APPLICATION OF DEHYDROGENASE ASSAY FOR SEDIMENT HAZARDOUS ASSESSMENT IN SONGKHLA LAKE BASIN

BONGKOCH TONGSADAYU

A THESIS SUBMITTED IN PATIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(TECHNOLOGY OF ENVIRONMENTAL MANAGEMENT)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2007

COPYRIGHT OF MAHIDOL UNIVERSITY

Copyright by Mahidol University

Thesis

Entitled

APPLICATION OF DEHYDROGENASE ASSAY FOR SEDIMENT HAZARDOUS ASSESSMENT IN SONGKHLA LAKE BASIN

.....Bongkoch Tongsadayu.....

Miss Bongkoch Tongsadayu

Candidate

.....

Asst. Prof. Patana Thavipoke, Ph. D.

Major-Advisor

.....

Assoc. Prof. Duangrat Inthorn, Ph. D.

Co-Advisor

.....

Lect.Chulaporn Kamnerdpetch, Dr. rer. nat

Co-Advisor

.....B. Mahaisavariya.....

Prof. Banchong Mahaisavariya, M. D.

Dean

Faculty of Graduate Studies

.....Kulvadee Kansuntisukmongkol.....

Lect. Kulvadee Kansuntisukmongkol, Ph.D.

Chair

Master of Science Programme in Technology of Environmental Management

Faculty of Environment and Resource Studies

Copyright by Mahidol University

Thesis

Entitled

APPLICATION OF DEHYDROGENASE ASSAY FOR SEDIMENT HAZARDOUS ASSESSMENT IN SONGKHLA LAKE BASIN

was submitted to the Faculty of Graduate Studies, Mahidol University
For the degree of Master of Science (Technology of Environmental Management)
on November 2, 2007

Bongkoch Tongsadayu

Miss Bongkoch Tongsadayu
Candidate

.....

Lect. Pichai Sonchaeng, Ph.D.
Chair

.....

Asst. Prof. Patana Thavipoke, Ph. D.
Member

.....

Assoc. Prof. Duangrat Inthorn, Ph. D.
Member

.....

Lect. Chulaporn Kamnerdpetch, Dr. rer. nat
Member

.....

Prof. Banchong Mahaisavariya, M. D.
Dean
Faculty of Graduate Studies
Mahidol University

.....

Asst. Prof. Sittipong Dilokwanich, Ph. D.
Dean
Faculty of Environment and Resource Studies
Mahidol University

Copyright by Mahidol University

ACKNOWLEDGEMENT

The success of this thesis would have not been possible if there had not been valuable support and assistance from the following people who contributed a great deal to the thesis's essence.

I would like to express my heartfelt thanks to my major adviser, Assist. Prof. Dr. Patana Thavipoke, for his kind encouragement, supports, much patient, as well as time for guiding, correcting and editing my thesis. Special thanks are also to other committee members: Dr. Chulaporn Kamnerdpetch for her kindness, guidance and support on this thesis. To Assoc. Prof. Dr. Duangrat Inthorn, I would like to extend my gratitude for her kindness and several helpful discussions. My deep appreciation goes to Dr. Pichai Sonchaeng for his support over a long period since my undergrad years, as well as for providing his valuable time to be my external examiner. I will remember his kindness with my heart.

I wish as well to sincerely thank the member of "Songkhla Project" Team, Rung, P' Poom, Gift, P' Best and Golf for their friendship and enjoying time, and last but not least, Mo for his encouragement, support, as well as consideration that he had extended to me during the course of this study.

From my ET' 31 friends, I experienced a wonderful friendship. My sincere thanks are especially extended to my dear friends Tae, Pond and Ann. I would also like to thanks P' Nuch, P' Gig and P' Moodang for their kindness and help.

This research was partially supported by a grant from the Post-Graduate Education, Training and Research Program in Environmental Science, Technology and Management under Higher Education Development Project of the Commission on Higher Education, Ministry of Education.

Most of all, I deeply appreciated my beloved family; my mother, my father and P' Pee for their infinite love, understanding, constant encouragement and financial support. They always offered the help and stayed beside me. Without them, I really could not achieve the graduation. They are all my life. Thank you.

Bongkoch Tongsadayu

Copyright by Mahidol University

APPLICATION OF DEHYDROGENASE ASSAY FOR SEDIMENT HAZARDOUS ASSESSMENT IN SONGKHLA LAKE BASIN

BONGKOCH TONGSADAYU 4736374 ENTM/M

M. Sc. (TECHNOLOGY OF ENVIRONMENTAL MANAGEMENT)

THESIS ADVISORS: PATANA THAVIPOKE, Ph.D. (ENVIRONMENTAL PROTECTION TECHNOLOGY), CHULAPORN KAMNERDPETCH Dr. rer. nat. (TECHNISCHE CHEMIE), DUANGRAT INTHORN Ph.D. (PHARMACEUTICAL SCIENCE)

ABSTRACT

Toxicity assessment of natural sediment samples collected from 37 stations in Songkhla Lake basin was conducted. For the purpose, dehydrogenase enzyme activity inhibition (DHA) method using bacteria *Bacillus cereus* TISTR 687 was chosen. Two different test phases, i.e. elutriate and whole sediment phases, were tested. Suitable test procedure and conditions, including bacterial suspension preparation, as well as pH and salinity levels, respectively were determined. In addition, physical and chemical properties of sediment samples were determined, including particle size distribution, pH, organic content, cation exchange capacity, salinity and electrical conductivity.

The results indicated that the time required to obtain suitable density of bacterial suspension was 2 hours 30 minutes, which represents growth in middle log phase (exponential phase). The pH levels 7 and 8 were found to be the most suitable pH range for the DHA method, while inhibition effects on enzyme activities of B. cereus were not observed at salinity up to 10 ppt. For sediment toxicity assessment, four toxicity levels were ranked using centroid hierarchical cluster analysis. Results obtained from whole sediment phase seemed to be able to indicate a higher number of toxic sediment samples than those from elutriate phase. From all the sampling stations, 14, 10 and 13 sites were found non toxic, slightly toxic and highly toxic, respectively. Most of those stations with apparent toxicity were located in tributaries of Songkhla Lake Basin. The results obtained from elutriate phase revealed a non toxic condition in most sediment samples. Sediment samples from 2 stations were found slightly toxic, while those from other 2 stations were found moderately toxic. Only one sample was considered highly toxic. Most of these stations were located in tributaries of Thale Luang area.

The overall relatively higher toxicity of sediment samples obtained from the tributaries of the Songkhla Lake Basin compared to samples from the main lake areas. Thale Noi, Thale Luang and Songkhla Lake implied the possibility of excessive human influence in the tributaries area. Although chemical contamination determination revealed low contamination levels mostly below the standard values in all stations, the results from dehydrogenase enzyme activity inhibition (DHA) method using *B. cereus* could indicate sites that are prone to produce serious environmental problems. Therefore, this biotest method should be incorporated into a monitoring plan in other risk areas so that potential health hazards to their population, as well as to the environment, could be lessened.

KEY WORDS: DEHYDROGENASE ASSAY/ SEDIMENT TOXICITY/ SONGKHLA LAKE BASIN

107 pp.

Copyright by Mahidol University

การใช้วิธี DEHYDROGENASE ASSAY ในการทดสอบความเป็นอันตรายของตะกอนดินในลุ่มน้ำทะเลสาบสงขลา (APPLICATION OF DEHYDROGENASE ASSAY FOR SEDIMENT HAZARDOUS ASSESSMENT IN SONGKHLA LAKE BASIN)

บงกช ทองสดายุ 4736374 ENTM/M

วท.ม. (เทคโนโลยีการบริหารสิ่งแวดล้อม)

คณะกรรมการควบคุมวิทยานิพนธ์: พัฒน ทวีโภค, Ph.D. (ENVIRONMENTAL PROTECTION TECHNOLOGY), จุฬาภรณ์ กำเนิดเพชร, Dr. rer. nat. (TECHNISCHE CHEMIE), ดวงรัตน์ อินทร, Ph.D. (PHARMACEUTICAL SCIENCE)

บทคัดย่อ

การศึกษาการประเมินความเป็นพิษของตะกอนดินธรรมชาติจากลุ่มน้ำทะเลสาบสงขลา รวมทั้งสิ้น 37 สถานีศึกษา โดยวิธีทดสอบการยับยั้งการทำงานของเอนไซม์ Dehydrogenase ของ แบคทีเรีย *Bacillus cereus* สายพันธุ์ TISTR 687 แบ่งเป็น 2 ลักษณะการศึกษา ได้แก่ การศึกษาความเป็นพิษของน้ำสกัดจากตะกอนดิน และ ความเป็นพิษของตะกอนดินทั้งหมด โดยทำการศึกษาปัจจัยที่เหมาะสมในการทดสอบ ได้แก่ วิธีการเตรียมตัวอย่างแบคทีเรีย ค่าความเป็นกรด-ด่าง และความเค็มที่เหมาะสมในการทดสอบ นอกจากนั้นยังทำการวิเคราะห์คุณสมบัติทางเคมีและทางกายภาพของตะกอนดิน เพื่อใช้เป็นข้อมูลในการประเมินความเป็นพิษของตะกอนดินร่วมกับผลการทดสอบทางชีววิทยา เช่น ขนาดอนุภาคของตะกอนดิน ค่าความเป็นกรด-ด่าง ค่าปริมาณอินทรีย์สาร ค่าความสามารถในการแลกเปลี่ยนประจุ ค่าความเค็ม และค่าการนำไฟฟ้า

จากผลการศึกษาพบว่า ช่วงเวลาที่จำเป็นต้องใช้ในการเตรียมตัวอย่างแบคทีเรียที่มีความหนาแน่นที่เหมาะสม สำหรับการทดลอง และอยู่ในระยะที่มีการเจริญเติบโตสูงสุด (exponential phase) คือ 2 ชั่วโมง 30 นาที โดยที่ระดับความเป็น กรด-ด่าง 7-8 และระดับความเค็มไม่เกิน 10 ส่วนในพัน จะตรวจไม่พบการยับยั้งการทำงานของเอนไซม์ Dehydrogenase ของแบคทีเรีย *B. cereus* ส่วนการจัดกลุ่มความเป็นพิษของตะกอนดิน ใช้วิธีการจัดกลุ่มแบบเป็นขั้นตอน (centroid hierarchical cluster analysis) จัดระดับความเป็นพิษของดินตะกอนได้เป็น 4 กลุ่ม พบว่า การทดสอบโดยใช้ตะกอนดินทั้งหมดมีแนวโน้มที่จะตรวจพบตะกอนดินที่ความเป็นพิษมากกว่าการทดสอบความเป็นพิษโดยใช้น้ำสกัดจากตะกอนดิน โดยจากสถานีศึกษาทั้งหมด ตรวจไม่พบความเป็นพิษ 14 สถานี ระดับความเป็นพิษในระดับต่ำ 10 สถานีศึกษา และ ระดับความเป็นพิษในระดับสูง 13 สถานีศึกษา โดยพบว่าสถานีที่ตรวจพบความเป็นพิษในตะกอนดินส่วนใหญ่พบอยู่ในลำน้ำสาขาที่ไหลลงสู่ทะเลสาบสงขลา ส่วนผลจากการทดสอบความเป็นพิษโดยใช้น้ำสกัดจากตะกอนดิน พบว่า ไม่สามารถตรวจพบความเป็นพิษในดินตะกอนจากสถานีศึกษาส่วนใหญ่ มีเพียง 2 สถานี ที่แสดงความเป็นพิษในระดับต่ำ 2 สถานีศึกษาที่แสดงความเป็นพิษในระดับปานกลาง และ มีเพียงสถานีเดียวที่แสดงความเป็นพิษระดับสูง โดยสถานีศึกษาที่แสดงความเป็นพิษโดยใช้น้ำสกัดส่วนใหญ่เป็นสถานีศึกษาในคลองสาขาที่ไหลลงสู่ทะเลหลวง

