organisms with low BAF ratios were clab *Leucosia haswelli*, and clam *Solen regularis*. These organisms tend not to accumulate chromium in their tissue.

Moreover, fish fed on small benthic animals in this loop had very low BAFs. In addition, chromium occurring in fish tissues was a net result of uptake, distribution and elimination of chromium which also showed as the lack of ability to accumulate chromium via this food chain.

When considering organisms and their trophic levels, lower trophic levels organisms contained high chromium concentrations. For instant, phytoplankton and zooplankton that directly received chromium from seawater and polychaetes *Perinereis* sp and bivalve *Tapes turgidus* that also fed on detritus. However, higher trophic level animals such as fish that fed on several types of foods contained less chromium concentrations. Thus, chromium transferred to the next trophic level via their food was not so significant. Therefore, chromium did not biomagnify in marine food web (Neff, 2002; UK marine sacs, 2005).

Mearns (1985) showed that there was no bioaccumulation of chromium in fish. Organisms that were directly in contact with the media (water and sediment) were the species that can accumulate chromium in their body at high level. The

same study by Mearns (1985) shown that annelids were able to accumulate chromium to 50 µg/g dry weight or at two order magnitude above of the control animals (0.05-0.15 µg/g dry weight).

4.1.8 Chromium Concentrations in Certified Reference Materials

To verify the accuracy of analysis, certified referenced materials were analyzed to compare chromium concentrations of true and found value. The percents recovery of chromium concentrations in the certified reference materials were in between 80-120% which could verify that analytical method was accepted. The results were shown in Table 4.6.

Table 4.6 Chromium concentrations in certified reference materials

Certified Reference Materials	Chromium Concentrations (µg/g dry weight)		
	Value ± Uncertainty	Found value	%Recovery
Sediment			
SRM 2704 Buffalo River Sediment	135 ± 5	109.08	80.8
SRM 1646 Estuarine Sediment	76 ± 3	63.6	83.68
Tissues			
IAEA 350 Tuna Homogenate	0.75 ± 0.2	0.78	104.07
CRM 278 Mussel Tissue	0.8 ± 0.85	0.85	106.52

4.2 Sediment Toxicity Test

Sediments used in this toxicity test come from two sources; field and spiked sediment. Eight test sets in this study are designated as Field Sed (field sediment), Field+Control (mixture of field sediment with clean sediment), Control (clean sediment), ConC 1, ConC 2, Con C 3, and ConC 4(clean sediment spiked with chromium concentration level 1, 2, 3 and 4, respectively). Total chromium concentrations of water, sediment and polychaete of each set were shown in Table 4.7.

During the experiment, water quality parameters; dissolved oxygen (DO), pH and salinity were measured every other day to ensure suitable conditions for test species (Appendix B-1). The suitable conditions of this species were DO > 4 mg/L, pH ~ 8, and salinity < 30‰. During the experiment, seawater was changed twice according to the guideline of sediment toxicity test (Vranken, 1989 and Puget Sound Water Quality Authority, US.EPA. 1997).

Table 4.7 Chromium concentrations of seawater, sediment, and polychaetes and survival and growth rate of polychaetes during the 15 day toxicity test

Test Set	Water	Cr Concentration (µg/g dry weight)				Survival		Growth Rate	
	(µg/L)	Total Cr	Bioavail	%avail	polychaete	No.	%	(day^{-1})	%
Field Sediment									
1.Field Sed	8.54	261.50	101.54	38.83	32.00	7	96.43	0.00906	66.61
2.Field+Cont	9.50	152.56	59.55	39.09	38.87	5	71.43	0.00811	59.62
Spiked Sediment									
3.Control	9.72	36.90	13.68	37.25	29.81	7	96.43	0.01047	76.95
4.ConC 1	9.67	54.34	16.06	29.60	13.38	5	76.19	0.01055	77.72
5.ConC 2	9.71	70.37	19.45	27.78	21.94	6	85.71	0.01095	80.55
6.ConC 3	9.69	136.24	21.75	16.01	35.84	6	89.29	0.01080	79.41
7.ConC 4	9.75	231.51	29.75	12.85	24.84	6	82.14	0.00921	67.73
8.Artificial Sand (Experimental Control)					3.89	7	100	0.01360	100

The highest concentration of total chromium was found in Field Sed set (261.50 µg/g). The total chromium concentrations of spiked sediment were between 36.90 to 231.51 µg/g. Total chromium concentrations in sea water were

approximately 9 to 10 µg/L. Again, the low chromium concentrations in seawater indicated that chromium mainly bound with sediment. Percentages of growth and survival rate compared with experimental control set were also shown in Table 4.7.

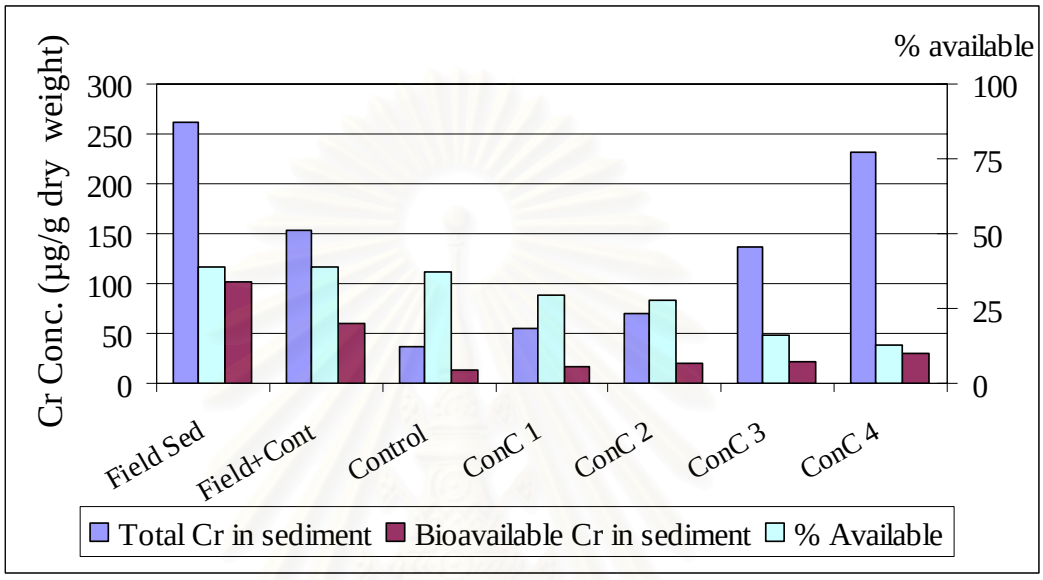

Figure 4.7 Sediment toxicity test: Chromium concentrations in sediment

Figure 4.7 showed chromium concentrations in sediment of each test set (set 1-7). Bioavailable fractions of sediment set in the field sediments (Field Sed. and Field+Cont) were similar at about 40% of total chromium concentration. For the spiked sediment set (Cont and ConC 1-4), decreased in bioavailable fraction with increasing spiked chromium concentration was observed.

