การประเมินความเสี่ยงของการปนเปื้อนโลหะหนักในปลาจากแม่น้ำกวง จังหวัดเชียงใหม่และลำพูน ประเทศไทย

นายชนกันต์ จิตมนัส

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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RISK ASSESSMENT OF HEAVY METAL CONTAMINATION IN FISH FROM MAE KUANG RIVER, CHIANGMAI AND LAMPHUN, THAILAND

Mr.Chanagun Chitmanat

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Environmental Management
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Thesis Title	RISK ASSESSMENT OF HEAVY METAL CONTAMINATION IN FISH FROM MAE KUANG RIVER, CHIANGMAI AND LAMPHUN, THAILAND
By	Mr.Chanagun Chitmanat
Field of Study	Environmental Management
Thesis Advisor	Associate Professor Siripen Trichaiyaporn, Ph.D.
Thesis Co-Advisor	Professor Mark Gregory Robson, Ph.D., M.P.H. Professor Piamsak Menasveta, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.... Dean of the Graduate School

(Associate Professor Pornpote Piumsomboon, Ph.D.)

THESIS COMMITTEE

........Ekawan........ Chairman

(Assistant Professor Ekawan Luepromchai, Ph.D.)

........Siripen........ Thesis Advisor

(Associate Professor Siripen Trichaiyaporn, Ph.D.)

........Mark G Robson........ Thesis Co-Advisor

(Professor Mark Gregory Robson, Ph.D., M.P.H.)

.... Thesis Co-Advisor

(Professor Piamsak Menasveta, Ph.D.)

.... Examiner

(Nyein Nyein Aung, Ph.D.)

........Boonsom Waraegsiri........ Examiner

(Associate Professor Boonsom Waraegsiri, Ph.D.)

........Ponlayuth Sooksamiti........ External Examiner

(Ponlayuth Sooksamiti, Ph.D.)

ชนกันต์ จิตมนัส : การประเมินความเสี่ยงของการปนเปื้อนโลหะหนักในปลาจากแม่น้ำกวง จังหวัดเชียงใหม่และลำพูน ประเทศไทย. (RISK ASSESSMENT OF HEAVY METAL CONTAMINATION IN FISH FROM MAE KUANG RIVER, CHIANGMAI AND LAMPHUN, THAILAND) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. ศิริเพ็ญ ตรัยไชยาพร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.ดร.เปี่ยมศักดิ์ เมนะเศวต, ศ.ดร.มาร์ค รอบสัน, 138 หน้า.

จุดประสงค์ของการศึกษานี้ คือ 1) ตรวจสอบคุณสมบัติของน้ำทางกายภาพและเคมีของแม่น้ำกวง 2) เปรียบเทียบปริมาณโลหะหนักแคดเมียม (Cd) ตะกั่ว (Pb) และสังกะสี (Zn) ในน้ำ ดินและปลา 3) ศึกษารูปลักษณ์ของ Cd, Pb และ Znในตะกอนดินจากแม่น้ำกวง 4) ตรวจสอบการตอบสนองของปลาที่อาศัยอยู่ในแหล่งน้ำที่มีการปนเปื้อนโลหะหนัก 5) ทดสอบความเป็นไปได้ในการให้อาหารผสมสาหร่ายไก (*Cladophora* และ *Microphora*) แก่ปลาดุกรัสเซียเพื่อป้องกันพิษจากตะกั่วและลดการสะสมตะกั่วในเนื้อเยื่อปลา

พบว่า ปริมาณ Pb และ Cd ที่ปนเปื้อนในน้ำอยู่ในระดับต่ำกว่าที่เครื่องมือตรวจวัดได้ แต่ปริมาณ Zn ที่ปนเปื้อนในน้ำอยู่ในช่วง 0.01 - 0.11 มิลลิกรัมต่อลิตร ความเข้มข้นของ Pb, Cd และ Zn ในตะกอนดินมีค่า 3.13 – 27.56, <0.02 – 0.43 และ 3.42 – 10.32 มิลลิกรัมต่อกิโลกรัม ตามลำดับ ปริมาณ Pb และ Cd ในปลาช่อน (*Channa striata*) เท่ากับ <0.05 – 2.13 และ <0.02 – 0.24 มิลลิกรัมต่อกิโลกรัม ในขณะที่ปริมาณของสังกะสีในเนื้อปลาช่อนเท่ากับ 3.37 – 12.19 มิลลิกรัมต่อกิโลกรัม

ตัวบ่งชี้ทางชีววิทยา เช่น อัตราส่วนระหว่างตับและน้ำหนักปลา ระดับกลูโคสในซีรั่ม เอนไซม์แคทาเลส และ ไลโซไซม์ สามารถใช้เป็นตัวบ่งชี้ถึงผลของการสัมผัสสารพิษที่ปนเปื้อนในแหล่งน้ำ อย่างไรก็ตามการใช้ตัวบ่งชี้ทางชีวภาพในการตรวจสอบภาคสนามอาจจะยากที่จะประเมินผลสำหรับแหล่งน้ำที่มีการปนเปื้อนของสารพิษหลากหลายชนิด ปลาดุกรัสเซียที่ได้รับอาหารผสมสาหร่ายไกมีการเจริญเติบโตสูงขึ้น ปริมาณเม็ดเลือดเพิ่มขึ้นและมีการทำงานของเอนไซม์ไลโซไซม์ดีขึ้น ($P < 0.05$) และหลังจากปลาดังกล่าวได้สัมผัสกับสารตะกั่วในน้ำ พบว่าปลาที่ได้รับสาหร่ายมีการทำงานของเอนไซม์ไลโซไซม์และแคทาเลสเพิ่มขึ้นกว่าปลาในชุดควบคุม ($P < 0.05$) การทดลองนี้ชี้ให้เห็นว่าอาหารผสมสาหร่ายไกมีส่วนช่วยป้องกันปลาดุกจากพิษของตะกั่ว แต่ไม่สามารถลดการสะสมตะกั่วในเนื้อปลา

ความรู้ที่ได้จากการศึกษาครั้งนี้จะให้ข้อมูลที่มีประโยชน์สำหรับการตรวจเฝ้าระวังการปนเปื้อนโลหะหนักในแม่น้ำ รวมทั้งใช้เป็นแนวทางในการพัฒนาอาหารปลาในการป้องกันความเครียดที่เกิดจากความเสื่อมโทรมของสภาวะแวดล้อม

สาขาวิชา.....การจัดการสิ่งแวดล้อม.........ลายมือชื่อนิสิต ....
ปีการศึกษา.............2553.....................ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..........................
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ....
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ....

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KEYWORDS : MAE KUANG RIVER / HEAVY METAL / RISK ASSESSMENT / CATFISH / KAI ALGAE

CHANAGUN CHITMANAT: RISK ASSESSMENT OF HEAVY METAL CONTAMINATION IN FISH FROM MAE KUANG RIVER, CHIANGMAI AND LAMPHUN, THAILAND. THESIS ADVISOR: ASSOC. PROF. SIRIPEN TRAICHAIYAPORN, Ph.D., THESIS CO-ADVISOR: PROF. PIAMSAK MENASVETA, Ph.D., PROF. MARK GREGORY ROBSON, Ph.D., M.P.H., 138 pp.

The objectives of this study aimed: i) to determine the physico-chemical status of water in the Mae Kuang River; ii) to compare the results of heavy metals including cadmium (Cd), lead (Pb), and zinc (Zn) in water, sediment, and fish tissues ; iii) to study the speciation of Cd, Pb, and Zn in sediments along Mae Kuang River; iv) to identify responses in exposed fish; v) to investigate a possible protective effect of Kai algae (*Cladophora* and *Microphora*) as fish supplementary diet against lead-induced toxicity in catfish.

Pb and Cd in water were below detection limits, while Zn concentrations in water ranged 0.01 – 0.11 ppm. The Pb, Cd and Zn concentrations in sediment were 3.13 – 27.56, <0.02 – 0.43 and 3.42 – 10.32 mg/kg, respectively. Pb and Cd residues in snakehead fish (*Channa striata*) were <0.05 – 2.13 and <0.02 – 0.24 mg/kg wet weight, while the concentrations of Zn in these fish were 3.37 – 12.19 mg/kg. Biological indices including hepatosomatic index, serum glucose, catalse, lysozyme activity could highlight some exposure effects in fish living in contaminated ecosystems, but their application of these effects in field biomonitoring may not be easily predictable in complex mixtures of contaminants. African fish fed with Kai showed increased growth, blood cells, and lysozyme ($P < 0.05$) compared with the control group. Following exposure with Pb, more lyzozyme and catalase activities were observed in the group fed the experimental diets than the control group ($P < 0.05$). These results indicate that Kai supplementation is useful in prevention catfish from Pb toxication but it could not reduce Pb accumulation.

The information gained from this research will be valuable for heavy metal contamination monitoring in river. In addition, it provides the guideline for the development of diet to mitigate fish stress from deteriorated environment.

Field of Study : Environmental Management	Student's Signature C Chitt

Academic Year : 2010	Advisor's Signature

Co-Advisor's Signature

Co-Advisor's Signature

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CHAPTER I

INTRODUCTION

1.1 Statement of Problems

The Mae Kuang River flows through large catchment areas into the Ping River, merges with others, and finally converges to the Chao Phraya River, a major river in Thailand. Currently, the surface water quality of Mae Kuang River is extremely deteriorating due to anthropogenic activities, industrialization expansion, agricultural farming activities, urbanization, animal and human excretions, and domestic wastes. An urban drain which generally collects industrial wastewaters is a major metal contamination source in densely populated area (Sörme and Lagerkvist, 2002). In addition, the Lamphun Northern Industrial Estate has released wastewater into this river. Some effluents are directly discharged into this river without treatment. There are limited data available on heavy metal pollution at this moment. For this reason, it is necessary to routinely monitor the water quality in order to get the data which can be used for effective management policies.

In addition to water quality examination, sediments which usually serve as a pool for pollutants (Fatoki and Mathabatha, 2001; McCready *et al*., 2006) should be simultaneously conducted. The distribution of metals in sediments adjacent to human settlement and industrial areas can provide researchers with evidence of the anthropogenic impact on ecosystems and, therefore, aid in assessing the risks associated with discharged human waste (Demirak *et al*., 2006). These trace metals are able to move towards the water column or accumulate in plants, aquatic animals, and consequently enter the food chain. The heavy metal concentration in fish tissues also reflects past exposure via water and/or food and it can demonstrate the current situation of the aquatic animals before toxicity affects the ecological balance of populations in the aquatic environment (Forstner and Wittmann, 1983). In certain regions of Mae Kuang River which are close to Lumphun Industrial Estates, some fish are often consumed without any information of this toxic content. In addition, indigenous fishes are used as bioindicators in this study. Heavy metal treatments showed significant reduction in carbohydrate and lipid contents in muscles as well as in gills in Indian major carps, *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* (Garg *et al*., 2009). Although reports on the use of fishes as biomonitors of heavy metal pollution can be widely found in the literature, such studies that had been done in this region are non-existent.

Catfish is one of economically cultured species in Thailand, and is increasingly being cultured. Commercial catfish production generates over 22 percent of the value of freshwater aquaculture production in 2007. As fish are an important food resource and heavy metals may accumulate in the body throughout the food chain leading to a serious threat to human health. Unfortunately, only a limited number of studies have determined metal concentrations in farmed fish. Calvi *et al.* (2006) observed higher levels of arsenic, lead, and zinc in farm-raised eel versus wild-caught eel. The purpose of this study also was to investigate the immunostimulatory effect of Kai in feed on aspects of the nonspecific immune response and to determine if Kai is able to stimulate protection against lead toxicity and prevent its accummulation in catfish. Watanuki *et al.* (2006) suggested that dietary *Spirulina* has immunostimulatory effects on the innate immune system of carp. Until now there are not many studies on the effects of heavy metals concerning immune competence of catfish. Thus, this investigation is the first to deal with immunomodulation in catfish exposed to sublethal concentrations of lead. This study proposes to determine the protective effects of the dietary Kai green algae (*Cladophora* and *Microphora*) in African catfish because Kai has been shown to exhibit antioxidant activity (Malaiwan and Peerapornpisal, 2007).

1.2 Theoretical basis of research

1) The heavy metals are able to accumulate in various fish tissues, such as muscle, liver, and gills.

2) There are differences in heavy metal residues and biomarker parameters in fishes at contaminated locations and different seasons.

3) The changes in enzymatic activities and immune responses are indicators of heavy metal exposure and the effects of heavy metals on aquatic organisms.

4) Health risks to human via dietary intake of fishes will be assessed by the target hazard quotients.

1.3 Research objectives

The objectives of this study aimed:

(i) to determine the physical and chemical status of water in the Mae Kuang River;

(ii) to compare the results of heavy metals including cadmium (Cd), lead (Pb), and zinc (Zn) in fish tissues, sediment, and water;

(iii) to study the speciation of Cd, Pb, and Zn in sediments along Mae Kuang River;

(iv) to identify biological responses including hepatosomatic index, Pack cell volume, blood glucose, serum protein, lysozyme, and antioxidant enzymes (catalase, superoxide dismutase, and glutathione-S-transferase) in exposed fish;

(v) to investigate a possible protective effect of Kai algae (*Cladophora* and *Microphora*) as fish supplementary diet against lead-induced toxicity in catfish, and

(vi) to conduct participation action in order to brain-storm all stakeholders to find management strategies for Mae Kuang River.

1.4 Scope of Study

This research contained three main parts as follows;

1.) **Part A** involved the determination of Cd, Pb, and Zn in sediments, water, and three indigenous fish species. This monitoring was conducted every month but fish sampling was conducted three times; in summer, rainy, and winter seasons. The changes in enzymes and immunity were examined only in snakehead fish which were caught and alive in contaminated sites.

2.) **Part B** was designed to test the possibility of the use of Kai as supplementary feed for catfish in order to protect fish from lead toxicity. African catfish were fed with Kai supplementary diet for 3 months and then challenged with various concentration of lead in water. The ability of protective effects in catfish was measured by the alterations of enzymes and immunity after lead exposure. In addition, the bioaccumulation of lead in catfish was determined to see if exposed catfish is safe for consumption or not.

3.) **Part C** was public hearing. The information from part A was used in meeting in order to give the update information about the current status of water quality in Mae Kuang River. The brain-storm discussion among stakeholders in order to solve this problem was conducted.

CHAPTER II

LITERATURE REVIEWS

2.1 Mae Kuang River

The Mae Kuang River with a length of approximately 112 kilometers is one of the most important water resources of the Northern Thailand. This river runs downstream towards the Ping River, which runs through provinces, merges rivers and finally converges to the Chao Phraya River, a major river in Thailand. This river has become severely degraded over recent years, and is particularly threatened by the expansion of industry, settlement and agriculture. A national alert has called for rehabilitation of the water quality of the Mae Kuang River since the water quality in this river was classified as class 3 – 4 witnessed incidences of suspicious fish deaths in these areas. This fresh surface water is fairly clean in rainy season so it can be used for consumption, but requires special water treatment process before using. However, it turns to be dark, smelly, and dry in summer. This river flows past the Lamphun industrial park where villagers suspect if the pollutants are released from this area.

2.2 Heavy metals

Heavy metals refer to metals with a density greater than 5 g/cm^3. They are naturally occurring constituents in the environment and vary in concentrations across geographic regions. As a result of fast-growing industrialization and not-well planned community expansion, contaminated toxic metals are continually elevated. Heavy metals released into natural waters can be a serious threat because of their possible bioaccumulation and biomagnifications in the food chain (Rashed, 2001).

The main pollution sources of heavy metals are different among the cities. Cadmium (Cd), lead (Pb), and zinc (Zn) are common constituents of industrial effluents which were targeted in this study because of their potential for human exposure and increased health risk. The pollution sources of Cd, Pb, and Zn may be mainly derived from industrial sources such as electroplating plant, spring factory, band steel factory, leather factory, petrochemical complex, etc. Furthermore, sources of Cd in agricultural soils may be also originated from pesticides and fertilizers.

2.2.1 Cadmium

Cadmium (Cd) is one of the most toxic heavy metals and a common environmental contaminant. It occurs in naturally high abundance in mining activities, in some sewage sludge, and in phosphate fertilizers. The uses of phosphate fertilizers in farm, sewage sludge, and industrial application of cadmium have been identified as a major cause of widespread dispersion into the environment and foodstuffs. Cd is also a by-product of zinc and lead mining and smelting (Klaassen, 2001).

Cd is able to enhance lipid peroxidation by increasing the production of free radicals in several organs, mainly lung and brain; likewise to interfere with the cellular mechanisms against oxidation (glutathione peroxidase and catalase) and this should be considered as a potential significant event in the generation of free radicals. As a result, tissue damage and cellular death are observed (Kumar *et al*., 1996; Casalino *et al*., 2002). Cd can generate free radicals also leading to the oxidation of nuclei acids and change in DNA repair mechanisms and alterations of membrane structure/function (Kumar *et al*., 1996; Hartwig *et al*., 2002). Cd is considered as a potential carcinogen and is associated with etiology of a number of diseases, especially cardiovascular, kidney, nervous system, blood as well as bone diseases (Järup and Åkesson, 2009). Cd has a long biological half-life, from 17 to 30 years in humans (Castro-Gonzalez and Mendez-Arment, 2008). Excessive cadmium in drinking and irrigating water resulted in itai-itai disease first characterized in Japan. People who had eaten contaminated rice suffered from bone disease. It is reported that river water contains dissolved cadmium ranging between less than 1 and 13.5 ng/L (WHO, 1998). The occupational levels of cadmium exposure prove to be a risk factor for chronic lung disease and testicular degeneration (Benoff *et al*., 2000).

2.2.2 Lead

Lead (Pb) is a highly toxic element. The presence of anthropogenic lead in aquatic systems is mostly due to the burning of leaded fuels, metal smelters and mining activities (Alleman *et al*., 2001). It is extremely stable in compound forms; as a result, it is the high-quality paint because of its cracking and peeling resistance. Pb in household paint was banned in Australia in 1920, by international convention in 1925, and by the United States in 1978. The phase-out of Pb in gasoline began in the mid-1970s in the United States; many other countries have also banned leaded gasoline. Subsequently, Thailand banned leaded gasoline in 1996. Although Pb emissions into the environment have declined markedly as a result of the decreased use of leaded gasoline, Pb is still a potential problem in aquatic systems because of industrial application. Pb is able to move into the aquatic systems after emitted into the atmosphere or soil. Both surface water and groundwater possibly contain considerable amounts of Pb derived from these sources. A residence time of Pb in surface waters was ~ 2 years, whereas in deep waters a residence time of a few hundred years was estimated (Capodaglio, 1990). The uptake and accumulation of lead by aquatic organisms from water and sediment are influenced by various environmental factors such as temperature, salinity, and pH, as well as humic and alginic acid content.

Exposure to Pb is associated with numerous adverse effects including cognitive dysfunction (Morgan *et al*., 2000), hearing impairment, and weak immunity (Wang *et al*., 2009). The accumulation of this metal in the bone marrow could result in adverse effects to progenitor cells and consequently lower the resistance to a variety of pathogens (Westerman *et al*., 1965). Impaired male reproductive function and depressed adrenal and pituitary function have been also noticed for lead poisoning (Erfurth *et al*., 2001). Pb exposure in children and adults can cause a wide spectrum of health problems, ranging from convulsions, coma, renal failure and death at the high end to subtle effects on metabolism and intelligence at the low end exposures (US Agency for Toxic Substances & Disease Registry, 1999). Furthermore, lead acetate and lead phosphate are listed as reasonably anticipated human carcinogens (NIEHS, 1994).

2.2.3 Zinc

Zinc (Zn) is an essential structural, catalytic and regulatory micronutrient for many enzymes and critical for protein synthesis, cell proliferation, growth, development and reproduction (Brandão-Neto *et al*., 1995). In addition, Zn is used to prevent rust and mix with other metals to make alloys including brass and bronze. Although Zn is necessary for life, this metal is also a problematic environmental toxicant (Hogstrand *et al*., 2002). Zinc is present only in the divalent state, Zn (II). Zn is normally found in association with other including as copper and lead. Increased Zn supply may reduce Cd absorption and accumulation and prevent the adverse actions of Cd, whereas Zn deficiency can enhance Cd accumulation and toxicity (Brzóska and Moniuszko-

Jakoniuk, 2001). In humans, prolonged excessive dietary intake of Zn can lead to deficiencies in iron and copper, nausea, vomiting, fever, headache, tiredness, and stomach pain (Dural *et al*., 2007). No reports on carcinogenicity of zinc and compounds on humans are available (Fosmire, 1990).

The largest natural emission of zinc to water results from erosion. Natural inputs to air are mainly due to igneous emissions and forest fires. The production for sulfidic zinc ores produces large amounts of sulfur dioxide and cadmium vapor. Coal and fuel combustion, waste disposal and incineration, and the use of zinc-containing fertilizers and pesticides also contain significant amounts of zinc.

However, Zn concentrations in freshwater and seawater are not sufficient to meet the requirement for aquatic organisms. Hence, Zn is regarded as an essential nutrient in fish and shrimp feeds (Li *et al*., 1995). Gatlin *et al*. (1991) reported that dietary Zn requirement of the red drum was between 20 and 25 mg/kg diet. Whereas, the requirement of Atlantic salmon for Zn is more than and equal 67 mg/kg diet (Maage and Julshamn, 1993).

2.3 Heavy metals contamination in water, sediment, and fish

Heavy metals present in rivers as a result of chemical leaching of bed rocks, water drainage, runoff from the banks, and discharge of urban and industrial wastewaters. Although these toxic metals are usually present at low concentration in aquatic ecosystem, deposits of anthropogenic origin have raised the metal accumulation, creating environmental problems in coastal zones, lakes and rivers. Low pH in waters keeps heavy metals in solution leading to an amplification of water contamination. Klavins *et al.* (2000) reported the world average of background concentrations for Cd, Cu, Pb, and Zn were 0.02, 1.00, 0.2, and 10 µg/L, respectively. The metal concentrations of surface waters from river Dzindi, Madanzhe and Mvudi in Thohoyandou, South Africa were: 1.6 – 9.3, 2.0 – 3.0, 10.5 – 20.1 and 2.1 – 2.5 µg/L for Cd, Cu, Pb and Zn, respectively (Okonkwo and Mothiba, 2005). These metal concentration ranges were below the international guidelines and acceptable concentrations for drinking water except the values for Cd and Pb. All metals, except for Pb, in water from Panyu-Nansha area of Pearl River estuary, China were below the safe values for aquatic life (Zhang *et al*., 2010). Total metals in water from the estuary of Tinto and Odiel rivers in Huelva (Spain), one of the most metallic polluted estuaries in Europe were ranging over following intervals: Zn: 49.8 – 381.6 µg/L; Cd: 0.7 – 8.9 µg/L; and Pb: 2.6 – 17.8 µg/L (Vicente-Martorell *et al*., 2009). Cd and Pb monitored along the Mae Kuang River in 2003 – 2004 were not detected - 0.0002 mg/L and not detected - 0.0002 mg/L, respectively (Chumroonrat, 2004). However, heavy metals can be transferred from stream waters to stream sediments through two main paths including precipitation (when pH of stream water rises) and adsorption. Decreasing in water turbulence and evaporation during summer also favor metal precipitation from waters to stream sediments (Concas *et al*., 2006).

Metals tend to accumulate in sediments where can be held up to a million times more metal than an equivalent volume of water. For this reason, concentrations of heavy metals in sediments were considerably higher than those obtained in river. Grain size is one of the controlling factors affecting natural concentrations of trace metals in sediments (Zhang *et al*., 2001). Fine-grained sediments tend to have relatively high metal concentrations due in part to the high specific surface area of the smaller particles. Background concentrations of Cd, Cu, Pb, and Zn from sediments in River were 0.38, 17, 14, and 67 mg/kg dry weight, respectively (Bervoets and Blust, 2003). Total concentrations of metals in the sediments from Gomti River, India were Cd 0.34 – 8.38, Pb 6.3 – 75.3, and Zn 3.1 – 101.7 mg/kg dry weight (Singh *et al*., 2005). Metal concentrations in sediments in Almendares River, Cuba ranged from 86.1 to 708.8 for Zn, 39.3 to 189.0 for Pb, and 1.0 to 4.3 for Cd mg/kg dry weight (Olivares-Rieumont *et al*., 2005). Surface sediments (0 – 5 cm) from 59 stations within the Yangtze River intertidal zone, China were sampled and determined for metal contamination in April and August 2005. The concentrations ranged: Cd, 0.12 – 0.75; Cr, 36.9 – 173; Cu, 6.87 – 49.7; Mn, 413 –1,112; Ni, 17.6 – 48.0; Pb, 18.3 – 44.1; and Zn, 47.6 – 154 mg/kg dry weight; respectively (Zhang *et al*., 2009).

Sediments can serve as temporary sinks from which some of the metal can enter ecological and human food webs through several routes (Fairbrother *et al*., 2007; Macdonald *et al*., 2000), primarily through accumulation in benthic organisms. These organisms include those that fully or partially live in the sediments (e.g., tubificids, chironomids, trichopteran larvae) or those that feed from the sediment bed (e.g., suckers, carp). Some aquatic organisms obtain these toxic substances from both pelagic and benthic routes (e.g., lake whitefish, walleye, snakehead), but due to high chemical concentrations in sediments, the benthic route can be the dominant route of uptake (Morrison *et al*., 1996). Moreover, bioaccumulation is to a large extent mediated by abiotic and biotic factors that influence metal uptake (Pyle *et al*., 2005). Due to the deleterious effects of metals on aquatic ecosystems, it is necessary to monitor their bioaccumulation in key species, because this will give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on organism health (Kotze *et al*., 1999). Natural background total zinc concentrations are usually more than 0.1 – 50 μg/L in freshwater, 0.0002 – 0.1 μg/L in seawater, 10 – 300 mg/kg dry weight in soil, up to 100 mg/kg dry weight in sediments.

Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from water (Mansour and Sidky, 2003). Furthermore, fish are one of the most indicative factors in freshwater systems, particularly for the estimation of the risk potential from human consumption. Heavy metals taken up by an organism are distributed to different organs of the fish because of the affinity between them. Fish accumulate heavy metals in higher concentrations in their tissues, mainly through ingestion of contaminated food or by environmental absorption along the gill surface (Kraal *et al*., 1995), with metals being accumulated mainly in metabolically active tissues (Kock and Hofer, 1998) including gills, liver, kidney, and digestive tract (Miliou *et al*., 1998). The gills are the uptake site of waterborne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (Jezierska and Witeska, 2001). Fish liver is the storage organ and thus was mostly used because of the fact that it accumulates the highest level of heavy metals (Amundsen *et al*., 1997; Papagiannis *et al*., 2004; Farkas *et al*., 2003; Yilmaz, 2003; Yilmaz *et al*., 2007) and is proportional to those present in the environment (Dural *et al*., 2007). Kidney and liver showed higher enrichment coefficients than gill, muscle and swim bladder (Kargin, 1998; Liu *et al*., 2001). It was also found that gonad showed a high enrichment coefficient for Zn (Wong *et al*., 2001). In fact, several works have reported that some fish species are far more sensitive to heavy metals toxic effects than mammals (Kelly *et al*., 1998).

The concentrations of Cd, Pb, Zn and Cr in gills were higher than that in muscle of *Leuciscus cephalus* from a stream in southwestern Turkey (Demirak *et al*., 2006). Begum *et al*. (2005) suggested that there is a need for continuous monitoring of heavy metal concentrations in edible freshwater fish in Bangladesh because the lead levels found in 3 species of fish in Dhanmondi Lake were twice the maximum level regulated by the European Union at 0.2 mg/kg wet weight or 0.96 μg/g dry weight. Farkas *et al*. (2003) measured the liver concentrations of cadmium in freshwater fish populating a low contamination site to exceed the tolerable levels for human consumption. Season (Kargin, 1996), length, weight, and age of organisms, physical and chemical status of water (Jezierska and Witeska, 2001) affected on metal accumulation in fish. Different fish species contained different metal levels in their tissues because of variations in feeding habits, habitats and behavior (Dural *et al*., 2007). Seasonal variations in metal concentrations may be caused by such factors as land drainage to the estuary, availability of food, temperature, and the reproductive cycle and condition of the organism (Wright and Mason, 1999).