จากผลค่าความเป็นพิษ ที่ส่วนใหญ่ตรวจพบในลำคลองสาขาของทั้งสามพื้นที่หลักของลุ่มน้ำทะเลสาบสงขลา ซึ่งได้แก่ ทะเลน้อย ทะเลหลวง และ ทะเลสาบสงขลา นั้น บ่งชี้ให้เห็นถึงผลกระทบที่เกิดจากกิจกรรมของประชาชน ที่มีต่อแหล่งน้ำสาขานั้น ๆ มากกว่าในพื้นที่หลัก แม้ว่าผลการตรวจการปนเปื้อนของสารเคมีต่าง ๆ ในดินตะกอนจะแสดงว่าส่วนใหญ่ยังอยู่ในเกณฑ์มาตรฐาน แต่จากการใช้การทดสอบการยับยั้งการทำงานของเอนไซม์ Dehydrogenase ของแบคทีเรีย *B. cereus* สามารถตรวจสอบพบจุดที่มีแนวโน้มว่าจะก่อปัญหาสิ่งแวดล้อมอย่างรุนแรงได้ จึงสมควรนำวิธีตรวจสอบความเป็นพิษทางชีววิทยานี้ไปใช้ในพื้นที่อื่นที่เสี่ยงต่อการเกิดปัญหาสิ่งแวดล้อมต่อไป เพื่อลดอันตรายที่อาจเกิดต่อสุขภาพของประชากร และสิ่งแวดล้อมในพื้นที่

107 หน้า

Copyright by Mahidol University

CONTENTS

		Page
ACKNOWLWDGEMENTS		**iii**
ABSTRACT		**vi**
LIST OF TABLES		**viii**
LIST OF FIGURES		**ix**
CHAPTER		
I	**INTRODUCTION**	1
	1.1 Statement of problem	1
	1.2 Objectives	2
	1.3 Hypothesis of the study	2
	1.4 Concept of the study	3
	1.5 Scopes of the study	4
	1.6 Expected result	4
II	**LITERATURE REVIEW**	5
	2.1 Sediment Contamination	6
	2.2 Chemical Mixture Interaction	6
	2.3 Toxicity Testing	9
	2.4 Soil and Sediment Toxicity Test	10
	2.5 Microbiological Toxicity Test System	11
	2.6 Enzymatic Activity	15
	2.7 Dehydrogenase Assay	16
	2.8 Songkhla Lake Basin	19
III	**MATERIALS AND METHODS**	25
	3.1 Materials and Chemical reagents	26
	3.2 Sediment Samples	27

Copyright by Mahidol University

CONTENTS (Continued)

		Page
	3.3 Microbiological Methods	33
	3.4 Toxicity Testing	34
	3.5 Data Analysis	36
IV	**RESULTS AND DISCUSSION**	37
	4.1 Method Standardization	37
	4.2 Sediment Properties	40
V	**CONCLUTION AND RECOMENDATION**	73
	5.1 Conclusion	73
	5.2 Recommendation	74
REFERENCE		75
APPENDIX		87
BIOGRAPHY		107

Copyright by Mahidol University

LIST OF TABLES

Table		Page
2-1	Components of an integrated approach to assess receiving water quality	8
2-2	Overviews of microbiotests with bacteria	14
3-1	The sampling stations in Songkhla Lake Basin.	31
3-2	Parameter and analytical methods used for determination of sediment quality.	33
4-1	Physical and chemical properties of sediment samples of Thalaynoy	42
4-2	Physical and chemical properties of sediment samples of Thalayluang	46
4-3	Physical and chemical properties of sediment samples of Songkhla Lake	51
4-4	Concentration range of 9 different heavy metals in sediment samples collected from 3 different areas, Thale Noi, Thale Luang, Songkhla lake, as well as their tributaries, in Songkhla Lake Basin (Pollution Control Department, 2006)	64
4-5	Toxicity levels of sediment samples as categorized by their inhibition effects on dehydrogynase activities of *Bacillus cereus*	65
4-6	Comparison of sediment toxicity levels resulting from three different toxicity assessment procedures observed from 37 sampling stations in Songkhla Lake Basin	71

Copyright by Mahidol University

LIST OF FIGURES

Figure		Page
1-1	Conceptual framework	3
2-1	Joint interaction of chemical mixtures	7
2-2	Chemical changes of resazurin and resulting color changes	19
2-3	Location map of Songkhla Lake Basin	21
3-1	The study flow chart.	25
3-2	The sampling station in Songkhla Lake.	29
3-3	The sampling station in the canals, wich flow into Songkhla Lake	30
4-1	Growth curve of bacteria (*Bacillus cereus*) incubated in incubate shaker at 37ºC, presented as viable cell count (CFUs/ml), as well as optical density (abs) at wavelength of 600 nm against incubation time (hr).	38
4-2	Relationships between varying pH levels of distilled control pH water and level of % Inhibition of dehydrogenase activity	39
4-3	Relationships between salinity and level of % Inhibition of dehydrogenase activity	40
4-4	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Noi (SK1, SK2 and SK3)	53
4-5	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Noi tributaries (PAC1 and PAC2)	53
4-6	Toxicity levels of sediment samples, both elutriate(25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang (SK4, SK5, SK6 and SK7)	54

Copyright by Mahidol University

LIST OF FIGURES (Continued)

Figure		Page
4-7	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang tributaries (RNC1, RNC2, MKC1, MKC2, TKC1 and TKC2)	55
4-8	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang tributaries (NTC1, NTC2, TNC1, TNC2, TCC1 and TCC2)	56
4-9	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang tributaries (SK8, SK9, SK10 and SK11)	57
4-10	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Songkhla Lake (SK12, SK13, SK14 and SK15)	58
4-11	Toxicity levels of sediment samples, both elutriate and whole sediment phases, collected from Songkhla Lake tributaries (PBC1, PBC2, PRC1 and PRC2)	59
4-12	Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Songkhla Lake tributaries (PMC1, PMC2, UTC1 and UTC2)	60
4-13	Toxicity levels of sediment samples collected from main Songkhla Lake resulted from elutriate phase	67
4-14	Toxicity levels of sediment samples collected from Songkhla Lake tributaries resulted from elutriate phase	68
4-15	Toxicity levels of sediment samples collected from main Songkhla Lake resulted from whole sediment phase.	69
4-16	Toxicity levels of sediment samples collected from Songkhla Lake tributaries resulted from whole sediment phase.	70

Copyright by Mahidol University

CHAPTER I
INTRODUCTION

1.1Statement of problem

Water pollution has become a major global environmental problem. The significant role that sediments play in aquatic ecosystems is also well known (Jenny, 2004). They serve as both a sink and source of organic and inorganic materials, which are critical cycling processes for organic matter and other critical elements (e.g., C, N, P and S). Decomposition processes of allocthonous and autochthonous inputs have been shown to occur primarily in the sediment. Since most chemicals of anthropogenic origin [e.g., pesticides, polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbon] tend to sorb to sediments and organic materials, they also end up concentrating in the sediment (David, 1994). Some of these chemicals pass through the environment without making any harm, while some of them cause temporary lesions and some are making irreversible damage (Jenny, 2004). When chemicals are released into the environment, they become chemical mixtures. Effects of interaction (e.g., addition, antagonism and synergism) among chemicals are difficult to assess. In sediment, toxicity of chemical mixtures are even more complex depending on several factors, including sediment characteristics (capacity controlling properties), sorption kinetics, as well as sediment deposition and erosion (Ahlf, 2006). Therefore, only chemical analyses data alone may underestimate the toxicity of each single chemical.

The way to find out about hazard and effects of such chemical mixtures to the environment is to perform assays on living materials (Jenny, 2004). Because of their rapid, ease of use, good reproducibility and low cost (Lui and Dutka, 1984), several bacterial tests are commonly used for the preliminary screening of sewage and industrial effluents for toxicity (Kilroy and Gray, 1995).The often applied microbial toxicity tests can be categorized as single species tests, biomass measurements, carbon and nitrogen transformations, enzymatic tests and tests measuring changes in microbial diversity (Beelen and Doelman, 1997).

Copyright by Mahidol University

From results of several studies, dehydrogenase enzyme activity (DHA) of microorganisms was demonstrated as one of the most sensitive parameter for evaluation of toxicity (Torslov, 1993). As a rule, the determination of DHA comprises incubation of a sediment sample in the presence of tetrazolium salt, which acts as an electron acceptor and is reduced to a colored formazan (Rossel et al., 1997). DHA assay with Resazurin as an oxidoreduction dye has been used successfully to determine toxicity of contaminants (Lui and Dutka, 1984) and their combined mixtures (Ahlf, 2006).

In the present study, inhibition of dehydrogenase enzyme activity (DHA) of bacteria (*Bacillus cereus*) was used as a test endpoint for assessing toxicity of natural sediments. Since dehydrogenases are involved in vital anabolic and catabolic reactions, they seem to be ideal for such toxicity studies (Lennette, 1985). For this method, resazurin dye is used as an electron acceptor (Colowisk, 1962). And the toxicity of sediment samples was presented in term of inhibition percentage.

1.2 Objectives

1.2.1 To study effects of natural sediment samples obtaining from Songkhla Lake Basin on dehydrogenase activities of *Bacillus cereus* TISTR 687

1.2.2 To compare the toxicity of sediment samples on dehydrogenase activities of *B. cereus* TISTR 687 resulting from two different test phases, i.e. elutriate and whole sediment.