Polychaetes in every set contained chromium at the same level in their bodies. In addition, chromium concentrations in polychaetes were at the same level of available chromium of each test set (Figure 4.8). Only Field Sed test that chromium concentration of polychaetes were less than of the bioavailable fraction.

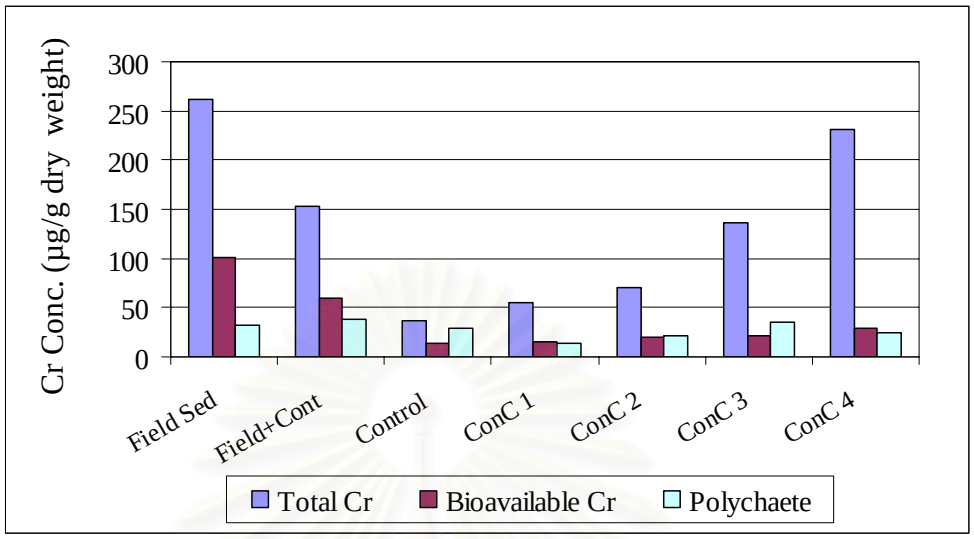

Figure 4.8 Sediment toxicity test: Comparisons of chromium concentrations in sediments and polychaetes

Chromium concentrations of polychaetes were not above 40 µg/g dry weight for every test set. In comparison, polychaetes in the normal habitat (artificial sand) contained only 4 µg/g dry weight of chromium in their bodies. Increasing of chromium in polychaetes at higher levels of chromium in sediment was not observed here. Moreover, among the same sediment test set chromium in polychaete still varied (Appendix B-2). Thus, polychaetes accumulated chromium regardless of chromium level in the sediment.

The numbers of survival of polychaetes in the experiment were between 5-7 individuals. The death numbers of tested organisms occurred during the first three day of the experiment due to the acclimation failure. After that, there was no further death. Thus, levels of chromium in the test were in the range that the test species were able to tolerate.

Interestingly, percentages of growth rate in Figure 4.9 showed the retardation of growth rate in both field and spiked sediment when comparing with the growth rate of the experimental control. The experiment control set yielded the highest growth rate (Figure 4.9). Polychaete in the experimental control set lived in artificial sand. Under this condition, there were no stress from sediment particle size (fine

particles) and chromium. When growing under the highest content of chromium in the field sediment, percent of growth rate was the lowest. The field sediment was represented the habitat of the study area. Thus, it can be concluded that the growth of polychaetes found in the study was reduced due to the stresses of chromium and particle size.

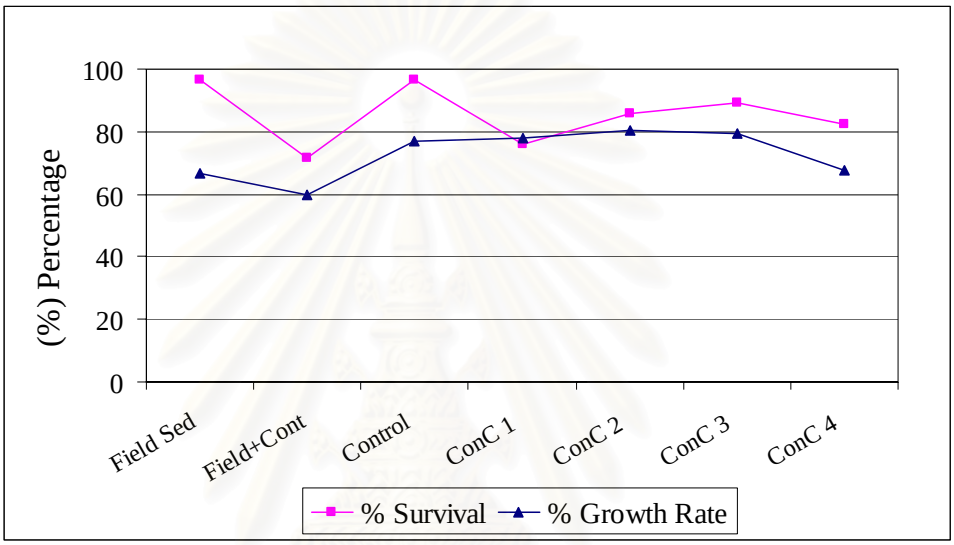

Figure4.9 Sediment toxicity test: Percentages of growth and survival Rates

Percentage of growth rate in most of the spiked sediment was about 80% as shown in Figure 4.9. The percentages of growth rate in ConC 1, ConC 2, and ConC 3 were quite similar where as, the percent of growth rate of ConC 4 set was substantially lower at 67 %. Thus, the reduction of growth rate in polychaete of spiked sediment set was due to the stress that introduced from contaminant.

In general, the response curve of sediment toxicity had dropped substantially when concentration of contaminant had increased (Ramachandran, 1997; Borgman, 2001; Moreno-Garrido, 2003). It is because organisms can tolerate to contaminant at a certain limit (Borgman, 2001; Moreno-Garrido, 2003). Above that limit, contaminant can cause death to the organisms. Moreover, mean effect concentration EC_{50} was also calculated and used as a value to compare the effect among the contaminant (Ramachandran, 1997).

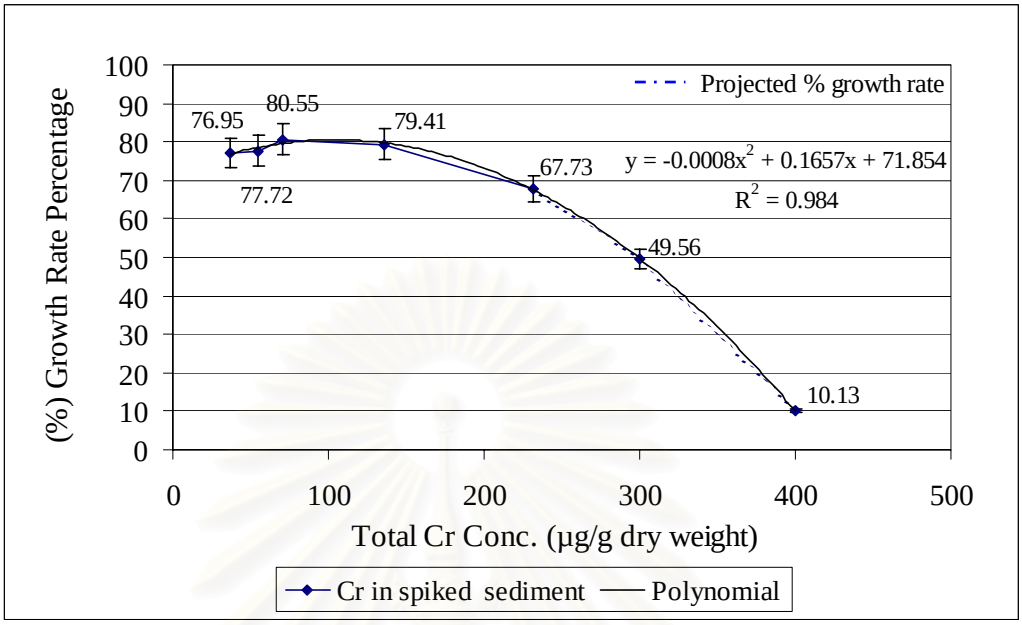

Figure 4.10 Interpolations of percentage of growth rate and chromium concentrations in spiked sediment with polynomial fitted curve