2.4 Effects of Cadmium, Lead and Zinc on Fish

Acute toxicity to aquatic organisms depends on the physical and chemical factors affecting speciation, complexation, and competition of metals for interaction at the biotic ligand (Fairbrother *et al*., 2007). High heavy metal contamination in water can retard fish development resulting in fish size alterations (Friedmann *et al*., 1996). The early life stages such as hatching time, larval development and juvenile growth is more sensitive to toxic metals than the mature stages.

Cd is a non-nutrient and nondegradable cumulative pollutant which is toxic to fish even at low concentrations. The harmful effects of Cd include bioaccumulation, mild anemia, osteoporosis, renal damage, and emphysema (Peraja *et al*., 1998). After entering into the organism through water, cadmium binds to albumins and erythrocytes in the blood and then is transferred into tissues and organs, where it is bound to proteins of low molecular mass producing metallothioneins by the induction of metallothionein mRNA synthesis (George *et al*., 1996). Cd increases the production of reactive oxygen species (ROS) in tissues and inhibits the activity of some enzymes of the antioxidative defense system (Šikic *et al*., 1997). ROS include a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H_2O_2), superoxide ($O_2\bullet-$) and hydroxyl radical ($OH\bullet$). ROS are formed and degraded by all aerobic organisms and can readily react with many biomolecules including proteins, lipids and lipoproteins and DNA (Nordberg and Arner, 2001). Lipid peroxidation is a well-established mechanism of cellular injury, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds (Romero *et al*., 1999). Aerobic organisms are protected against free radicals by antioxidants including endogenously synthesized compounds such as reduced glutathione (GSH), superoxide dismutase (SOD) and nitric oxide (NO) (Hudecova and Ginter, 1992). Cd accumulates in sensitive organs like gills, liver, and kidney of fish in an unregulated manner (McGeer *et al*., 2000). The acute toxicity of cadmium to aquatic organisms is variable in fish species, and is related to the free ionic concentration of the metal.

Zn is a micronutrient involving in an active role in many enzyme systems; however, it is able to be hazardous to aquatic organisms at high concentrations (Vallee and Falchuk, 1993; Wood, 2001). Zn is homeostatically regulated in the liver and kidney, and mostly accumulated in the skin, muscle and bone of fish (Glynn, 1991). Zinc increases the toxicity of cadmium to aquatic invertebrates. Zn^{2+} and Ca^{2+} are divalent cations and could conceivably compete for the same transport mechanisms (Gómez *et al*., 1998). There were differences in Zn toxicity on fish due to different pH, hardness and alkalinity as well as fish stages and species. Zn toxicity on Cnesterodon decemmaculatus were strongly affected by water chemistry, decreasing with increasing hardness, alkalinity, and conductivity (Gómez *et al*., 1998). High levels of zinc leads to gill damage involving the separation of epithelium, enlargement of central and marginal channels, occlusion of central blood spaces, and results in decreased oxygen consumption, the ability to transport ions across the gill surface and an increase in hypoxia ventilation frequency (Lloyd, 1992).

Pb is also one of the most commonly used metals in industry and its toxicity is important partly due to its persistence in the environment (Gurer-Orhan *et al*., 2004). As a result, this metal may progressively increase in water, sediments or in biological tissues to levels above natural background. Pb is known to adversely affect many organs and systems in aquatic organisms and humans, where the hematological system is one of the important targets. Pb is shown to induce changes in the composition of red blood cell (RBC) membrane proteins, induce lipid peroxidation, and inhibit hemoglobin synthesis. Acute lead poisoning in human results in severe distortion in kidney, reproductive system, brain, and nervous system. Lead poisoning from environmental exposure is believed to cause mental retardation in children. Some *in vitro* and *in vivo* animal studies suggested that lead-induced oxidative damage contributes to RBC damage in lead intoxication. This was supported by several human studies where the disrupted antioxidant balance was demonstrated in lead-exposed workers (Sugawara *et al*., 1991). Waterborne Pb exerts toxic effects in fish through disturbance of ionoregulatory mechanisms, evident in the disruption of Ca^{2+} balance and the interference with Na^{+} and Cl^{-} regulation (Rogers *et al*., 2003).

2.5 Speciation of heavy metals

Total metal contents in sediments cannot provide sufficient information about mobility, bioavailability and toxicity of metals. Metals deposited on the sediment can be present as particles or associated with sediment components. Metals can be mobilized from solid phases due to changes in pH or redox conditions and complexation with organics, and subsequently transported in the aquatic phase (Heier *et al*., 2009). Sequential extraction can provide information about the identification of the main binding sites, the strength of metal binding to the particulates and the phase associations of trace elements in sediment. This could help us to better understand the geochemical processes governing heavy metal mobilization and potential risks induced (Yuan *et al*., 2004). In unpolluted soils or sediments trace metals are mainly bound to silicates and primary minerals forming relatively immobile species, whereas in polluted ones trace metals are generally more mobile and bound to other soil or sediments phases (Rauret, 1998).

Speciation refers to the distribution of metal species in a particular sample or matrix or species distribution (Templeton *et al*., 2000). The metals exist as aqueous (or dissolved) species bound to colloids and those bound to sediment particles through an exchangeable binding process. This pool is often referred to as the "exchangeable" or "labile" pool. The second pool consists of metals found within the mineral matrix of the sediment solids. This pool is largely unavailable to biota, and its release will occur over geologic time scales, through diagenetic processes. Because the latter pool is largely unavailable, only the exchangeable pool of metals is considered. Note that the exchangeable pool will be composed of naturally occurring metals that are released into solution as a result of weathering and diagenetic processes as well as metals released into the environment as a result of anthropogenic activities. The exchangeable pool of metals is subject to speciation in the aqueous phase (e.g., within the pore water) and sorption to solid phases, where sorption is a general term that includes adsorption (the accumulation of matter at the solid–water interface or a two-dimensional process) and absorption (inclusion in a three-dimensional matrix) (Stumm and Morgan, 1996). In the aqueous phase, metal will react or bind with dissolved ligands according to the pH, Eh, ionic strength, and abundance of ligands. The binding capacity of the selected metals to the organic matter decreases in the following order: Zn > Pb > Cd (Soares *et al*., 1999).

Referring to partitioning patterns for Zn, Cd, Cu, and Pb in sediment samples from Kolleru lake, India indicated that all metals were more associated with mobilisable fraction (exchangeable and carbonate bound), these metals are more available to aquatic life (Sekhar *et al*., 2004). Fish gills may accumulate bioavailable trace elements, and metal accumulation on gills reflects the speciation of metals in water (Rosseland *et al*., 1992). Although cadmium occurs in the aquatic organism and marine environment only in trace concentrations, it was found to be associated with more labile fraction and the salinity can affect the speciation of this metal, and bioaccumulation is affected both by temperature and salinity (Ray, 1986). Lead is the least mobile and is present mainly in the residual fraction in amounts over 90% in most of the studies (Morillo *et al*., 2002).

2.6 Risk assessment

Risk assessment is referred to as the process of assessing magnitudes and probabilities to the adverse effects of human activities or natural catastrophes (Suter, 1993). It consists of eight steps, which are defined as follows (Van Leeuwen and Hermens, 1995):

2.6.1 Hazard identification is the identification of the adverse effects, which may be caused by chemicals. The likelihood of harm due to exposure distinguishes risk from hazard.

2.6.2 Effect assessment is the estimation of the relationship between dose or level of exposure to chemicals and the incidence and severity of an effect. Most of the experiments are carried out in order to determine a no effect level (NEL), which can be converted to a predicted no effect level (PNEL) or a predicted no effect concentration (PNEC) for other species.

2.6.3 Exposure assessment is the estimation of concentrations or doses to which human populations or environmental compartments are or may be exposed. For existing chemicals exposure can be assessed by measuring concentrations, while for new chemicals a predicted environmental concentration (PEC) can be estimated.

2.6.4 Risk characterization is an integration of the first three steps of the risk assessment process in order to estimate the incidence and severity of the deleterious effects likely to occur due to actual or predicted exposure to chemicals. For newly developed chemicals the PEC/PNEC ratio, i.e. the risk quotient, can be determined. The risk quotient, combined with uncertainty factors, links the risk analysis to the risk management by quantifying the hazards and risks for specific situations (Duke and Taggart, 2000; Jager *et al*., 2001).

2.6.5 Risk classification is the evaluation of risks in order to decide if risk reduction is required. Generally, risk classification is performed using two risk levels, in which the upper limit is the maximum permissible level (MPL) and the lower limit is the negligible level (NL).

2.6.6 Risk – benefit analysis is the drawing up of a balance sheet of the respective risks and benefits of a proposed risk – reduction action.

2.6.7 Risk reduction is taking measures to protect man and/or the environment from the risks identified. A risk reduction may be achieved by defining safety standards, such as the acceptable daily intake (ADI).

2.6.8 Monitoring is a repetitive observation for defined purposes of one or more chemical or biological elements. During a problem formulation chemical and biological monitoring (BM) of ambient waters may indicate deviations from the normal (alarm and trend function), thus triggering problem recognition. During the risk analysis stage chemical monitoring (CM) of receiving waters as well as selected effluents can help in exposure characterization, while BM may be used to predict ecological effects (instrument function and early-warning). During the risk management stage monitoring will help by verifying control strategy results and in checking compliance (control function).

2.7 Human health risk

Although essential metals provide some components of an important biochemical or enzymatic reaction for human and animals, a number of nonessential toxic metals may generate hardzard. World Health Organisation (WHO, 1998) drinking water guideline values for the dissolved Cd, Pb, and Zn concentrations are 3, 10, and 3,000 µg/L. Increased heavy metal concentrations especially mercury, cadmium and lead have been observed in freshwater fish in open waters (Castro-Gonzalez and Mendez-Arment, 2008). Heavy metal bioaccumulation in fish tissues is influenced by biotic and abiotic factors including fish biological habitat, chemical form of metal in the water, water temperature and pH value, dissolved oxygen concentration,water transparency, as well as by fish age, gender, body mass, and physiologic conditions (Has-Schon *et al*., 2006).

For carcinogenic contaminants, the observed or predicted exposure concentrations are compared with thresholds for adverse effects or the toxicant reference value as determined by dose–effect relationships (Solomon *et al*., 1996). The probability risk assessment technique has been adopted to fully utilize available exposure and toxicity data (Hall *et al*., 1999; Wang *et al*., 2002). For carcinogens, risks are estimated as the incremental probability of an individual to develop cancer over a lifetime. Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000). Target carcinogenic risk (TR) was calculated using the following equation;

$$TR = \frac{EF \ X \ ED \ X \ FIR \ X \ C \ X \ CSFo \ X10^{-3}}{WAB \ X \ TA}$$

Where EF is exposure frequency (365 days/year);

ED is the exposure duration (70 years), equivalent to the average lifetime;

FIR is the food ingestion rate (fish: 36 g/person/day; FAO, 2005);

C is the metal concentration in fish (mg/kg);

CSFo is the oral carcinogenic slope factor from the Integrated Risk Information System (US EPA, 2010) database (8.5×10^{-3} $(mg/kg/day)^{-1}$ for Pb).

WAB is the average body weight (60 kg);

TA is is the number of days over which the exposure is averaged (365 days per year × ED for non-carcinogenic effects and 25,500 days (70 years × 365 days/year) for carcinogenic effects).

However, these methods are only applied to quantify the magnitude of health risks of carcinogenic pollutants, but not for quantifying non-cancer risks. Current non-cancer risk assessment methods do not provide quantitative estimates on the probability of experiencing non-cancer effects from contaminant exposures. These are typically based on the target hazard quotient (THQ), which is a ratio of determined dose of a pollutant to the dose level (a Reference Dose or RfD). If the ratio is less than 1, there will not be any obvious risk. Conversely, an exposed population of concern will experience health risks if the dose is equal to or greater than the RfD. The method for the determination of THQ was provided in the U.S. EPA Region III risk-based concentration table (US EPA, 2000). It was further assumed that the ingested dose is equal to the absorbed pollutant dose as stated in the U.S. EPA guidance (US EPA, 1989).

Chien *et al.* (2002) determined health risks associated with heavy metals based on the target hazard quotients (THQs), which can be derived from concentrations of heavy metals in fish consumed in four districts (Dong Li, Xi Qing, Jin Nan, and Bei Chen) and the urban area of Tianjin, China. They applied the following equation and assumed that cooking has no effect on the toxicity of heavy metals in food;

$$THQ = \frac{EF \times ED \times FIR \times C \times 10^{-3}}{RFD \times WAB \times TA}$$

Where EF is exposure frequency (365 days/year);

ED is the exposure duration (70 years), equivalent to the average lifetime;

FIR is the food ingestion rate (fish: 36 g/person/day; FAO, 2005);

C is the metal concentration in fish (mg/kg);

RFD is the oral reference dose

(Cd = 1 X 10^{-3}, Pb = 0.004, and Zn = 0.3 mg/kg/day) (US EPA, 2000);

WAB is the average body weight (60 kg);

TA is the averaging exposure time for non-carcinogens (365 days/year X ED).

It was found that no THQ values over 1 through the fish consumption suggesting that health risks for the local inhabitants in Dong Li, Xi Qing, Jin Nan, and Bei Chen Districts, and the urban area of Tianjin, China associated with heavy metals exposure is not significant (Chien *et al*., 2002). Vieira *et al*. (In press) analyses non-carcinogenic risks from exposure to metals through fish consumption of three commonly consumed and commercially valuable fish (sardine, chub and horse mackerel) captured from the Northeast and Eastern Central Atlantic Ocean in Portuguese waters and indicated that these fishes were safe for human consumption in terms of the amounts of Cd and Pb found in muscles (THQ values <1).

The Turkish legislation establishes maximum levels for human consumption as following; 0.1 mg/kg for Cd, 1.0 mg/kg for Pb, 20.0 mg/kg for Cu, and 50 mg/kg for Zn (Anonymous, 1996). Food and Agricultural Organization limits for Cd and Pb 0.5 mg/kg, for Cu and Zn 30 mg/kg ((Food and Agriculture Organization) FAO, 1983).There is also legislation in other countries regulating the maximum concentration of metals. For example, Spanish legislation limits the levels for Cd at 1 mg/kg, and Pb at 2 mg/kg

(Usero *et al*., 2003). Referring to the UK Food Standards Committee report, Zn levels in food should not exceed 50 mg/kg (Cronin *et al*., 1998). The recommended dietary allowance for zinc in humans is 15 mg/d for men, 12 mg/d for women, 10 mg/d for children, and 5 mg/d for infants (Nord *et al*., 2004). The World Health Organization has recommended that dietary Pb should not exceed 0.3 μg/g (wet weight basis), and with a recommended limit of 450 μg of Pb per day for adults. Cd is not an essential element, and the World Health Organization/Food and Agricultural Organization (WHO/FAO) has determined a maximum tolerable daily intake of 55 μg/(person·d).

2.8 Heavy metal biomarkers

Currently, there has been a growing concern over the increase in heavy metal contamination affecting the aquatic environment and ultimately how it would affect human health. The release of heavy metals into the aquatic environment is known to cause detrimental effects to the environment and to the living organisms, giving a significant interest to the study of biomarkers in aquatic organisms for toxic metal contamination. A biomarker is referred to as a chance in molecular, biochemical and/or cellular components induced by exposure to one or more chemical pollutants, which can be measured in biological tissues and fluids (Depledge *et al*., 1995). The use of biomarkers as measured biological responses in organisms is important to simplify and reduce costs of biological monitoring. This method could provide early-warning signals of effects at later response levels. The use of stress indices has been recently proposed to evaluate the effects of metals on aquatic organisms. Two major sources of antioxidant enzymes and metallothioneins (MTs) are important biomarkers due to their critical function in maintaining appropriate redox potentials and viability in the cell (Schlenk and Rice, 1998).

WHO (1993) subdivided biomarkers into three classes:

(1) biomarkers of exposure: covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism;

(2) biomarkers of effect: including measurable biochemical, physiological or other changes within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease;

(3) biomarkers of susceptibility: indicating the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance, including genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure.

The different biomarker groups used to determine the impact of toxic xenobiotics on fish are discussed as following;

(a) Biotransformation enzymes: the activity of these enzymes in fish may be induced or inhibited upon exposure to xenobiotics. Two major types of enzymes involved in xenobiotic biotransformation are distinguished:

- Phase I enzymes; The first phase of metabolism involves oxidation, reduction or hydrolysis (Goeptar *et al*., 1995). For the majority of xenobiotic compounds the phase I reactions are catalyzed by microsomal monooxygenase (MO) enzymes, also known as the mixed-function oxidase. Most oxidative phase I biotransformations in fish are catalyzed by these cytochrome P450-dependent Mos (Van der Oost *et al*.., 2003). In vitro, Cu^{2+} and Pb^{2+} decreased the cytochrome P450 (Cyt P450 content) in the carp liver microsomes (Henczová *et al*., 2008). Cyt P450 enzymes are a large and ubiquitous family of heme containing proteins found in vertebrates, invertebrates, plants, and microorganisms that catalyze the oxidative biotransformation of diverse lipophilic xenobiotic and endogenous compounds including steroids, fatty acids, drugs, and organic pollutants. A significant increase in cyt P450 levels in fish was observed in 53% of the laboratory studies and 51% of the field studies, while strong increases (> 500% of control) were observed in 3 and 6% of the laboratory and field studies, respectively (Van der Oost *et al*.., 2003).

- Phase II enzymes and cofactors; these involve conjugation of the xenobiotic parent compound or its metabolites with an endogenous ligand. The majority of the phase II type enzymes catalyze these synthetic conjugation reactions, thus facilitating the excretion of chemicals by the addition of more polar groups (e.g. glutathione (GSH) and glucuronic acid (GA)) to the molecule (Commandeur *et al*., 1995). Some xenobiotic compounds possess the requisite functional groups (such as -COOH, -OH or -NH_2) for direct metabolism by conjugative phase II enzyme systems, while others are metabolized by an integrated process involving prior action of the phase I enzymes (George, 1994). Phase II enzymes can play an important role in homeostasis as well as in detoxification and clearance of many xenobiotic compounds. The major pathway for electrophilic compounds and metabolites is conjugation with GSH, while for nucleophilic compounds conjugation with GA is the major route (George, 1994).

The conjugation of electrophilic compounds (or phase I metabolites) with GSH is catalyzed by the glutathione S-transferases (GSTs), a multigene superfamily of dimeric, multifunctional, primarily soluble enzymes (Van der Oost *et al*.., 2003). GST is involved in phase II reactions, catalysing the conjugation of the tripeptide glutathione (GSH) with xenobiotics (e.g., PAHs and PCBs) and metals (Stegeman *et al*., 1992). GST seems to play important roles in both etoxification and bioactivation reactions. These enzymes are mainly located in the cytosolic fraction of the liver (Sijm and Opperhuizen, 1989). The total GST activity is usually examined by using the artificial substrate 1-chloro-2,4-dinitrobenzene (CDNB). Attempts to detect chemically induced activities of GSTs in freeliving fish also yielded conflicting results. A higher level of GST activity in Mediterranean deep-seafish was affected by urban and industrial waste water (Porte *et* *al*., 2000). However, GST activities in most studies are not significant differences between fish from control and polluted sites (Van der Oost *et al*.., 2003). A significant increase in GST activity was observed in only 33% of the laboratory studies and in 33% of the field studies, while no strong increases (> 500% of control) were reported in any of the laboratory and field studies considered (Van der Oost *et al*.., 2003).

(b) Oxidative stress parameters: Many environmental contaminants (or their metabolites) have been shown to exert toxic effects related to oxidative stress (Winston and Di Giulio, 1991). Oxygen toxicity is defined as injurious effects due to cytotoxic reactive oxygen species (ROS), also referred to as reactive oxygen intermediates (ROIs), oxygen free radicals or oxyradicals (Di Giulio *et al*., 1989a). Of particular interest are the reduction products of molecular oxygen which may react with critical cellular macromolecules, possibly leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and, ultimately, cell death (Winston and Di Giulio, 1991). The activities of the antioxidant enzymes, which defend the organisms against ROS, are critically important in the detoxification of radicals to non-reactive molecules. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-dependent peroxidase (GPOX) and glutathione reductase (GRED) are defence systems that tend to inhibit oxyradical formation. SOD, CAT and GPOX are important in the detoxification of radicals to nonreactive molecules. Glutathione-S-transferase (GST), and catalase (CAT) are biochemical biomarkers for heavy metal monitoring. There are numerous low-molecular-weight antioxidants, such as GSH, b-carotene (vitamin B), ascorbate (vitamin C), and (Stegeman *et al*., 1992).

The SODs are a group of metalloenzymes that catalyse the conversion of reactive superoxide anions (O_2^-) to yield hydrogen peroxide (H_2O_2), which in itself is an important ROS as well. H_2O_2 is subsequently detoxified by two types of enzymes: CATs and glutathione dependent peroxidases (GPOXs). SODs are considered to play a pivotal antioxidant role; their importance is indicated by their presence in all aerobic organisms examined (Stegeman *et al*., 1992). Most techniques for the measurement of SOD activity are indirect assays in which an indicating scavenger competes with endogenous SOD for O_2^-. A unit of SOD activity is defined as the amount that causes 50% inhibition of the reduction of the scavenger under specified conditions (Stegeman *et al*., 1992). In eight of the 11 field studies considered, however, a significant increase of hepatic SOD activity was observed, i.e. in brown bullhead, carp, dab, grey mullet, Nile tilapia, red mullet, sardine and spot from polluted environments (Van der Oost *et al*., 2003).

CATs are hematin-containing enzymes reduce hydrogen peroxide (H_2O_2), which is metabolized to molecular oxygen (O_2) and water. Unlike some peroxidases that can reduce various lipid peroxides as well as H_2O_2, CATs can only reduce H_2O_2 (Filho, 1996). A commonly employed assay for the measurement of CAT activity follows the disappearance of exogenous H_2O_2 spectrophotometrically (Stegeman *et al*., 1992).

A significant increase in CAT activity was observed in 20% of the laboratory studies and 55% of the field studies, while a strong increase (> 500% of control) was not observed in any of the laboratory or field studies considered. Similar as for SOD, more CAT responses were observed in the field than in lab studies (Van der Oost *et al*., 2003).

(c) The stress proteins comprise a set of abundant and inducible proteins involved in the protection and repair of the cell against stress and harmful conditions including high or low temperature, ultraviolet light, oxidative conditions, anoxia, salinity stress, heavy metals, and xenobiotics such as teratogens and hepatocarcinogens (Di Giulio *et al*., 1995). Special groups of stress proteins are the metallothioneins (MTs), which are inducible by both essential and toxic heavy metals (Viarengo *et al*., 2000), and the heat shock proteins, (HSPs).

MTs constitute a family of low-molecular-weight, cysteine-rich proteins functioning in the regulation of the essential metals Cu and Zn, and in the detoxication of these and other, non-essential, metals such as Cd and Hg (Roesijadi and Robinson, 1994). The cellular interactions involving MTs are expected to follow two general lines, the first being the interception and binding of metal ions that are initially taken up by the cell and the second being the removal of metals from nonthionein ligands that include cellular targets of toxicity. The latter may represent a detoxication function for structures, which have been reversibly impaired by inappropriate metal binding (Van der Oost *et al*., 2003). The role of MTs in sequestering metals is well established, while their induction by exposure to a wide variety of metals (e.g. Cd, Cu, Zn, Hg, Co, Ni, Bi, and Ag) is associated with their protective function (Stegeman *et al*., 1992; Viarengo *et al*., 2000). The capacity for MT induction is greatest in tissues that are active in uptake, storage and excretion, e.g. the small intestine, liver and gills of fish (Roesijadi and Robinson, 1994). The potency of Hg > Cd > Ag > Zn to induce MT was found in carp gill tissue (Cosson, 1994). However, nutrition and water quality (pH, temperature, salinity and dissolved oxygen) can affect stress protein responses, these factors should be monitored during laboratory experiments and field collections.

(d) Haematological parameters: Several haematological parameters in fish are potential effect biomarkers. The leakage of specific enzymes (e.g. transaminases) into the blood may be indicative of the disruption of cellular membranes in certain organs (Moss *et al*., 1986). Although less specific, other haematological parameters, like hematocrit, hemoglobin, protein and glucose, may be sensitive to certain types of pollutants as well (Van der Oost *et al*., 2003).

(e) Immunological parameters: A large number of environmental chemicals have the potential to impair components of the immune system. The immune system biomarkers in fish have a potential for pollution biomonitoring (Wester *et al*., 1994). Several immunological parameters, e.g. white blood cell (leukocyte) and lymphocyte status (measured as blood cell or differential counts), non-specific defence factors (such as lysosomal activity and levels of acute phase proteins in body fluids), weight and morphology of leukocyte producing organs (such as spleen, thymus and kidney), melanomacrophage centers (number, size and histopathological examination), macrophage function (chemotaxis, phagocytosis, pinocytosis and chemiluminescence), increased susceptibility to bacterial infections may potentially be used as biomarkers in fish (Van der Oost *et al*., 2003).

(f) Reproductive and endocrine parameters: The impact of xenobiotic compounds on reproductive and endocrine effects has attracted growing interest in recent years. Since a decreased reproductive capability in feral fish may in the long run threaten the survival of a large number of susceptible species, these parameters certainly deserve thorough examination (Van der Oost *et al*., 2003). Hormone regulation may be impaired as a consequence of exposure to environmental pollutants (Spies *et al*., 1990).

(g) Genotoxic parameters: The exposure of an organism to genotoxic chemicals may stimulate a cascade of events (Shugart *et al*., 1992): formation of structural alterations in DNA, procession of DNA damage and subsequent expression in mutant gene products, and diseases (e.g. cancer) resulting from the genetic damage. The detection and quantification of various events in this sequence may be employed as biomarkers of exposure and effects in organisms exposed to genotoxic substances in the environment. Apoptosis or programmed cell death is a physiological and irreversible process in tissue homeostasis that leads to DNA fragmentation of multiples of 180 – 200 basepairs (Van der Oost *et al*., 2003). Apoptosis could be demonstrated by an increased number of small DNA fragments in liver of dab exposed to PCBs and cadmium (Piechotta *et al*., 1999).

(h) Physiological and morphological parameters: The actual measurement of adverse effects or of the consequences of those effects may also be used as biomarkers. Determination of adverse effects can be performed histopathologically, by investigating lesions, alterations or tumour formation (neoplasms) in fish tissues. In addition, the liver somatic index (LSI) and the condition factor (CF) are morphological parameters that are often determined in field research. A significant increase in LSI values was observed in 38% of the laboratory studies and 43% of the field studies, while strong increases (> 500% of control) were not observed, while (Van der Oost *et al*., 2003). A significant increase in CF values was observed in none of the laboratory studies and 17% of the field studies, while strong increases (>500% of control) were not observed. . Although the condition of the liver and of the whole body, as measured with the LSI and CF values, are not very sensitive and may be affected by non-pollutant factors (e.g. season, disease, nutritional level), they may serve as an initial screening biomarker to indicate exposure and effects or to provide information on energy reserves (Mayer *et al*., 1992).

The bioaccumulation of certain persistent environmental contaminants in animal tissues may be considered to be a biomarker of exposure to these chemicals (WHO, 1993). As both overestimation and underestimation of effects may occur, laboratory observations on biomarkers must always be validated with field research (Van der Oost *et al*., 2003). In addition, biomarker data from field studiess may provide an important index in the 'real world' exposure.

2.9 Heavy metal biotransformation and detoxification

When these heavy metals enter the human and animal bodies, it cannot be metabolized or broken down. As a result, they generate toxicity. This makes heavy metal biotransformation and detoxification important. Biotransformation of xenobiotic chemicals often involves enzymes that have a relatively low degree of substrate specificity when compared with enzymes involved in the metabolism of constitutive compounds (Vermeulen, 1996). Metabolism in an organism is able to cause the toxicity of a foreign compound to be either beneficial (detoxication) or harmful (bioactivation). Aquatic organisms have two major ways to protect themselves and get rid of toxicants by either excretion in its original form (the parent compound) or biotransformation (Van der Oost *et al*., 2003). Biotransformation generally leads to the formation of a more hydrophilic compound which is more easily excreted than the parent compound (Vermeulen, 1996). Liver involves in the biotransformation of foreign compounds because of its function, position and blood supply (Van der Oost *et al*., 2003). Toxicity is usually mitigated due to a detoxication reaction while the excretion is generally increased. Antioxidant defense systems (Fig. 1) consist of catalase (CAT), superoxide dismutase (SOD), xanthine oxidase (XOD), glutathione peroxidase (GPX) and glutathione S-transferase (GST) (Basha and Rani, 2003).