1.3 Hypothesis of the study

Various sediment samples collection from Songkhla Lake Basin have different effects on enzyme dehydrogenase activity of *B. cereus* TISTR 687

Copyright by Mahidol University

1.4 Concept of the study

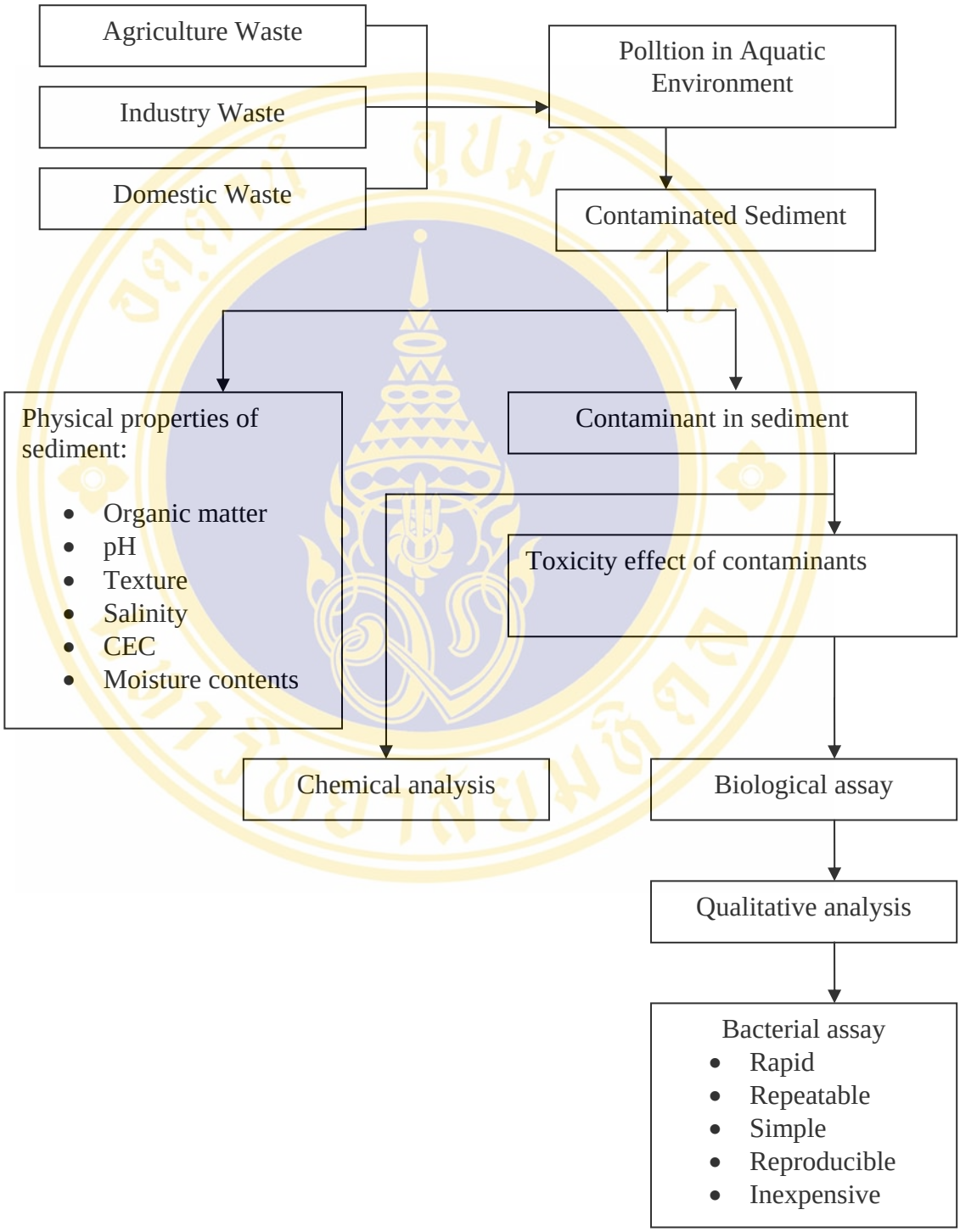


Figure 1-1 Conceptual framework

Copyright by Mahidol University

1.5 Scope of the study

1.5.1 A pure culture of *Bacillus cereus* TISTR 687 was obtained from Bangkok Microbiological Resource Centers (MIRCENs), Institute of Scientific and Technological Research (TISTR), Thailand

1.5.2 Whole sediment samples as well as their extracts was used for all the toxicity studies

1.5.3 Sediment samples were collected from Songkhla Lake Basin

1.6 Expected results

1.6.1 Possibility of using Dehydrogenase Assay for assessing toxicity of natural sediment samples

1.6.2 A simple method for routine application of predicting toxicity of sediment samples.

Copyright by Mahidol University

CHAPTER II
LITERATURE REVIEW

The industrialization of our society has led to an increased production and emission of natural chemical substances, and for most chemicals nothing or very little is known about their effects when being discharged into nature. Many of these chemicals will end up in the soil and sediment. Various soil and sediment constituents have a great capacity to retain chemicals, especially those with a polar molecule or positively charged divalent and trivalent ions. Consequently, the soil and sediment is a net sink for all kinds of chemicals, and concentrations are often considerably higher than in any other environmental compartment. This situation may lead to smaller or larger impacts on the functioning of soil ecosystems. Important ecological functions of the soil are those associated with organic matter decomposition, mineralization of nutrients, and synthesis of substances. For that reason, an increasing need exists for methods to assess the side effects of these chemicals on soil ecosystems. (OECD, 1989). Many of the sediments in rivers, lakes, and oceans have been contaminated by pollutants. Many of the contaminants were released for years ago while other contaminants enter our water every day. Some contaminants flow directly from industrial and municipal waste dischargers, while others come from polluted runoff in urban and agricultural areas. Still other contaminants are carried through the air, landing in lakes and streams far from the factories and other facilities that produced them. Source of pollution such as water run off and sediments are now recognized as long-term, widespread pollutant source of aquatic system. Substantial impacts on the ecosystem from sediment associate-contaminate range from direct effect on benthic communities to substantial contributions to contaminants load and effect on upper trophic levels through food chain contaminations (McCarty and Secord, 1999)

Copyright by Mahidol University

2.1 Sediment Contamination

Sediment contamination primarily occurs because many chemicals bind to organic or inorganic particles that eventually settle to the bottom of river, lake, stream reservoir, estuaries or marine water. This type of contamination is the presence of man-made chemicals or other alteration to the natural sediment environment. This type of contamination typically arises from rupture of underground storage tanks, application of pesticides and herbicides, percolation of contaminated surface water to subsurface strata, leaching of wastes from landfills or direct discharge of industrial wastes to the sediment. The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, herbicides, lead and other heavy metals. The occurrence of this phenomenon is correlated with the degree of industrialization and intensity of chemical usage (Crossley, 1970). The microbial, meiofauna and macrofauna which reside in sediments process organic matter and serve as food sources for higher trophic levels including humans. Loss of any biological community in the ecosystem is the cause of direct and indirectly affects other components of the system. Effects on ecosystem processes have been very dramatic in areas affected by both acid precipitation and acid mine drainage. However, in most areas receiving pollutant loading, the effect are difficult to observe and require use of variety of assessment tools, such as benthic macroinvertebrate community analysis, chemical testing, quantification of habitat characteristics and toxicity testing (David, Kirkby and Noble, 1994).

2.2 Chemical Mixture Interaction

Literally, thousands of chemicals, mostly as mixtures, are found in the environment. These mixtures could be simple or complex in their content or composition (Borgert**,** 2002). Chemical mixtures in the environment can be and generally are complex, consisting of parent compounds, reaction or transformation products along with other residues and potentially inert materials. Exposure of biota to combinations of pollutants may sometimes result in unexpected consequences, i.e., significantly lower or greater toxic response than a simple summation of the response

Copyright by Mahidol University

induced by the mixture components taken individually. Such effects, the consequence of a phenomenon termed as toxicological interactions, may be beneficial (one chemical affording protection against the toxic effects of another) or hazardous (one chemical increasing the toxicity of the other).

The toxicological interactions are frequently described using terms like additive, antagonism and synergism. Toxicological interactions were show in Figure 2-1.

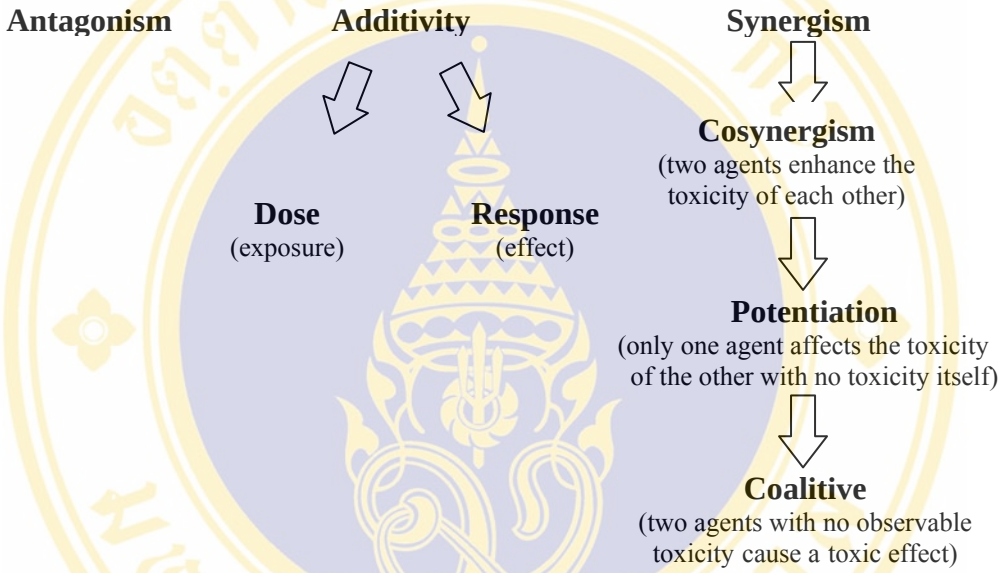


Figure 2-1 Joint interaction of chemical mixtures
(From: http://www.uoguelph.ca/cntc/publicat/toxtalk/toxtalk_fall99.shtml)

Landis and Yu (1995) provided definition for each terminology as follows:

Additivity

This occurs when the toxicity of chemical mixtures are greater than the exposure to each chemical individually, due to the sum of the toxic effects acting together. For example, the combination of one chemical with a toxicity level 1, with another compound also having a toxicity of 1 would equal a toxicity level 2.

Antagonism

The characteristic property of a mixture of toxicants that exhibits a less-than-additive total toxic effect For example, the mixture of two compounds with a toxicity level of 1 each might give a toxicity level of 1.5.

Copyright by Mahidol University

Synergism

Occurs when the toxicity of chemical mixtures are greater than expected on the basis of exposure to each chemical individually. Simply put, a combination of two compounds with individual toxicity levels of 1 might yield a toxicity level of 10.

Real sediments are always contaminated with a mixture of chemicals, which may act simply additively or a combination of single substances which may act synergistically or may be less toxic than expected by a simple addition of the effects (Liß and Ahlf, 1997). A wide variety of methods exist by which sediment contamination can be assessed (Table 2-1).

Table 2-1 Components of an integrated approach to assess receiving water quality

Control Approach:	What It Provides:	What It Doesn't Provide:
Chemical-Specific	- Human health protection - Complete toxicology - Straight forward treatability - Familiarity with control - Persistency coverage - Regulatory ease	- All toxics present - Bioavailability - Interactions of mixtures (e.g., additivity) - Poor trend analysis - Accurate toxicology (false assumptions)
Toxicity	- Aggregate toxicity - All toxics present - Bioavailability - Accurate toxicology - Good trend analysis - Lab or *in situ* testing	- Human health protection - Complete toxicology (few species may be tested) - Simple treatability - Persistency coverage
Bioassessment	- Actual receiving water effects - Trend analysis - Severity of impact - Total effect of all sources	- Critical flow effects - Straight forward - interpretation of results - Cause of impact - Differentiation of sources

From: USEPA (1990)

Copyright by Mahidol University

2.3 Toxicity Testing

Toxicity is the potential of a substance to cause adverse effect on living organisms (Ahlf, 2006). Toxicity tests are used to detect and predict the deleterious effects of chemicals on populations, communities and ecosystems. Toxicity testing of complex environmental mixtures of regulatory importance should be performed for hazard identification and to generate comparative potency estimates of human risk.

2.3.1 Toxicity-based approach

Toxicity tests directly measure toxic effects. Toxicity testing involves the measurement of a biological effect (e.g., death) associated with exposure to complex mixtures in instances when the mechanisms of the observed effect are not readily apparent and the specific causes of the effect are often unknown. The toxicity-based approach was developed for measuring and regulating the toxicity of complex effluents discharged to surface waters (U.S. EPA, 1985).