In addition, in this experiment, EC_{50} can not be set because the effective result did not reach to 50% affected to the test organisms. Thus, the percentages of growth rate of polychaete and chromium concentrations of spiked sediment were then used to calculate the percentages of growth rate when the concentrations of chromium were higher than in the experiment. Figure 4.10 showed the percentage of growth rate of spiked sediment when interpolated with polynomial regression. It was projected that percentages of growth rate of polychaetes in the sediment contained 300 and 400 µg/g dry weight of chromium would be 49.56 % and 10.13 %, respectively. However, these numbers can be served as a trend of toxic effect of chromium toxicity in this study only. To establish the 50 % effect concentration of chromium sediment toxicity test, the future study is needed.

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTION

5.1 Conclusions

This study investigated chromium concentrations in seawater, sediment and organisms in the study area at Bangpoo, Muang District, Samutprakarn Province. The area received waste discharge from tannery industries. The results showed that chromium concentrations in seawater were in the normal range (<5 µg/L). However, sediments at the sampling sites near shoreline had high chromium concentrations (as high as 527µg/g). Nine species of benthic organisms were found during the study. Nereid worm *Perinereis* sp, which was found at every sampling site, had high chromium concentrations (300-1200 µg/g). Bivalve, *Tapes turgidus*, gastropod *Cerithidea* sp. and *Sipunculida* sp. contained relatively high chromium in their bodies (100-600 µg/g). However, fish, mussels and cockles had low chromium concentrations (3 µg/g dry weight).

Bioaccumulation factors were used to determine the ability of organisms to accumulate chromium. Benthic organisms were the group that can accumulate highest chromium concentrations where as fish, mussels and cockle had less ability to accumulate chromium. The lower trophic level organisms such as plankton and polychaetes accumulate chromium better than the higher trophic level organisms such as fish. Thus, chromium did not biomagnify along the food web in the study site.

A sediment toxicity test was also conducted by observing the effect of chromium in sediment towards the test animals, *Perinereis Nuntia*. The test was 15 days and used field and spiked sediment. The results showed that high chromium concentrations in sediment reduced the growth rate of *Perineris. nuntia*. Chromium concentration in sediment at 231.51 µg/g dry weight can reduce the percent of

growth to 67.73 % of the growth of the control. Moreover, *Perineris nuntia* can accumulate chromium regardless to the concentration of chromium in sediment.

From the study of chromium contamination in marine ecosystem at Bangpoo, Muang district, Samutprakarn province, sediment in this area contained relatively high chromium concentrations(as high as 527 µg/g). This concentration was above the soil quality standard established for habitat and agriculture uses (300 µg/g of hexavalent chromium) (PCD, 2005). Moreover, the sediment toxicity test was also showed that there was the effect of chromium on growth rate of the test organisms. The chromium concentrations of 231.51 µg/g dry weight can reduce the percent of growth rate to 67.73 % of control. Thus, government should establish the concentration levels of the toxic effects of heavy metal in sediment to aquatic organisms and set the marine sediment quality standard in order to control the pollution discharged into the sea and to prevent the deterioration of marine ecosystems.

5.2 Future Direction

This work had been studied the sediment quality in terms of concentration of toxicants and the biological effects (toxicity test). The microcosm should be conducted to compare the results and give more information of chromium effect to the marine organisms. However, chromium was the only contaminant investigated in this study. Thus, the toxicity tests of multi-contaminants effects to marine organisms are necessary to get a better understanding of complex conditions of the real ecosystem.

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APPENDICES

APPENDIX A

Appendix A

Table A-1 Total Chromium Concentrations in Seawater

STATION	Cr concentration (µg/L)				MEAN
	SET A		SET B		
	rep 1	rep 2	rep 1	rep 2	
1	1.60	1.56	1.50	1.58	1.56 ± 0.07
2	2.44	2.22	2.65	2.53	2.46 ± 0.08
3	3.09	3.00	2.99	2.90	2.99 ± 0.07
4	1.67	1.84	1.89	1.79	1.80 ± 0.07
5	3.05	2.99	3.11	3.02	3.04 ± 0.08
6	1.44	1.59	1.67	1.70	1.60 ± 0.04
7	4.19	4.62	4.56	4.82	4.55 ± 0.06
8	2.36	2.08	2.44	2.30	2.30 ± 0.07
9	1.89	1.73	2.00	1.83	1.86 ± 0.08
10	2.77	2.86	2.89	2.96	2.87 ± 0.06
11	1.60	1.72	1.78	1.78	1.72 ± 0.04
12	1.52	1.39	1.39	1.44	1.44 ± 0.06

Note: Set A and B were the duplicate samples collected at each station

Appendix A

Table A-2 Chromium Concentration in Sediment

STATION	Total Cr Concentration (µg/g dry weight)				Total Bioavailable Cr Conc(µg/g dry weight)				% available		
	A	B	C	mean	A	B	C	mean	A	B	C
1	213.19	266.91	314.94	265.01 ± 0.39	115.52	172.72	198.60	162.28 ± 0.02	54	65	63
3	299.14	339.79	368.61	335.85 ± 0.66	222.88	251.88	248.10	240.95 ± 0.02	75	74	67
5	402.34	352.90	494.44	416.56 ± 0.33	269.68	239.66	329.84	279.73 ± 0.02	67	68	67
6	119.23	138.60	135.97	131.27 ± 0.97	85.59	75.31	65.55	75.48 ± 0.04	72	54	48
7	320.51	312.04	258.81	297.12 ± 0.33	242.28	220.20	182.03	214.84 ± 0.04	76	71	70
8	111.17	104.85	91.36	102.46 ± 0.65	51.87	43.51	47.95	47.78 ± 0.02	47	42	52
9	475.87	654.51	451.33	527.24 ± 0.66	402.96	442.86	311.99	385.94 ± 0.02	85	68	69
10	180.52	103.42	131.16	138.37 ± 0.33	117.19	54.80	80.50	84.16 ± 0.04	65	53	61
11	26.94	30.53	27.42	28.30 ± 0.33	15.74	16.18	14.28	15.40 ± 0.06	58	53	52
12	87.12	96.03	83.64	88.93 ± 0.66	46.15	55.70	45.60	49.15 ± 0.04	53	58	55