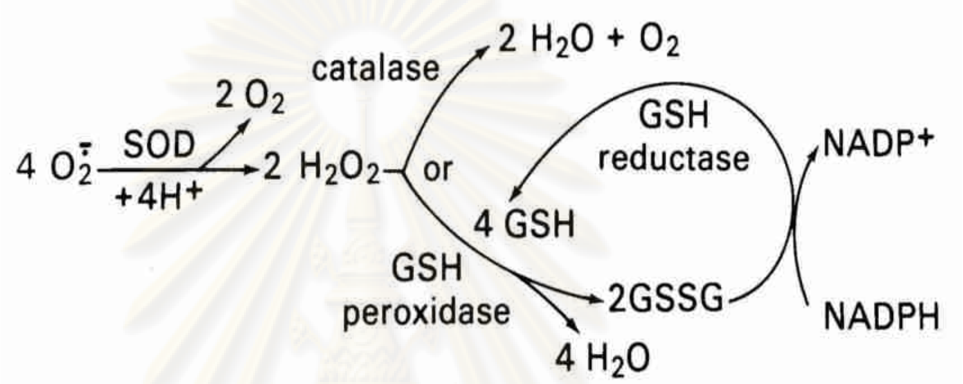

Figure 1 Antioxidant defense – enzymes (Proctor and Reynolds, 1984).

Three important intracellular enzymes constitute antioxidant defense; superoxide dismutase (SOD), catalase, and the GSH peroxidase/GSSG reductase system. SOD catalyzes the dismutation of superoxide, catalase the conversion of hydrogen peroxide to H_2O and O_2, while GSH peroxidase transfers electrons from GSH to reduce peroxides to water. The oxidized glutathione produced (GSSG) is re-reduced back to GSH by glutathione reductase utilizing NADPH (Proctor and Reynolds, 1984).

Glutathione (l-γ-glutamyl-cysteinyl-glycine) is a tripeptide that is mainly present in cells in its reduced form (GSH), which basically acts as an intracellular reductant and nucleophile. It functions in the synthesis of proteins and DNA, amino acid transport, maintenance of the thiol-disulfide status, free radical scavenging, signal transduction, as an essential cofactor of several enzymes, as a non-toxic storage form of cysteine, and as a defence against oxidizing molecules and potentially harmful xenobiotics such as metals (Elia *et al*., 2003). It was suggested that GSH content showed both increases and decreases in fish tissues exposed to metals due to their organ-specific responses (Pena *et al.,* 2000).

The glutathione-S-transferase (GST) is an enzyme that plays an important role in the cell protection from reactive electropiles. There are two functional categories of GST including detoxication enzyme and binding protein. GST is able to protect a cell from electrophilic xenobiotic and endogenous compounds including the product of cytochrome P450 metabolism (West, 1990). GST catalyzes toxic substances with reduced GSH, which neutralizes their electrophilic sites and renders the product more soluble, thus facilitating in the elimination step.

Glutathione (GSH in its reduced form) is considered one of the most important antioxidant agents involved in protection of cell membranes from lipid peroxidation by scavenging oxygen radicals (yielding glutathione disulfide, GSSG) (Meister, 1989). Moreover, glutathione is the cofactor of many enzymes catalyzing the detoxification and excretion of several toxic compounds. Among these enzymes, the glutathione peroxidases (including Se-dependent as well as Se-independent enzymes), through reduction of both hydrogen peroxide and organic hydroperoxides (ROOH + 2GSH +

ROH + H_2O + GSSG), provide an efficient protection against oxidative damage and free radicals. Another group of enzymes, glutathione S-transferases, acts as the catalyst of a very wide variety of conjugation reactions of glutathione with xenobiotic compounds containing electrophilic centers.

Microalgae produced peptides capable to bind heavy metals leading to prevent or neutralize their potential toxic effect (Cobbett and Goldsbrough, 2002). *Chlorella* is unicellular green algae with a great source of minerals, vitamins, and proteins. Its high levels of chlorophyll work as an effective heavy metal detoxification agent and is used extensively in colon cleansing processes. This alga has the ability to attach itself to heavy metal toxins. *Chlorella vulgaris* extract had ablity to restore the immunosuppressive effects induced by lead (Queiroza, *et al*., 2003).

2.10 Kai algae

Kai is one of the most delicious freshwater green algae. Taxonomic identification within the genus *Cladophora* is difficult because of high morphological variation under different ecological conditions (Khuantrairong, 2010). It is classified in Division Chlorophyta including *Cladophora glomerata* Kützing, *Cladophora* sp., *Microspora floccosa* (Vaucher) Thuret, *Microspora pachyderma* (Will) Lagerheim, and *Microspora* spp. (Peerapornpisal, 2008). It is abundant in Nan River in Nan Province and Mekong River at Chiang Rai Province during winter and summer.

The nutritional values of *Cladophora* spp. compose of 28% protein, 6.81% fat, 20.80% ash, 13.19% moisture, and 30.34% carbohydrate (percent in dry weight) (Peerapornpisal, 2009). It also contains selenium, an essential trace element required in the diet for normal growth and physiological function of fish (Wang and Lovell, 1997 and Lin and Shiau, 2007). Selenium (Se) also serves as a component of the enzyme glutathione peroxidase which protects cell membranes against oxidative damage. In addition, Se has been shown to have a protective effect in some mammals such as rat against toxic levels of heavy metals (Soudani *et al.*, *In press*). Lin and Shiau (2007) determined the effects of Se supplementation with (0, 0.8, or 1.6 mg Se kg^{-1} diet) on the oxidative stress of grouper, *Epinephelus malabaricus*, fed high dietary copper (20 mg Cu^{2+} kg^{-1} diet) for 8 weeks. They found that high dietary Se (1.6 mg kg^{-1} diet) supplementation reduced this oxidative stress and improved the fish immune response.

Nuisance growth of the attached, green alga *Cladophora* was considered to have been abated by phosphorus management programs mandated under the Great Lakes Water Quality Agreement (Auer, *et al.*, 2010). *Cladophora glomerata* was applied for heavy metal monitoring in the river Danube, Hungary (Oertel, 1993). The heavy metal concentration in these algae significantly differs according to its developmental stages and spatial (vertical) position. The continuously growing *Cladophora* can be considered one of the most significant factors which directly affects the reduction in heavy metal concentration; however, other physical-chemical parameters including flooding, light condition, temperature, conductivity and redox potential seem to be the most important factors influencing indirectly the heavy metal uptake and accumulation.

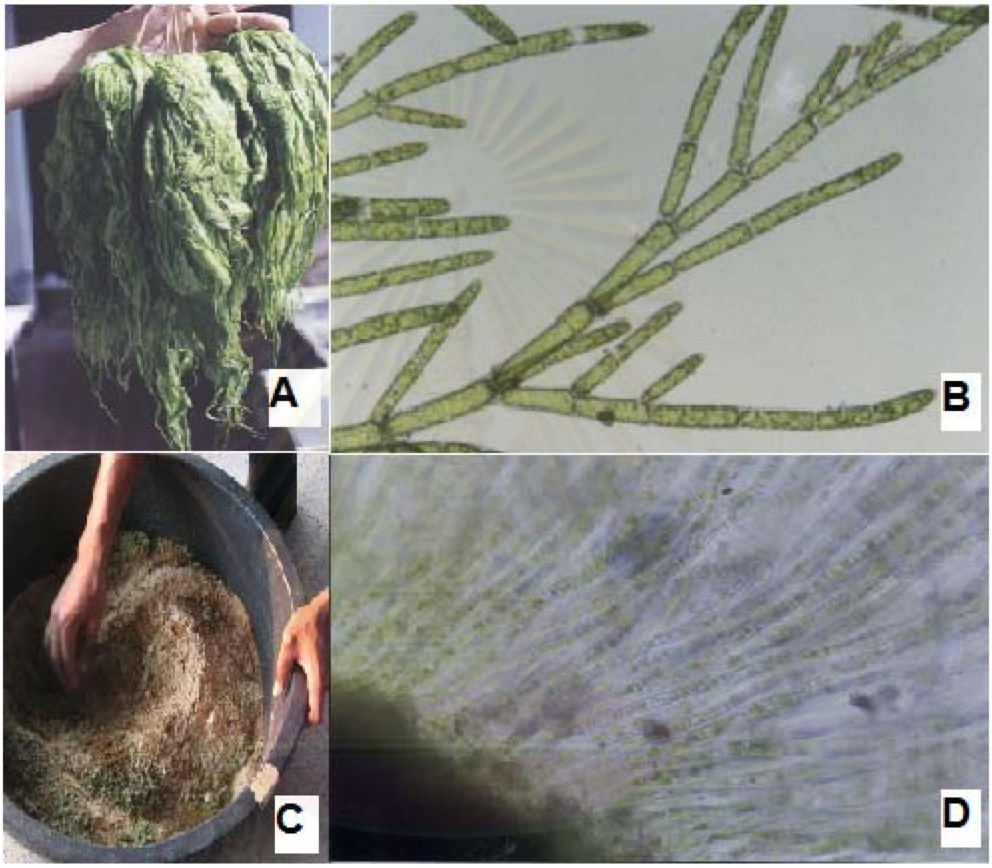

Figure 2 Kai algae (A) Kai harvested from Nan River; (B) Kai, *Cladophora glomerata*; (C) Kai additive feed preparation (D) *Microspora* spp. (Trichaiyaporn *et al*., 2010)

2.11 Effects of algae on fish immunity

Heavy metal contamination in aquatic environment is a serious problem worldwide due to their incremental accumulation in the food chain and continued persistence in the ecosystem. The acute and chronic lead poisoning also causes pathophysiological changes in tissues, and morphological changes in bone marrow cells, and necrosis in proximal tubular cells, reduces glomerular filtration rate and dysfunction in kidney. Lead toxicity induces changes in the composition of red blood cell (RBC) membrane proteins and lipids that inhibit haemoglobine synthesis, insufficient erythrocyte production, and reduce red cell survival (Ancheva *et al*., 2003).

Aquatic plants and algae have all attracted considerable attention for the capacity to eliminate heavy metal. Algae-based biotechnologies for pollution control, which employs suspended biomass of common green algae (*Chlorella, Scenedesmus, Cladophora*), cyanobacteria (*Spirulina, Oscillatoria, Anabaena*) or consortia of both, have been used for the removal of inorganic nutrients (Hoffmann, 1998). *Spirulina plantensis* is a blue-green filamentous cyanobacterium containing high-quality protein and other nutritional components such as vitamins, minerals, essential fatty acids and β-carotene (Hayashi *et al*., 1998). Recently, *Spirulina* has been speculated to be associated with modulation of the host immune system. Zhang *et al*. (2001) stated that *Spirulina* increased the haemoglobine concentration, RBC and white blood cell (WBC) counts, and erythropoiesis during chemotherapy. Watanuki *et al.* (2006) suggested that dietary *Spirulina* has immunostimulatory effects on the innate immune system of carp. This study proposes to determine the protective effects of the dietary Kai green algae (*Cladophora* and *Microphora*) in African catfish.

2.12 Participation action and public hearing on river management

Management of water resources is a particularly challenging and difficult task, where the complexities arising from the functioning of hydrological cycles and biological systems are combined with the multiple perspectives, needs, values and concerns associated with the use of water for human purposes (Antunes *et al*., 2009).The major initiatives in water management in Thailand including Chao Phraya River, Thachin River, Ping River, has been decentralization of management from central government to some form of local water authority, with varying degrees of stakeholder participation. Results of study on Involvement of stakeholders in the water quality monitoring and surveillance system in Zimbabwe indicate that there is very limited stakeholder participation despite the presence of adequate supportive structures and organizations (Nare *et al*., 2006). They also suggested that stakeholder participation and ownership of resources needs to be encouraged through participatory planning, and integration between the three government departments (water, environment and health).

Stakeholders are all those people, institutions and organizations that are affected by the actions considered, and are self-regarding rational actors (Castelletti and Soncini-Sessa, 2006). A participatory decisionmaking procedure does not require only gathering information and opinions from the stakeholders (informative participation), but should make them act as they were decision makers, who have to negotiate the final decision (active involvement). A responsibility for everyone involved in risk management is risk communication. This is an ongoing process at the local level and usually involves a government agency, represented by risk managers, industry and other stakeholders, and the public at large. The objective of risk communication is to maximize the transparency of every activity related to the risk through interaction with the broadest range of interested parties. This objective includes risk identification, analysis, assessment, implementation of the decision, and subsequent monitoring. It is important that the communication process is begun as soon as possible, preferably with an announcement of the project itself.

Risk communication is carried out in a variety of ways. Productive communication is invariably conducted at public hearings when, in theory, everyone listens carefully to each other without any prejudgment of the issue. But this is not always the case, and it is important for the risk managers representing government agencies at such hearings to maintain public trust by their independence and impartiality. Good communication is also achieved by regularly circulating published materials. Some aspects of risk assessment are scientific and very technical, and therefore it is important that the data and all methods of collection, any models and assumptions that have been applied, and any conclusions drawn are reviewed by peers.

Mae Kuang River represents an ecosystem affecting from industrial, agricultural, and anthropogenic activities, and this aquatic ecosystem has been progressively degrading. The heavy metal contamination has been an important factor in the decline of water and sediments quality and may adversely affect fish health and human who consume these fishes. In this work, the indigenous fish will be sampled and assess metal concentrations of Cd, Pb, and Zn in water, sediments and their bioaccumulation in various fish tissues, such as muscle, liver, and gills. Furthermore, the ablity of Kai supplementary diet to prevent catfish from lead toxicity and accumulation in various tissues were investigated.

CHAPTER III

MATERIALS AND METHODS

Part A: Monitoring of Cadmium, Lead, and Zinc contaminated in water, sediment, and fish in Mae Kuang River

(A.1) Study area

The study was carried out in Kuang River, Thailand. The study sites were shown in Figure 3.

Site 1 is located upstream far from Chiangmai and Lamphun Municipalities. It generally had good water quality and was used as reference site.

Site 2 is closed to East Lamphun industrial park. The effluent from this area runs into central wastewater treatment on the West side.

Site 3 is closed to industrial discharge from Lamphun industrial park treatment ponds.

Site 4 is opposite Haripoonchai temple and Lamphun Downtown. There are a lot of restaurant along this river.

Site 5 is predominantly agricultural area located below Lamphun Municipality.

Site 6 is the station that Mae Kuang River merges to Ping River.

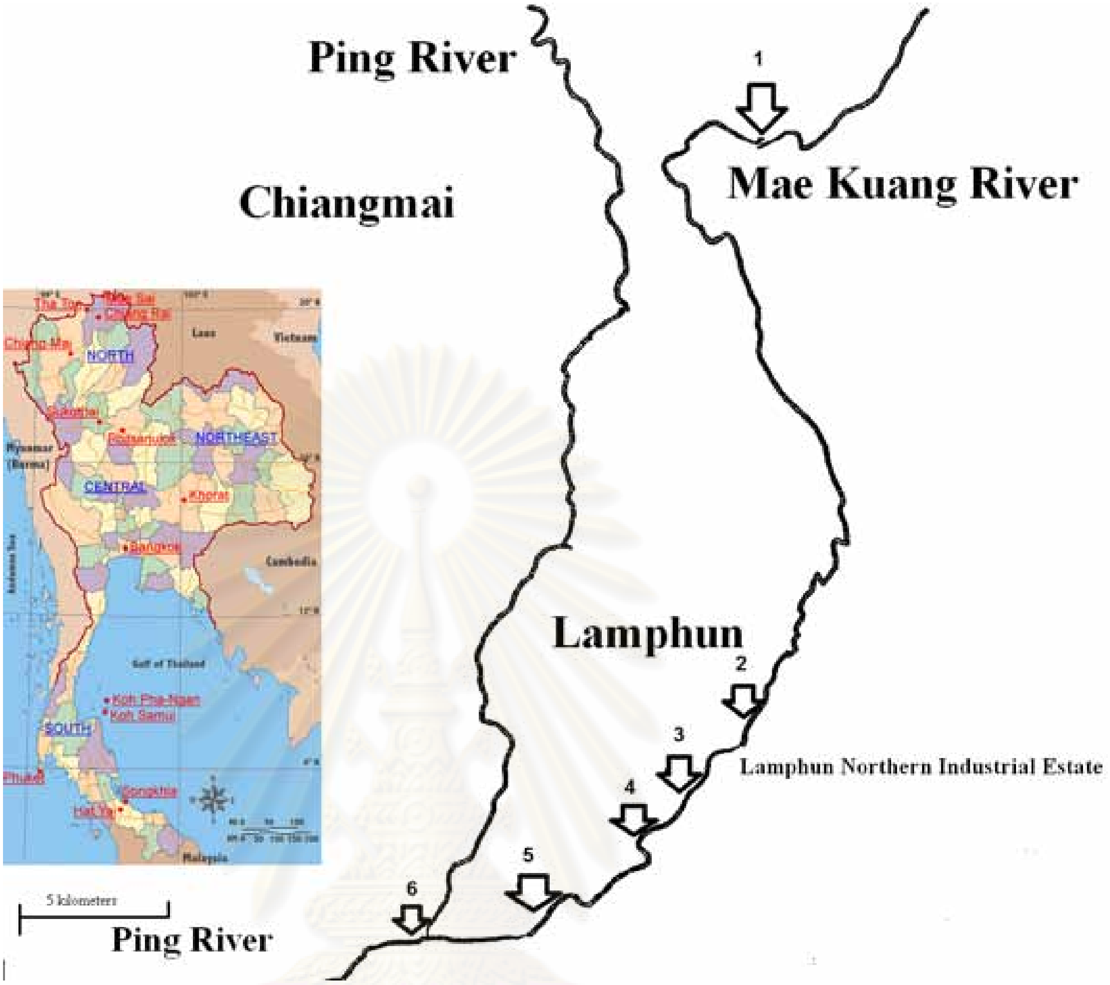

Figure 3 Map of the sampling stations in the Mae Kuang River

(A.2) Sampling protocol

The samples of water, sediment, and fish were collected from different sites during June 2008 to July 2009.

A.2.1 Water

Water samples at a depth of 30 cm below the surface were collected once a month from each station and kept in clean acid-washed polyethylene bottles. Samples were acidified with 10% HNO_3, placed in an ice box and brought to the laboratory.

Physical and some chemical parameters of water samples including temperature, dissolved oxygen, pH, and conductivity were also examined using a Multi-Probe System (YSI 556 MPS) at the time of sample collection. Other parameters such as BOD, COD, ammonia, nitrate, and phosphate were immediately analyzed upon laboratory arrival according to the procedures by APHA, AWWA and WEA (1998).

A.2.2 Sediment

Surface bottom sediment (0 – 5 cm) samples were collected monthly from six stations with the use of an Ekman grab sampler. Samples were transferred to acid-washed plastic bags and placed in a cooler at 4 °C, and then transported to the laboratory. Sediment samples were subsequently air dried for 4 days until they reached constant weight.

A.2.3 Fish

Three indigenous fish species, individually 50 – 200 g in weight, (Fig. 4) including Jullien's mud carp (*Henicorhynchus siamensis*), Smith's barb (*Puntioplites proctozysron*), and snakehead (*Channa striatus*) were caught from selected sampling sites by local fishermen using electrofishing and netting. Jullien's mud carp is omnivorous, pelagic feeding on phytoplankton and aquatic insects. Smith's barb is omnivorous, feeding on detritus of other animals, as well as on phytoplankton. Snakehead is a voracious carnivore feeding mainly on live animals. The sample size was 5 fishes for each station. Sampling was done every four months.

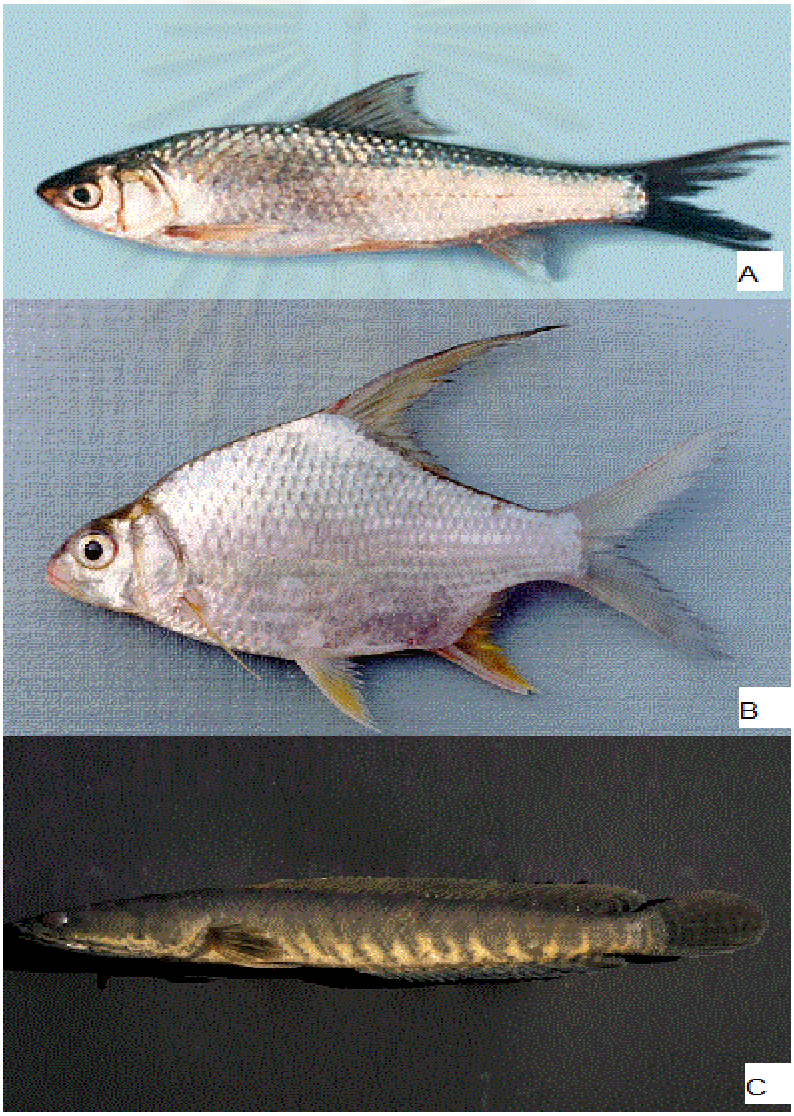

Figure 4 A) Jullien's mud carp (*Henicorhynchus siamensis*), B) Smith's barb (*Puntioplites proctozysron*), and C) snakehead (*Channa striatus*)

(A.3) Metal preparation and analysis

A.3.1 Cadmium, Lead, and Zinc analysis in water

The method was followed the AOAC Official Method 974.27. Briefly, transfer aliquot of well mixed water sample to beaker and add 3 mL HNO_3. Cover with watch glass, heat and evaporate to dryness in fume hood. Filter with Whatman® filter paper and dilute with deionized water to 50 mL.

A.3.2 Sediment preparation for heavy metal analysis

Acid digestion for total heavy metal analysis

A 5 g air-dried sample of sediments was ground and passed through a 1 mm sieve. The *aqua regia* method (Hseu, 2004) was applied to analyze the total contents of Cd, Pb, and Zn. Briefly, one gram of sediment samples were treated with 4 ml of an oxidising mixture (HNO_3: HCl = 3:1) and 6 ml HF in a 250 ml digestion beaker. The mixture was boiled gently for 30 – 45 min to oxidize all easily oxidizable matter. After cooling, 5 ml of 70% $HClO_4$ was added and the mixture was boiled gently until dense white fumes appeared. After cooling, 20 ml of distilled water was added and the mixture was boiled further to release any fumes. The solution was cooled, further filtered through Whatman® No. 42 filter paper and diluted to 50 ml by deionized water.

Sequential extraction procedure

Sequential extractions were performed in triplicate using procedure described by Tessier *et al.* (1979) and modified by Vicente-Martorell *et al.* (2009). The extraction steps and the reagents used in the sequential extractions are summarized in Table 1. Briefly, 1 g of air-dried sediment samples were placed in 50 ml polycarbonate centrifuge tubes, mixed in a stepwise fashion with various reagents, and the suspensions equilibrated as described in Table 1. Following equilibration, the solution and solid phases were separated by centrifugation at 1200 Xg for 10 min. The supernatants were filtered through a 0.45 μm membrane, and the solid residues were preserved for the subsequent extractions.

The extracts were stored at −4 °C in acid washed polyethylene bottle until analyzed by AAS.

A.3.3 Fish tissue preparation for heavy metal analysis

For preparation of fish tissues, the method of the Association of Official Analytical Chemists (AOAC) was used. This method involved the digestion of the sample in an open beaker on a hot plate prior to use, all glassware were previously soaked in dilute nitric acid for 24 h and then rinsed with deionized water. Fresh gills, muscle and pooled liver samples from each site were weighed, wrapped in aluminium foil, and frozen at −20 °C prior to digestion.

A.3.4 Heavy Metal Analysis

Cd, Pb, and Zn were analyzed by Graphite Atomic Absorption Spectrophotometry (using Varian Spectra AA-220FS). The wavelengths were 228.8 nm for Cd, 217.0 nm for Pb, and 213.9 nm for Zn. Data provided were the average of three

Table 1 Reagents and conditions applied in sequential extraction procedure. Method based on Tessier *et al.* (1979) as modified by Vicente-Martorell *et al.* (2009).

Phase	Extraction reagent	Extraction Conditions
Exchangeable	1 M $MgCl_2$ at pH 7	Shaken at room temperature for 1 h
Carbonate	1 M NaOAc adjusted to pH 5 with HOAc	Shaken at room temperature for 5 h
Reducible	0.04 M $NH_2OH.HCl$ in 25% (v/v) HOAc	Shaken at 96°C for 6 h
Oxidizable	0.02 M HNO_3 + 30% H_20_2 (adjusted to pH 2 with HNO_3). On cooling add 3.2 M NH_4OAc in 20% (v/v) HNO_3	Shaken occasionally at 85°C for 5 h then shaken on addition of NH_4OAc for a further 0.5 h
Residual	HNO_3, HF, $HClO_4$	As for total metal analysis

replicates. The detection limits of the method were 0.005 ppm Cd, 0.01 ppm Pb, and 0.015 ppm Zn.

(A.4) Enzyme assays

The following enzyme assays were used to determine the effects of pollutants in Kuang River on fish and also examine the protective effects of Kai supplementary feed against lead toxicity in African catfish.

A.4.1 Catalase (CAT)

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decomposition of 10 mM H_2O_2 according to Aebi (1974) at 240 nm ($\varepsilon = 40\ M^{-1}\ cm^{-1}$.) in a medium (final volume 3 ml) containing 50 mM KH_2PO_4 (pH 7.0) and approximately 150 μg of proteins. Reactions were initiated by adding 0.1 ml of extract to 2.9 ml of the reaction mixture, and the decrease in absorbance was measured for 30 s intervals during 2 min. Results were related to the soluble protein.

A.4.2 Superoxide dismutase (SOD)

The superoxide anion production was measured An SOD activity was measured by the indirect method according to Atli and Canli (2010) with some modifications. This involved the inhibition of cytochrome c reduction at 550 nm for 1 min. The reaction buffer contained 50mM potassium phosphate buffer (pH 7.8), 0.1mM EDTA, 10 mM cytochrome c, 0.05 mM hypoxanthine and the supernatant.The reaction started by adding 1.87 mU/ml Xanthine oxidase in a final volume of 1mL. A unit of an SOD activity was defined as the amount of enzyme that causes 50% inhibition of cytochrome c reduction and was expressed as unit/mg protein.

A.4.3 Glutathione S-transferases (GST)

Gill GST activity was determined at 340 nm by the method of Habig *et al.* (1974), adapted to microplate as described in Booth *et al.* (2000), using 0.1 ml of homogenate and 0.2 ml of the reaction mixture (GSH at 10mM and CDNB at 60mM).

(A.5) Determination of immunological parameters

A.5.1 Serum lysozyme content measurement

The serum lysozyme content was determined by a turbidimetric method (Ellis, 1990). The substrate used was *Micrococcus lysodeikticus* (0.25 mg/mL of 0.05 M PBS; pH 7.4). Briefly, serum with 250 μL *M. lysodeikticus* was added to every well of a 96-well microtiter plate for 10 min at 37 °C. The absorbance at 450 nm was read at 1 min and then at 1-min intervals. A unit of lysozyme activity is defined as the amount of sample causing a decrease in absorbance of 0.001/min.