Some toxicants may interfere with a process essential to reproduction or growth. Some responses are much more sensitive than other (e.g., enzyme inhibition versus lethality) and should not be considered equally in evaluating the significance of the ecosystem impact (David, Kirkby and Noble, 1994). For example, carboxin acts by blocking the succinate branch of the respiratory pathway at the iron – sulphur protein of the dehydrogenase complex. Oligomycin and rutamycin strongly inhibit a limited number of yeast and fungal species. They also act on animal cells but not on bacteria. Additionally, Oligomycin reduces the oxygen consumption of intact cells and also of some mitochondrial preparations. But antibiotic has no effect on the oxygen consumption of submitocondrial particles, which lack the oxidative phosphorylation mechanism (Franklin and Snow, 1989).

2.3.2 Chemistry-based approach

Chemical analyses and laboratory-generated water quality (or air, soil, or sediment) criteria are used to estimate toxicity. For example, if concentrations of specific chemicals in surface waters (or air, soil, or sediment) exceed criteria values, then the concentrations are considered to be toxic.

Both may be appropriate for assessing the toxicity of environmental media contaminated by hazardous wastes. However, it is now generally considered

Copyright by Mahidol University

that for complex chemical mixtures of unknown composition, such as hazardous waste site samples, the toxicity-based approach is better for estimating potential toxicity. Toxicity testing may also be applied to the evaluation of metabolic interactions of certain chemical mixtures and elucidation of interaction thresholds (Wright, 2002). Additionally, toxicity testing is to establish the potential impact of chemicals on biota in the environment. The information gained can then be used to manage the treatment or release of chemicals.

2.4 Soil and Sediment Toxicity Test

Because of the paucity of data concerning sediment toxicity, tests designed specifically to evaluate the toxicity of sediments are in their early stages of development. Currently, protocols exist for chronic toxicity tests only (Bitton, Jung, and Koopman, 1994). As research continues, new tests should become available to investigators.

Sediment Toxicity tests have been performed with a wide variety of freshwater and marine organisms (Burton, 1991). A large number of other species have been used for determining the toxicity of sediments, ranging on bacteria to fish and amphibian.

These tests have applied to both water column and benthic species with measured endpoints, including survival, growth, reproduction, larval development, enzyme inhibition and luminescence of test species.

Techniques to assess were developed specifically for testing benthic organisms such as bivalves, polycheates and echinoderm with whole sediment. Other tests were developed by modifying methods developed for effluent toxicity testing and applying them to whole sediments, elutriate, pore water or extracts (David, Kirkby and Noble, 1994). The use of pore water is based on the assumption that chemicals associated with the solid phase are largely unavailable and therefore sediment toxicity can be estimated by measuring or modeling the pore water concentration.

Copyright by Mahidol University

Extraction Procedures

Two simple commonly used extraction procedures, water extraction and organic solvent extraction, are performed sequentially on the same sediment sample.

Water Extraction

Elutriates (water extracts) represent only a part of multiple contamination, due to the different solubility of each contaminant in water. Water elutriation could underestimate the types and concentrations of bioavailability organic contamination present (Ongley *et al.,* 1988).

Solvent Extraction

Different solvents such as dimethylsulfoxide (DMSO), methanol, ethanol and acetone can be used for extraction of organic contaminants (Kwan and Dutka, 1995). One problem when using solvents is the possible toxicity of the solvent itself. Hence the maximum allowable concentration of the extract solvent in the test must first be measured (Brouwer *et al*., 1990) and the possible synergistic interactions between contaminants must also be considered (Gunkel *et al.,* 1993). Aqueous extraction could be selective in multiple compound contaminations in solid samples, due to the different solubility of especially organic compounds in water (Ongley *et al*., 1988). The use of assays employing direct contact of test organisms with the sample could better assess the bioavailability and toxicity of the contaminants in solid samples (Rönnpagel *et al*., 1995).

On the other hand, solvent extracts enhance removal of organic contaminants and indicate the maximum toxicity potential. Moreover, even with low solvent concentrations, the results can be influenced by synergistic action between contaminants and the solvent and it is possible to quantify the contribution of the solvent influence (Gunkel *et al.,* 1993).

2.5 Microbiological Toxicity Test System

Any test for a toxic chemical requires an indicator organism. A compound is defined as being toxic if it damages living material. The tests used to define toxicity legally use vertebrate (e.g., rats, mice, dogs, rabbits, fish and amphibian) or

Copyright by Mahidol University

invertebrate (e.g., *Daphnia,* polycheate, echinoderm, nematode, amphipod). These tests are complicated and expensive. They require highly skilled personnel to carry out. It is necessary to determine if the animal died from the toxin or from other causes. Damage to the animal is usually determined by the death of the animal. Many toxins may damage the animal but may not kill the animal. Damage to the animal is not determined in these tests, only death. Animal tests require days if not weeks to carry out. In addition, animal rights advocates object to using animals for this purpose. There is an obvious need for a simple, fast, and inexpensive method to assay for toxicity of chemicals (Ames, McCann and Yakasai, 1975). It would be useful to have a simple initial test to determine if a chemical were toxic analogous to this test for chemical mixture.

2.5.1 Microbiotest

Short-term Microbial Tests are based on inhibition of activities of bacteria, algae, and fungi, and are versatile and cost-effective assessment tools (Bitton and Dutka, 1986; Liu and Dutka, 1984; Rönnpagel, JanBen and Ahlf, 1998). Because they are simple, rapid, and relatively inexpensive procedures, they are readily adaptable to miniaturization and automation to characterize the extent of contamination. Microbial test methods have been developed that assess the toxicity of domestic and industrial effluents, discharges, and waste products. However, with the increasing awareness of the long-term effects of chemicals discharged into aquatic systems and landfill sites, recent research efforts have been directed to the development of short-term bioassay tests to alert regulatory and monitoring agencies, as well as dischargers, of the presence of toxicants in effluents and the aquatic ecosystem (Bulich 1979; Dutka and Kwan, 1988). In addition, many of the microbial functional responses assays represent important ecosystem processes and microbial tests, i.e. Microtox, have been applied in the field to evaluate these processes.

2.5.2 Bacteria Assay

Microorganisms such as bacteria are very suitable for toxicity testing. Their small size makes it possible to make assays on millions of individuals simultaneously and thereby avoid uncertainties due to individual variations. As

Copyright by Mahidol University

bacteria have short generation times, sometimes as short as 20 minutes, test times can be reduced considerably compared to tests on larger organisms. Bacteria are easy and cheap to handle. And staff with any advanced training is generally able to perform the tests. They incorporate toxicants in their metabolism faster than higher organisms do. Most importantly, there are no ethical problems connected to the use of microorganisms in toxicity testing. (Bitton and Dutka, 1986)

In addition to the direct measurement of toxicity by growth rate or cell viability, various microbial test methods are based on the assessment of biochemical properties of the bacteria. These biochemical indicators of toxic results include the ATP concentration (e.g., ATP-TOX system test) (Schüürmann and Markert, 1998), bioluminescence assays, microbial growth assays and enzymatic activity (e.g., the ATPase activity, the dehydrogenase activity and the activity of enzymes, phosphatases, urease and esterases). A summary of important aspects of various microbial tests is given in Table 2-2.

Copyright by Mahidol University

Table 2-2 Overviews of microbiotests with bacteria.

Test	Organism	Principle	Standardization Applications	Reference
Microtox Microtox Solid Phase	*Vibrio fischeri*	inhibion of luminescence after exposure and filtration	soil, sediment and wastes water	Tung, *et al*. 1990
Direct contact luminescence bacteria test	*Vibrio fischeri*	inhibition of luminescence after exposure and centrifugation	sediment, wastes water	Brouwer, *et. al.* 1995 and Thomulka, et al.
Luminescent bacteria flash test during exposure	*Vibrio fischeri*	kinetic measurement of luminescence inhibition	wastes soil, sediment	Lappalainen, *et al*. 2001
Toxi-Chromo Pad test Sediment chromotest	*Escherichia coli*	β-galactosidase synthesis colorimetric Inhibition after or during exposure	sediment, soil	Kwan, 1995
MetPAD MetPLATE	*Escherichia coli*	β-galactosidase synthesis/ colorimetric	soil, sediment and wastes water	Dutka and Gorie, 1988
Bacillus cereus contact test	*Bacillus cereus*	inhibition of Dehydrogenase Activity	sediment, soil and wastes water	Rönnpagel, *et al*. 1995
ATP concentrate luminescence	*Escherichia coli*	measurement of concentration of ATP	sediment	Xu and Dutka, 1987
Size of bacteria laser technique	*Bacillus subtilis*	measurement of growth	water	Felkner, et al. 1988

Modified From: Ahtiainen (2002) and Schüürmann and Markert (1998)

Copyright by Mahidol University

2.6 Enzymatic Activity

Since enzymes are key catalysts for metabolic reactions in cells, their inhibition by environmental toxicants could be the underlying cause of toxicity to the cells. Enzyme inhibition as a basis for toxicity testing has been explored for a wide range of enzymes with special emphasis on the dehydrogenase enzymes (Bitton and Koopman 1986; Christensen et al. 1982). Other enzymes studied include ATPases, esterases, phosphatases, amylase, protease, beta-glucosidase, urease, and luciferase (Obst et al., 1988). Although enzymes are quite sensitive to heavy metals, they generally display little sensitivity to organic toxicants.

The inhibition of enzyme activity or the inhibition of enzyme induction and *de novo* synthesis is assayed in some test procedures. The inhibition of *Escherichia coli* dehydrogenase activity is measured in a rapid ECHA screening test (Dutka and Gorrie 1989) and the inhibition of β-galactosidase activity in MetPAD (Bitton, Campbell and Koopman, 1992) and MetPLATE (Bitton *et al*. 1994). Inhibition of the hydrolysis of fluorescein diacetate (FDA) by several enzymes (proteases, lipases and esterases) and the measurement of fluorescence was applied in a test procedure presented by Haig and Rennie (1994). The inhibition of enzyme induction and *de novo* synthesis are considered to be more sensitive phenomena than the inhibition of enzyme activity (Ongley *et al*. 1988) or other metabolic activity in bacterial cells (Reinhartz *et al.* 1987). The ToxiChromo- test is based on inhibition of the activity of the lac-operon and of synthesis of β-galactosidase enzyme (Reinhartz *et al.,* 1987). Inhibition of the induction of α-glucosidase in *Bacillus licheneformis* has also been reported to be especially sensitive to hydrophobic substances (Dutton *et al*. 1990). The greater sensitivity is caused by the difference of the cell wall of this gram-positive bacterium compared to *E. coli*.

A variety of techniques are available to measure changes in dehydrogenase activity as a result of chemical effect on microorganisms. These include measuring color changes of tetrazolium dyes and resazurin, and the direct inhibition of specific dehydrogenase enzymes (Bitton and Koopman 1986).

Copyright by Mahidol University

2.7 Dehydrogenase Assay

A dehydrogenase is an enzyme that oxidizes a substrate by transferring one or more protons and a pair of electrons to an acceptor, usually NAD/NADP or a flavin coenzyme such as FAD or FMN. Aerobic respiration process requires dehydrogenase to remove hydrogen from substrate and obtain carbon dioxide from piruvate ($CH_3COCOOH$), especially in tricarboxylic acid cycle. In fact many different dehydrogenase enzymes involved in respiration, removing hydrogen atoms during glycolysis and the Krebs cycle (e.g. pyruvate dehydrogenase, isocitrate dehydrogenase, and alpha-ketoglutarate dehydrogenase) (Penry and Saley, 1997).