Note: A, B, and C were the field triplicate sediment samples

Appendix A

Table A-3 Total Chromium Concentrations in Planktons

Table A-3.1 Chromium Concentrations in Phytoplankton

STATION	Total Cr Concentration (µg/g DW)				MEAN
	set A		set B		
	rep 1	rep 2	rep 1	rep 2	
1	8.61	10.34	45.75	43.26	26.99 ± 0.35
3	27.81	29.31	32.13	28.64	29.47 ± 0.25
4	10.40	12.10	8.25	8.21	9.74 ± 0.18
5	29.50	28.98	25.29	29.34	28.28 ± 0.16
6	26.79	25.69	14.85	15.11	20.61 ± 0.28
7	34.90	30.50	26.34	24.63	29.09 ± 0.20
8	18.76	17.54	12.38	11.96	15.16 ± 0.21
9	42.12	38.39	36.13	35.68	38.08 ± 0.11
10	25.25	26.31	28.86	22.72	20.79 ± 0.20
11	23.25	25.36	23.83	24.06	24.13 ± 0.22
12	19.20	18.79	10.41	11.03	14.86 ± 0.18

Table A-3.2 Chromium Concentrations in Zooplankton

STATION	Total Cr Concentration (µg/g DW)				MEAN
	set A		set B		
	rep 1	rep 2	rep 1	rep 2	
1	22.75	23.56	7.20	7.12	15.16 ± 0.21
3	13.54	14.28	27.94	26.59	20.59 ± 0.18
4	12.76	11.54	11.18	12.06	11.88 ± 0.24
5	13.69	12.85	14.78	12.57	13.47 ± 0.12
6	13.89	15.76	14.98	13.82	14.61 ± 0.15
7	57.12	55.98	47.10	148.01	77.05 ± 0.14
8	74.12	75.23	87.56	94.23	82.79 ± 0.24
9	74.78	73.51	64.32	66.68	69.82 ± 0.15
10	31.68	31.74	26.39	25.07	28.72 ± 0.13
12	21.07	20.79	32.83	29.82	26.13 ± 0.17

Note: Set A and B were the duplicate samples collected at each station

Appendix A

Table A-4 Chromium Concentrations of Benthic Organisms

Benthic Organisms			Chromium Concentration (µg/g DW)		
Station 1					
Bivalves	ridged enus clam	*Tapes turgidus*	602.19	±	2.41
Polychaetes		*Perinereis* spp.	739.81	±	3.78
Station 3					
Bivalves	ridged venus clam	*Tapes turgidus*	566.37	±	5.24
Gastropods	*Cerithidea*	*Cerithidea* sp.	216.65	±	7.41
Polychaetes		*Perinereis* spp.	124.37	±	6.92
Station 5					
Polychaetes		*Perinereis* spp.	303.82	±	8.68
Station 6					
Bivalves	ridged venus clam	*Tapes turgidus*	72.04	±	7.02
Crabs		*Dotilla wichmani*	293.35	±	4.12
Gastropods		*Cerithidea* sp.	564.36	±	5.24
Polychaetes		*Perinereis* spp.	217.32	±	9.29
Shrimp		*Acetes* sp.	577.06	±	5.62
Station 7					
Bivalves	ridged venus clam	*Tapes turgidus*	270.13	±	8.87
Polychaetes		*Perinereis* spp.	460.12	±	5.26
Station 8					
Bivalves	ridged venus clam	*Tapes turgidus*	88.24	±	4.42
Crabs		*Dotilla wichmani*	12.33	±	1.05
Gastropods		*Cerithidea* sp.	71.27	±	6.87
Peanut worms		*Sipunculida*	31.82	±	3.42
Polychaetes		*Perinereis* spp.	663.75	±	8.83
Bivalves	razor clam	*Solen regularis*	41.65	±	2.58
Station 9					
Polychaetes		*Perinereis* spp.	1243.39	±	13.54
Station 10					
Bivalves	ridged venus clam	*Tapes turgidus*	127.70	±	5.43
Cockles		*Arca granulosa*	13.63	±	0.66
Crabs		*Dotilla wichmani*	10.50	±	0.85
Gastropods		*Cerithidea* sp.	27.93	±	3.91
Peanut worms		*Sipunculida*	198.28	±	6.88
Polychaetes		*Perinereis* spp.	592.82	±	7.30

Appendix A

Table A-4 (cont.) Chromium Concentrations of Benthic Organisms

Benthic Organisms		**Chromium Concentration (µg/g DW)**			
Station 11					
Bivalves	ridged venus clam	*Tapes turgidus*	56.89	±	2.72
Crabs		*Leucosia haswelli*	3.88	±	0.43
Polychaetes		*Perinereis* spp.	42.46	±	2.16
Station 12					
Bivalves	ridged venus clam	*Tapes turgidus*	53.71	±	2.52
Crabs		*Dotilla wichmani*	12.10	±	1.37
Gastropods		*Cerithidea* sp.	23.94	±	3.57
Peanut worms		*Sipunculida*	40.16	±	3.67
Polychaetes		*Perinereis* spp.	304.45	±	8.72

Appendix A

Table A-5 Chromium Concentrations in Green Mussels *Mytilus viridis*

Size	Chromium Conc. (µg/g dry weight)				Average shell size	
	set	rep1	rep2	mean	Length(mm)	Width(mm)
Size 1	A	2.24	2.85	2.55 ± 0.05	75	32
Size 1	B	3.38	4.34	3.86 ± 0.05	71	29
Size 2	A	3.06	4.47	3.77 ± 0.04	60	26
Size 2	B	2.64	3.62	3.13 ± 0.03	60	26
Size 2	C	2.51	4.18	3.35 ± 0.00	65	29
Size 3	A	2.69	5.57	4.13 ± 0.05	58	26
Size 3	B	3.34	3.42	3.38 ± 0.03	58	25
Size 3	C	2.61	4.49	3.55 ± 0.05	57	26
Size 3	D	3.20	4.28	3.74 ± 0.05	53	23
Size 3	E	3.61	5.64	4.63 ± 0.05	52	23
Size 4	A	3.07	3.61	3.34 ± 0.04	44	22
Size 4	B	2.66	3.99	3.33 ± 0.03	45	23
Size 5	A	2.93	3.74	3.34 ± 0.04	32	18
Size 5	B	2.55	2.81	2.68 ± 0.03	31	16

Note: set A, B, C, D and E were sub sets of mussels with similar size

Appendix A

Table A-6 Chromium Concentrations in Bloody Cockles *Arca granulose*

Size	Chromium Conc. (µg/g dry weight)				Average Shell Size	
	set	rep1	rep2	mean	Length(mm)	Width(mm)
Size 1	A	2.19	2.11	2.15 ± 0.06	45.8	32
Size 1	B	2.93	2.79	2.86 ± 0.04	42.2	32
Size 2	A	3.36	3.14	3.25 ± 0.04	38.3	28
Size 2	B	3.04	3.07	3.06 ± 0.05	38.7	29
Size 2	C	3.03	3.17	3.10 ± 0.03	37.5	29
Size 3	A	2.77	2.45	2.61 ± 0.05	34.3	24
Size 3	B	2.13	2.22	2.18 ± 0.06	33.7	24
Size 3	C	2.03	2.21	2.12 ± 0.04	34.0	23
Size 3	D	2.51	2.0	2.56 ± 0.03	33.3	24
Size 4	A	2.01	2.13	2.07 ± 0.06	28.0	23
Size 4	B	2.32	2.53	2.43 ± 0.04	30.0	23