A.5.2 Hematological parameters

Blood sample analysis included blood cell count and hematocrit.

(A.6) Target hazard quotient (THQ)

The health risk from consumption of fish by local inhabitants was assessed based on the target hazard quotient (THQ). This THQ methodology offers an indication of the risk level due to pollutant exposure. The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed population is unlikely to experience obvious adverse effects. This method is available in US EPA Region III Risk based Concentration table (USEPA, 2000) and it is described by the following equation:

$$THQ = \frac{EF \times ED \times FIR \times C \times 10^{-3}}{RFD \times WAB \times TA}$$

Where EF is exposure frequency (365 days/year);

ED is the exposure duration (70 years), equivalent to the average lifetime;

FIR is the food ingestion rate (fish: 36 g/person/day; FAO, 2005);

C is the metal concentration in fish (mg/kg);

RFD is the oral reference dose

(Cd = 1 X 10^{-3} mg/kg/day, Pb = 0.004 mg/kg/day) (US EPA, 1997, 2000);

WAB is the average body weight (60 kg);

TA is the averaging exposure time for non-carcinogens (365 days/year X ED).

In addition, in order to assess the level of heavy metal contamination in Mae Kuang River, the peak concentrations measured in water, sediment, and fish were compared with toxicity data for standard test organisms and water quality criteria (US EPA, 1986).

Part B: Protective Effects of Kai Additive Feed against Lead Toxicity in African Catfish, *Clarias gariepinus*

(B.1) Kai preparation

Kai was obtained from local markets in Tambol Tha Wang Pha, Amphur Pua, Nan province.

(B.2) Experimental design and fish rearing

Healthy African catfish (150 – 200 g) were obtained from a local supplier and reared in aerated laboratory tanks for 2 weeks prior to experiments. They were fed with 0%, 10% and 15% of Kai supplementary diets for 3 months, each of which had three replicates of 50 fish/tank. The parameters of protective mechanisms as mentioned above in A.4 were performed every month after Kai administration.

Group	Feed supplements
Control	Basal Diet (0% Kai Supplement)
1	Basal Diet + 10% Kai Supplement
2	Basal Diet + 15% Kai Supplement

(B.3) Waterborne Pb challenge and sampling

Catfish were divided into groups (10 fish each) and subjected to heavy metal treatment. The fish were exposed to 0 ppm of Pb (negative control), ½ LC50, and the concentration of Pb that found in Mae Kuang River. After this exposure period catfish (n = 3 per tank) were collected three times (namely Days 7, 15, and 30). In addition one fish from each tank was sampled before the start of the exposed trial, representing a control group at Day 0 (n = 9). Fish were killed by cervical dislocation, their livers, kidneys and gills were removed and stored at -20 °C. The protective enzyme and immunity levels as well as genotoxicity were evaluated (details in A4, A5, and A6). Actual dissolved Pb concentration and lead concentration in fish muscle were measured using graphite furnace atomic absorption spectrophotometry (GFAAS; Spectra AA-220, Varian, Australia) to determine if fish is safe for consumption or not.

Part C: An informal round-table for stakeholders

All stakeholders including those directly involved and who benefit, as well as agencies with management responsibilities and the general public concerned with rural development and aquatic environment management were invited to share experiences in Mae Kuang monitoring and management. A half-day discussion for a proposed policy-oriented round-table on Mae Kuang River Management as part of the Maejo fisheries and aquaculture conference was held in the beginning of December 2009 in Chiangmai.

Statistics

All values were expressed as mean ± S.D. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test. A value of $P < 0.05$ was considered statistically significant. Correlations between different parameters were investigated. Data was analyzed using SPSS statistical package program version 15.0.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Physical and chemical status of water in the Mae Kuang River

The general water chemistry in the Mae Kuang River in winter, summer and rainy seasons is listed in Table 2. Water temperature ranged 22.13 – 31.86 °C; pHs were 6.71 – 8.97; Dissolved Oxygen (DO) were from 1.07 – 7.26 mg/L; ammonia varied from 0.01 to 0.15 mg/L; nitrite and nitrate were 0.01 – 0.85 mg/L and 0.01 – 1.75 mg/L, respectively; orthophosphorus were 0.01 – 0.71 mg/L; electrical conductivity values were 140 – 350 µs/cm; COD varied extremely from 2.86 – 5.28 mg/L; and BOD varied from 0.19 – 3.22 mg/L. There were no significant differences among stations for pH, water temperature, ammonia, and COD ($P > 0.05$). On the other hand, seasonal variation in DO, BOD, and Total-P was found. Water temperatures were high in summer and lower in winter because of annual cycle characteristics. The water temperature was higher than the previous study (20 – 35 °C) by Lertsri (1998). COD is commonly used for municipal and industrial waste determination. The higher values of COD were recorded at stations 2 and 3 where might affect from industrial sites. Most environmental scientists in each plant concern only their own effluents to meet the standard requirement, but they do not pay attention on the overall wastewaters from all industries. The BOD in the Mae Kuang River varied from 1.54 – 2.18 mg/L in the rainy season, from 2.19 to 2.81 mg/L in the winter season, and from 1.50 to 1.97 mg/L in the summer season.

Table 2. Physical-chemical parameters in water from Mae Kuang River during June 2008 – July 2009

season	station	pH	Dissolved oxygen (mg/L)	T (°C)	BOD (mg/L)	COD (mg/L)	NH_3 (mg/L)	PO_4 (mg/L)
Rainy	1	7.42 ± 0.47a	3.68 ± 1.54 a	27.70 ± 0.67a	1.54 ± 0.59 a	3.34 ± 0.69a	.0382 ± 0.02a	.0995 ± 0.05a
	2	7.44 ± 0.43a	4.42 ± 0.41ab	28.15 ± 0.86a	2.01 ± 0.38 ab	3.41 ± 0.77a	.0333 ± 0.02a	.2106 ± 0.12b
	3	7.41 ± 0.43a	4.60 ± 0.67ab	28.53 ± 0.97a	2.18 ± 0.34 b	3.62 ± 0.62a	.0529 ± 0.02a	.1412 ± 0.08ab
	4	7.38 ± 0.40a	4.37 ± 0.68ab	28.54 ± 0.96a	2.02 ± 0.20 ab	3.50 ± 0.56a	.0392 ± 0.02a	.1389 ± 0.05ab
	5	7.48 ± 0.41a	4.11 ± 0.50ab	28.45 ± 1.33a	1.78 ± 0.58 ab	3.42 ± 0.52a	.0447 ± 0.02a	.1447 ± 0.04ab
	6	7.45 ± 0.40a	4.95 ± 0.30 b	28.75 ± 1.07a	1.93 ± 0.51 ab	3.38 ± 0.61a	.0453 ± 0.03a	.1594 ± 0.07ab
Winter	1	8.97 ± 2.17c	6.36 ± 0.80 c	22.92 ± 1.54c	2.62 ± 0.44cd	3.11 ± 0.69c	.0679 ± 0.05c	.6339 ± 0.70c
	2	8.15 ± 0.10c	6.25 ± 0.70cd	23.43 ± 1.30c	2.81 ± 0.41d	3.25 ± 0.38c	.0729 ± 0.05c	.7073 ± 0.63c
	3	8.02 ± 0.13c	5.62 ± 0.52 cde	24.04 ± 1.53c	2.58 ± 0.40cd	3.37 ± 0.46c	.1503 ± 0.24c	.6577 ± 0.65c
	4	8.08 ± 0.15c	5.47 ± 0.74 de	24.18 ± 1.50c	2.39 ± 0.23cd	3.37 ± 0.56c	.0740 ± 0.04c	.6793 ± 0.70c
	5	7.99 ± 0.11c	4.80 ± 0.80 e	24.38 ± 1.53c	2.19 ± 0.43c	3.31 ± 0.63c	.0830 ± 0.04c	.6728 ± 0.70c
	6	8.09 ± 0.22c	6.17 ± 0.71cd	24.54 ± 1.63c	2.74 ± 0.27d	3.36 ± 0.44c	.0686 ± 0.04c	.7312 ± 0.67c
Summer	1	7.41 ± 0.47f	2.21 ± 1.02g	29.63 ± 1.52g	1.79 ± 0.38f	2.86 ± 1.50f	.0112 ± 0.02f	.1743 ± 0.15f
	2	7.66 ± 0.46f	2.22 ± 1.17g	29.62 ± 1.08g	1.96 ± 0.36g	3.49 ± 1.50f	.0127 ± 0.02f	.1242 ± 0.11f
	3	7.85 ± 0.79f	2.08 ± 0.59g	29.61 ± 1.22g	1.97 ± 0.45g	3.01 ± 1.07f	.0177 ± 0.01f	.1795 ± 0.23f
	4	7.64 ± 0.55f	2.05 ± 0.87g	29.92 ± 0.77g	1.92 ± 0.41g	2.93 ± 1.73f	.0208 ± 0.02f	.1773 ± 0.24f
	5	7.58 ± 0.51f	1.90 ± 1.42g	29.97 ± 1.72g	1.67 ± 0.26f	2.21 ± 0.87f	.0187 ± 0.02f	.2791 ± 0.38f
	6	7.59 ± 0.40f	1.91 ± 0.89g	30.16 ± 1.70g	1.50 ± 0.49f	3.61 ± 1.07f	.0109 ± 0.01f	.2305 ± 0.30f

*Different letters in same column in each season indicate significant differences at $P < 0.05$ (ANOVA).

Increased values of BOD in stations 2, 3, and 4 were observed in this River because of pollutant combination including urban and industrial sewage. In summer, the water flow velocity decreased dramatically, while there were still a lot of high organic drainages without treatment. As a result, DO were in unacceptable levels in summer and subsequently resulted in fish death.

Surface inland water quality standards in Thailand were classified into five classes. Class 1 refers to extra clean natural water without any effluent that is able to be used for domestic consumption after simple disinfection, for recreation, or for aquatic organism breeding and conservation. Class 2 refers to clean water that can be used as domestic water after treatment, for recreational purposes or for fishing, farming, aquatic organism conservation, swimming. Class 3 includes polluted water, which can be used after water improvement. This water can also be used for agricultural purpose. Class 4 includes polluted water, which can only be used as industrial water after treatment. Class 5 refers to heavily polluted water that can be only used for navigation. Referring to pH, BOD and DO parameters, water in the Mae Kuang River was classified as class 3 – 4, according to Thailand National Environmental Quality Act (1992). That means this water can also be used for consumption after water improvement and disinfection. However, the sewage treatment processing must be carried out and sources of pollution must be reduced. Drought crisis, industrial development, and uncontrolled city expansion make this problem more serious. Mechanical aeration and sewage treatment is recommended to reduce this problem. A high nutrient disposal also causes massive water hyacinth expansion, affects the aquatic organisms and increases the cost of tap water preparation. Community participation must be applied for river clean-up. Prasopkeatpoka (2008) surveyed the willingness to pay of the local residents was about 5 US Dollars/month for the Mae Kuang River conservation. In my opinion, this rate might be too high for poor people especially in the inflation situation.

Rapid flow in the rainy season caused flooding on both banks water lacks of water for agriculture were common in the dry season. The water quality changes all the time due to the amount of water, the growth of aquatic plants, the difference in life styles of people who live along the river, etc. In rainny season, the rain usually dilutes the deteriorated water while the water quality in summer is worser because of less water. Some factories are closed at nights and during weekend. As a result, there might have a variation among working hours, working days, and holidays.

Pb and Cd concentrations in water were below detection limits, while the concentrations of Zn in water were 0.01 – 0.11 mg/L (Table 3). There were not different seasonal variations in zinc concentrations in water in this study. Chumroonrat *et al.* (2004) reported Pb in water collected from Mae Kuang River near Lamphun industrial park during 2003 – 2004 was below detection limit in rainy and winter seasons while the maximum (0.0024 mg/L) was found in summer. Pb, Cd, and Hg in surface water of the Ping River were below than detection limit (Traichaiyaporn and Chitmanat, 2008).

Table 3. Heavy metal concentration in water (mg/L), sediment (mg/kg), fish muscle (mg/kg) from Mae Kaung River during June 2008 – July 2009

(All results are given as mean value standard deviation of three determinations.)

Samples	Zn	Pb	Cd
Rainy			
Water			
Site 1	0.03 ± 0.01a	<0.005	< 0.002
Site 2	0.03 ± 0.00ab	<0.005	< 0.002
Site 3	0.06 ± 0.01bc	<0.005	< 0.002
Site 4	0.07 ± 0.03c	<0.005	< 0.002
Site 5	0.04 ± 0.00abc	<0.005	< 0.002
Site 6	0.04 ± 0.01abc	<0.005	< 0.002
Sediment			
Site 1	6.17 ± 0.17a	10.81 ± 1.49a	0.14 ± 0.02ab
Site 2	6.34 ± 0.81a	16.51 ± 3.45b	0.16 ± 0.10ab
Site 3	10.55 ± 0.29b	18.24 ± 0.87b	0.33 ± 0.04c
Site 4	6.10 ± 0.29a	17.65 ± 5.82b	0.24 ± 0.06bc
Site 5	4.21 ± 0.37c	13.40 ± 0.82b	0.07 ± 0.06a
Site 6	1.12 ± 0.12d	4.86 ± 0.46b	0.04 ± 0.05a
Fish			
Site 1	9.25 ± 0.96ab	1.48 ± 0.51a	0.08 ± 0.02a
Site 2	8.36 ± 1.55a	1.70 ± 0.69a	0.14 ± 0.02a
Site 3	12.19 ± 1.07b	1.79 ± 0.18a	0.15 ± 0.05a
Site 4	8.33 ± 1.05a	1.56 ± 0.59a	0.09 ± 0.01a
Site 5	7.50 ± 0.65a	1.09 ± 0.15a	0.10 ± 0.05a
Site 6	6.63 ± 1.98a	1.04 ± 0.31a	0.07 ± 0.02a

Different letters in same column in each season indicate significant differences at $P < 0.05$ (ANOVA).

Table 3 (Continued). Heavy metal concentration in water (mg/L), sediment (mg/kg), fish muscle (mg/kg) from Mae Kaung River during June 2008 – July 2009 (All results are given as mean value standard deviation of three determinations.)

Samples	Zn	Pb	Cd
Winter			
Water			
Site 1	0.04 ± 0.01a	<0.005	< 0.002
Site 2	0.07 ± 0.04a	<0.005	< 0.002
Site 3	0.08 ± 0.02a	<0.005	< 0.002
Site 4	0.05 ± 0.01a	<0.005	< 0.002
Site 5	0.07 ± 0.01a	<0.005	< 0.002
Site 6	0.04 ± 0.01a	<0.005	< 0.002
Sediment			
Site 1	1.86 ± 0.03a	4.68 ± 0.19a	< 0.02
Site 2	2.05 ± 0.10a	6.47 ± 0.83b	0.11 ± 0.03a
Site 3	5.00 ± 0.38c	10.36 ± 0.51c	0.17 ± 0.01a
Site 4	6.32 ± 0.36d	12.78 ± 1.94c	0.15 ± 0.08a
Site 5	1.63 ± 0.10a	4.07 ± 0.20a	0.08 ± 0.01a
Site 6	3.87 ± 0.51b	9.67 ± 0.88c	< 0.02
Fish			
Site 1	4.07 ± 1.32b	0.41 ± 0.15a	0.14 ± 0.05a
Site 2	4.82 ± 0.45b	1.16 ± 0.38ab	0.14 ± 0.08a
Site 3	4.46 ± 0.63b	1.56 ± 0.42bc	0.11 ± 0.05a
Site 4	3.37 ± 0.50a	2.13 ± 0.21bc	0.07 ± 0.06a
Site 5	4.60 ± 1.21b	2.37 ± 0.24c	0.24 ± 0.12a
Site 6	6.06 ± 1.11c	0.49 ± 0.38a	0.21 ± 0.02a

Different letters in same column in each season indicate significant differences at $P < 0.05$ (ANOVA).

Table 3 (Continued). Heavy metal concentration in water (mg/L), sediment (mg/kg), fish muscle (mg/kg) from Mae Kaung River during June 2008 – July 2009

(All results are given as mean value standard deviation of three determinations.)

Samples	Zn	Pb	Cd
Summer			
Water			
Site 1	0.03 ± 0.01ab	<0.005	< 0.002
Site 2	0.07 ± 0.03cd	<0.005	< 0.002
Site 3	0.07 ± 0.01d	<0.005	< 0.002
Site 4	0.06 ± 0.01bcd	<0.005	< 0.002
Site 5	0.02 ± 0.01a	<0.005	< 0.002
Site 6	0.04 ± 0.01abc	<0.005	< 0.002
Sediment			
Site 1	4.70 ± 0.56a	6.73 ± 0.37a	< 0.02
Site 2	6.24 ± 0.19a	23.34 ± 1.66c	0.14 ± 0.02a
Site 3	14.16 ± 5.11b	12.13 ± 4.62b	0.19 ± 0.03ab
Site 4	6.92 ± 0.32a	6.42 ± 0.67a	0.21 ± 0.03ab
Site 5	3.38 ± 0.31a	5.09 ± 0.26a	0.25 ± 0.03b
Site 6	5.77 ± 0.89a	26.44 ± 1.12c	< 0.02
Fish			
Site 1	7.07 ± 1.26a	1.40 ± 0.07a	0.09 ± 0.01ab
Site 2	8.32 ± 1.95a	1.43 ± 0.31a	0.18 ± 0.06ab
Site 3	10.02 ± 1.15a	1.65 ± 0.03a	0.21 ± 0.05b
Site 4	7.70 ± 2.16a	1.58 ± 0.61a	0.11 ± 0.07ab
Site 5	7.17 ± 0.94a	1.22 ± 0.17a	0.12 ± 0.04ab
Site 6	6.07 ± 2.14a	1.07 ± 0.34a	0.06 ± 0.03a
Background concentrations in water* (world average)	10	0.2	0.02
Tolerance level in fish**	50	0.5	0.1

* Klavin *et al.* (2000)

** Demirak *et al.* (2006)

*** Different letters in same column in each season indicate significant differences at P < 0.05 (ANOVA).

Similar to study of Bordalo *et al*. (2001), Cd in water was not always found in the Bangpakong River, one of the most important rivers in the Eastern Thailand. The levels of zinc, lead, and cadmium in this study should not present any hazard for fish. However, villagers usually believe the fish death phenomenon in Mae Kuang River is caused by heavy metals from industrial effluents. For this reason, they always ignore to reduce or treat their own wastewater after daily uses. The right information should be delivered to public.

4.2 Metal concentrations in sediment

Pb, Cd, and Zn concentrations in sediment were 3.13 – 27.56, < 0.02 – 0.43, 3.42 – 10.32 mg/kg, respectively (Table 3). Heavy metals tend to be trapped by bottom sediments (Dauvalter, 1998). In some cases, sediments hold greater than 99% of total quantity of a metal present in an aquatic system (Netpae and Phalaraksh, 2009). Generally, content of metal in sediment were higher than content of metal in water and fish. The heavy metal concentrations were 100 - 10,000 times greater in the sediment than in the water (Yi *et al*., 2008). The mean concentrations of Cd, Pb, and Zn in sediment from sampling sites near industrial community were greater than those in sediments from the upstream reference site. Especially, Pb is usually used in a large number in industrial process. Clearly, Zn, Cd, and Pb levels in sediment were much lower than global standards. However, there were great variations in heavy metal concentrations due to a large water flows and sediment in this river. Rauf *et al.* (2009) suggested that a big part of heavy metals in sediments were likely to release back to water compartment; for this reason, special attention must be given to the remobilization issue. Both grain size and organic matter content are important factors affecting the metal distribution in sediments (Chen *et al*., 2007).

4.3 Metal concentrations in fish

Jullien's mud carp (*Henicorhynchus siamensis*), Smith's barb (*Puntioplites proctozysron*), and snakehead (*Channa striatus*) were most abundant indigenous fish species. Differences in metal accumulation between fish species were observed due to different physiology, size, behavior, and feeding habits. No Cd and Pb residues were found in *Henicorhynchus siamensis* and *Puntioplites proctozysron* flesh, while the concentrations of Zn in these fish were 4.57 – 6.58 mg/kg. Cd and Zn concentrations in muscles of pelagic fish species were lower than for benthic fish species (Romeo *et al*., 1999).

The levels of zinc, lead, and cadmium in muscles of snakehead fish (*C. striatus*) are given in Table 3. The highest Zn (12.19 mg/kg wet weight) concentration was detected in rainy season and Cd (0.24 mg/kg) and Pb (2.37 mg/kg) were highest in summer and winter seasons, respectively. A fluctuation of heavy metal concentrations in fish was observed since different temperature in each season affects the metal uptake and fish metabolism. Heavy rainfall increases metal concentrations in water by agricultural waste runoff leading to higher metal accumulation in wet seasons (Dural *et al*., 2007). Priprem *et al*. (2007) stated that the average metal concentrations in fish tissues were Zn > Pb> Cd, which were similar to our investigation.

Although Pb is toxic metal, it poorly accumulates in fish muscle (Erdogrul and Erbilir, 2006). This study conflicted with Mzimela *et al*., (2003) which reported that Pb concentrations in fish reflected increased concentrations in water. As there was no metal detection in these fish muscles (*H*. *siamensis* and *P*. *proctozysron*), they cannot be used as bioindicators for metal pollution in aquatic environment.

Zn, Cd and Pb were examined in fish muscle because of its importance for human consumption; however, the liver and gill were determined as these organs tend to accumulate metals (Marcovecchio *et al*., 1991). This study showed metal accumulation was higher in liver and gills (Table 4). Similar results were reported in Turkmen *et al.* (2008). These organs are also good indicators of chronic exposure to heavy metals because they are the site of metal metabolism (Dural *et al*., 2007). Jezierska and Witeska (2001) stated that metal concentrations increase in gills especially at the beginning of exposure, before the metal enters other parts of organism. Futhermore, the liver is often considered a good monitor of water pollution with metals since it is highly active in the uptake and storage of heavy metals. As a result, their concentrations are proportional to those present in the environment.

Table 4 Mean and standard deviations of Zn, Pb, and Cd (mg/kg tissue) in the tissues of examined species and comparison of different seasons and tissues

Samples	Zn	Pb	Cd
Rainy			
Muscle			
Site 1	9.25 ± 0.961ab	1.48 ± 0.51a	0.08 ± 0.02a
Site 2	8.36 ± 1.55a	1.70 ± 0.69a	0.14 ± 0.02a
Site 3	12.19 ± 1.07b	1.79 ± 0.18a	0.15 ± 0.05a
Site 4	8.33 ± 1.05a	1.56 ± 0.59a	0.09 ± 0.01a
Site 5	7.50 ± 0.65a	1.09 ± 0.15a	0.10 ± 0.05a
Site 6	6.63 ± 1.98a	1.04 ± 0.31a	0.07 ± 0.02a
Liver			
Site 1	20.88± 0.46f	1.51± 0.07cd	0.19± 0.02de
Site 2	19.26± 2.1f	1.74± 0.08de	0.26± 0.05e
Site 3	24.32± 1.03g	1.85± 0.07de	0.24± 0.07e
Site 4	17.36± 3.65de	1.66± 0.31e	0.20± 0.02de
Site 5	15.65± 0.78cd	1.25± 0.13c	0.16± 0.02ce
Site 6	12.77± 0.67c	1.28± 0.04c	0.11± 0.02c
Gill			
Site 1	31.27± 1.60i	3.14± 0.07i	0.73± 0.08h
Site 2	30.17± 1.58i	3.12± 0.10j	0.80± 0.03h
Site 3	33.00± 1.46i	3.47± 0.12j	0.78± 0.04h
Site 4	30.94± 1.38i	3.36± 0.11j	0.75± 0.06h
Site 5	30.97± 3.83i	2.780.12h	0.820.08h
Site 6	23.44±1.00h	2.60± 0.19h	0.77± 0.05h

*** Different letters in same column in each season indicate significant differences at $P < 0.05$ (ANOVA).

Table 4 (Continued). Mean and standard deviations of Zn, Pb, and Cd (mg/kg tissue) in the tissues of examined species and comparison of different seasons and tissues

Samples	Zn	Pb	Cd
Winter			
Muscle			
Site 1	4.07 ± 1.32b	0.41 ± 0.15a	0.14 ± 0.05a
Site 2	4.82 ± 0.45b	1.16 ± 0.38ab	0.14 ± 0.08a
Site 3	4.46 ± 0.63b	1.56 ± 0.42bc	0.11 ± 0.05a
Site 4	3.37 ± 0.50a	2.13 ± 0.21bc	0.07 ± 0.06a
Site 5	4.60 ± 1.21b	2.37 ± 0.24c	0.24 ± 0.12a
Site 6	6.06 ± 1.11c	0.49 ± 0.38a	0.21 ± 0.02a
Liver			
Site 1	8.74± 0.44e	0.54± 0.12c	0.35± 0.16de
Site 2	9.16± 0.14f	0.99± 0.02d	0.23± 0.03cd
Site 3	8.66± 0.11e	1.65± 0.13e	0.23± 0.05cd
Site 4	7.42± 0.11c	2.00± 0.01f	0.14± 0.01c
Site 5	8.13± 0.09d	2.60± 0.17g	0.43± 0.11e
Site 6	11.16± 0.11g	0.78± 0.22d	0.46± 0.05e
Gill			
Site 1	12.54± 0.19ij	1.33± 0.11h	0.88± 0.08hi
Site 2	13.23± 0.11j	2.93± 0.10i	0.98± 0.04i
Site 3	12.37± 0.16ij	3.73± 0.23j	0.72± 0.16h
Site 4	11.15± 0.95h	4.01± 0.17k	0.75± 0.11h
Site 5	11.85± 0.19hj	4.00± 0.12jk	0.95± 0.05i
Site 6	12.23± 0.90ij	1.27± 0.14h	0.86± 0.10i

*** Different letters in same column in each season indicate significant differences at $P < 0.05$ (ANOVA).

Table 4 (Continued). Mean and standard deviations of Zn, Pb, and Cd (mg/kg tissue) in the tissues of examined species and comparison of different seasons and tissues

Samples	Zn	Pb	Cd
Summer			
Fish muscle			
Site 1	7.07 ± 1.26a	1.40 ± 0.07a	0.09 ± 0.01ab
Site 2	8.32 ± 1.95a	1.43 ± 0.31a	0.18 ± 0.06ab
Site 3	10.02 ± 1.15a	1.65 ± 0.03a	0.21 ± 0.05b
Site 4	7.70 ± 2.16a	1.58 ± 0.61a	0.11 ± 0.07ab
Site 5	7.17 ± 0.94a	1.22 ± 0.17a	0.12 ± 0.04ab
Site 6	6.07 ± 2.14a	1.07 ± 0.34a	0.06 ± 0.03
Liver			
Site 1	18.48± 2.20de	1.44± 0.05c	0.15± 0.01d
Site 2	20.39± 0.56e	1.67± 0.14d	0.28± 0.01h
Site 3	23.80± 0.34f	1.97± 0.12e	0.33± 0.01g
Site 4	18.83± 0.55de	1.87± 0.13e	0.17± 0.01e
Site 5	17.17± 1.15cd	1.33± 0.10c	0.21± 0.02f
Site 6	16.30± 0.86c	1.23± 0.10c	0.08± 0.01c
Gill			
Site 1	28.8± 50.50ij	3.28± 0.06i	0.81± 0.07i
Site 2	29.52± 0.54ij	3.58± 0.06j	0.82± 0.07i
Site 3	31.36± 1.88j	3.90± 0.01k	0.94± 0.07j
Site 4	28.63± 0.95ij	3.62± 0.34jk	0.73± 0.07i
Site 5	28.08± 1.71i	3.69± 0.06jk	0.79± 0.07i
Site 6	26.60± 2.46i	3.05± 0.14i	0.70± 0.07i

*** Different letters in same column in each season indicate significant differences at $P < 0.05$ (ANOVA).

The highest cadmium concentrations were found in the liver of red gurnard, while the lowest cadmium concentrations were always found in muscle tissues of the fishes (Canli and Atli, 2003). Usero *et al*. (2004) noted that metal concentrations in fish muscles were significantly lower than those found in fish livers and enrichment factors in the livers for Zn, Cd and Pb were around 5. No correlation was reported, however, between metal concentration in the water and fish muscles (Dural *et al*., 2007). Hence, the concentrations of heavy metals in water, sediments, and fish from sampling stations near industrial park were quite higher than other sampling sites. As toxic metals including Cd and Pb were not detected in water samples, sometimes people neglect to pay attention on accumulation of these toxic substances in sediment and fish.