The dehydrogenase assay with resazurin as an oxidoreduction dye was used for environmental monitoring. Dehydrogenases are involved in vital anabolic and catabolic reactions and seem ideal for use in such toxicity studies. In this toxicity method, resazurin acts as an electron acceptor. Toxicity measurements are based on the quantitative reduction of resazurin to resorufin by microbial dehydrogenase and it is measured spectrophotometrically. Chemical reducing substances (aliphatic and aromatic alcohols) in water inhibit microbial dehydrogenase. This inhibition causes decreased reduction of resazurin, which is related with the toxicity of these chemicals (Genc and Özturk, 1998). The reduction of resazurin to resorufin by microbial dehydrogenase is shown earlier in Figure 2-2

There are many methods for calculation of inhibitory concentration. Commonly used value are LOEL (Lowest Observed Effect Level), NOEC (No Observed Effects Concentration), MIT (Minimum Inhibitory Concentration) and IC_{50} (the concentration at which 50% Inhibition is obtained). The IC_{50} is the concentration at which 50% Inhibition of biological activity (growth, enzyme activity, number of living individuals, etc.) is found. In most cases, this value must be interpolated from the obtain data, as much luck is needed if the exact IC_{50} should be among the tested concentrations in an assay. The interpolation is made between the two concentrations closest under and above 50% inhibition and inhibition cure are assumed to form a straight line between these two points (Jenny, 2004).

The toxicities of soils and sediments could be tested directly and with high sensitivity with the procedure presented by Rönnpagel *et al*. (1995). They used the

Copyright by Mahidol University

bacterium *Bacillus cereus* as test organism assessing the inhibition of dehydrogenase activity measured by rezasurin reduction (Liu and Strachan 1981).

Moreover, Wlodarczyk, Stepniewski, and Brzezinska (2005) studied the relationship between soil dehydrogenase activity and aeration status. Dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in the soil. It increases under anaerobic conditions. This tendency has been observed in model experiments (with soils incubated at diminished oxygen concentration or under soil flooding), as well as under natural field. The anaerobic respiration is less efficient than aerobic (with utilization O_2 as terminal electron acceptor). Increased dehydrogenase activity under prolonged soil hypoxia suggests that the facultative and anaerobic members of the microbial community become more important in the total soil respiration.

Strotmann, Butz and Bias (1993) used the dehydrogenase assay for monitoring the performance of six laboratory-scale sewage treatment plants. The dehydrogenase activity measured proved to be a reliable monitoring parameter, although it did not always positively correlate with the simultaneously estimated respiration activity. With the help of the dehydrogenase assay, the toxicity of different phenolic compounds was determined. It was found that the toxicity of 2.4-dichlorophenol, 3.5-dichlorophenol, and 2.4-dinitrophenol was clearly dependent on the pH. These data were supported by shock loading experiments in laboratory-scale sewage treatment plants.

Experiments from Rönnpagel, JanBen and Ahlf (1997) compared sensitivity of *Arthrobacter globiformis* and *Bacillus cereus* by dehydrogenase assay. Both organisms show a similar sensitivity pattern (same ranking of substances) but in each particular case, different levels of effect concentrations. With these results it seems that testing with both test organisms does not give important supplementary information.

Experiments conducted by Collins and Stotzky (1992) showed a charge conversion of bacteria cells due to heavy metal adsorption. The resulting positive net charge can impact physiological functions of cells and their reaction with other cells or non living particles. This can be a reason for overestimating the risk of copper

Copyright by Mahidol University

contamination when an exceptionally sensitive Gram-positive test bacterium is applied in the contact assay.

2.7.1 Organism

The bacteria, *Bacillus cereus,* which will be used as a test organism for assessing toxicity of natural sediment in this study, is a gram-positive, facultative aerobic rod-shaped endospore-forming bacteria. Its cell wall might be more permeable to hydrophobic organic compounds than the cell wall of the gram-negative bacteria used in most tests. Most *Bacillus* species including *B. cereus* are motile by means of peritrichous flagella. Their colonies have a variable appearance but are usually 3-8 mm in diameter, raised, irregular, with a grayish "frosted glass" appearance and undulate margins (Granum, 1994).

B. cereus occurs ubiquitously in soil and in many raw and processed foods, such as rice, milk and dairy products, spices, and vegetables. Many strains of *B. cereus* are able to produce toxins and cause distinct types of food poisoning. Concerns over *B. cereus* contamination have increased because of the rapidly expanding number of chilled foods that may be pasteurized but may still contain viable spores (Carlin et al., 2000). Spores from *B. cereus* can germinate and outgrow during storage, even at low temperatures (Granum, 1994). Major efforts to battle this increasing problem are focusing on determining the causes of the spore's resistance and the mechanisms of germination.

Bacillus cereus is one of the major food-borne pathogenic bacteria and a common contaminant of food and dairy products. That is the causative agent of two types of food poisoning, the emetic and diarrheal forms. The mechanism of the pathogenesis of *B. cereus* has not yet been fully clarified. The vegetative bacterial cells can produce a number of virulence factors, including enterotoxins, of which at least three have been characterized (Granum, 1994).

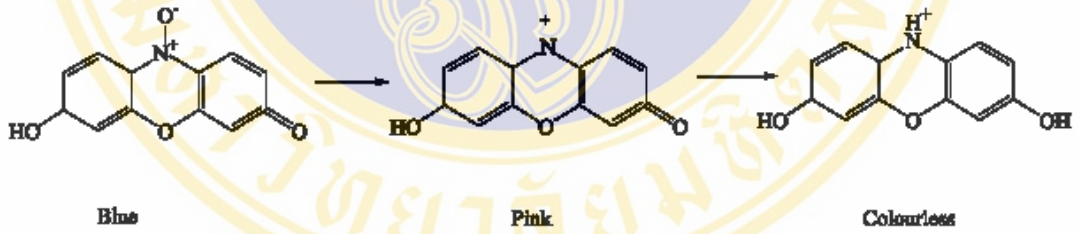
2.7.2 Resazurin Dye

A blue compound, 7-hydroxy-3H-phenoxazin-3-one 10-oxide, used as a redox indicator in the reductase test of milk and also as a pH indicator (orange at 3.8, violet at 6.5). Resazurin is an oxidoreduction dye, indicating the

Copyright by Mahidol University

bacterial dehydrogenase activity. This dye is light sensitive and stable at 2 - 8°C for 12 months. Supplied as a ready to use solution in a 0.2 mm filtered, buffered solution (Ansar Ahmed, 1994). Resazurin can be used to trace electron transport activity. These dyes act as artificial hydrogen acceptors and upon reduction change color allowing easy quantification with a spectrophotometer (Sunahara *et al.*, 1998).

The molecular of resazurin is blue in its original form but changes color in two steps by bacterial dehydrogenase activity to the pink colored compound resorufin, which determined fluorometically at the excitation wavelength 560/590 nm. and next to the colorless dihydroresorufin (Conn, 1961). Resazurin is used to detect the dehydrogenase activity in a sample containing microorganisms (Torslove, 1993) and is widely used in the diary industry. The resorufin intermediate is fluorogenic and the reaction can thus followed by fluorimetry. It can also be registered by eye as the colors are easily detected, but visual inspections could lead to a certain amount of error (Grabrielson, 2004). In this case, resazurin acts as a hydrogen acceptor by respiration process of *B. cereus*.



resazurin resorufin dihydroresorufin

Figure 2-2 Chemical changes of resazurin and resulting color changes (Sunahara *et al.*, 1998)

2.8 Songkhla Lake Basin

Songkhla Lake Basin is one of Thailand's most precious natural treasures, which is widely recognized for its natural quality, biodiversity and economic potential. There ecosystem comprises four interconnected lakes (Thale Sap Songkhla, Thale Sap, Thale Luang and Thale Noi), ranging from saline to freshwater, located on

Copyright by Mahidol University

7°10'-7°50'N, l00°05'-100°40'E where the east coast of southern Thailand (Figure 2-3). The lake area covers 1,081 sq. km and discharges through Thale Sap into the Gulf of Thailand. More than 20 streams flow into the lakes from a catchment area covering 6,938 sq. km. A great many small villages containing approximately 80,000 persons are scattered around the lake shore, most of these on the eastern side (NESDB and NEB, 1985).

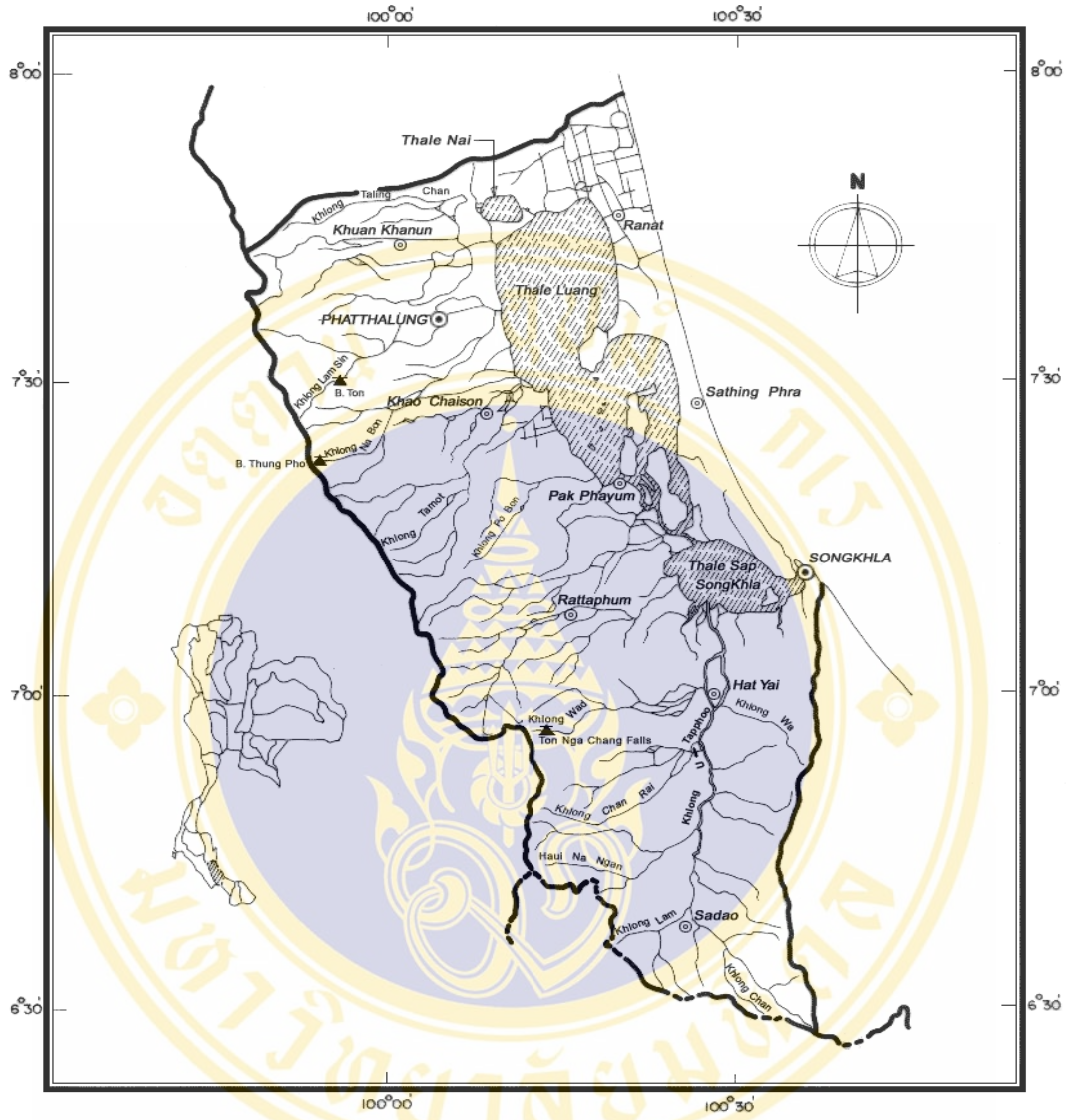
Songkhla Lake is influenced by run-off from the steep, mainly forested mountains to the west and by the introgression of salt water from the sea. Water quality varies greatly throughout the different parts of the lake; there is much seasonal and spatial variation in salinity and turbidity (Nachaingmai, 1991)

In the lake, the salinity was 5.3 ppt in November to only 0.7 ppt in January, following input of heavy rainfall. The turbidity is highest near the shoreline and increases to a maximum of 95-100 FTU in January. During the drier months, March to August, it is around 30-50 FTU. The average depth of the lake is about 1.0-1.5 m over most areas, and the maximum depth, 2.5 m. There are, however, considerable fluctuations in water level. At the Thale Noi Area, the average depth in December, at the height of the monsoon, is l.46m. This declines to about 0.5m by August. The average tidal range at the mouth of the overflow channel is approximately 0.5m, the maximum, 1 .3 m.