Note: set A, B, C, and D were sub sets of cockles with similar size

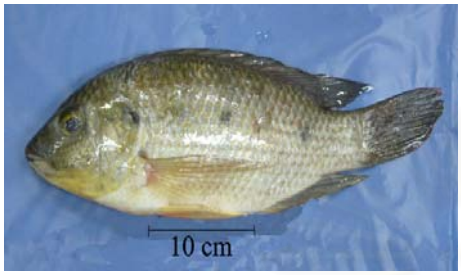
Appendix A

Table A-7 Chromium Concentrations in Fish

Group	Common name	Scientific Species	Total Chromium Concentrations(µg/g dry weight)			Average size	
			rep 1	rep 2	mean	Length(cm)	Weight(g)
F1G1	Java Tilapia	*Oreochromis mossambicus*	2.96	3.08	3.02 ± 0.04	22.1	210.0
F1G2	Java Tilapia	*Oreochromis mossambicus*	3.00	2.95	2.98 ± 0.05	22.4	241.7
F1G3	Java Tilapia	*Oreochromis mossambicus*	3.07	2.89	2.98 ± 0.03	24.3	275.0
F2G1	Java Tilapia	*Oreochromis mossambicus*	2.99	2.99	2.99 ± 0.03	18.8	140.0
F2G2	Java Tilapia	*Oreochromis mossambicus*	2.99	3.12	3.06 ± 0.04	19.8	166.7
F2G3	Java Tilapia	*Oreochromis mossambicus*	3.01	3.24	3.13 ± 0.04	21.0	196.7
F3G1(S)	Threadfin	*Eleutheronema tetradactylum*	2.82	3.73	3.28 ± 0.04	18.1	40.0
F3G2(L)	Threadfin	*Eleutheronema tetradactylum*	3.01	2.85	2.93 ± 0.03	15.5	15.0
F4	SandWhiting	*Sillago sihama*	3.34	3.01	3.18 ± 0.05	13.0	15.0
F5	Sea Catfish	*Plotosus lineatus*	3.30	3.12	3.21 ± 0.05	19.0	30.0
F6	Soldier Crocker	*Nibea soldado*	3.43	3.10	3.27 ± 0.03	11.0	10.0
F7G1	Greenback Mullet	*Liza subviridis*	2.62	2.93	2.78 ± 0.03	10.0	10.0
F7G2	Greenback Mullet	*Liza subviridis*	1.34	3.00	2.17 ± 0.04	13.0	30.0
F7G3	Greenback Mullet	*Liza subviridis*	2.99	3.07	3.03 ± 0.05	15.0	60.0

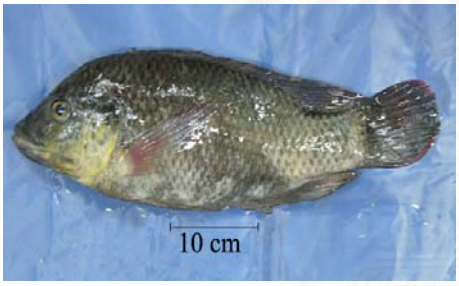
Appendix A

Figure A-1 Pictures of Fish from Field Study (10 cm scale)



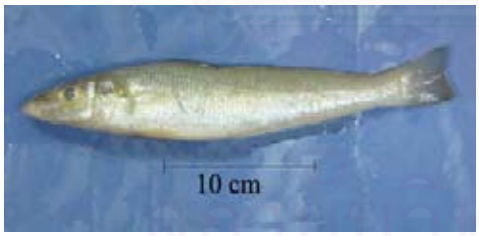
Type 1 Java Tilapia
Oreochromis mossambicus (Female)

Type 5 Sea Catfish
Plotosus lineatus

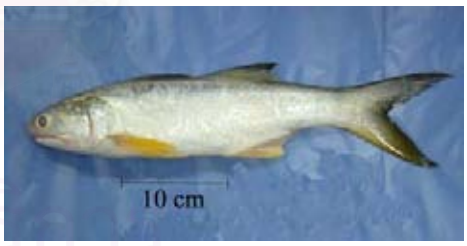


Type 2 Java Tilapia
Oreochromis mossambicus (Male)

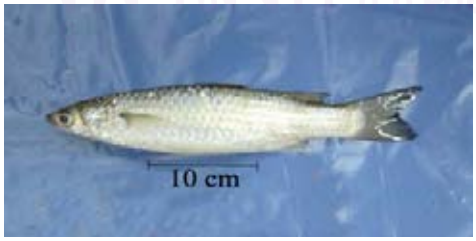
Type 6 Soldier Crocker
Nibea soldado



Type 3 Threadfin
Eleutheronema tetradactylum



Type 7 Greenback Mullet
Liza subviridis



Type 4 SandWhiting
Sillago sihama

APPENDIX B

APPENDIX B

Sediment Toxicity Test

Table B-1 Chromium Concentration in Sediment and Seawater

ID	Sediment Cr Conc. (µg/g dry weight)	Bioavailable Cr (µg/g dry weight)	%available	Seawater Cr Conc. (µg/L)
Field Set 1	255.68 ± 0.69	100.22 ± 0.03	39.19	8.42 ± 0.05
Field Set 2	261.34 ± 0.50	101.10 ± 0.02	38.69	8.78 ± 0.04
Field Set 3	262.37 ± 0.36	100.31 ± 0.02	38.23	8.33 ± 0.03
Field Set 4	266.61 ± 0.32	104.54 ± 0.04	39.21	8.64 ± 0.06
Field Cont Set 1	158.87 ± 0.55	60.02 ± 0.02	37.78	9.56 ± 0.04
Field Cont Set 2	153.22 ± 0.40	60.58 ± 0.02	39.54	9.42 ± 0.04
Field Cont Set 3	155.79 ± 0.42	58.70 ± 0.01	37.68	9.54 ± 0.02
Field Cont Set 4	142.38 ± 0.23	58.91 ± 0.02	41.37	9.48 ± 0.04
Control Set 1	37.61 ± 0.39	14.22 ± 0.04	37.82	9.57 ± 0.07
Control Set 2	33.10 ± 0.43	12.84 ± 0.06	38.79	9.71 ± 0.07
Control Set 3	35.07 ± 0.37	13.46 ± 0.04	38.38	9.74 ± 0.08
Control Set 4	41.82 ± 0.33	14.22 ± 0.02	34.00	9.85 ± 0.04
ConC 1 Set 1	56.27 ± 0.67	16.52 ± 0.02	29.36	9.62 ± 0.06
ConC 1 Set 2	56.69 ± 0.36	16.12 ± 0.04	28.43	9.70 ± 0.07
ConC 1 Set 3	49.63 ± 0.32	15.53 ± 0.02	31.29	9.66 ± 0.08
ConC 1 Set 4	54.79 ± 0.45	16.05 ± 0.03	29.30	9.71 ± 0.06
ConC 2 Set 1	72.11 ± 0.66	19.43 ± 0.02	26.94	9.69 ± 0.06
ConC 2 Set 2	77.26 ± 0.33	19.20 ± 0.04	24.85	9.69 ± 0.04
ConC 2 Set 3	63.40 ± 0.35	19.33 ± 0.01	30.49	9.78 ± 0.06
ConC 2 Set 4	68.73 ± 0.25	19.83 ± 0.02	28.85	9.68 ± 0.04