The ranges of levels of Cd, Pb, and Zn in muscles of herbivorous fish from the Pong and Chi Rivers collected during late 2002 until early 2003 were 0.002 – 0.014, 0.05 – 0.91, and 26.8 – 88.9 mg/kg wet weight, respectively while The ranges of levels of Cd, Pb, and Zn in muscles of carnivorous fish from these Rivers were 0.002 – 0.022, 0 – 1.42, and 24.27 – 45.10 mg/kg wet weight, respectively (Priprem *et al*., 2007). The higher contamination of Cd, Pb, and Zn in fishes caught along the Sakaekrang River was found in 2006 than 2005 (Petpiroon *et al*., 2008). Average concentrations of freshwater fish from this river were 0.16, 0.32, and 6.06 mg/kg, respectively. The heavy metal contamination in fish varies in different fish, time, and places. Petpiroon *et al.* (2009) reported that the average concentrations of Cd, Pb, and Zn in freshwater fish collected from Phetchburi River in 2008 were 0.11, 1.02, and 5.53 mg/kg, respectively. These were within standard levels according to notification of Ministry of Public Health (No. 98) B.E. 2529, as such they are safe for consumption. However, a potential health risk may generate in near future because of the unplanned rapid expansion of agricultural, provincial, and industrial development.

Other marine species such as crab may contain cadmium more than 10 mg/kg, whereas the hepatopancreas of spanner crabs (*Ranina ranina*) contained cadmium upto 14.9 mg/kg (Rayment, 1995). The Cd concentration in two bivalve molluscs species, *Modiolus barbatus* and *Tapes decussates*, captured in the north Adriatic sea of Italy were greater than 2 mg/kg wet weight (Storelli and Marcotrigiano, 2001). The Cd levels in offal of dugongs and turtles ranged from 7 to 76 mg/kg wet weight (Dight and Gladstone, 1993; Gordon *et al*., 1998).

4.4 Correlation between fish, water and sediment

The correlation between metal pairs in water, sediment and muscle of *Channa striatus* was statistically tested. The Pearson's correlation coefficient matrix for the element pairs was performed, the correlation coefficient of Zn between sediment and fish muscle was found to be significant (Table 5). However, muscle Zn was negatively correlated with pH and BOD in water. At low pH, free ionic metal species tend to dominate total metal concentration, and at high pH, metals tend to form inorganic complexes with hydroxides and carbonates.

Metal concentrations in water positively correlated with ones in fish tissue (Svobodova *et al*., 1996). In this study, there was no correlation between Zn in water with either its metal in sediment or fish. The Cd concentration in water gave no indication of the safety of aquatic organisms as human food; for this reason, heavy metal contamination is an important consideration when aquatic organisms are harvested from natural water sources (Ruangsomboon and Wongrat, 2006).

Table 5 Pearson Correlation (PC) coefficient matrix between the trace and major elements and fine fractions of sediments

		pH	BOD	COD	Zn Water	Zn Sediment	Zn Fish	Pb Sediment	Pb Fish	Cd Sediment	Cd Fish
pH	Pearson Correlation	1									
	Sig. (2-tailed)										
BOD	Pearson Correlation	.738*	1								
	Sig. (2-tailed)	.000									
COD	Pearson Correlation	-.081	.164	1							
	Sig. (2-tailed)	.750	.516								
Zn-W	Pearson Correlation	.201	.436	.363	1						
	Sig. (2-tailed)	.423	.070	.139							
Zn-S	Pearson Correlation	-.301	-.240	.100	.262	1					
	Sig. (2-tailed)	.224	.337	.693	.293						
Zn-F	Pearson Correlation	-.665*	-.493*	.074	-.039	.691*	1				
	Sig. (2-tailed)	.003	.037	.771	.878	.002					
Pb-S	Pearson Correlation	-.375	-.317	.602*	.141	.480*	.388	1			
	Sig. (2-tailed)	.125	.200	.008	.577	.044	.111				
Pb-F	Pearson Correlation	-.332	-.175	.107	.397	.365	.152	.106	1		
	Sig. (2-tailed)	.178	.488	.671	.102	.136	.546	.675			
Cd-S	Pearson Correlation	-.260	-.060	-.214	.069	.602*	.553*	.344	.185	1	
	Sig. (2-tailed)	.370	.840	.462	.816	.023	.040	.228	.526		
Cd-F	Pearson Correlation	.339	.399	-.046	.377	.145	.094	-.145	.110	.033	1
	Sig. (2-tailed)	.168	.101	.856	.123	.565	.712	.565	.664	.911	

* Correlation is significant at the 0.05 level (2-tailed).

4.5 Speciation of heavy metals in surface sediment

The mobility and bioavailability of the metals varied with the properties of sediment including particle size, organic matter, pH, redox potential, and water flow (Segura *et al*., 2006). Heavy metals may be recycled within the sediment compartment and back to the water column by chemical and biological processes (Ip *et al*., 2007). The accumulation of metal contaminants in sediments can pose serious environmental problems to the surrounding areas. Heavy metal contamination in sediment could affect the water quality, the bioassimilation, and metal bioaccumulation in aquatic organisms, resulting in potential long-term implications on human health and ecosystem.

Metals in the sediments bound to different fractions with different strengths. Sediment which can release in exchangeable and acid soluble fractions less than 1% of the total metal will be considered safe for the environment. The sequential extraction data are described as percentage of zinc, cadmium, and lead (Fig. 5, 6, and 7, respectively). In this study, Zn, Cd, and Pb associated mainly to residual fraction. So it should not be available for remobilization.

This study indicated that zinc was mainly bound to residual fractions. This fraction ranged over 30 – 65% (Fig. 5). These results are consistent with Davutluoglu *et al.* (2010) who stated that most of Zn in surface sediments of Akyatan Lagoon, Turkey would be immobile because of its mostly high residual fraction (82%). Different to these

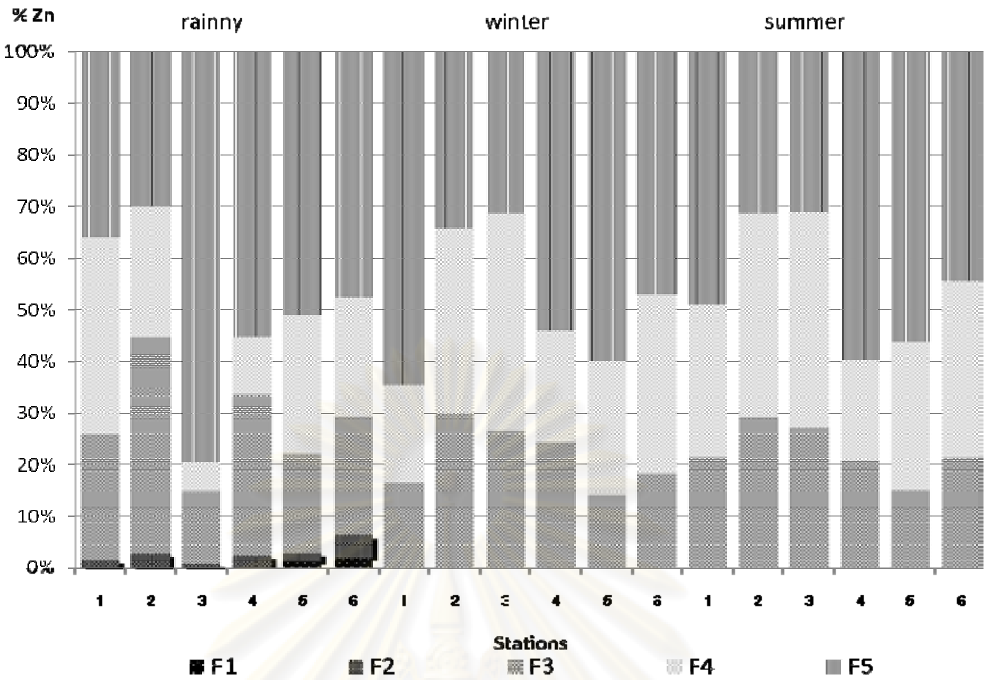

Fig. 5 Fractionation profile of Zn in sediments along the Mae Kuang River (S1 to S6 stations) obtained in the three seasons of the year using the sequential extraction scheme:

F1 (exchangeable); F2 (carbonates); F3 (reducible); F4 (oxidizable); and F5 (residual)

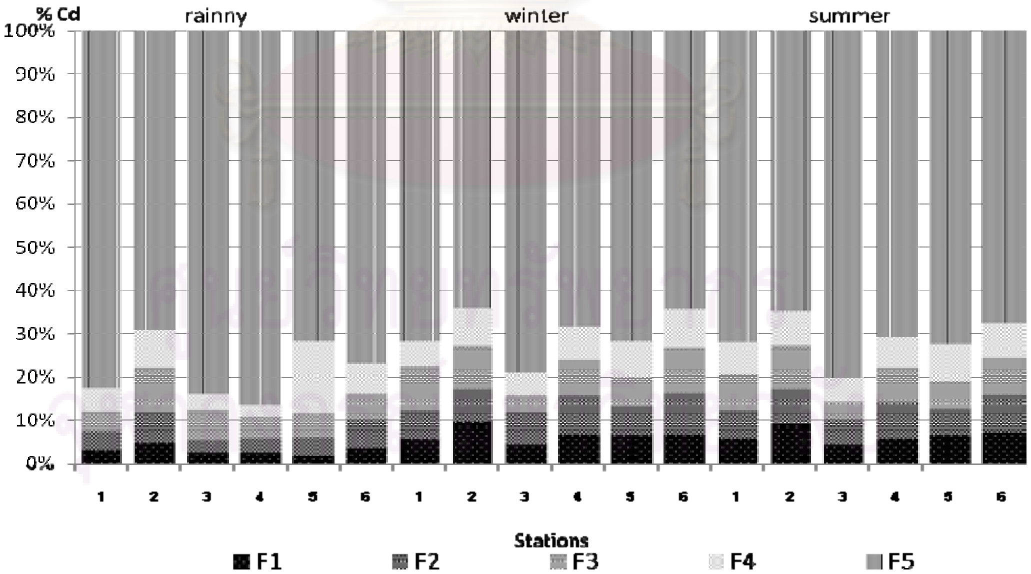

Fig. 6 Fractionation profile of Cd in sediments along the Mae Kuang River (S1 to S6 stations) obtained in the three seasons of the year using the sequential extraction scheme:

F1 (exchangeable); F2 (carbonates); F3 (reducible); F4 (oxidizable); and F5 (residual)

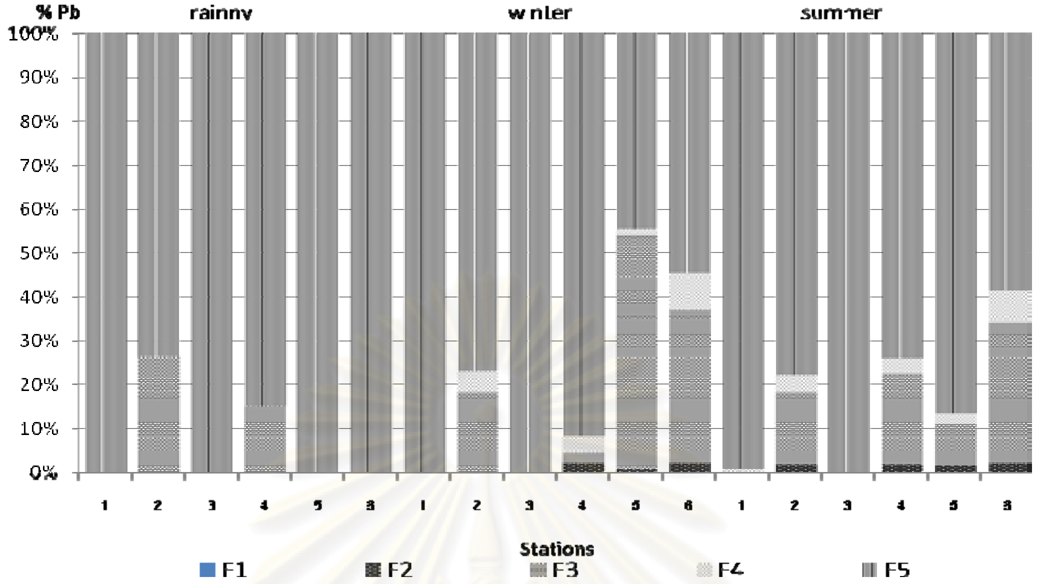

Fig. 7 Fractionation profile of Pb in sediments along the Mae Kuang River (S1 to S6 stations) obtained in the three seasons of the year using the sequential extraction scheme: F1 (exchangeable); F2 (carbonates); F3 (reducible); F4 (oxidizable); and F5 (residual)

observations, Zn showed a high mobility appearing mainly bound to most labile fractions (Vicente-Martorell *et al*., 2009). It seems there were not different in speciation of Zn among the sampling sites and seasons.

Although the average total content of cadmium in sediments was the lowest in three heavy metals, it appeared some bound to most exchangeable fraction (Fig. 6). The fraction containing the most cadmium was the species bond to residual fraction (F5) in the sediments. A different result was found by other previous studies showing that Cd was the most mobile metal mainly bound to weak acid and carbonates (Vicente-Martorell *et al*., 2009; Morillo, *et al*., 2004. Although the mean total amount of cadmium in the sediments was lower than that of other metals, the Cd contamination into rivers should be managed. As the amounts increase, the active fractions would be easily released, and would then cause adverse effects to the aquatic environment and fish consumers.

The average total content of Pb in the sediment samples was the highest in the three metals determined. According to this study, Pb was the least labile metal because of its strong association with the residual fraction of sediment (up to almost 100%) (Fig. 7). As a result, this metal might present a less serious contamination risk than more mobile (bioavailable) Cd. Different results was found in other studies showing that F3 was the fraction containing the most Pb in the sediment samples of many rivers and lakes (Relic *et al*., 2005; El-Azim and El-Moselhy, 2005; Zhu *et al*., 2001; Feng *et al*., 2004).

According the results, the availability patterns of metals in sediment were as follows: Cd > Zn > Pb. This fish has a benthic behaviour and could be more affected by sediment. Therefore, the “bio” availability of this metal would depend on Cd bound to organic matter instead of exchangeable fraction of Cd that showed the highest percentage. These correlations showed that the species studied had different uptake of heavy metals, so this way pelagic fish are more sensible to level of heavy metal from waters, while benthic (bottom-dwelling) fish can be affected by heavy metals from sediments.

4.6 Health risks from fish consumption

The daily ingestion rates of Thai, American, and Japanese were 32, 50, and 69 kg/person/year, respectively (Bangkokhealth.com, 2011), while adult inhabitants living along Mae Kuang River areas, the daily ingestion rate of fish consumption was approximately 26 kg/person/year. Mean concentration of Pb and Cd in captured snakehead fish (*Channa striata*) were <0.05 – 2.13 and <0.02 – 0.24 mg/kg wet weight, while the concentrations of Zn in these fish were 3.37 – 12.19 mg/kg. Referring to Target carcinogenic risk (TR) equation and the oral carcinogenic slope factor from the Integrated Risk Information System database for Pb (US EPA, 2010), it was found that the carcinogenic risk for fish consumption was high (up to 10^{-2}). On the other hand, because of no target hazard quotients (THQs) of Pb, Cd, and Zn, valued over 1 through the fish consumption, it suggests that health risks for the local inhabitants along the Mae Kuang River is low. Morevoer, most people living along the Mae Kuang River consume fish from other places. Because fish captured in that river has been dramatically reduced. Virulhakul and Suntipiriyaporn (2006) reported that only less than 1% of fish samples collected in Thailand during 1974 – 2005 was higher than allowable limits for Pb and Cd.

International Standards for edible portion of freshwater fish based on Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products, Food and Agriculture Organization of the United Nations (1983) were 40 – 100, 2 – 10, and 0.05 – 2 ppm (wet weight) for Zn, Pb, and Cd, respectively. The Joint FAO/World Health Organization suggested the provisional tolerable weekly intakes are 0.007 and 0.025 mg/kg body weight for cadmium and lead, respectively (Castro-González and Méndez-Armenta, 2008). As there are different in standard levels, it is difficult to justify that snakehead fish from some sample site were safe for consumption or not. It could inform local population that eating fish from the Mae Kuang River is at your own risk. This result was in agreement with recommendation of French which is to consume fish from various sources and avoid eating fish captured from heavily contaminated sites especially in Adour-Garonne Rivers (Shinn *et al*., 2009). The permissible limits proposed by the FAO, WHO and Turkish legislation established the following maximum levels for the metals which consumption is not permitted: 0.1 mg/kg for Cd, 50 mg/kg for Zn, and 0.5 mg/kg for Pb (Demirak *et al*., 2006).

Excessive Zn intake will cause poisoning, nausea, acute stomach pains, diarrhea and fever (Qiao-qiao *et al*., 2007). The recommended dietary allowance for zinc in humans is 15 mg/d for men, 12 mg/d for women, 10 mg/d for children, and 5 mg/d for infants (Nord *et al*., 2004). Lead is a neurotoxin that causes behavioral deficits in vertebrates, decreases in survival and growth rates, causes learning disabilities, and metabolism (Qiao-qiao *et al*., 2007). The World Health Organization has recommended that dietary Pb should not exceed 0.3 μg/g (wet weight basis), and with a recommended limit of 450 μg of Pb per day for adults. Cd is not an essential element, and the World Health Organization/Food and Agricultural Organization (WHO/FAO) has determined a maximum tolerable daily intake of 55 μg/(person·d) (Qiao-qiao *et al.*, 2007).

4.7 Biological Responses of Striped Snakehead Fish, *Channa striata*, Exposed to Heavy Metal Contaminants in the Mae Kuang River

Mean concentrations of Cd, Pb, and Zn in snakehead muscle samples are presented in Table 5. Heavy metal fluctuations in fish were observed because different temperature in each season affects the metal uptake and fish metabolism. Heavy rainfall increases metal concentrations in water by agricultural waste runoff leading to higher metal accumulation in wet seasons (Dural *et al.*, 2007). Priprem *et al.* (2007) stated that the average metal concentrations in fish tissues were Zn > Pb> Cd, which were similar to our investigation. Moreover, variability of metal levels in fish depends on feeding habits (Watanabe et al., 2003), age, size and length of the fish (Linde *et al.*, 1998; Al-Yousuf *et al.*, 2000) and their habitats (Canli and Atli, 2003). Season (Kargın, 1996), physical and chemical status of water (Jezierska & Witeska, 2001) also affect an accumulation of metals in the tissue. Seasonal changes of metal concentrations in fish may result from intrinsic factors such as growth cycle and reproductive cycle and from changes in water temperature. Knowledge of heavy metal concentrations in fish is important both with respect to nature management and human health.

Many of the existing methods for the measurement of heavy metals are highly relatively expensive and required sophisticated instruments. The high cost of analysis is an important barrier for heavy metal monitoring. As a result, it is necessary to develop simple, affordable and accurate methods for scientific monitoring and assessment. One possible approach for heavy metal assessment would be to deploy biomarker and bioindicator methods.

4.7.1 Changes in hepatosomatic index and blood haematocrit

Hepatosomatic index (HSI) is associated with liver energetic reserves and metabolic activity (Pyle *et al*., 2005). HSI of fish from sites 2 and 3 were a little bit lower than other sites. However, there was no clear evident of an association between heavy metal contents and changes in the fish HSI of *Channa striata* in this study (Fig. 8A). As a result, it may not be a useful indicator of physiological disturbance in metal-contaminated fish. The same result was reported for yellow perch (*Perca flavescens*) by Giguere *et al*. (2004) who concluded that HSI is not a good biomarker of metal exposure.

Changes in the hematological values reflect alteration of fish physiology due to stress and pollutant exposure. There was not much different in blood haematocrit (Hct) or percent cell packed (%PCV) in each site (Fig. 8B). The slightly increased in %PCV in fish from polluted stations that have affected from industrial park and Lamphun downtown compared to the reference site. The reason for an increase in %PCV possibly

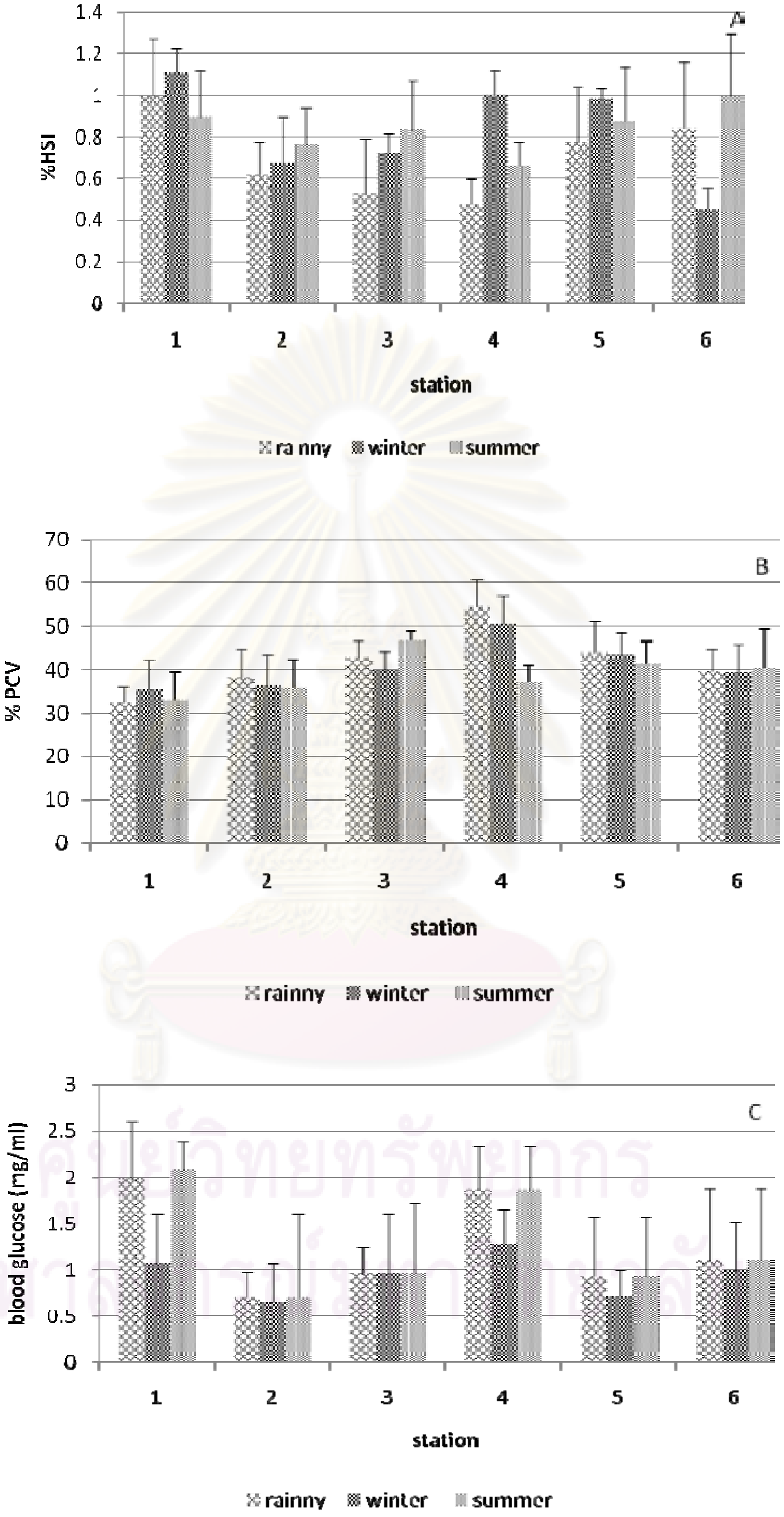

Fig. 8 Mean values of Hepatosomatic index (%HSI; A), Percent Cell Packed (%PCV; B), and blood glucose (C) of Striped Snakehead Fish, *Channa striata* measured at six stations situated in the Mae Kuang River

due to changes in blood parameters which result in erythrocyte swelling, or by release of large red blood cells from the spleen. A significant decrease in total leukocyte counts was noticed in polluted sites. Changes in leukocyte counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of the fish. Studies on yellow perch (*Perca flavescens*) report similar findings after exposure to different contaminants in the St. Lawrence River, Canada (Dautremepuitsa *et al*., 2009).

4.7.2 Changes in blood glucose and protein

Carbohydrate is stored as glycogen in both muscles and liver, but in the liver, it constitutes the first line of defense when the blood glucose concentration becomes low (Miranda *et al*. 2008). It is well established that physiological and biochemical parameters in the blood and tissues of fish could change when exposed to pollutants (Cicik and Engin, 2005). The presence of heavy metals in the liver and muscle could change the glycogen reserves in fish by affecting the biochemical activities in tissues. It was found that blood glucose of fish caught from sites 2 and 3 were quite lower than other sites (Fig. 8C). It implied that fish were exposed to pollutant; however, the tolerance levels should be further investigated.

The total proteins measured in fish serum sampled along Mae Kuang River were not significantly different (Fig. 9A). For this reason, serum protein cannot be used as biomarker for heavy metal assessment in this study. Catalase (CAT) activity (Fig. 9B) was relatively increased in fish sampled at polluted sites. To protect themselves from

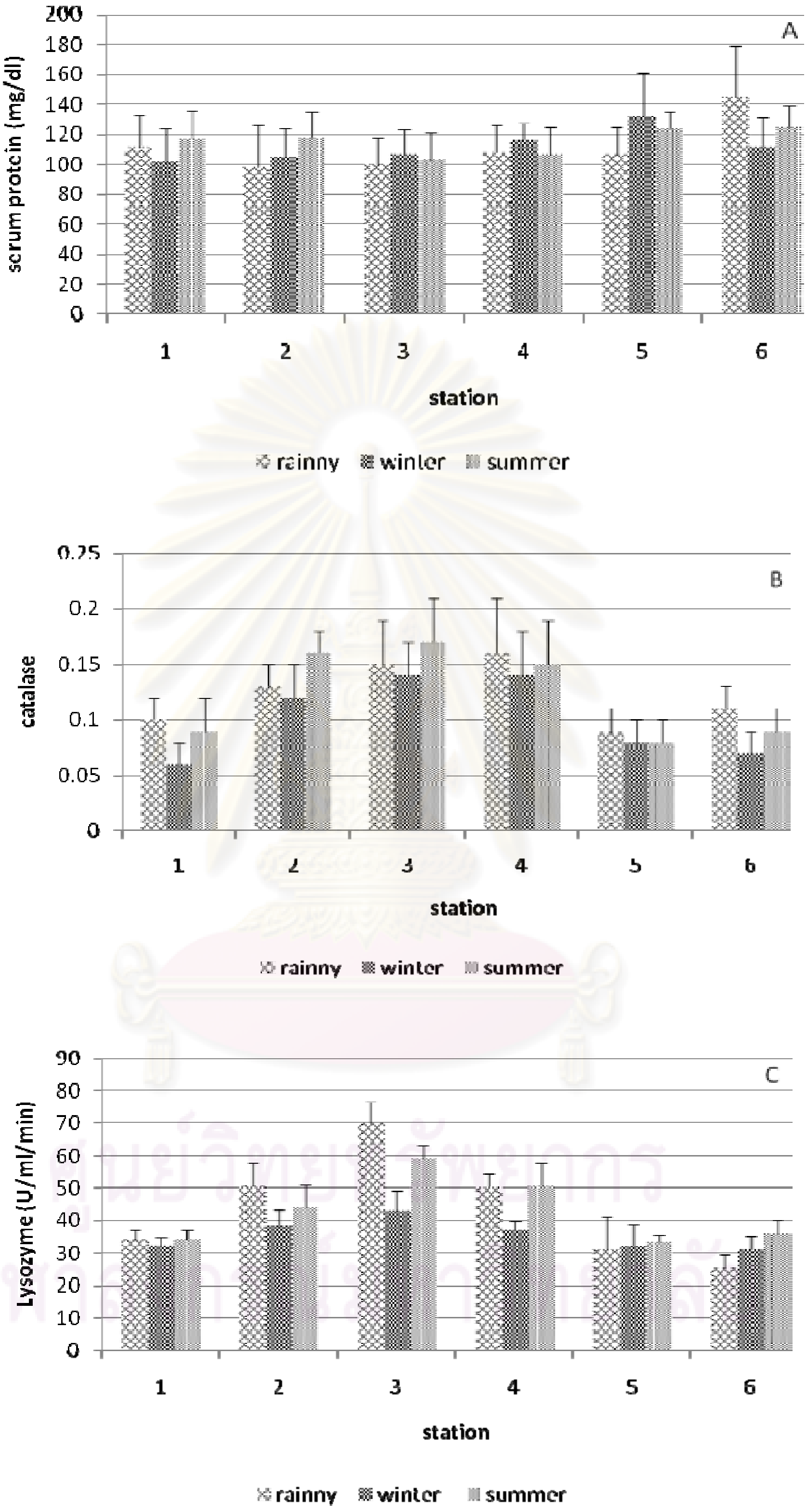

Fig. 9 Serum protein (A), Catalase (B), and Lysozyme (C) of Striped Snakehead Fish, *Channa striata* sampled at different sites along the Mae Kuang River. The values are expressed as mean ± standard deviation.

free radicals against such stress, antioxidant enzymes including catalase showed significantly increased activity in both tissues. CAT activity was significantly higher in worms exposed to copper (Geracitano *et al*., 2002). CAT activity in the liver of *Brachydanio rerio* increased with the Cu concentration and the duration of exposure (Paris-Palacios *et al*., 2000). There were high correlations of CAT activities and Cd residue in seabream liver (Ferreira *et al*., 2008). However, opposite result was observed activity in cultured seabream fish with higher metal accumulation in liver (Fernandes *et al*., 2007). CAT was either induced or inhibited by heavy metals depending on species and exposure dosages (Van der Oost *et al*., 2003; Gravato *et al*., 2006).