2.8.1 Climatic conditions

The climatic conditions of Songkhla Lake Basin are tropical monsoonal climate with an average annual rainfall of 2,094 mm. There is, however, a great deal of variation in the annual rainfall; in 1983, only 983 mm were recorded. Some 60% of the rain falls during the northeast monsoon, from October to December. The wettest month is usually December, while the driest months are from January to April (Nachaingmai, 1991). The mean relative humidity is 79%, with a minimum of 76% in March and a maximum of 84% in November. The mean daily temperature is 27.6°C (maximum 28.7°C in April, minimum 26.6°C in December). Prevailing winds are easterly during November to April and southwesterly during June to October.

Copyright by Mahidol University



LEGEND

STREAMFLOW MEASURING STATION WHERE SEDIMENT SAMPLES ARE TAKEN AND METEOROLGICAL STATION.

STREAMFLOW MEASURING STATION AND METEOROLGICAL STATION.

GAGE HEIGHT RECORDING STATION AND METEOROLGICAL STATION.

STREAMFLOW MEASURING STATION WHERE SEDIMENT SAMPLES ARE TAKEN.

STREAMFLOW MEASURING STATION.

GAGE HEIGHT RECORDING STATION.

INTERNATIONAL BOUNDARY

BASIN BOUNDARY

MAE NAM

Figure 2-3 Location map of Songkhla Lake Basin

Copyright by Mahidol University

2.8.2 Principal vegetation

Marshall (1984) reported a total of 19 species of aquatic plants that has been collected from the Kukut area of Thale Sap. The chief emergents and lake shore plants include *Scirpus litoralis, Paspalum vaginatum, Eleocharis dulcis, Fimbristylis ferruginea, Cyperus distans* and *C. stoloniferus.* In addition, there are small areas, where a mangrove-type community dominates. The prominent species are *Sonneratia caeseolaris, Rhizophora apiculata, Nypa fruticans* and *Acanthus ebracteatus*. In Songkhla lake, this community occupies waters with a much lower salinity than is typical. Some other lake shore plants, such as *Melaleuca leucadendra, Hibiscus tiliatus* and *Olax scandens*, are more typical of sandy, coastal conditions. The chief submerged and floating aquatic species are *Chara* sp, *Ceratophyllum demersum, Hydrilla verticillata, Lemna purpusilla, Najas* spp, *Pistia stratiotes* and *Potamogeton malayanus*. The principal vegetation in surrounding areas is rice paddy and *Borassus* palms with some areas of *Melaleuca* scrub.

2.8.3 Fauna

Several hundred species of fish and other aquatic animals inhabit the lake, of which 100 species are abundant. Of 66 species collected in the vicinity of the Thale Noi, 26 were freshwater species, 28 brackish to brackish/marine and 12 fresh/brackish or fresh/brackish/marine (Marshall, 1984). Approximately 140 species of birds have been recorded from the Thale Noi Area (Thamrongwang et al., 1999). Larger species, such as egrets, are present only as winter visitors. The most abundant breeding or apparently resident species are *Tachybaptus ruficollis*, *Ixobrychus sinensis, I. cinnamomeus, Dendrocygna javanica, Nettapus coromandelianus, Gallinula chioropus* and *Porphyrio porphyrio*. The lake is important as the only known breeding site for *Himantopus himantopus* in the Malay Peninsula. Wintering concentrations of over 2,000 each of *Dendrocygna javanica* and *Nettapus coromandelianus* have been recorded, together with a few hundred *Anas querquedula.*

At least 19 species of Odonata have been recorded among seven aquatic insect orders in total. The macrobenthic fauna is dominated by Nereidae, Gammaridae and Chironomidae. The overall species diversity of Crustacea is very high. A few River Terrapins *Batagur baska* may still be present. The species formerly

Copyright by Mahidol University

nested in large numbers at Khao Chaison, on the western shore of Thale Sap. Otters, probably *Lutra perspicillata*, are reportedly present on the lake.

2.8.4 Land use

The Basin contains a mixture of land uses ranging from wet and dry season rice, rubber plantations, tropical evergreen forests, wetlands and Melaleuca swamp and settled areas comprising villages with home gardens and mixed orchards. Land use of Songkhla lake basin is chiefly fishing for molluscs, crustaceans and fish, together with some aquaculture. A variety of traps and nets are used. Aquaculture is beginning to be developed, with small areas of ponds for the culture of *Machrobrachium rosenbergii* and for sea-bass (Anon, 1984). There are some cattle grazing around the lake. Local people utilize various aquatic plants such as *Eichhornia* and *Ceratophyllum* for animal fodder and others such as *Eleocharis* and *Scirpus* for weaving mats etc. Surrounding areas are extensively farmed. Over most of the lake basin (1,648 sq.km) there is one crop of rainfed, wet-season rice per year. Field preparation takes place from August, with most planting in October and the crop being harvested in March. Some 808 sq.km are given over to irrigated rice cultivation. The palm *Borassus flabellifer* is cultivated as a source of sugar and for its fruits. Some caves in limestone islands and other outcrops are important as sources of birds' nests and bat guano. These are leased from the government by a private company and therefore do not directly benefit the local people. There is also a small amount of tin mining in the basin.

Thamrongwang et al. (1999) conducted a study on physical and chemical water qualities in Songkhla Lake basin between February 1995 to January 1996. Klong-wad, Klong-Ou-Tapoa, Klong-Toey and the end at the mouth of Klong-Ou-Tapoa. The results from this study indicated that water flowed through the mouth of the Songkhla Lake area had higher temperature (28.9 ^{o} C), electrical conductivity (EC, 7,318.3 μ siemen/cm.), total solid (3,406.9 ppm) and As contents (17.5 ppm) than other places, whereas water discharged from the upstream of Klong Ou-Ta Pao area had the highest level of turbidity and Zn contents with average values of 112.2 NTU and 44 ppb,. The highest level of pH (6.8) and Dissolved Oxygen (DO, 8.1 ppm) were found in water flowed from Tone-Nga-Chang area. Water flowed through the

Copyright by Mahidol University

end of Klong U Ta Pao area contained the highest amount of hardness and Cu contents values of 668.4 ppm and 14 ppb. Songkhla Lake basin are still being in acceptable based on standard level for natural water of Thailand except turbidity, EC, total solid, DO, Pb and As contents in some areas, whereas Tone-Nga-Chang (natural forest) and Klong-Wad canal stand discharged water of better quality.

Office of the National Environment Board Syn. (1990) study on saline intrusion through a coast into the Songkhla Lake was conducted by measuring ground receptivity with Schlumberger configuration in Ranot district of Songkhla province. The number of measuring points was 14. The second ground layer of less than 2.5 ohm receptivity and of 26-110 m thick is clearly observed. This layer lies continuously from the Songkhla Lake to the Gulf of Thailand. The ground resistivity of 2.5 ohm is equivalent to 2.2 ppt salinity of NaCl solution, whereas the salinities of the Songkhla Lake and of the Gulf of Thailand are 0.5 and 19 ppt respectively. The study results show that the boundary of the saline intrusion in the coast is much far away from the Songkhla Lake.

Experiment from Nachiangmai (1991) was undertaken in order to study the water quality of the Outer Songkhla Lake Basin covering the period of August 1978 through January 1979. The indices of water quality, i.e., the values of pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), nitrate, phosphate and salinity were measured. The means and SD. of these values were as follows: pH 7.8±0.15, DO 6.8±0.3 mg/l, BOD 2.0±0.4 mg/l, nitrate 6.2±0.69 mg/l, phosphate 0.13±0.04 mg/l and salinity 19.4±3.8 ppt. From the results of the study, the water quality in the basin was found not to be seriously affected by pollution and other man-made activities. It seemed that the salinity had slightly increased. The salinity intrusion into the basin was, however, found to be partially due to the amount of rainfall on the catchments area.

Copyright by Mahidol University

CHAPTER III
MATERIALS AND METHODS

The experimental research was conducted in three main parts. The first part involved with test optimization. The second part covered chemical and physical properties determination of natural sediment samples. These included particle size distribution, pH, organic content, CEC, salinity and electrical conductivity. The last part was mainly toxicity assessment of natural sediment samples, using the inhibition of dehydrogenase enzyme activity of *Bacillus cereus* as toxicity endpoint. Toxicity of both elutriate, as well as whole sediment phase were observed. Natural Sediment samples used for the purpose were collected from 37 stations around Songkhla Lake Basin. The overall study was performed as shown in Figure 3-1