APPENDIX B

Sediment Toxicity Test

Table B-1 (cont.) Chromium Concentration in Sediment and Seawater

ID	Sediment Cr Conc. (µg/g dry weight)	Bioavailable Cr (µg/g dry weight)	% available	Seawater Cr Conc. (µg/L)
ConC 3 Set 1	127.18 ± 0.39	21.36 ± 0.02	16.79	9.66 ± 0.05
ConC 3 Set 2	137.19 ± 0.33	21.85 ± 0.04	15.93	9.71 ± 0.04
ConC 3 Set 3	146.32 ± 0.67	21.40 ± 0.02	14.63	9.65 ± 0.03
ConC 3 Set 4	134.26 ± 0.23	22.39 ± 0.03	16.67	9.72 ± 0.06
ConC 4 Set 1	226.46 ± 0.43	31.29 ± 0.02	13.82	9.62 ± 0.04
ConC 4 Set 2	223.15 ± 0.36	26.73 ± 0.04	11.98	9.73 ± 0.04
ConC 4 Set 3	235.66 ± 0.42	29.71 ± 0.02	12.61	9.81 ± 0.02
ConC 4 Set 4	240.77 ± 0.37	31.26 ± 0.04	12.99	9.83 ± 0.04

APPENDIX B

Sediment Toxicity Test

Table B-2 Chromium Concentration in Polychaete *Perinereis nuntia*

ID	polychaete Cr Conc.(µg/g dry weight)			Survival(total 7)		Growth	
				survival	%survival	rate(day-1)	% Growth Rate
Field Set 1	38.45	±	2.41	6	85.71	0.0078	57.07
Field Set 2	14.89	±	1.05	7	100	0.0100	73.43
Field Set 3	61.50	±	2.72	7	100	0.0099	73.01
Field Set 4	13.15	±	0.85	7	100	0.0086	62.96
Field Cont Set 1	30.82	±	2.55	4	57.14	0.0075	55.36
Field Cont Set 2	52.60	±	2.32	5	71.43	0.0014	10.03
Field Cont Set 3	15.07	±	1.52	4	57.14	0.0105	77.26
Field Cont Set 4	56.96	±	2.63	7	100	0.0130	95.82
Control Set 1	7.83	±	0.96	6	85.71	0.0081	59.26
Control Set 2	6.34	±	0.53	7	100	0.0104	76.27
Control Set 3	41.68	±	2.64	7	100	0.0093	68.43
Control Set 4	63.39	±	3.78	7	100	0.0141	103.85
ConC 1 Set 1	6.40	±	0.54	6	85.71	0.0104	76.68
ConC 1 Set 2	5.18	±	0.52	5	71.43	0.0106	77.94
ConC 1 Set 3	3.71	±	0.45	6	85.71	0.0104	76.62
ConC 1 Set 4	38.22	±	1.37	4	57.14	0.0108	79.66
ConC 2 Set 1	3.83	±	0.23	6	85.71	0.0101	74.58
ConC 2 Set 2	17.63	±	1.02	7	100	0.0113	82.97
ConC 2 Set 3	61.41	±	2.32	6	85.71	0.0106	77.79
ConC 2 Set 4	4.90	±	0.66	5	71.43	0.0118	86.86

APPENDIX B

Sediment Toxicity Test

Table B-2 (Cont.) Chromium Concentration in Polychaete *Perinereis nuntia*

ID	polychaete Cr Conc.(µg/g dry weight)			Survival(total 7)		Growth	
				survival	%survival	rate(day-1)	% Growth Rate
ConC 3 Set 1	11.24	±	1.03	6	85.71	0.0105	77.54
ConC 3 Set 2	53.86	±	2.31	7	100	0.0071	52.14
ConC 3 Set 3	69.04	±	2.74	5	71.43	0.0114	83.90
ConC 3 Set 4	9.20	±	0.32	7	100	0.0142	104.05
ConC 4 Set 1	17.98	±	1.35	7	100	0.0100	73.72
ConC 4 Set 2	15.55	±	1.53	5	71.43	0.0074	54.33
ConC 4 Set 3	36.72	±	1.20	6	85.71	0.0121	88.63
ConC 4 Set 4	29.11	±	0.85	5	71.43	0.0074	54.24

APPENDIX B

Sediment Toxicity Test

Table B-3 Water Parameters during Sediment Toxicity Test; Day 1

Status	Day 1 of experiment					
Test Date	July 15, 2005					
Time of measurement	9.00					
	Water					
ID	temperature (°C)	pH	DO (mg/L)	DO (%)	Salinity (‰)	Conductivity (mS/cm)
Field Set 1	28.6	7.99	5.38	70.14	25.8	40.3
Field Set 2	28.7	8.01	5.31	69.23	25.8	40.3
Field Set 3	28.9	8.07	6.17	80.44	26.0	40.6
Field Set 4	28.6	8.05	5.79	75.49	25.3	39.6
Field Cont Set 1	28.7	8.03	5.64	73.53	25.7	37.4
Field Cont Set 2	28.7	8.03	6.10	79.53	25.7	37.4
Field Cont Set 3	28.9	8.05	6.19	80.70	26.2	38.2
Field Cont Set 4	28.7	8.07	6.16	80.31	25.9	37.7
Control Set 1	28.4	8.09	5.59	72.88	27.3	42.5
Control Set 2	28.8	8.14	6.05	78.88	26.7	41.6
Control Set 3	28.7	8.11	6.38	83.18	26.4	41.2
Control Set 4	28.4	8.15	6.02	78.49	26.0	40.7
ConC 1 Set 1	28.2	8.11	5.91	77.05	26.7	41.6
ConC 1 Set 2	28.3	8.10	5.74	74.84	26.5	41.3
ConC 1 Set 3	28.4	8.14	6.36	82.92	25.6	40.1
ConC 1 Set 4	28.5	8.13	6.41	83.57	26.6	41.6
ConC 2 Set 1	28.6	8.12	5.70	74.32	26.1	40.8
ConC 2 Set 2	28.7	8.08	5.86	76.40	26.1	40.8
ConC 2 Set 3	28.7	8.07	6.23	81.23	25.6	40.0
ConC 2 Set 4	28.9	8.09	5.86	76.40	25.8	40.4
ConC 3 Set 1	29.1	8.11	5.56	72.49	25.6	41.2
ConC 3 Set 2	29.0	8.19	6.48	84.49	25.3	39.9
ConC 3 Set 3	29.2	8.19	5.72	74.58	25.5	39.7
ConC 3 Set 4	29.3	8.07	6.66	86.83	26.4	40.1
ConC 4 Set 1	29.2	8.01	5.19	67.67	27.5	42.8
ConC 4 Set 2	28.7	8.08	6.14	80.05	25.8	40.5
ConC 4 Set 3	28.9	8.02	5.44	70.93	25.5	40.0
ConC 4 Set 4	29.0	8.07	6.24	81.36	26.5	41.4