4.7.3 Changes in lysozyme levels

Lysozyme activity is one of the non-specific immunity reactions that is able to disrupt bacteria cell wall. An increase in lysozyme activities in fish sampled at the contaminated localities compared to those from the reference site was observed (Fig. 9C). Exposure of rainbow trout to municipal effluents resulted in elevated plasma lysozyme concentrations (Salo *et al*., 2007). High lysozyme activity detected in head kidney suggested immune responses in fish exposed to sublethal concentrations of metals as well (Zelikoff *et al*., 2000). However, lysozyme response maybe affect by species, sex, tissue, and type of pollutant (Dautremepuitsa *et al*., 2009).

4.7.4 Changes in antioxidant enzymes

Superoxide dimutase (SOD) is an oxidoreductase which accelerates the conversion of superoxide anion radical (O_2^-) to H_2O_2; the efficacy of SOD as an antioxidant relies on its cooperation with CAT (Parihar *et al*., 1997). SOD activities were decreased in fish captured from polluted sites in comparison to reference site (Fig. 10A). Achuba and Osakwe (2003) suggested that a decrease in SOD activity can generate cell damage because of an imbalance in toxic oxygen radical production.

An increased of GST activity in gills of snakehead caught in sites 2, 3, and 4 indicated GST could be one of the biomarkers for assessing effluent impact (Fig. 10B). Similarly, GST activity of caged mussels was significantly increased in downstream of municipal effluent discharge (Gagné *et al*., 2004). Maria *et al*. (2003) also reported a significant increase in liver GST activity of eels (*Anguilla anguilla* L) exposed to Pulp and paper mill effluents.

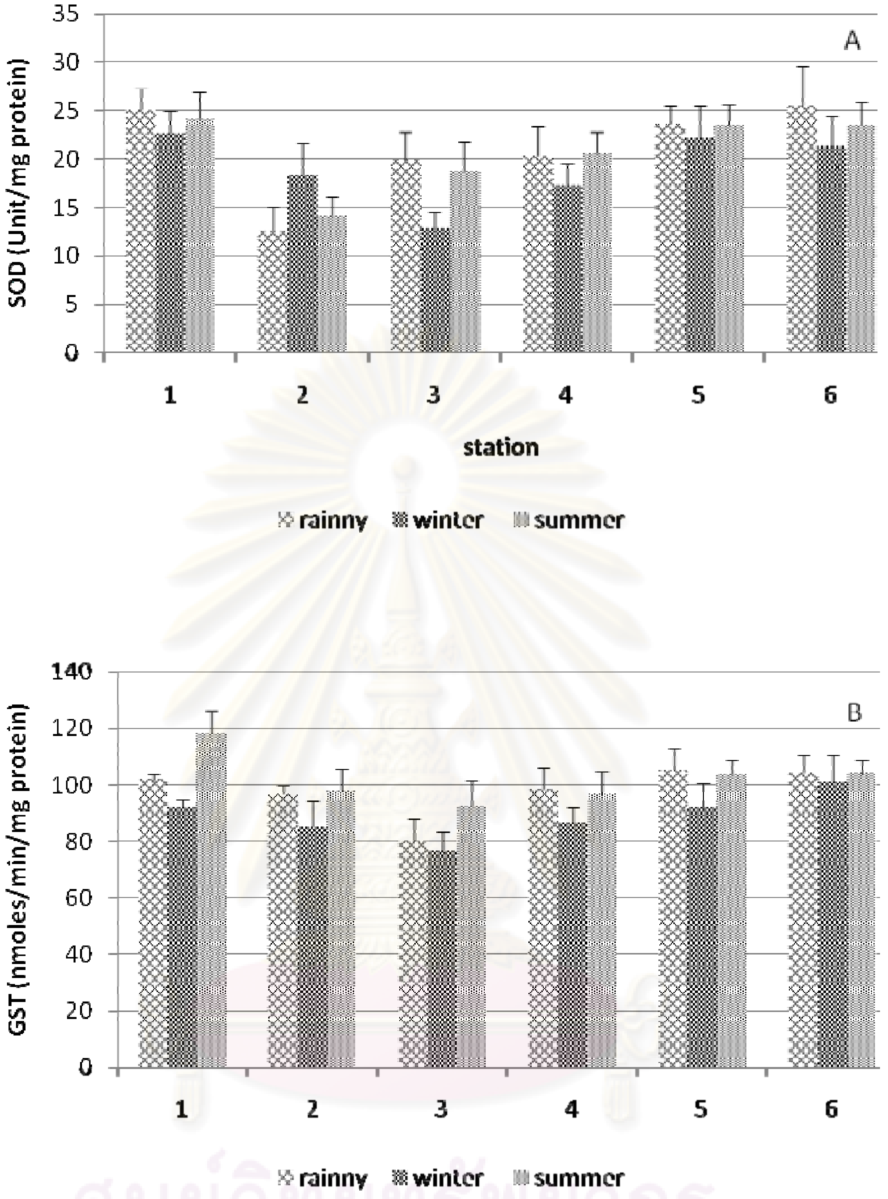

Fig. 10 Superoxide dismutases, SOD (A) and Glutathione-S-Transferase, GST (B) of Striped Snakehead Fish, *Channa striata* sampled at different sites along the Mae Kuang River. The values are expressed as mean ± standard deviation.

4.8 Stakeholder Participation in Mae Kuang River Management

The Faculty of Fisheries Technology and Aquatic Resources provided the brain-storming discussion among stakeholders to solve this serious situation. This was held as part of the 4th Maejo fisheries and aquaculture conference on December 8, 2009 in Chiangmai. Both quality and quantity information about current status of Mae Kuang River was presented. Only 50 people from different institutions attended this meeting which indicated insufficient stakeholder involvement. The current status of water quality was presented at the discussion but many people did not have access to this information. In addition to the poor quality of water, a water shortage and aquatic weed growths were also big issues in this discussion. Participants requested to find the key organization to take care of these problems including fund raising and expanding community participation. Although many institutions are involved in the Mae Kuang River use and management, there is too little coordination. Managing the risk to human and aquatic organisms from contaminated heavy metals involves regular monitoring of contaminants, assessing risks, biomonitoring levels of toxic metals in media and biota, regulations and enforcement, source reduction, and direct management (Burger, 2008). In addition, local communities in various areas have to develop their own strategies to ensure improvement of water resources for sustainable use. Water treatment in each household and community must be constructed in order to reduce the nutrient load in this river. In summary, the conflicts in Kuang River management will get worse and worse if neglected, does not get a serious effort to solve the problem, and wait for others to help instead of the stakeholders becoming active and working on the problem themselves.

4.9 Protective Effects of Kai Additive Feed against Lead Toxicity in African Catfish, *Clarias gariepinus*

The growth of African catfish fed on the diets supplemented with Kai was significantly increased (Table. 6). For this reason, this alga incorporation in catfish feed is suitable. However, the cost must be reduced. In addition, this study showed Kai, one of antioxidant nutrients, rebalanced the impaired oxidative stress in abating lead toxicity. Hematolgical, immunological, and antioxidant parameters of African catfish fed with control and Kai supplementary diets are shown in Table 7.

Table 6 Average weight (g) of African Catfish fed with diets containing various levels of dried Kai algae for 30 – 90 days

Group*	Final weight (g)		
	Day 30	Day 60	Day 90
1	84.83 ± 8.75a	96.50 ± 1.32c	115.83 ± 4.25d
2	85.00 ± 3.12a	113.17 ± 13.42c	122.33 ± 7.29de
3	106.33 ± 14.37b	117.67 ± 12.91c	131.57 ± 6.65e

* Group 1: Basal Diet (0% Kai Supplement)
Group 2: Basal Diet + 10% Kai Supplement
Group 3: Basal Diet + 15% Kai Supplement

** Different letters in each column indicate significant differences (Duncan's test, $P < 0.05$).

Although the red blood cells (RBC) trend to increase; however, there were not significantly different ($P > 0.05$). However, White blood cell (WBC) and the hematocrit (Ht or HCT) or the percentages of packed cell volume (%PCV) values were significantly increased in catfish with 15% Kai feeding. Lysozyme is one of non-specific immune responses. Serum lysozymes were not significantly different. Catfish which received 15% *Cladophora* sp. supplementation showed the best record of white blood cell count and hematocrit. Catfish fed with Kai diets showed a little bit increased levels of superoxide dimutase (SOD) and catalase (CAT) enzyme while there was not significantly different in Glutathione *S*-transferase (GST) activity ($P > 0.05$) compared with the control group. SOD catalyzes the dismuation of superoxide radical to hydrogen peroxide that can help to protect against cell destruction, while catalase decomposition of hydrogen peroxide to water and oxygen (Tjalkens *et al*., 1998). GSTs are a group of enzymes that catalyse the chemical conjugation of reduced glutathione, to a variety of electrophilic compounds (Lauren *et al*. 1989). GST seems to play important roles in both detoxification and bioactivation reactions generated by heavy metals (Napierska *et al*., 2006). A dose-dependent effect in hematological and immunological parameters as well as antioxidant enzymes was seen in catfish fed with Kai supplementary diets.

The lethal concentration of lead on African catfish (6 g in average weight) was approximately 0.6 ppm and all catfish were dead after 10 ppm lead chloride exposure (Olifa *et al*., 2003). No mortalities were observed in this study after Pb exposure. These results are similar with Alves and Wood (2006) who observed no mortalities and growth effects when juvenile rainbow trouts were fed with pellets amened with 0.3 – 500 µg Pb/g for 42 days. It is also consistent with Mount *et al.* (1994) who showed no effects of brine shrimp contaminated with 170 Pb/g on survival and growth of rainbow trout. Although background level of Pb in water is less than 0.02 ppb (Förstner and Wittmann, 1983), the highest concentration of Pb that was found in Mae Kuang River was 4 ppb (0.004 ppm). Results of hematological, immunological, and antioxidant contents of catfish exposed to various concentrations of lead are shown in Table 8 and 9.

Lead (Pb) is heavy metal that can cause damage to the erythrocyte membrane resulting in hemolysis and leading to reduction in hematocrit value (Simsek *et al*., 2009; Ancheva *et al*., 2003). Referring to RBC and WBC production in this study, it seems Kai supplementary diets could prevent the toxicity from lead exposure in African catfish. This presence of WBC might be associated with high phagocytic activity. The occurrence of phagocyte cells was found in neotrophical fish after Pb exposure (Rabitto *et al*., 2005). Algae have been reported for RBC and WBC improvement. For example, A significant ($p \leq 0.05$) increase in WBC was observed in rohu, *Labeo rohita* fed with 0.1 – 1.0 g *Euglena viridis* / kg of feed on day 60 (Das *et al*., 2009). In addition, post-challenge with *A. hydrophila*, a significant ($p \leq 0.05$) difference in the WBC was observed in rohu fed with 0.1 – 1.0 g *Euglena viridis* / kg of feed. Furthermore, the dietary $CaCO_3$ is able to reduce Pb burdens in juvenile rainbow trout exposed to environments contaminated with waterborne Pb (Alves and Wood, 2006).

An increase in lysozyme activity was found in Rainbow trout (Oncorhynchus mykiss) after a 10 min handling stressor (Demers and Bayne, 1997), while there was a significant decline in the serum lysozyme activity of the crowding stressed fancy carp (Yin *et al*., 1995). This present data show lysozyme activity increased after Pb exposure. It appeared to be dose-dependent of algae feeding. The elevated lysozyme might be good for catfish to fight with pathogens.

A decrease in catalase and superoxide dismutase (SOD) activities occurred after 7, 15, and 30 days of Pb exposure. Pb exposure has a dose response relationship with changes in antioxidant enzyme activities that include SOD and glutathione-S-transferase (GST) (Adonaylo and Oteiza, 1999). The GST is a cytosolic enzyme involved in the detoxification of a range of xenobiotic compounds by conjugation to GSH which is essential in the maintenance of normal physiological processes (Daggett *et al*., 1998). Pb can induce SOD activities in rainbow trout (Ates *et al*., 2008). Changes in SOD levels have been also detected in goldfish exposed to various degrees of oxygen tension (Lushchak *et al*. 2001). Dietary Kai affected RBC, WBC, %PCV. Significantly increased in WBC and %PCV observed in catfish fed with Kai supplementary diets after lead exposure. As the SOD–CAT system provides the first defense against oxygen toxicity, increase in the activities of CAT and SOD was usually seen in fish exposed to pollutants

Table 7 Effects of Kai on hematological and immunological parameters of African catfish after feeding for 30, 60 and 90 days

		Feeding period (days)		
		30	60	90
RBC ($X10^6$/μl)	0% Kai	2.17 ± 0.49	2.08 ± 0.11	2.27 ± 0.28
	10% Kai	2.12 ± 0.22	2.32 ± 0.25	2.22 ± 0.17
	15% Kai	2.24 ± 0.22	2.14 ± 0.06	2.34 ± 0.08
WBC ($X10^3$/μl)	0% Kai	3.53 ± 0.22 a	3.42 ± 0.37	3.73 ± 0.18 a
	10% Kai	3.43 ± 0.19 a	3.49 ± 0.20	4.40 ± 0.21 b
	15% Kai	4.75 ± 0.27 b	3.58 ± 0.10	4.80 ± 0.18 b
%PCV	0% Kai	43.17 ± 0.86 c	45.17 ± 0.55 c	45.67 ± 1.17 c
	10% Kai	44.76 ± 1.20 c d	46.35 ± 0.40 c	46.67 ± 0.30 c
	15% Kai	45.66 ± 0.16 d	50.09 ± 0.47 d	50.33 ± 0.62 d
Lysozyme (unit/min)	0% Kai	0.004 ± 0.001	0.005 ± 0.001 e	0.005 ± 0.001
	10% Kai	0.009 ± 0.002	0.011 ± 0.002 f	0.012 ± 0.001
	15% Kai	0.008 ± 0.053	0.014 ± 0.003 f	0.024 ± 0.009
CAT (mmol/min/mg protein)	0% Kai	11.13 ± 0.04 g	11.09 ± 0.07 g	11.33 ± 0.41 g
	10% Kai	11.67 ± 0.22 g h	12.33 ± 0.52 g h	12.67 ± 0.34 gh
	15% Kai	12.32 ± 0.53 h	11.93 ± 0.35 g	12.33 ± 0.66 h
SOD (unit/mg protein)	0% Kai	214 ± 7.0 i	233 ± 15.5	243 ± 6.6 i
	10% Kai	215 ± 6.0 i	243 ± 3.6	253 ± 7.8 i j
	15% Kai	255 ± 6.5 j	257 ± 11.1	275 ± 12.5 j
GST (nmol/min/mg protein)	0% Kai	133.33 ± 5.51	135.67 ± 10.02	135.33 ± 6.81
	10% Kai	127.67 ± 14.15	137.33 ± 6.66	137.67 ± 14.15
	15% Kai	130.33 ± 7.64	130.67 ± 6.68	140.33 ± 7.64

*Different letters in same column indicate significant differences at $P < 0.05$ (ANOVA), while there were no differences in the parameters without letters ($P > 0.05$).

Table 8 Effects of Kai on hematological and immunological parameters of African catfish after 90-day feeding and lead exposure (0.004 ppm)

		Days after Pb exposure		
		7	15	30
RBC	0% Kai	2.24 ± 0.22	2.30 ± 0.28	2.18 ± 0.15
	10% Kai	2.11 ± 0.13	2.33 ± 0.09	2.34 ± 0.16
	15% Kai	2.33 ± 0.08	2.43 ± 0.25	2.34 ± 0.15
WBC	0% Kai	3.15 ± 0.12 a	3.21 ± 0.02 a	3.16 ± 0.02 a
	10% Kai	3.14 ± 0.10 a	3.29 ± 0.15 a	3.46 ± 0.20 a b
	15% Kai	4.20 ± 0.14 b	3.74 ± 0.25 b	4.09 ± 0.51 b
%PCV	0% Kai	44.75 ± 3.87	43.71 ± 2.72 c	44.23 ± 1.49 c
	10% Kai	48.67 ± 0.49	47.72 ± 0.39 d	47.48 ± 0.78 d
	15% Kai	49.05 ± 0.81	48.26 ± 0.18 d	47.04 ± 0.70 d
Lysozyme	0% Kai	0.003 ± 0.001	0.004 ± 0.001 e	0.003 ± 0.001
(unit/min)	10% Kai	0.008 ± 0.002	0.009 ± 0.002 f	0.011 ± 0.001
	15% Kai	0.005 ± 0.004	0.012 ± 0.002 f	0.013 ± 0.003
CAT	0% Kai	8.51 ± 0.67 g	8.52 ± 0.67g	9.83 ± 0.65 g
(mmol/min/mg	10% Kai	9.12 ± 0.17 g h	9.81 ± 0.48 g h	10.23 ± 0.15 g h
protein)	15% Kai	10.32 ± 0.52 h	10.58 ± 0.34 h	10.91 ± 0.51 h
SOD	0% Kai	207 ± 7.6	210 ± 11.5	207 ± 5.9
(unit/mg	10% Kai	198 ± 12.1	201 ± 3.6	208 ± 3.5
protein)	15% Kai	212 ± 3.0	207 ± 9.2	217 ± 11.5
GST	0% Kai	165.67 ± 18.66	137.77 ± 8.87	136.74 ± 1.93
(nmol/min/mg	10% Kai	156 ± 7.04	130.50 ± 11.05	136.02 ± 2.92
protein)	15% Kai	167.67 ± 10.52	136.34 ± 11.03	136.28 ± 8.11

*Different letters in same column indicate significant differences at $P < 0.05$ (ANOVA), while there were no differences in the parameters without letters ($P > 0.05$).

Table 9 Effects of Kai on hematological and immunological parameters of African catfish after 90-day feeding and lead exposure (0.3 ppm)

		Days after Pb exposure		
		7	15	30
RBC	0% Kai	2.23 ± 0.15	2.21 ± 0.16	2.15 ± 0.18
	10% Kai	2.13 ± 0.14	2.31 ± 0.07	2.28 ± 0.15
	15% Kai	2.29 ± 0.18	2.36 ± 0.21	2.28 ± 0.06
WBC	0% Kai	3.01 ± 0.08 a	2.97 ± 0.09 a	3.04 ± 0.10 a
	10% Kai	3.00 ± 0.14 a	3.16 ± 0.06 a b	3.19 ± 0.15 a
	15% Kai	3.27 ± 0.03 b	3.35 ± 0.23 b	3.57 ± 0.10 b
%PCV	0% Kai	41.94 ± 1.21 c	41.91 ± 0.48	41.12 ± 1.21 c
	10% Kai	45.58 ± 0.58 d	44.65 ± 1.32	43.80 ± 1.06 c d
	15% Kai	45.55 ± 0.73 d	43.98 ± 2.04	44.32 ± 0.99 d
Lysozyme	0% Kai	0.002 ± 0.001 e	0.003 ± 0.001 e	0.002 ± 0.001
(unit/min)	10% Kai	0.006 ± 0.003 e f	0.007 ± 0.003 e f	0.010 ± 0.001
	15% Kai	0.003 ± 0.001 f	0.011 ± 0.002 f	0.009 ± 0.008
CAT	0% Kai	7.11 ± 0.37 g	7.03 ± 0.57 g	7.21 ± 0.54 g
(mmol/min/mg	10% Kai	7.49 ± 0.69 g	7.56 ± 0.53 g h	7.08 ± 0.81 g
protein)	15% Kai	9.46 ± 0.48 h	9.58 ± 1.38 h	9.89 ± 1.03 h
SOD	0% Kai	158 ± 4.9 i	192 ± 21.4	194 ± 8.1
(unit/mg	10% Kai	117 ± 12.5 i j	189 ± 18.6	203 ± 12.3
protein)	15% Kai	207 ± 8.2 j	194 ± 7.0	207 ± 7.8
GST	0% Kai	193.67 ± 8.87	189.30 ± 8.24 k	160.93 ± 1.99 k
(nmol/min/mg	10% Kai	185.33 ± 8.42	163.51 ± 15.06 k l	146.88 ± 7.14 k
protein)	15% Kai	175.00 ± 20.15	152.16 ± 14.81 l	148.89 ± 4.68

*Different letters in same column indicate significant differences at $P < 0.05$ (ANOVA), while there were no differences in the parameters without letters ($P > 0.05$).

(Pandey *et al*., 2003). This study reveals Kai showed a protective effect against lead toxication by enhancement of antioxidant enzymes.

S. platensis, a blue-green alga, is rich in proteins, lipids and carbohydrates, as well as the elements such as zinc, magnesium, manganese, selenium and some vitamins including beta-carotene, riboflavin, cyanocobalamin, a-tocopherol and a-lipoic acid. *S. platensis* is also reputed to be an external source of the vital antioxidant enzyme SOD (Upasaniand Balaraman, 2003).

The protective effect of *S. platensis* and *P. ginseng* against cadmium-induced oxidative stress in this study could also be either direct by inhibiting lipid peroxidation and scavenging free radicals (Mei *et al*., 1994), or indirect through the enhancement of the activity of GSH peroxidase and superoxide dismutase, the enzymatic free radicals scavengers in the cells (Abdel-Wahhab and Ahmed, 2004). These characteristics can be attributed to the high levels of antioxidants such as vitamins, minerals, proteins, lipids, carbonhydrates, carotenoids and phyocyanin reported in *S. platensis* and *P. ginseng* (Upasani and Balaraman, 2003; Attele *et al*., 1999).

4.10 Effect of Kai Supplementary Diet on Lead Accumulation in African Catfish, *Clarias gariepinus*

Although oxidative stress responses in African catfish were observed after 0.004 ppm Pb exposure, the Pb concentrations in muscle, gill, and liver were below the detection limit. On the other hand, there were no significant differences in Pb concentrations in all catfish tissues after exposure to 0.3 ppm Pb waterborne. Atli and Canli (2008) reported that there was no regulation of cadmium and lead occurred in fish.

Elevated Kai dietary levels had slightly decreased Pb in liver (Fig. 9C), slightly increase of Pb concentrations were found in fish muscle and gills (Fig. 9A and 9B). In contrast to Alves and Wood (2006) who found elevated dietary Ca^{2+} was clearly protective against the bioaccumulation of Pb in rainbow trout. Meyer *et al.* (2008) recommended three key strategies to prevent lead poisoning: identify sources, eliminate or control sources, and monitor environmental exposures and hazards.

To date, *Spirulina platensis* supplemental treatment has been proved for antioxidative, antiviral, and anticancer properties. However, little is known about Kai algae, this information will be beneficial for aquaculture practices. Further study is needed. For example, the active ingredients those provide antioxidant enzymes and immune stimulation. These protective effects might be good for catfish to cope with cold stress or diseases instead.

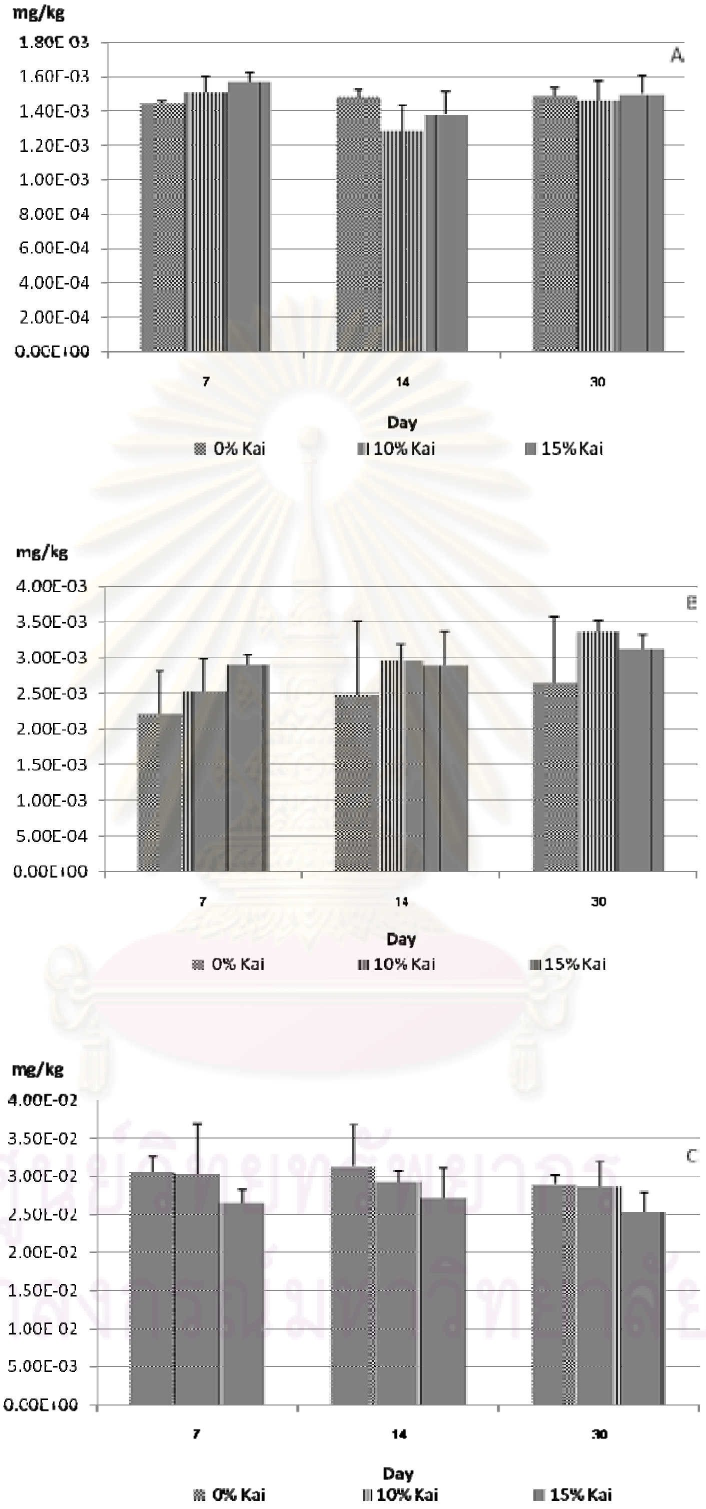

Fig. 11 Pb concentration in muscle (a), gills (b), and liver (c) of African catfish after lead exposure (0.3 ppm) over 30 days

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study illustrated the current situation of heavy metal contamination in the Mae Kuang River. In addition, the biological responses in heavy metal exposed fish were investigated. Subsequently, a possible protective effect of Kai algae (*Cladophora* and *Microphora*) as fish supplementary diet against lead-induced toxicity in catfish was determined. The summary results are shown as following;

1. Anthropogenic pollution from urban sewage, industrial effluent and agricultural runoff was clearly seen between the sampling stations 2 and 4 of the Mae Kuang River. Seasonal variations and flooding affected water flow rate and subsequently led to pollutant dilution. Even though heavy metal contents in water and sediments were below the acceptable levels, a hazardous possibility may develop depending on the rapid expansion of urban and industrial development in the near future. In addition, drought and water shortage can make this polluted river worsen if the pollution sources are not reduced.