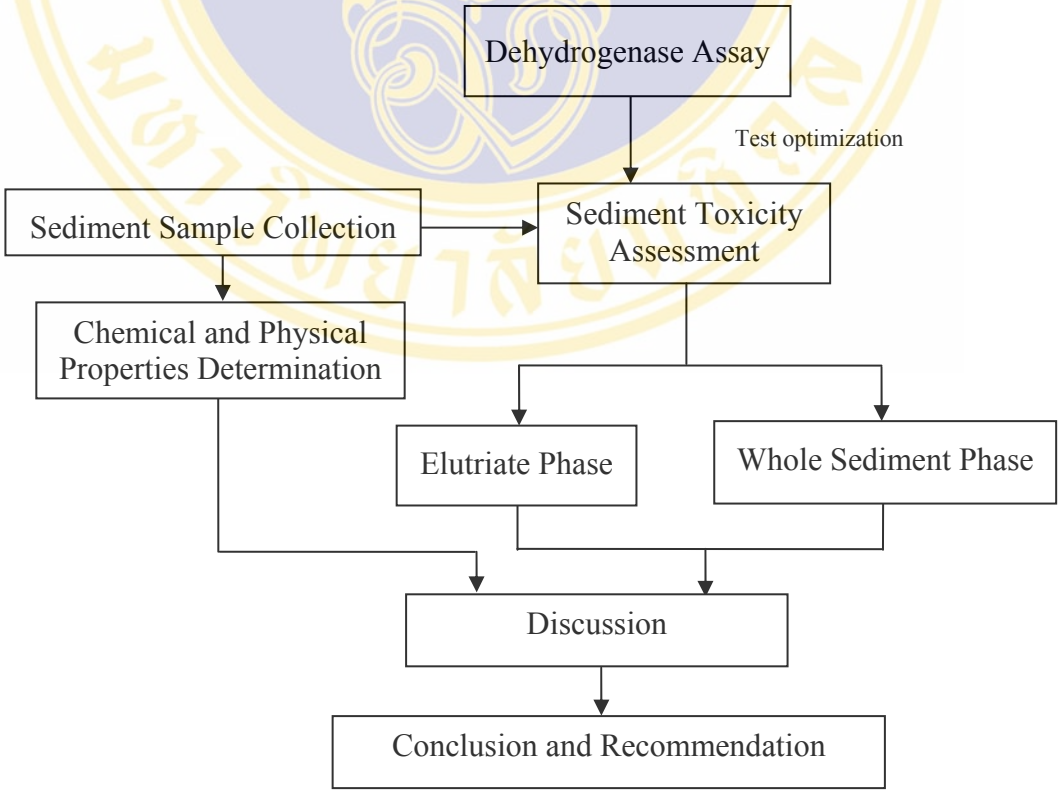


Figure 3-1 The study flow chart

Copyright by Mahidol University

3.1 Materials and Chemical reagents

3.1.1 Materials

1) Ekman Grab (size 15 x 15 cm)
2) Autoclave (Hirayama H-300MII)
3) UV/ VIS (Jasco, Model V-530)
4) Micropipettes with tip (Boeco, Model 9215000)
5) Vortex Mixture (Scientific Industries, Inc, Model G-560E)
6) Hot plate (Labinco, Model L355)
7) Centrifuge (Hettich, Model Universal 16)
8) pH meter (Orion, EA940)
9) Refrigerator 4 ºC (LG, Model GR-B247DBZA)
10) Freezer -40ºC (Sanyo, Model MDF 592)
11) Incubating shaker (Gyromax ™, Model 737R)
12) Incubator (Termarks, KB4115)
13) Membrane filters (pore size 2.5 μm)
14) Nilon Sieve 100 (pore size 100 μm)
15) Soil hydrometer (ASTM 152H hydrometer)
16) Glass wares

3.1.2 Chemical reagents

1) Nutrient broth
2) Nutrient agar
3) Resazurin ($C_{12}H_6NO_4Na$)
4) Sodium hydroxide (NaOH)
5) Hydrochloric acid (HCl)
6) Deionized water
7) Sodium hydrogencarbonate ($NaHCO_3$)
8) Amyl alcohol ($C_5H_{11}OH$)
9) Potassium hydrogen phthalate (KHP)
10) Potassium dichromate ($K_2Cr_2O_7$)
11) Ferrous Ammonium Sulphate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$)
12) Phenanthroline indicator

Copyright by Mahidol University

13) Conc. H_2SO_4
14) NH_4OAc
15) $[(NH_4)_2C_2O_4 \cdot H_2O]$
16) NH_4OH
17) NH_4Cl
18) $AgNO_3$
19) Sodium chloride (NaCl)
20) H_3BO_3
21) Sodium Hexametaphosphate
22) $Fe\ (NO_3)_3.9H_2O$
23) Potassium hydroxide (KOH)
24) Calcium carbonate ($CaCO_3$)
25) Quartz powder
26) Bentonite
27) Goethite
28) Manganese Oxide (MnO_4)
29) Seafresh Chitosan Powder (85 % DAC)

3.2 Sediment samples

For comparison, artificial sediment was prepared and used as reference sediment. Toxicity effects on respiration activity of *Bacillus cereus* was tested with natural sediment samples collected from Songkhla Lake Basin.

3.2.1 Preparation of artificial sediments

Laboratory sediment toxicity tests generally include the use of control sediment samples. Which refer to sediment samples that essentially free of contamination and are used routinely to assess the acceptability of a test (USEPA, 1994). For this study, an artificially sediment (Rönnpagel, et al., 1995) was utilized for the purpose. Its formulation was 58 % quartz powder, 30 % bentonite, 5 % chitin, 5 % goethite and 2 % manganese-IV oxide.

Copyright by Mahidol University

3.2.2 Natural sediment samples

For the present study, natural sediment samples were collected using Ekman Grab, size 15 x 15 cm. The totals of 37 sampling stations in the Songkhla Lake Basin were shown in Figure 3-2 and Figure 3-3. The information on locality of each station was provided in Table 3-1.

Collected sediment samples were stored at 4ºC until used. For each sediment samples, dry weight, particle size distribution, pH, organic contents, CEC, as well as salinity and electrical conductivity were determined as described in Table 3-2.