Table B-4 Water Parameters during Sediment Toxicity Test; Day 3

Status	Day 3 of experiment					
Test Date	July 17, 2005					
Time of measurement	10.30					
	Water					
ID	temperature (°C)	pH	DO (mg/L)	DO (%)	Salinity (‰)	Conductivity (mS/cm)
Field Set 1	31.0	7.88	5.19	67.67	27.0	42.1
Field Set 2	30.1	7.92	5.29	68.97	26.8	41.8
Field Set 3	30.8	8.03	5.08	66.23	27.1	42.2
Field Set 4	31.1	8.05	5.02	65.45	25.6	40.1
Field Cont Set 1	29.8	8.07	5.80	75.62	26.4	41.2
Field Cont Set 2	30.1	8.13	5.42	70.66	25.6	40.2
Field Cont Set 3	29.9	8.09	5.91	77.05	26.7	41.8
Field Cont Set 4	30.4	8.12	5.66	73.79	25.9	40.6
Control Set 1	30.1	8.11	4.65	60.63	28.1	43.6
Control Set 2	30.4	8.12	5.09	66.36	26.5	41.5
Control Set 3	30.2	8.13	5.38	70.14	27.8	43.2
Control Set 4	30.3	8.14	6.14	80.05	27.0	42.2
ConC 1 Set 1	29.6	8.12	5.68	74.05	27.2	42.4
ConC 1 Set 2	29.3	8.13	5.87	76.53	28.3	44.0
ConC 1 Set 3	29.8	8.15	5.81	75.75	27.0	42.1
ConC 1 Set 4	29.5	8.18	5.68	74.05	28.3	43.8
ConC 2 Set 1	30.0	8.16	6.34	82.66	28.0	43.5
ConC 2 Set 2	29.4	8.15	6.08	79.27	29.2	43.7
ConC 2 Set 3	29.9	8.10	6.14	80.05	26.6	41.5
ConC 2 Set 4	29.8	8.09	5.72	74.58	26.4	41.3
ConC 3 Set 1	30.2	8.15	5.61	73.14	28.1	43.6
ConC 3 Set 2	30.1	8.16	6.13	79.92	27.9	43.3
ConC 3 Set 3	30.0	8.18	6.24	81.36	28.0	43.5
ConC 3 Set 4	30.3	8.10	5.87	76.53	27.2	42.4
ConC 4 Set 1	32.0	7.97	6.03	78.62	27.8	43.3
ConC 4 Set 2	31.2	8.05	6.11	79.66	27.4	42.7
ConC 4 Set 3	30.5	8.07	5.83	76.01	27.6	43.0
ConC 4 Set 4	30.5	8.08	5.67	73.92	27.3	42.5

Table B-5 Water Parameters during Sediment Toxicity Test; Day 6

Status	Day 6 of experiment					
Test Date	July 20, 2005					
Time of measurement	15.30					
	Water					
ID	temperature (°C)	pH	DO (mg/L)	DO (%)	Salinity (‰)	Conductivity (mS/cm)
Field Set 1	31.4	8.11	5.24	72.78	27.9	43.3
Field Set 2	32.4	8.08	4.87	67.64	27.8	43.2
Field Set 3	32.6	8.22	5.73	79.58	28.1	43.6
Field Set 4	32.8	8.23	5.72	79.44	26.9	41.9
Field Cont Set 1	31.3	8.20	5.63	78.19	27.4	42.8
Field Cont Set 2	31.0	8.25	5.28	73.33	26.8	41.9
Field Cont Set 3	30.8	8.15	5.77	80.14	27.9	43.4
Field Cont Set 4	30.7	8.14	5.89	81.81	27.0	42.1
Control Set 1	30.3	8.17	5.65	78.47	28.3	43.9
Control Set 2	30.9	8.19	5.57	77.36	28.1	43.6
Control Set 3	30.4	8.20	4.94	68.61	28.4	44.1
Control Set 4	30.9	8.21	5.65	78.47	27.9	43.4
ConC 1 Set 1	30.5	8.17	5.65	78.47	28.2	43.8
ConC 1 Set 2	29.9	8.18	5.85	81.25	29.1	45.0
ConC 1 Set 3	30.5	8.20	6.03	83.75	28.2	43.8
ConC 1 Set 4	30.3	8.20	5.31	73.75	28.9	44.7
ConC 2 Set 1	30.3	8.21	5.82	80.83	28.8	44.5
ConC 2 Set 2	29.9	8.16	5.67	78.75	28.8	44.5
ConC 2 Set 3	30.1	8.09	5.15	71.53	27.6	43.0
ConC 2 Set 4	30.3	8.11	5.53	76.81	27.6	42.9
ConC 3 Set 1	31.6	8.31	5.57	77.36	29.1	45.0
ConC 3 Set 2	31.6	8.33	5.89	81.81	28.8	44.6
ConC 3 Set 3	31.0	8.32	5.57	77.36	27.9	43.3
ConC 3 Set 4	31.2	8.13	5.04	70.00	28.2	43.7
ConC 4 Set 1	33.2	8.24	5.63	78.19	29.2	45.2
ConC 4 Set 2	32.0	8.14	5.03	69.86	28.5	44.1
ConC 4 Set 3	31.5	8.20	5.14	71.39	28.5	44.1
ConC 4 Set 4	31.4	8.23	5.58	77.50	28.5	44.2