2. Speciation analysis of sediment provided information of metal availability. The difference in heavy metals fractions showed that the mobility could vary affecting the toxicity of these metals. In this study, Zn, Cd, and Pb associated mainly to residual fraction. So it should not be available for remobilization.

3. The heavy metal concentrations varied among fish species. Moreover, Pb levels in snakehead fish from some sites were higher than acceptable standard for consumption. The appropriate risk management recommendation to help local communities is to avoid eating too much snakehead fish from this river.

4. Biological changes such as glucose, lysozyme, and percent blood cell packed in snakehead Fish, *Channa striata* exposed to contaminants in the Mae Kuang River can serve as possible biomarkers for fish exposed to heavy metal contamination. By making a comparative study of these responses with the reference sites, it was possible to correlate the levels of pollution and induction of immunological responses and antioxidant enzymes. However, these effects may not be predictable in complex mixtures of contaminants. In addition, the adverse effects of oxidative stress on physiological responses such as growth, reproduction, survival cannot be overlooked. Also, seasonal pollution fluctuations may affect the biomarker level. Available information concerning biomarkers in rainbow trout, which are more extensively used in oxidative stress studies, could also be expanded to include other indigenous species.

5. Kai additive feeding tended to protect African Catfish, *Clarias gariepinus*, against lead toxicity in water. These protective effects may be due to the radical scavenging activity of its components. As a result, these algae are quite valuable in the treatment of lead toxicity; however, it was not able to prevent catfish from Pb

accummulation in tissues. This information could provide valuable knowledge on the fish culture without chemical and antibiotic usage.

6. The involvement of local communities in Mae Kuang River management was quite low. However, community participation should be considered as mandatory in any development projects and local communities should be viewed as equal development partners who should fully participate in the planning, implementation and benefit sharing for any water related development projects.

Outcomes gained from this research include:

1. The update information on water quality status and heavy metal contamination in water, sediment, and fish from the Mae Kuang River.
2. Kai algae (*Cladophora* and *Microphora*) is able to be used as supplementarty feed for African catfish to enhance both immune and antioxidant systems.
3. Strengthening on Mae Kuang River research network

5.2 Recommendations

During this study as well as the analysis processes several other ideas turned up that could be of interest and worthwhile to investigate more thoroughly.

1. Further research is required to clarify how contaminants, both organic and inorganic, can affect the biomarker level, and whether the signal due to chemical contaminants can be separated from background variations and used for monitoring purposes.

2. Studies should be designed to address effects of annual variations, nutritional status, and life stages in all of these species if they are used in biomonitoring.

3. Kai supplementary diets can be used for fish meal subsitution, growth promotion, and immune enhancement; however, the suitable levels must be investigated for practical application.

4. Future work should also focus on identifying which specific active ingredients are attributed during oxidative stress. Elevated dietary Kai might enhance immune systems that could deal with serious diseases and deteriorated water quality.

5. In order to design more effective and appropriate participatory processes, research is needed to better understand and prioritise the factors that make stakeholder participation lead to stronger and more durable decisions in different contexts. Future research needs to evaluate whether decisions emerging from participatory processes are perceived to be more holistic and representative of diverse values and needs, and whether this has the capacity to enhance public trust in the decision-making process.

6. The researchers should provide the risk assessment information to local communities. The announcement board should be utilized to inform people who consume aquatic organisms from this river about the risk of heavy metal hazard along this river.

REFERENCES

Achuba, F.I.; and Osakwe, S.A. Petroleum – induced free radical toxicity in African catfish *(Clarias gariepinus)*. Fish Physiology and Biochemistry 29(2003): 97 – 103.

Aebi, H. Catalases. In: Bergmeyer, H.U. (ed.), Methods of Enzymatic Analysis, Vol. 2, pp: 673 – 684. New York: Academic Press, 1974.

Alleman, L.Y.; and others. Isotopic evidence of contaminant lead in the South Atlantic troposphere and surface waters Deep Sea Research Part II: Topical Studies in Oceanography 48(2001): 2811 – 2827.

Amundsen, P.; and others. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Science of The Total Environment 201(1997): 211 – 224.

Ancheva, M.; Metcheva, R.; and Teodorova, S. A comparative analysis of the heavy metal loading of small mammals in different regions of Bulgaria II: chromosomal aberrations and blood pathology. Ecotoxicology and Environmental Safety 54(2003): 188 – 193.

Antunes, P.; Kallis, G.; Videira, N.; and Santos, R. Participation and evaluation for sustainable river basin governance. Ecological Economics 68(2009): 931 – 939.

Atli, G.; and Canli, M. Responses of metallothionein and reduced glutathione in a Freshwater fish *Oreochromis niloticus* following metal exposures. Environmental Toxicology and Pharmacology 25 (2008): 33 – 38.

Auer, M.T.; and others. Great Lakes *Cladophora* in the 21st century: same algae—different ecosystem. Journal of Great Lakes Research 36(2010): 248 – 255.

Bangkokhealth.com. Omega-3 in freshwater fish. [Online] Available from http://www.oknation.net/blog/print.php?id=278133 [2011, February 28]

Benoff, S.; Jacop, A.; and Hurley, I. R. Male Infertility and Environmental Exposure to Lead and Cadmium. Human Reproduction Update 6(2000): 107 – 121.

Bervoets, L.; and Blust, R. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. Environmental Pollution. 126(2003): 9 – 19.

Brandão-Neto, J.; Stefan, V.; Mendonça, B.B.; Bloise, W.; and Castro, A.V. The essential role of zinc in growth. Nutrition Research 15(1995): 335 – 358.

Brzóska, M. M.; and J. Moniuszko-Jakoniuk. Interactions between cadmium and zinc in the organism. Food and Chemical Toxicology 39(2001): 967 – 980.

Burger, J. Assessment and management of risk to wildlife from cadmium. Science of The Total Environment 389(2008): 37 – 45.

Canli, M.; and Atli, G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution 121(2003): 129 – 136.

Capodaglio, G. Lead Speciation in Surface Waters of the Eastern North Pacific. Marine Chemistry 29(1990): 221 – 233.

Casalino, E.; Calzaretti, G.; Sblano, C.; and Landriscin, C. Molecular inhibitory mechanism of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 179 (2002): 37 – 50.

Castelletti, A.; and Soncini-Sessa, R. A procedural approach to strengthening integration and participation in water resource planning. Environmental Modelling & Software 21(2006): 1455 – 1470.

Castro-Gonzalez, M.I.; and Mendez-Arment, M. Heavy metals: Implications associated to fish consumption Environmental Toxicology and Pharmacology 26 (2008): 263 – 271.

Chien, L.C.; and others. Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Science of The Total Environment 285 (2002): 177 – 185.

Chumroonrat, N. Study on heavy metals contamination in the Kuang river case study: Lumphun Province. Rajamangala University of Technology Lanna, Payap Campus Research Report, 2004.

Cobbett, C.; and Goldsbrough, P. Phytochelatin and metallothioneins: Roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology 53(2002): 159 – 182.

Commandeur, J.N.M.; Stijntjes, G.J.; and Vermeulen, N.P.E. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. Pharmacological Reviews 47(1995): 271 – 330.

Concas, A.; Ardau, C.; Cristini, A.; Zuddas, P.; and Cao, G. Mobility of heavy metals from tailings to stream waters in a mining activity contaminated site. Chemosphere 63(2006): 244 – 253.

Cosson, R.P. Heavy metal intracellular balance and relationship with metallothionein induction in the gills of carp after contamination by Ag, Cd, and Hg following pre-treatment with Zn or not. Biological Trace Element Research 46(1994): 229 – 245.

Cronin, M.; and others. Trace metal concentrations in deep sea fish from the North Atlantic. Marine Environmental Research 45 (1998): 225 – 238.

Davies, B.E. Inter-relationships between soil properties and the uptake of Cd, Cu, Pb and Zn from contaminated soils by radish (*Raphanus sativus*, L). Water, Air, & Soil Pollution 63(1992): 331 – 342.

Davutluoglu, O.; Seckin, G.; Kalat, D.G.; Yilmaz, T.; and Ersu, C.B. Speciation and implications of heavy metal content in surface sediments of Akyatan Lagoon–Turkey. Desalination 260 (2010): 199 – 210.

Demirak, A.; Yilmaz, F.; Tuna, A.L.; and Ozdemir, N. Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. Chemosphere 63 (2006): 1451 – 1458.

Depledge, M.H.; Aagaard, A.; and Gyorkos, P. Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. Marine Pollution Bulletin 31(1995): 19 – 27.

Dight, I., Gladstone, W. Pilot Study Final Report. Torres Strait Baseline Study, Great Barrier Reef Marine Park Authority, 1993.

Di Giulio, R.T.; Benson, W.H.; Sanders, B.M.; and van Veld, P.A. Biochemical mechanisms: metabolism, adaptation, and toxicity. In: Rand, G.M. (Ed.), Fundamentals of Aquatic Toxicology: Effects, Environmental fate, and Risk Assessment, pp. 523 – 562. London, UK: Taylor and Francis, 1995.

Di Giulio, R.T.; Washburn, P.C.; Wenning, R.J.; Winston, G.W.; and Jewell, C.S. Biochemical responses in aquatic animals: a review of determinants of oxidative stress. Environmental Toxicology & Chemist 8(1989): 1103 – 1123.

Duke, L.D.; and Taggart, M. Uncertainty factors in screening ecological risk assessments. Environmental Toxicology & Chemistry 19(2000): 1668 – 1680.

Dural, M.; Goksu, M.Z.L.; and Akif Ozak, A. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chemistry 102 (2007): 415 – 421.

Ellis, A. E. Lysozyme Assays. Techniques in Fish Immunology, pp. 101 – 103. Fair Haven, USA: SOS Publications, 1990.

El-Nekeety, A.A.; El-Kady, A.A.; Soliman, M.S.; Hassan, N.S.; Abdel-Wahhab, M.A. Protective effect of *Aquilegia vulgaris* (L.) against lead acetate-induced oxidative stress in rats. Food and Chemical Toxicology 47(2009): 2209 – 2215.

Erfurth, E.M.; and others. Effects of lead on the endocrine system in lead smelter workers. Archives of Environmental Health 56 (2001): 449 – 455.

Fairbrother, A.; Wenstel, R.; Sappington, K.; and Wood, W. Framework for Metals Risk Assessment. Ecotoxicology and Environmental Safety 68(2007): 145 – 227.

Filho, D.W. Fish antioxidant defenses - a comparative approach. Brazilian Journal of Medical and Biological Research 29(1996): 1735 - 1742.

Fosmire, G. J. Zinc toxicity. American Journal of Clinical Nutrition 51(1990): 225.

Friedmann, A.S.; Watzin, M.C.; Brinck-Johnsen, T.; and Leiter, J.C. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology 35(1996): 265 – 278.

George, S.G. Enzymology and molecular biology of phase II xenobiotic-conjugating enzymes in fish. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular perspectives. pp. 37 – 85. Lewis Publishers, CRC press, 1994.

George, S.G.; Todd, K.; and Wright, J. Regulation of metallothionein in teleosts: induction of MTmRNA and protein by cadmium in hepatic and extrahepatic tissues of a marine flatfish, the turbot (*Scophthalmus maximus*). Comparative Biochemistry and Physiology - Part C 113(1996): 109 – 115.

Gatlin III, D.M.; O'Connell, J.P.; and Scarpa, J. Dietary zinc requirement of the red drum, *Sciaenops ocellatus*. Aquaculture 92(1991): 259 – 265.

Goeptar, A.R.; Scheerens, H.; and Vermeulen, N.P.E. Oxygen reductase and substrate reductase activity of cytochrome P450. Critical Reviews in Toxicology 25(1995): 25 – 65.

Gómez, S.; Villar, C.; and Bonetto, C. Zinc toxicity in the fish *Cnesterodon decemmaculatus* in the Paraná River and Rio de La Plata Estuary. Environmental Pollution 99(1998): 159 – 165.

Gordon, A.N.; Pople, A.R.; and Ng, J. Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland. Australian Journal of Marine & Freshwater Research 49(1998): 409 – 414.

Gurer-Orhan, H.; Sabýr, H.U.; and Özgünes, H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology 195 (2004): 147 – 154.

Hall, L.W.; and Anderson, R.D. A Deterministic Ecological Risk Assessment for Copper in European Saltwater Environments. Marine Pollution Bulletin, 38(1999): 207 – 218.

Hartwig, A.; and others. Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms. Environmental Health Perspectives 110(2002): 797 – 799.

Has-Schon, E.; Bogut, I.; and Strelec, I. Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). Archives of Environmental Contamination and Toxicology 50(2006): 545 – 551.

Heier, L.S.; and others. Speciation of lead, copper, zinc and antimony in water draining a shooting range—Time dependant metal accumulation and biomarker responses in brown trout (*Salmo trutta* L.). Science of The Total Environment 407(2009): 4047 – 4055.

Henczová, M.; Deér, A.K.; Filla, A.; Komlósi, V.; and Mink, J. Effects of Cu^{2+} and Pb^{2+} on different fish species: Liver cytochrome P450-dependent monooxygenase activities and FTIR spectra. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 148(2008): 53 – 60.

Hogstrand, C.; Balesaria, S.; and Glover, C.N. Application of genomics and proteomics for study of the integrated response to zinc exposure in a non-model fish species, the rainbow trout. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 133(2002): 523 – 535.

Hseu, Z. Evaluating heavy metal contents in nine composts using four digestion methods. Bioresource Technology 95(2004): 53 – 59.

Hudecova, A; and E. Ginter. The influence of ascorbic acid on lipid peroxidation in guinea pigs intoxicated with cadmium. Food and Chemical Toxicology. 30 (1992): 1011 – 1013.

Ip, C.C.M.; Li, X.; Zhang, G.; Wai, O.W.H.; and Li, Y. Trace metal distribution in sediments of the Pearl River Estuary and the surrounding coastal area, South China. Environmental Pollution 147(2007): 311 – 323.

Jager, T.; Vermeire, T.G.; Rikken, M.G.J.; and Van der Poel, P. Opportunities for a probabilistic risk assessment of chemicals in the European Union. Chemosphere 43(2001): 257 - 264.

Järup, L.; and Åkesson, A. Current status of cadmium as an environmental health problem. Toxicology and Applied Pharmacology 238(2009): 201 – 208.

Jezierska, B.; and Witeska, M. Metal toxicity to fish. University of Podlasie. Monografie No. 42, 2001.

Kargin, F. Metal concentrations in tissues of the freshwater fish *Capoeta barroisi* from the Seyhan River (Turkey). Bulletin of Environmental Contamination Toxicology 60(1998): 822 – 828.

Kargin, F. Seasonal Changes in Levels of Heavy Metals in tissues of *Mullus barbatus* and *Sparus aurata* collected from Iskenderun Gulf (Turkey). Water, Air and Soil Pollution 90(1996): 557 – 562.

Khuantrairong, T. Enhancement of carotenoid production and nutritional value of an alga Kai (*Cladophora* sp.) under mass culture. Ph.D. Thesis, Department of Biology, Faculty of Science, Chiang Mai University, 2010.

Kumar, A.; Agarwal, K.; and Seth, P.K. Oxidative stress-mediated neurotoxicity of cadmium Toxicology Letters 89 (1996): 65 – 69.

Klaassen, C.D. Toxicology, 6th edition The McGraw-Hill Companies, New York, 2001.

Klavins, M.; Briede, A.; Rodinov, V.; Kokorite, I.; Parele, E.; and Klavina, I. Heavy metals in Rivers of Latvia. Science of The Total Environment 262(2000): 175 – 183.

Kock, G.; and Hofer, R. Origin of cadmium and lead in clear softwater lakes of high-latitude, and their bioavailability and toxicity to fish. In: T. Braunbeck, D.E. Hinton and B. Streit, Editors, Fish ecotoxicology, pp. 64 – 77. Birkhause Verlag, Boston, 1998.

Kotze, P.; Preez, H. H.; and van Vuren, J. H. J. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants river, Mpumalanga, South Africa. Water SA 25(1999): 99 – 100.

Kraal, M.H.; Kraak, M.H.S.; De Groot, C.J.; and Davids, C. Uptake and tissue distribution of dietary and aqueous cadmium by carp, *Cyprinus carpio*. Ecotoxicology and Environmental Safety 31(1995):179 – 183.

Lertsri, T. Monitoring of general water quality and some heavy metals in water samples from Kuang River in 1998. Master's Thesis (Teaching Chemistry), Chiang Mai University. 104 p., 1998.

Li, A.; Li, J.; Lei, Q.; Wang, F.; and Chuang, J. Application of different forms of trace minerals in feed for fish and prawn. In: Li, A. and Shiau, S., Editors, 1995. The Proceeding of the Second Symposium of World's Chinese Scientists on Nutrition and Feeding of Finfish and Shellfish, Ocean University of Qingdao Press, Qingdao, China, pp. 33 – 46 (in Chinese with English abstract). Cited in Tan, B.; and Mai, K. Zinc methionine and zinc sulfate as sources of dietary zinc for juvenile abalone, *Haliotis discus hannai* Ino. Aquaculture 192(2001): 67 – 84.

Lin, Y.; and Shiau, S. The effects of dietary selenium on the oxidative stress of grouper, *Epinephelus malabaricus*, fed high copper. Aquaculture 267(2007): 38 – 43.

Liu, C.; Tao, S.; and Long, A. Accumulations of lead and cadmium in goldfish, *Carassius auratus*. Acta Hydrobiologica Sinica 25(200l): 344 – 349.

Lloyd, R. Pollution and Fresh Water Fish. England : Blackwell Scientific Publications Ltd., 1992.

Maage, A.; and Julshamn, K. Assessment of zinc status in juvenile Atlantic salmon (*Salmo salar*) by measurement of whole body and tissue levels of zinc. Aquaculture 117(1993): 179 – 191.

Macdonald, R. W.; and others. Contaminants in the Canadian Arctic: 5 years of progress in understanding sources, occurrence and pathways. The Science of The Total Environment 254(2000): 93 – 234.

Magwere, T.; Naik, Y.S.; and Hasler, T.A. Effects of chloroquine treatment on antioxidants enzymes in rats liver and kidney. Free Radical Biology and Medicine 22(1997): 321 – 327.

Mansour, S. A.; and Sidky, M. M. Ecotoxicological studies. 3: Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. Food Chemistry 78(2002): 15 – 22. Cited in Rauf, A.; Javed, M.; and Ubaidullah, M. Heavy metal Levels in three major carps (*Catla catla*, *Labeo rohita, and Cirrhina mgrigala*) from the river Ravi, Pakistan. Pakistan Veterinary Journal 29(2009): 24 – 26.

Marcovecchio, J.E.; Moreno, V.J.; Pérez, A. Metal accumulation in tissues of sharks from the Bahia Blanca estuary, Argentina. Marine Environmental Research 31(1991): 263 – 274.

Mayer, F.L.; and others. Metabolic products as biomarkers. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, pp. 5 – 86. Chelsea, MI, USA: Lewis Publishers, 1992.

McGeer, J.C.; Szebedinszky, C.; McDonald, D.G.; and Wood, C.M. Effects of chronic sublethal exposure to waterborne Cu, Cd, or Zn in rainbow trout 2: tissue specific metal accumulation. Aquatic Toxicology. 50(2000): 245 – 256.

Meyer, P.A.; Brown, M.J.; and Falk, H. Global approach to reducing lead exposure and poisoning. Mutation Research 659(2008): 166 – 175.

Miliou, H.; Zaboukas, N., and Moraitou-Apostolopoulou, M. Biochemical composition, growth, and survival of the guppy, *Poecilia reticulata*, during chronic sublethal exposure to cadmium. Archives of Environmental Contamination and Toxicology. 35 (1998): 58 – 63.

Morgan, D. A.; Levitsky, B.; and Strupp, J. Effects of chronic lead exposure on learning and reaction time in a visual discrimination task. Neurotoxicology and Teratology 22(2000): 337 – 345.

Morillo, J.; Usero, J., and Gracia, I. Heavy metal distribution in marine sediments form the southwest coast of Spain. Chemosphere 55 (2004): 431 – 442.

Morillo, J.; Usero, J., and Gracia, I. Partitioning of metals in sediments from the Odiel river (Spain). Environment International 28(2002): 263 – 271.

Morrison, H.A.; Gobas, F.A.; Lazar, R.; and Haffner, G.D. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. Environmental Science & Technology 30(1996): 3377 – 3384.

Moss, D.W.; Henderson, A.R.; and Kochmar, J.F. Enzymes; principles of diagnostic enzymology and the aminotransferases. In: Tietz, N.W. (Ed.), Textbook of Clinical Chemistry, pp. 663 – 678. Philadelphia, PA : Saunders, 1986.

National Institute of Environmental Health Sciences (NIEHS). Annual Report on Carcinogens. National Toxicology Program. Research Triangle Park, NC., 1994.

Nord, L.G.; Craig, D.A.; and Bobby, G.W. Lead, zinc, copper, and cadmium in fish and sediments from the Big River and Flat River Creek of Missouri's old lead belt. Environmental Geochemistry and Health 26(2004): 37 – 49.

Nordberg, J.; and Arner, E.S.J. Reactive oxygen species, antioxidants and mammalian thioredoxin system. Free Radical Biology and Medicine 31(2001): 1287 – 1312.

Oertel, N. The applicability of *Cladophora glomerata* (L.) Kütz in an active bio-monitoring technique to monitor heavy metals in the river Danube. Science of The Total Environment 134(1993): 1293 – 1304.

Okonkwo, J.O.; and Mothiba, M. Physico-chemical characteristics and pollution levels of heavy metals in the rivers in Thohoyandou, South Africa. Journal of Hydrology 308(2005): 122 – 127.

Olivares-Rieumont, S.; Rosa, D.; Lima, L.; Graham, D.W.; Alessandro, K.D.; Borroto, J.; Martinez, F., and Sa´nchez, J. Assessment of heavy metal levels in Almendares River sediments—Havana City, Cuba. Water Research 39 (2005): 3945 – 3953.

Papagiannis, I.; Kagalou, I.; Leonardos, J.; Petridis, D.; and Kalfakakou, V. Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). Environment International 30(2004): 357 – 362.

Parihar, M.S.; and others. Responses of superoxide dismutase, glutathione peroxidase and reduced glutathione antioxidant defenses in gills of the freshwater catfish (*Heteropneustes fossilis*) to short-term elevated temperature. Journal of Thermal Biology 22(1997): 151 – 156.

Peerapornpisal, Y. Edible Freshwater Macroalgae in Northern Thailand. Journal of Fisheries Technology Research 2(2008): 178 – 189. [in Thai]

Peraja, M.A.: Ayala-Fierro, F.; Barber, D.; Casarez, R.; and Rael, L.T. Effects of micro nutrients on metal toxicity. Environmental Health Perspectives. 106(1998): 203 – 216.

Perin, G.; and others. Heavy metal speciation in the sediments of Northern Adriatic Sea — a new approach for environmental toxicity determination, in: T.D. Lekkas (Ed.), Heavy metal in the environment, vol. 2, pp. 454 – 456, 1985.

Petpiroon, P.; Rungsritsak, D; and Sangpak, J. The distribution and bioconcentration factors of heavy metals in freshwater organisms from the Petchburi River. Thai Fisheries Gazette 62(2009): 529 – 540.

Piechotta, G.; and others. Apoptosis in dab (*Limanda limanda*) as possible new biomarker for anthropogenic stress. Ecotoxicology and Environmental Safety 42(1999): 50 – 56.

Porte, C.; Escartin, E.; Garcia, L.M.; Sole´, M.; and Albaige´ s, J. Xenobiotic metabolizing enzymes and antioxidant defences in deep-sea fish: relationship with contaminant body burden. Marine Ecology Progress Series 19(2000): 259 – 266.

Priprem, A.; Sripanidkulchai, B.; Wirojanagud, W.; and Chalorpunrut, P. Heavy metals in freshwater fish along Pong and Chi Rivers. KKU Research Journal 12(2007): 420 – 430.

Proctor, P.H.; and Reynolds, E.S. Free radicals and disease in man. Physiological Chemistry and Physics and Medical NMR 16 (1984): 175 – 195. [Online] Available from: http://www.drproctor.com/rev/84/84rev.htm [2010, May].

Pyle, G.G.; Rajotte, J.W.; and Couture, P. Effects of industrial metals on wild fish populations along a metal contamination gradient. Ecotoxicology and Environmental Safety 61(2005): 287 – 312.

Qiao-qiao, C.; Guang-wei, Z.; and Langdon, A. Bioaccumulation of heavy metals in fishes from Taihu Lake, China. Journal of Environmental Sciences 19(2007): 1500 – 1504.

Queiroza, M.L.S.; Rodriguesa, A.P.O.; Bincoletto, C.; Figueireˆdoa, C.A.V.; and Malacrida, S. Protective effects of *Chlorella vulgaris* in lead-exposed mice infected with *Listeria monocytogenes*. International Immunopharmacology 3 (2003): 889 – 900.

Rashed, M.N. Monitoring of environmental heavy metals in fish from Nasser Lake. Environment International 27(2001): 27 – 33.

Rauret, G. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. Talanta 46 (1998): 449 – 455.

Rashed, M. N. Monitoring of environmental heavy metals in fish from Nasser Lake. Environment International 27(2001): 27 – 33.

Ray, S. Bioaccumulation of cadmium in marine organisms. Experientia 50(1986): 65 – 75.

Rayment, G.E. Sources of cadmium in agricultural products. National Cadmium Workshop. DPIE/NFA, Canberra, Australia, 1995.

Regoli, F.; and Principato, G. Glutathione, glutathione-dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers Aquatic Toxicology 31(1995): 143 – 164.

Roesijadi, G.; and Robinson, W.E. Metal regulation in aquatic animals: mechanisms of uptake, accumulation and release. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular Perspectives, pp. 387 – 420. Lewis Publishers, CRC press, 1994.

Rogers, J.T.; Richards, J.G.; and Wood, C.M. Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology. 64 (2003): 215 – 234.

Romeo, M.; Siau, Y., and Sidoumou, Z. Heavy metal distribution in different fish species from the Mauritania coast. The Science of the Total Environment 232(1999): 169 – 175.

Ruangsomboon; S. and Wongrat, L. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (*Chlorella vulgaris*), zooplankton (*Moina macrocopa*), and the predatory catfish *Clarias macrocephalus*×*C. gariepinus*. Aquatic Toxicology 78 (2006): 15 – 20.

Segura, R.; Arancibia, V.; Zúñiga, M.C.; and Pastén, P. Distribution of copper, zinc, lead and cadmium concentrations in stream sediments from the Mapocho River in Santiago, Chile. Journal of Geochemical Exploration 91 (2006): 71 – 80.

Sekhar, K.C.; Chary, N. S.; Kamala, C. T.; Raj, D. S.; and Rao, A.S. Fractionation studies and bioaccumulation of sediment-bound heavy metals in Kolleru lake by edible fish. Environment International 29(2004): 1001 – 1008.

Shugart, L.R.; others. DNA alterations. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, pp. 155 – 210. Chelsea, MI, USA: Lewis Publishers, 1992.

Šikic, R.V.; and others. Activities of superoxide dismutase and catalase in erythrocytes and transaminases in the plasma of carps (*Cyprinus carpio* L.) exposed to cadmium. Physiological Research 46(1997): 391 – 396.

Sijm, D.T.H.M.; and Opperhuizen, A. Biotransformation of organic chemicals by fish: enzyme activities and reactions. In: Hutzinger, O.(Ed.), Handbook of Environmental Chemistry Reactions and Processes, vol. 2, pp. 163 – 235. Berlin: Springer, 1989.

Simsek, N.; Karadeniz, A.; Kalkan, Y.; Keles, O.N.; Unal, B. *Spirulina platensis* feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. Journal of Hazardous Materials 164(2009): 1304 – 1309.

Singh, K.P.; Mohan, D.; Singh, V.K.; and Malik, A. Studies on distribution and fractionation of heavy metals in Gomti river sediments—a tributary of the Ganges, India. Journal of Hydrology 312 (2005): 14 – 27.

Solomon, K.R.; and others. Ecological risk assessment of atrazine in North American surface waters. Environmental Toxicology & Chemistry 15(1996): 31 – 74.

Soares, H. M. V. M.; Boaventura, R. A. R.; Machado, A. A. S. C. ; and Esteves da Silva, J. C. G. Sediments as monitors of heavy metal contamination in the Ave river basin (Portugal): multivariate analysis of data. Environmental Pollution 105(1999): 311 – 323.

Soudani, N.; Amara, I.B.; Sefi, M.; Boudawara, T.; and Zeghal, N. Effects of selenium on chromium (VI)-induced hepatotoxicity in adult rats. Experimental and Toxicologic Pathology, In Press.

Spies, R.B.; and others. Sublethal responses of *Platichtus stellatus* to organic contamination in San Francisco Bay with emphasis on reproduction. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination, pp. 87 – 122. Boca Raton, FL, USA: Lewis Publishers, 1990.

Stegeman, J.J.; Brouwer, M.; Di Giulio, R.T.; Fo¨ rlin, L.; Fowler, B.A.; Sanders, B.M.; and Van Veld, P.A.. Enzyme and protein synthesis as indicator of contaminant exposure and effect. In: Huggett, et al. (Eds.), Biomarkers—Biochemical, Physiological and Histological Markers of Anthropogenic Stress, SETAC Special Publication Series, pp. 235–334. Boca Raton, FL: Lewis Publisher, 1992.

Storelli, M.M.; and Marcotrigiano, G.O. Consumption of bivalve molluscs in Italy: estimated intake of cadmium and lead. Food Additives & Contaminants 18(2001): 303 – 307.

Struzynska, L.; Sulkowski G.; Lenkiewicz A, Rafalowska U. Lead stimulates the glutathione system in selective regions of rat brain. Folia Neuropathology 40(2002): 203 – 209.

Sugawara, E.; Nakamura, K.; Miyake, T.; Fukumura, A.; and Seki, Y. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. British Journal of Industrial Medicine 48 (1991): 239 – 242.

Suter, G.W. Ecological Risk Assessment, 538 p. Boca Raton, FL, USA: Lewis Publishers, 1993.

Svobodova, Z.; and others. Evaluation of the effect of chemical substances, preparation, wastes and waste waters to organisms in the aquatic environment. Bulletin VURH Vodnany 32(1996): 76 – 96.

Stumm, W.; and Morgan, J.J. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3rd ed. New York: Wiley, 1996.

Templeton, D.M.; and others. Guidelines for terms related to chemical speciation and fractionation of elements. Pure and Applied Chemistry 72(2000): 453 – 470.

Tessier, A.; Campbell, P.G.C.; and Bisson, M. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51(1979): 844 – 851.

Traichaiyaporn, S.; Waraegsiri, B.; and Promya, J. *Cladophora* (Kai) culture for the Mekong Giant Catfish feed (Phase 2). Final report submitted to Thailand Research Fund (RDG 5020065), 2010.

Traichaiyaporn, S. Heavy metal contamination in edible macroalgae in Northern Thailand. Final report, The Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Chiangmai University, 2011.

US Agency for Toxic Substances and Disease Registry. Lead. Toxicological profiles. Atlanta: Centers for Disease Control and Prevention. PB/99/166704, 1999.

United States Environmental Protection Agency (USEPA). Risk-Based Concentration Table. [Online] Available from: http://www.epa.gov/reg3hwmd/risk/human/index.htm [2010, May].

Upasani, C.D.; and Balaraman, R. Protective effect of *Spirulina* on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phytotherapy Research 17(2003): 330 – 334.

US EPA. Risk-based concentration table. Philadelphia PA: United States Environmental Protection Agency, Washington DC, 2000.

US EPA. Risk assessment guidance for superfund, Human Health Evaluation Manual Part A, Interim Final vol. I, United States Environmental Protection Agency, Washington (DC) EPA/540/1-89/002, 1989.

Usero, J.; Izquierdo, C.; Morillo, J.; and Gracia, I. Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. Environment International 29(2003): 949 – 956.

Vallee, B.L.; and Falchuk, K.H. The biochemical basis of zinc physiology. Physiological Reviews. 73 (1993): 79 – 118.

Van der Oost, R.; Beyer, J.; Vermeulen, N.P.E. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology 13(2003): 57 – 149.

Van Leeuwen, C.J.; and Hermens, J.L.M. Risk Assessment of Chemicals: an Introduction. Dordrecht, Netherlands: Kluwer Academic Publishers, 1995.

Vermeulen, N.P.E. Role of metabolism in chemical toxicity. In: Ioannides, C. (Ed.), Cytochromes P450: Metabolic and Toxicological Aspects, pp. 29 – 53. Boca Raton, FL, USA: CRC Press, 1996.

Viarengo, A.; Burlando, B.; Ceratto, N.; and Panfoli, I. Antioxidant role of metallothioneins: a comparative overview. Cell and Molecular Biology 46(2000): 407 – 417.

Vicente-Martorell, J.J.; Galindo-Riaño, M.D.; Garcia-Vargas, M.; and Granado-Castro, M.D. Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted estuary. Journal of Hazardous Materials 162 (2009): 823 – 836.

Vieira, C.; Morais, S.; Ramos, S.; Delerue-Matos, C.; and Oliveira, M.B.P.P. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption. Food and Chemical Toxicology *In Press*.

Virulhakul, P; and Suntipiriyaporn, S. Heavy metal contamination in fish and fishery products of Thailand. Thai Fisheries Gazette 59(2006): 115 – 125.

Wang, C.; and Lovell, R.T. Organic selenium sources in diets for channel catfish (*Ictalurus punctatus*). Aquaculture 152(1997): 223 – 234.

Wang, Q.; and others. Adverse health effects of lead exposure on children and exploration to internal lead indicator. Science of The Total Environment 407(2009): 5986 – 5992.

Wang, X.L.; Tao, S.; Dawson, R.W.; and Xu, F.L. Characterizing and comparing risks of polycyclic aromatic hydrocarbons in a Tianjin wastewater-irrigated area. Environmental Research 90(2002): 201 – 206.

Weeks, B.A.; and others. Immunologiocal biomarkers to assess environmental stress. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, pp. 211 – 234. Chelsea, MI, USA: Lewis Publishers, 1992

West, I.C. What determines the substrate specificity of the multi-drug-resistance pump? Trends in Biochemical Sciences 15(1990): 42 – 46.

Wester, P.W.; Vethaak, D.; and van Muiswinkel, W.B. Fish as biomarkers in immunotoxicology. Toxicology 86(1994): 213 – 232.

Westerman, M.P.; Pfitzer, E.; Ellis, L.D.; and Jensen, W.N. Concentrations of lead in bone in plumbism. The New England Journal of Medicine 23(1965): 1246 – 1250.

Glynn, A. Cadmium and zinc kinetics in fish: studies on waterborne ^{109}Cd and ^{65}Zn turnover and intracellular distribution in minnows *Phoxinus phoxinus*. Pharmacology & Toxicology 69(1991): 485 – 491.

Winston, G.W.; and Di Giulio, R.T. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquatic Toxicology 19(1991): 137 – 161.

Wong, C.K.; Wong, P.P.K.; Chu, L.M. Heavy metal concentrations in marine fishes collected from fish culture sites in Hong Kong. Archives of Environmental Contamination and Toxicology 40(2001): 60 – 69.

Wood, C.M. Toxic responses of the gill. In: D. Schlenk and W.H. Benson (Editors), Target Organ Toxicity in Marine and Freshwater Teleosts, pp. 1 – 87. London : Taylor & Francis, 2001.

World Health Organization.Guidelines for drinking-water quality, vol.2. Geneva: WHO, p. 281 – 283, 1998.

WHO International Programme on Chemical Safety (IPCS). Biomarkers and risk assessment: concepts and principles. Environmental Health Criteria 155, World Health Organization, Geneva, 1993.

Wright, P.; and Mason, C.F. Spatial and seasonal variation in heavy metals in the sediments and biota of two adjacent estuaries, the Orwell and the Stour, in eastern England. The Science of the Total Environment 226(1999): 139 – 156.

Yuan, C.; Shi, J.; Lui, J.; Liang, L.; and Jiang, G. Speciation of heavy metals in marine sediments from the East China Sea by ICP-MS with sequential extraction. Environment International 30(2004): 769 – 783.

Zhang, H.; and others. Heavy metals in water, soils and plants in riparian wetlands in the Pearl River Estuary, South China. Procedia Environmental Sciences 2 (2010): 1344 – 1354.

Zhang, W.; and others. Heavy metal contamination in surface sediments of Yangtze River intertidal zone: An assessment from different indexes. Environmental Pollution 157 (2009): 1533 – 1543.

Zhang, W.; and others. China's Yangtze Estuary: I. Geomorphic influence on heavy metal accumulation in intertidal sediments. Geomorphology 41(2001): 195 – 205.

APPENDICES

APPENDIX A

WATER QUALITY STANDARDS

Surface Water Classification and Objectives (Pollution Control Department, 2001)

Classification and Objectives	
Classification	Condition and Beneficial Usage
Class 1	Extra clean fresh surface water resources used for : (1) conservation not necessary pass through water treatment process require only ordinary process for pathogenic destruction (2) ecosystem conservation where basic organisms can breed naturally
Class 2	Very clean fresh surface water resources used for : (1) consumption which requires ordinary water treatment process before use (2) aquatic organism of conservation (3) fisheries (4) recreation
Class 3	Medium clean fresh surface water resources used for : (1) consumption, but passing through an ordinary treatment process before using (2) agriculture
Class 4	Fairly clean fresh surface water resources used for : (1) consumption, but requires special water treatment process before using (2) industry
Class 5	The sources which are not classification in class 1-4 and used for navigation.

Surface Water Quality Standards (Pollution Control Department, 2001)

Surface Water Quality Standards

Parameter[1/]	Units	Standard Value for Class[2/]					Methods for Examination
		Class 1	Class 2	Class 3	Class 4	Class 5	
1. Colour,Odour and Taste	-		n'	n'	n'	-	-
2. Temperature	C°	n	n'	n'	n'	-	Thermometer
3. pH	-	n	5-9	5-9	5-9	-	Electrometric pH Meter
4. Dissolved Oxygen (DO)[2/]/2	mg/l	n	6.0	4.0	2.0	-	Azide Modification
5. BOD (5 days, 20°C)	mg/l	n	1.5	2.0	4.0	-	Azide Modification at 20°C , 5 days
6. Total Coliform Bacteria	MPN/100 ml	n	5,000	20,000	-	-	Multiple Tube Fermentation Technique
7. Fecal Coliform Bateria	MPN/100 ml	n	1,000	4,000	-	-	Multiple Tube Fermentation Technique
8. NO_3 -N	mg/l	n	5.0			-	Cadmium Reduction
9. NH_3 -N	mg/l	n	0.5			-	Distillation Nesslerization
10.Phenols	mg/l	n	0.005			-	Distillation,4-Amino antipyrene
11.Copper (Cu)	mg/l	n	0.1			-	Atomic Absorption -Direct Aspiration

Surface Water Quality Standards (Continued)

Surface Water Quality Standards							
Parameter[1/]	Units	Standard Value for Class[2/]					Methods for Examination
		Class 1	Class 2	Class 3	Class 4	Class 5	
12.Nickle (Ni)	mg/l	n	0.1			-	Atomic Absorption -Direct Aspiration
13.Manganese (Mn)	mg/l	n	1.0			-	Atomic Absorption -Direct Aspiration
14.Zinc (Zn)	mg/l	n	1.0			-	Atomic Absorption -Direct Aspiration
15.Cadmium (Cd)	mg/l	n	0.005* 0.05**			-	Atomic Absorption -Direct Aspiration
16.Chromium Hexavalent	mg/l	n	0.05			-	Atomic Absorption -Direct Aspiration
17.Lead (Pb)	mg/l	n	0.05			-	Atomic Absorption -Direct Aspiration
18.Total Mercury (Total Hg)	mg/l	n	0.002			-	Atomic Absorption-Cold Vapour Technique
19.Arsenic (As)	mg/l	n	0.01			-	Atomic Absorption -Direct Aspiration
20.Cyanide (Cyanide)	mg/l	n	0.005			-	Pyridine-Barbituric Acid

APPENDIX B

Table B1 Absorbance of standard cadmium

Cd Concentration (mg/L)	Replication			Mean ± S.D.
	1	2	3	
0	0.000	0.000	0.000	0.000 ± 0.000
0.5	0.039	0.041	0.037	0.039 ± 0.002
1.0	0.063	0.058	0.074	0.065 ± 0.008
1.5	0.097	0.099	0.095	0.097 ± 0.002
2.0	0.154	0.129	0.110	0.131 ± 0.022
2.5	0.155	0.157	0.161	0.158 ± 0.003
3.0	0.182	0.164	0.159	0.168 ± 0.012
4.0	0.232	0.238	0.255	0.242 ± 0.012
5.0	0.301	0.299	0.303	0.301 ± 0.002

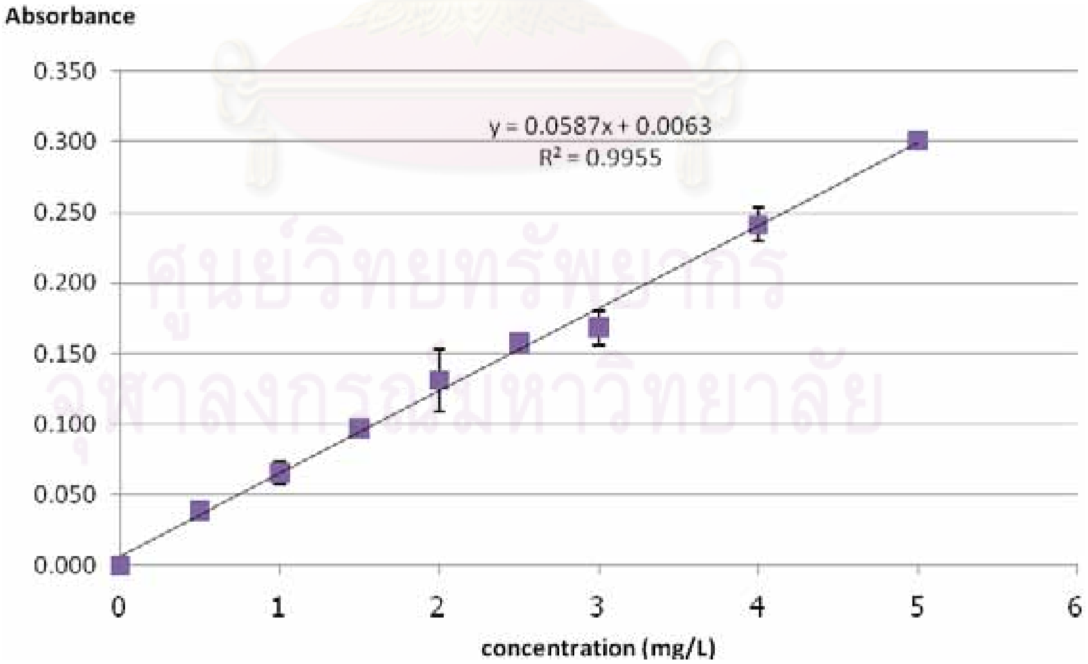

Figure B1 Calibration curve of standard cadmium

Table B2 Absorbance of standard lead

Pb Concentration (mg/L)	Replication			Mean ± S.D.
	1	2	3	
0	0.000	0.000	0.000	0.000 ± 0.000
0.5	0.007	0.008	0.007	0.007 ± 0.001
1.0	0.016	0.014	0.015	0.015 ± 0.001
1.5	0.023	0.021	0.024	0.023 ± 0.002
2.0	0.028	0.031	0.029	0.029 ± 0.002
2.5	0.036	0.039	0.037	0.037 ± 0.002
3.0	0.046	0.044	0.045	0.045 ± 0.001
4.0	0.062	0.059	0.057	0.059 ± 0.003
5.0	0.070	0.071	0.077	0.073 ± 0.004

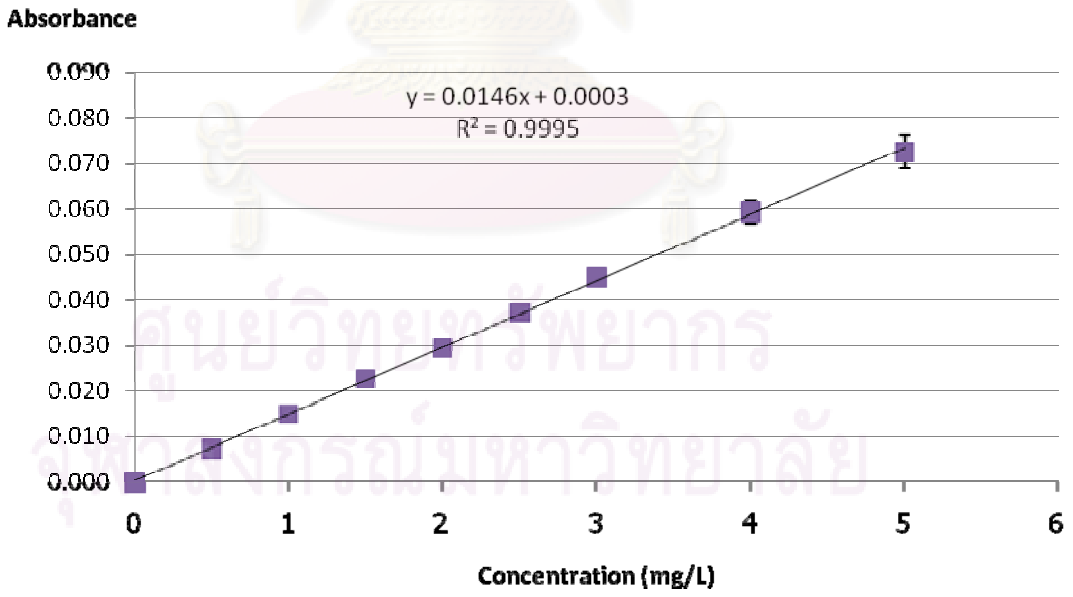

Figure B2 Calibration curve of standard lead

Table B3 Absorbance of standard zinc

Zinc concentration (mg/L)	Replication			Mean ± S.D.
	1	2	3	
0	0.000	0.000	0.000	0.000 ± 0.000
0.5	0.023	0.019	0.021	0.021 ± 0.002
1.0	0.036	0.035	0.037	0.036 ± 0.001
1.5	0.057	0.060	0.062	0.060 ± 0.003
2.0	0.077	0.076	0.079	0.077 ± 0.002
2.5	0.095	0.097	0.098	0.097 ± 0.002
3.0	0.114	0.112	0.117	0.114 ± 0.003
4.0	0.151	0.152	0.149	0.151 ± 0.002
5.0	0.189	0.191	0.192	0.191 ± 0.002

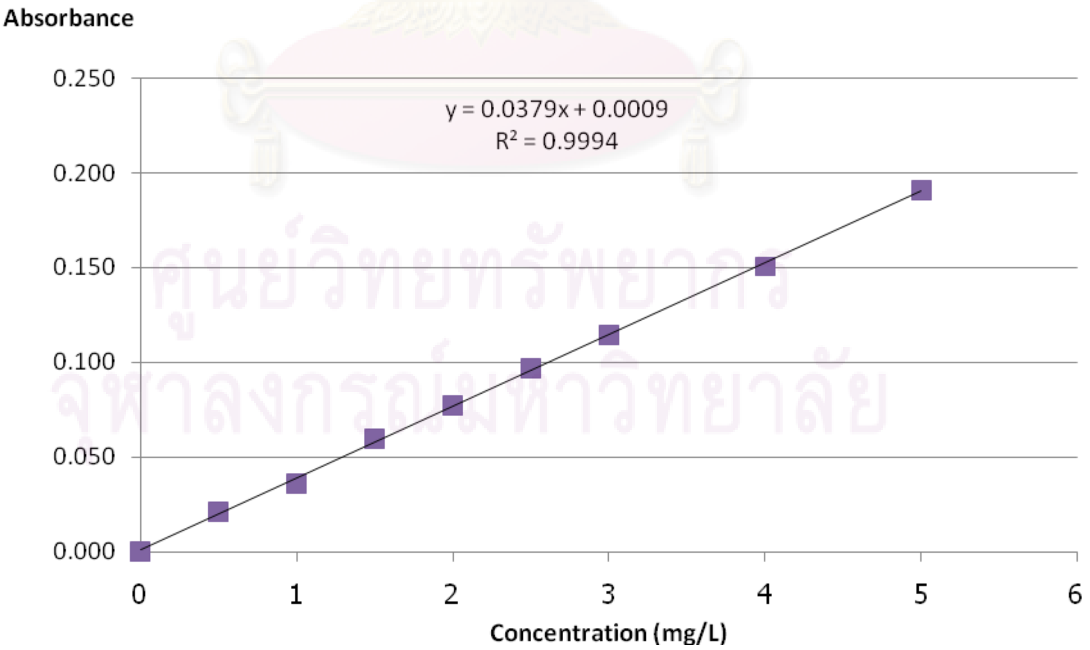

Figure B3 Calibration curve of standard zinc

APPENDIX C

National Center of
Excellence for
Environmental
and Hazardous
Waste Management

No...............

Date/month/year..................

Questionnaire

Risk assessment on fish consumption and participatory action for Mae Kuang management

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Section 1 Socio-economic status

1. Home Address..

Subdistrict....................................District...

Province..Tel...

2. Age

☐ 10 – 20 y/o ☐ 20 – 30 y/o ☐ 40 – 50 y/o ☐ 60 – 70 y/o

3. Sex ☐ Male ☐ Female

4. Weight.............................kg

5. Marriage status

☐ Single
☐ Married (stay together)
☐ Divorced
☐ married (live separately)

6. Education

☐ Primary school or lower
☐ Diploma
☐ Master's degree or higher
☐ Secondary school
☐ Bachelor's degree

7. Profession

☐ Student
☐ Farmer
☐ civil servant
☐ own business
☐ work for private company
☐ Others, please specific.................................

8. Household income/ year (last year data)

☐ 50,000 Baht/year or less
☐ 50,001 – 100,000 Baht/year
☐ 100,001 – 150,000 Baht/year
☐ 150,001 – 200,000 Baht/year
☐ 200,001 – 250,000 Baht/year
☐ 250,001 Baht/year or more

9. Debt

☐ No

☐ Yes From

- ☐ Loss from agriculture activities
- ☐ Daily life spending
- ☐ Land or/and house investment
- ☐ Education
- ☐ Others, please specific.................................

10. How long have you lived in Mae Kuang River vicinity?..........................Years

11. Have you ever eaten fish from Mae Kung River?

☐ No ☐ Yes 11.1 Eat fish but I dont' know where fish come from

11.2 Eat fish that come from Mae Kung River

12. Do you think eating fish from Mae Kung River is safe?

- ☐ Yes
- ☐ No, I will not eat fish from Mae Kuang if I know.
- ☐ I don’t care.

13. What kinds of fish do you consume? (can answer more than one)

13.1 Snake head (*Channa striatus*)...kg/week

13.2 Jullien’s mud carp (*Henicorhynchus siamensis*)..........................kg/week

13.3 Smith’s barb (*Puntioplites proctozysron*).....................................kg/week

13.4 Common carp (*Cyprinus carpio)*...kg/week

13.5 Others, please specific.. kg/week

14. Do you have any social management status?

☐ No ☐ Yes 1..

2...

3...

Section 2 Participation in Mae Kuang management and conservation

15. Currently, do you and your family take advantages from Mae Kuang River?

☐ No

☐ Yes from………… (can select more than 1)

- ☐ Agriculture and/or aquaculture purposes
- ☐ Fishing for foods
- ☐ Recreation fishing
- ☐ Food business
- ☐ Others, please specific.................................

16. Do you know about Mae Kuang problem?

☐ No

☐ Yes, such as
- ☐ Discharge from houses, shops, and buildings
- ☐ Industrial effluent
- ☐ Agricultural chemical runoffs
- ☐ No well-planned land use
- ☐ Forest/ open Fire
- ☐ Livestocks
- ☐ Soil erosion
- ☐ Garbage throwed into river
- ☐ Water hyacinth expansion

17. What do you think about Mae Kuang situation at present?

☐ Very polluted ☐ Polluted ☐ Not change a lot

18. Have you been asked to join activities related to Mae Kuang management and conservation from community leaders?

☐ No

☐ Yes, from

☐ Headman of a tambon ☐ Village headman

☐ Local community ☐ Teacher

☐ Public heath volunteer

☐ Others, please specific.................................

19. Are you one of members who get involved in Mae Kuang management and conservation?

☐ No

☐ Yes

1...

2...

3...

20. Since last year, have you been talking or discussing about Mae Kuang problem, management and conservation?

☐ No

☐ Yes, about.................................times / year

21. Have you participated in Mae Kuang management and conservation activities?

☐ No

☐ Yes, which activities…………

1...

2...

3...

22. Do you think you are needed to participate in Mae Kuang management and conservation activities?

☐ Strongly needed ☐ needed ☐ somewhat needed

23. Have you ever participated in planning for Mae Kuang management and conservation activities?

☐ Forest plantation

☐ Rule and regulation setting for Mae Kuang utilization

☐ Water use planning

☐ Fish conservation site planning

☐ Brain-storm for seminar or meeting relating to Mae Kuang River

☐ Waste management in local community

☐ Planning for wastewater treatment

24. Have you ever particpated in working or joining the Mae Kuang management and conservation activities? (can select more than 1 item)

- ☐ Tree plantation
- ☐ Fish release and fish feeding
- ☐ Volunteering as a narrator for Mae Kuang management and conservation
- ☐ Fund raising for Mae Kuang management and conservation
- ☐ Finding more people to participate in Mae Kuang management and conservation
- ☐ Wastewater treatment
- ☐ Garbage management

25. Have you ever evaluated the acitivities for the Mae Kuang management and conservation? (can select more than 1 item)

- ☐ Setting guidelines for Mae Kuang management and conservation project evaluation
- ☐ Problem solution after project evaluation

26. Problems related the Mae Kuang management and conservation that you always face (can select more than 1 item)

- ☐ Trespass
- ☐ Water usage conflice
- ☐ Wastewater discharge
- ☐ Garbages and flooding from garbage
- ☐ Water hyacinth expansion
- ☐ Soil erosion
- ☐ Shallow river
- ☐ Fish death and reduction

27. How would you like government or other stakeholders to involve this problem?

☐ Raise a campaign to use water wisely
☐ Separate the area for communities to take a responsity on their own area from upstream to downstream
☐ Continued water quality monitoring
☐ Provide effluent treatment for households and communities
☐ Training about water treatment and river conservation
☐ Promote to take water hyacinth out of river
☐ Tree plantation

Section 3: Any suggestions for participation in Mae Kuang management and conservation from community leaders?

....
....
....
....
....
....

Thank you very much for your kind co-operation.

BIOGRAPHY

Chanagun Chitmanat was born in Nakhon Si Thammarat. He finished Pathom 4 and then he moved to Amphur Ron Piboon where he stayed on a farm with his grandmother. Here he furthered his education in the small rural town. He finished the secondary school (Mathayom 3) from Ron Piboon Krietwasoontarapiwat before moving back to Nakhon Si Thammarat city and there finished his high school studies (Matayom 6) at Benchama Rachutit Secondary School. He passed the entrance examination to begin the study in the Department of Aquatic Sciences, Faculty of Natural Resources, Prince of Songkhla University, Hat Yai.

After graduation, he worked for Aquastar shrimp hatchery as fisheries scientist for 2 years. In 1993, he worked as a Biological scientist in viral diseases of cattle, Animal Health Institute, Ministry of Agriculture and Cooperation, Thailand. Later, he won the Royal Thai Government scholarship to study for a master's degree in the United States of America. During a TOEFL preparation and university application, he worked as an Environmental scientist in Water Quality Management Department, Ministry of Science, Technology, and Environment, Thailand. In the United States of America he earned his Master's Degree in Fisheries and Allied Aquaculture from Auburn University in Alabama. Subsequent to his Auburn degree he entered, completed, and received the master's degree program in Medical Microbiology from The University of Georgia, U.S.A.

In 2001, Chitmanat returned to Thailand and began his work as lecturer in the Faculty of Fisheries Technology and Aquatic Resources, Maejo University. He was promoted to the position of assistant professor at the same time that he received the scholarship for further study at Chulalongkorn University.