Table B-6 Water Parameters during Sediment Toxicity Test; Day 9

Status	Day 9 of experiment					
Test Date	July 23, 2005					
Time of measurement	8.30					
	Water					
ID	temperature (°C)	pH	DO (mg/L)	DO (%)	Salinity (‰)	Conductivity (mS/cm)
Field Set 1	28.3	8.19	6.79	88.2	28.1	43.6
Field Set 2	28.2	8.19	5.65	73.4	27.8	43.2
Field Set 3	28.0	8.18	6.15	79.9	27.5	42.7
Field Set 4	28.6	8.21	6.22	80.8	26.8	41.8
Field Cont Set 1	27.3	8.02	6.61	85.8	27.4	42.7
Field Cont Set 2	27.1	8.09	6.90	89.6	27.1	42.2
Field Cont Set 3	27.2	8.08	6.19	80.4	27.6	42.9
Field Cont Set 4	27.2	8.08	6.72	87.3	27.3	42.4
Control Set 1	27.3	8.13	6.03	78.3	28.1	43.6
Control Set 2	27.4	8.13	6.23	80.9	27.7	43.1
Control Set 3	27.3	8.13	6.75	87.7	28.4	44.0
Control Set 4	27.4	8.14	6.03	78.3	27.5	42.8
ConC 1 Set 1	27.6	8.12	6.02	78.2	27.6	42.9
ConC 1 Set 2	27.3	8.13	6.35	82.5	28.7	44.4
ConC 1 Set 3	27.2	8.15	5.96	77.4	27.9	43.4
ConC 1 Set 4	27.4	8.16	6.07	78.8	28.2	43.8
ConC 2 Set 1	27.6	8.15	6.35	82.5	28.2	43.7
ConC 2 Set 2	27.3	8.16	6.07	78.8	28.3	43.8
ConC 2 Set 3	27.6	8.16	6.28	81.6	27.3	42.5
ConC 2 Set 4	27.8	8.10	6.07	78.8	27.0	42.0
ConC 3 Set 1	27.9	8.15	6.65	86.4	27.9	42.4
ConC 3 Set 2	27.8	8.18	6.46	83.9	28.1	43.6
ConC 3 Set 3	27.7	8.19	6.82	88.6	28.2	43.8
ConC 3 Set 4	27.8	8.17	6.76	87.8	27.8	43.1
ConC 4 Set 1	28.5	8.17	6.81	88.4	28.6	44.3
ConC 4 Set 2	28.2	8.18	6.65	86.4	27.8	43.2
ConC 4 Set 3	28.1	8.17	6.52	84.7	28.0	43.5
ConC 4 Set 4	28.0	8.19	6.56	85.2	27.8	43.2

Remark Changed Water

Table B-7 Water Parameters during Sediment Toxicity Test; Day 12

Status	Day 12 of experiment					
Test Date	July 26, 2005					
Time of measurement	9.00					
	Water					
ID	temperature (°C)	pH	DO (mg/L)	DO (%)	Salinity (‰)	Conductivity (mS/cm)
Field Set 1	28.8	8.23	6.76	88.60	28.2	43.8
Field Set 2	28.8	8.30	6.32	82.83	27.4	42.6
Field Set 3	28.9	8.25	6.30	82.57	28.2	43.8
Field Set 4	29.3	8.31	6.37	83.49	26.4	41.2
Field Cont Set 1	30.0	8.16	6.50	85.19	27.3	42.6
Field Cont Set 2	29.1	8.25	6.49	85.06	27.1	42.2
Field Cont Set 3	29.3	8.15	6.51	85.32	27.1	42.2
Field Cont Set 4	29.2	8.16	6.48	84.93	27.1	42.2
Control Set 1	29.5	8.23	6.18	81.00	27.1	42.3
Control Set 2	29.3	8.21	6.45	84.53	27.2	42.4
Control Set 3	29.0	8.22	6.34	83.09	26.8	41.8
Control Set 4	29.1	8.25	6.20	81.26	27.0	42.1
ConC 1 Set 1	29.3	8.14	6.46	84.67	27.1	42.2
ConC 1 Set 2	28.8	8.20	6.40	83.88	27.3	42.5
ConC 1 Set 3	28.7	8.19	6.55	85.85	27.5	42.8
ConC 1 Set 4	29.2	8.23	6.34	83.09	27.2	42.4
ConC 2 Set 1	29.5	8.22	6.09	79.82	27.1	42.2
ConC 2 Set 2	29.2	8.19	6.09	79.82	27.6	42.8
ConC 2 Set 3	29.5	8.18	5.55	72.74	26.9	41.9
ConC 2 Set 4	29.5	8.16	6.18	81.00	27.1	42.3
ConC 3 Set 1	29.8	8.21	6.51	85.32	27.0	42.1
ConC 3 Set 2	29.5	8.22	6.62	86.76	27.2	42.3
ConC 3 Set 3	29.5	8.22	6.47	84.80	27.4	42.6
ConC 3 Set 4	29.7	8.20	6.36	83.36	27.4	42.6
ConC 4 Set 1	30.0	8.23	6.36	83.36	27.6	42.9
ConC 4 Set 2	29.4	8.24	6.50	85.19	27.3	42.6
ConC 4 Set 3	29.6	8.22	6.55	85.85	27.2	42.4
ConC 4 Set 4	29.6	8.22	6.78	88.86	27.4	42.7

Table B-8 Water and Sediment Parameters during Sediment Toxicity Test; Day 15

Status	Day 15 End of experiment					
Test Date	July 29, 2005					
Time of measurement	8.30					
	Water				Sediment	
ID	temperature (°C)	pH	Salinity (‰)	Conductivity (mS/cm)	temperature (°C)	pH
Field Set 1	29.5	8.30	29.8	46.1	29.0	7.12
Field Set 2	29.4	8.32	28.3	43.9	29.2	7.15
Field Set 3	29.3	8.29	29.8	45.9	29.1	7.14
Field Set 4	28.9	8.30	26.9	42.0	28.9	7.19
Field Cont Set 1	28.6	8.18	28.3	43.8	28.5	7.00
Field Cont Set 2	28.4	8.22	28.3	43.9	28.5	7.05
Field Cont Set 3	28.5	8.11	27.9	43.3	28.6	7.05
Field Cont Set 4	28.5	8.07	28.0	43.5	28.9	7.02
Control Set 1	29.0	8.21	28.1	43.6	27.7	7.11
Control Set 2	28.9	8.22	28.3	43.9	28.0	7.17
Control Set 3	28.8	8.22	28.2	43.7	28.3	7.11
Control Set 4	29.0	8.22	27.8	43.1	28.4	7.14
ConC 1 Set 1	28.6	8.15	27.7	43.1	28.3	7.17
ConC 1 Set 2	28.3	8.21	28.9	44.8	28.4	7.18
ConC 1 Set 3	28.4	8.18	29.2	45.1	28.7	7.14
ConC 1 Set 4	28.9	8.24	27.9	43.3	28.6	7.15
ConC 2 Set 1	28.8	8.21	28.7	44.5	28.3	7.01
ConC 2 Set 2	28.4	8.15	28.9	45.1	28.4	7.05
ConC 2 Set 3	28.6	8.15	27.7	43.0	28.2	7.05
ConC 2 Set 4	28.8	8.15	27.0	42.0	28.6	7.04
ConC 3 Set 1	29.5	8.26	28.1	43.6	28.7	7.30
ConC 3 Set 2	29.3	8.25	29.4	45.4	28.5	7.41
ConC 3 Set 3	29.2	8.25	29.8	45.9	28.7	7.37
ConC 3 Set 4	29.3	8.25	29.3	45.3	29.0	7.43
ConC 4 Set 1	30.3	8.25	29.8	46.0	28.8	7.25
ConC 4 Set 2	29.3	8.26	29.5	45.6	28.9	7.20
ConC 4 Set 3	29.4	8.24	28.5	44.2	28.9	7.21
ConC 4 Set 4	29.4	8.24	29.0	44.8	29.0	7.28

BIOGRAPHY

Sujaree Bureekul was born on August 13th, 1980 in Chiang Mai, Thailand. She went through K-12 in Phitsanulok, Thailand. She received a Bachelor of Science degree in Marine Science from Chulalongkorn University, Bangkok, Thailand in 2001. She started her Master degree in Environmental Management, at the National Research Center for Environmental and Hazardous Waste Management, Chulalongkorn University in 2003.