Copyright by Mahidol University

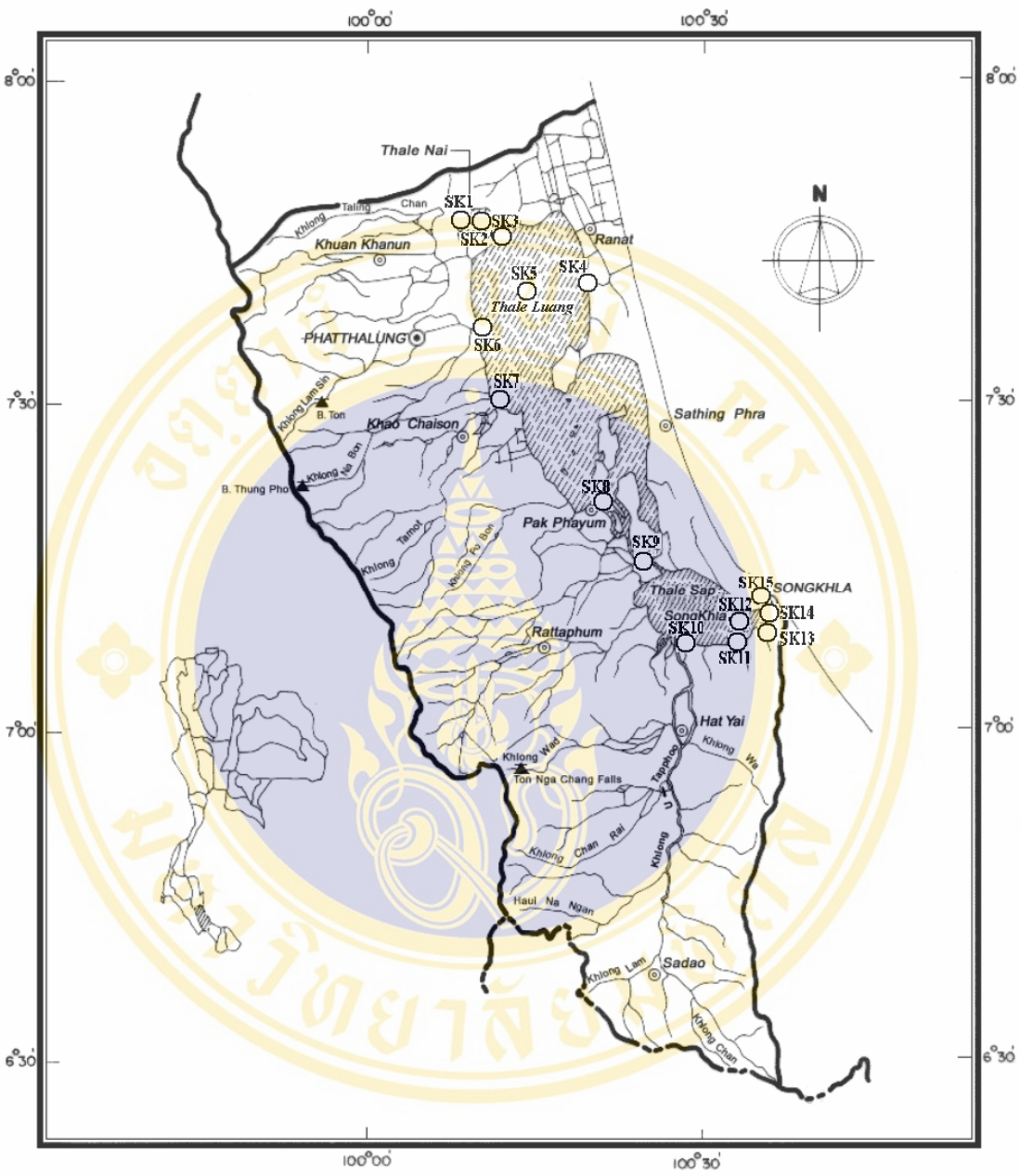


Figure 3-2 The sampling stations in Songkhla Lake

Copyright by Mahidol University

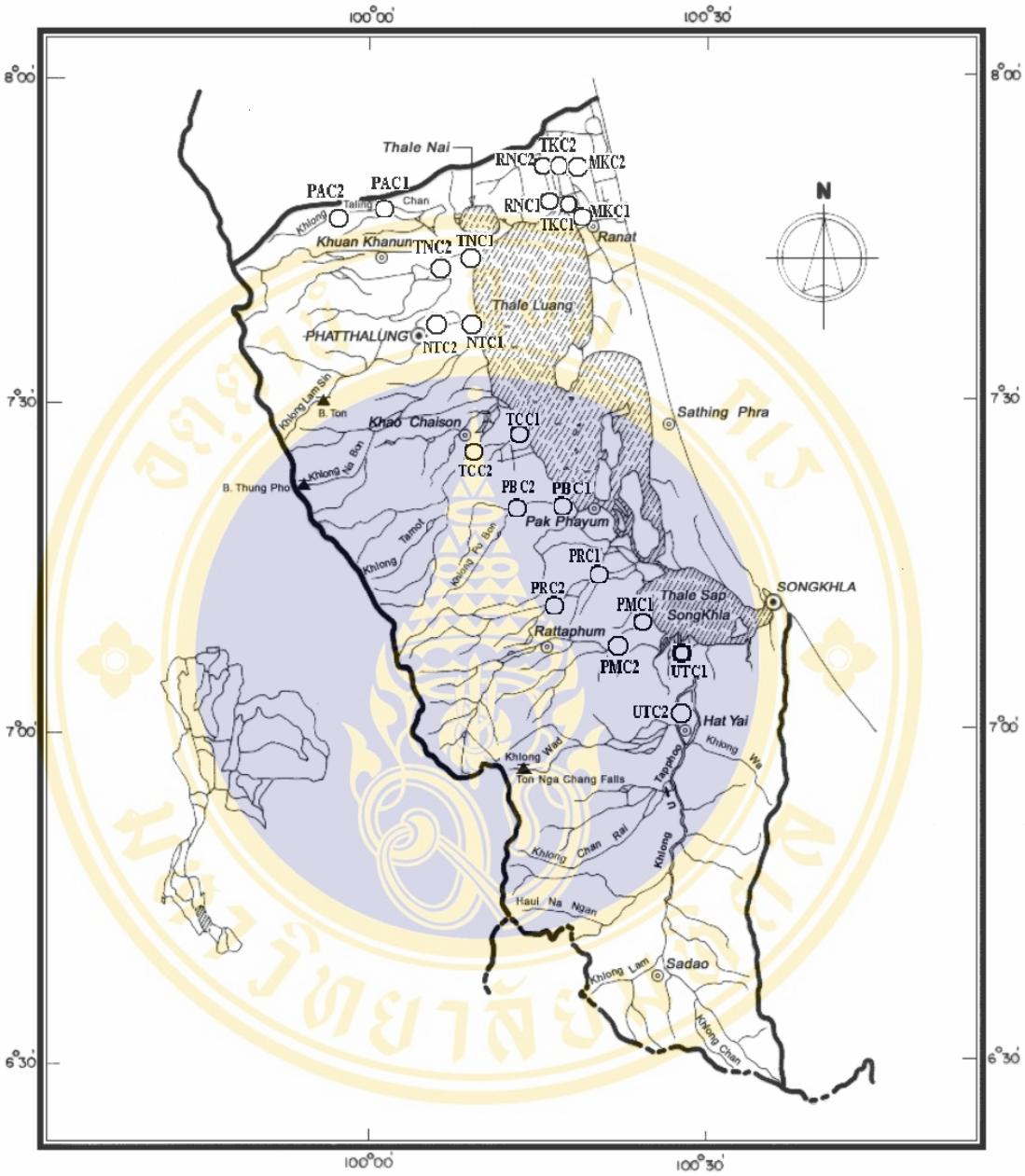


Figure 3-3 The sampling stations in the canals, which flowed into Songkhla Lake

Copyright by Mahidol University

Table 3-1 The sampling stations in Songkhla Lake Basin

Sampling Station	UTM		Location
	Latitude	Longitude	
SK01	0624656	0860768	Ban Thale Noi, Kuan Kanoon, Patthalung
SK02	0624428	0860205	Thale Noi, Kuan Kanoon, Patthalung
SK03	0630201	0860449	Khlong Nang Riam, Kuan Kanoon, Patthalung
SK04	0646563	0850265	Pak Khlong Ban Rong, Ranot, Patthalung
SK05	0636865	0850048	Thale Luang, Ranot, Patthalung
SK06	0628584	0844526	Pak Klong Lam Pam, Muang, Patthalung
SK07	0636541	0829261	Laem Chong Tanon, Khao Chaison, Patthalung
SK08	0648771	0812391	Ban Pak Payun, Pak Payun, Patthalung
SK09	0659708	0801019	Ban Pak Ja, Khuan Niang, Patthalung
SK10	0661831	0791609	Pak Khlong U-Tha Pao, Hat Yai, Songkhla
SK11	0671022	0789868	Pak Khlong Pha Wong, Muang, Songkhla
SK12	0672818	0793621	Kho Yor, Muang, Songkhla
SK13	0675811	0793839	Pak Khlong Som Rong, Muang, Songkhla
SK14	0674394	0794764	Wat Suwankiri, Muang, Songkhla
SK15	0674451	0799224	Outlet of Songkhla Lake, Muang, Songkhla

Copyright by Mahidol University

Table 3-1 The sampling stations in Songkhla Lake Basin (continued)

Sampling Station	UTM		Location
	Latitude	Longitude	
PAC1	0603987	0866002	Sa Pan Pa Payom, Pa Payom, Patthalung
PAC2	0595912	0864479	Wat Klong Yai School, Pa Payom, Patthalung
NTC1	0611768	0839032	Na Tom Weir, Muang, Patthalung
NTC2	0619163	084018	Ban Tam Nan, Muang, Patthalung
TCC1	0635394	0825139	Sa Pan Ban Pak Pon, Bangkaew, Patthalung
TCC2	0626305	0819713	Kaeng Hoo Rae, Bangkaew, Patthalung
PBC1	064322	0809299	Pak Klong Pa Bon Bridge, Pak Payun, Patthalung
PBC2	0641436	0808297	Sa Pan Yo Tha Ti Kan, Pak Payun, Patthalung
PRC1	0643244	0799107	Wat Khuan Peng, Pa Bon, Patthalung
PRC2	0636684	0795032	Klong Pru Por, Pha Bon, Patthalung
PMC1	0655468	0793878	Yotha Thikan Bridge, Klong Poom, Khuan Piang, Patthalung
PMC2	0644097	0789132	Klong Poom Bridge, Rat Ta Poom, Patthalung
UTC1	06621314	0785676	Wat Sa Pan Koo Tao, Klong U-Tha Pao,Hat Yai, Songkhla
UTC2	0661299	0773746	Wat Hat Yai Nai Pier, Klong U-Tha Pao, Hat Yai, Songkhla
MKC1	0645074	0854240	Pak Klong Ma Ha Kan, Ranot, Songkhla
MKC2	0645761	0854473	Ban Ma Ha Kan, Ranot, Songkhla
RNC1	0643956	0858715	Pak Klong Ranot, Ranot, Songkhla
RNC2	0645903	0859811	Ban Ton Somrong, Ranot, Songkhla
TKC1	0639605	0862112	Pak Klong Ta Kriea, Ranot, Songkhla
TKC2	0639605	086331	Wat Kaset Chonlatee, Ranot, Songkhla
TNC1	0626591	0854157	Ban Pak Pra, Kuan Kanoon, Patthalung
TNC2	0619192	0855353	Ban Talad Pak Klong, Kuan Kanoon, Patthalung

Copyright by Mahidol University

Table 3-2 Parameter and analytical methods used for determination of sediment quality

Parameter	Method	Reference
1. Particle size distribution	Pipette method.	USDA (1996)
2. pH	Soil suspension 1:1, pH meter	Eckert and Sims (1996)
3. Organic matter (OM)	Walkley and Black wet dichromate oxidation method	Walkley and Black (1947)
4. Cation Exchange Capacity (CEC)	Determination of CEC with ammonium acetate at pH 7	Chapman (1965)
5. Salinity and electrical conductivity	Removal of soluble cations	Raymont and Higginson (1996)

3.3 Microbiological Methods

For this study, Dehydrogenase (DHA) Assay using *Bacillus cereus* adapted from a method described by Liβ (1997) was chosen for the purpose of sediment toxicity assessment.

3.3.1 Bacteria sample

Lyophilized culture of *Bacillus cereus* TISTR 687 was obtained from Bangkok Microbiological Resource Centers (MIRCENs), Institute of Scientific and Technological Research (TISTR), Thailand.

3.3.2 Culture medium

3.3.2.1 Nutrient agar

Nutrient agar was used for preparation of agar slants. Contents per liter of deionized water were as follows:

Peptic digest animal tissue	5 g
Yeast extract	1.5 g
Peptone	1.5 g
Sodium chloride	5 g
Agar	15 g

Copyright by Mahidol University

The prepared nutrient agar solution was filled into each 15 ml screw-cap tube before autoclaved 15 minutes at 121ºC and 15 lb/ in^2 pressure. Bacteria sample from 3.1.1 was cultured on these nutrient agar slants for stock cell culture preparation.

3.3.2.2 Nutrient broth

Nutrient broth was used for active cell culture. Contents of nutrient broth per liter of distilled water were as follows:

Meat extract	1 g
Yeast extract	2 g
Peptone	5 g
Sodium chloride	5 g

The solution was autoclaved 15 minutes at 121ºC and 15 lb/ in^2 pressure.

3.3.3 Bacterial cell culture

One loop of bacteria stock cells (3.3.2.1) on agar slant was inoculated into 100 ml nutrient broth. The culture was then incubated in an incubator shaker at 37 ºC and 150 rpm. After 12 hours incubation period, inoculums were drawn and cultured in fresh nutrient broth (NB) with initial optical density of 0.1. Their growth was allowed to reach early log phase. This would subsequently used for preparation of cell suspension. The optical density (OD_{600nm}) of cell suspension was adjusted according to Liβ (1997), which was ($3.5\text{-}4.5x10^7$ cells / ml).

3.4 Toxicity Testing

Several toxicity tests were based on inhibition of dehydrogenase activity (Mosher *et al*., 2003). For the present study, Dehydrogenase (DHA) Assay using *Bacillus cereus* adapted from Liβ (1997) was applied. This method was based on inhibition of dehydrogenase activity using resazurin dye.

Dehydrogenase was one of the most frequently used enzymes for oxidation reactions (Ahlf, 2006). Toxicity tests were based mainly on the reduction of resazurin

Copyright by Mahidol University

by microbial dehydrogenase, in which hydrogen was removed from the substrate. If the dye solution was put into contact with cell suspension, the color would change from blue to pink or colorless (Lui, 1983). The dehydrogenase assay was determined calorimetrically, measuring the absorbance of the sample at the wavelength of 610 nm, in which the maximum absorbance of unreduced resazurin could be observed.

3.4.1 Elutriate phase

A portion of sediment was extracted with very high quality deionized-filtered water. The sediment sample was mixed with water in a 1:2 (w:w) ratio, shaken vigorously for 2 hours and then centrifuged at 10,000 rpm in a refrigerated centrifuge for 45 minutes. The supernatant was used immediately or frozen until required (Liß and Ahlf, 1996). The procedure was as follows:

Two ml of cell suspension were transferred into a 30 ml screw–cap tube containing 2 ml of extract sediment samples and 2 ml of deionized water. The tubes were incubated under dark condition in an incubating shaker at 25ºC and 150 rpm. As experimental control, 2 ml of extract obtained from artificial sediment (3.2.1) was added to the bacterial suspension instead of the extract from each sediment sample. After 2 hours incubation, 600 µl freshly prepared resazurin solution (1:1 deionized water, v:v) was added to each sample. They were incubated further for 25 min. Dehydrogenase reaction was stopped by an addition of 1 ml potassium hydrogen phthalate solution (0.05 M, pH 3.5) and 5 ml amyl alcohol. The samples were then centrifuged at 3,500 rpm for 5 minutes. The clear amyl alcohol supernatant containing unreduced resazurin was drawn into a 15 ml tube containing 2 mg of $NaHCO_3$ and shaked vigorously. After allowed to stand for 10 min, each sample was centrifuged at 3,000 rpm for 10 minutes. The amount of unreduced resazurin was measured spectrophotometrically against amyl alcohol at 610 nm.

For all elutriate assay, five concentrations, i.e. 100%, 75%, 50% and 25% and 0 % of sediment extract were prepared with deionized water.

3.4.2 Whole sediment phase

Two ml of cell suspension was transferred into a 15 ml screw – cap tube containing 2 g of sediment samples and 2 ml of deionized water. The samples

Copyright by Mahidol University

were then incubated under dark condition in an incubating shaker at 25ºC and 150 rpm. For experimental control, 2 g artificial sediment (3.2.1) was used instead of the sediment samples. After 2 hours incubation, 600 µl freshly prepared resazurin solution (1:1 deionized water, v:v) was added to each sample. After 2 hours incubation, 600 µl resazurin solution was added to each sample. Determination of unreduced resazurin was done as described in 3.4.1.

3.5 Data Analysis

3.5.1 Inhibition percentage

The inhibition percentage was used for assessing toxicity levels of sediment samples. It could be determined as follows:

$$Inhibition = 100 - \frac{(\Delta Abs.control \times 100)}{Abs.sample}$$

3.5.2 Statistical analysis

The average, standard deviations (SD) and % coefficient of variation (% CV) of the results were determined. The differences of values were compared using analysis of variance (one-way ANOVA). A centroid hierarchical cluster analysis was used to determine, which locations were toxicologically similar (Sunahara, *et al*, 1998 and Alf, 1992). Spearman correlation was used to analyze correlation between different chemical and physical properties of sediment samples and their relative toxicity (Rönnpagel, *et al.*, 1998). All of the statistical analysis was performed with a computer package program SPSS10.5 for Windows.

Copyright by Mahidol University

CHAPTER IV
RESULTS AND DISCUSSION

Toxicity assessment of natural sediment samples were performed using dehydrogenase enzyme assay (DHA). For the purpose, bacteria *Bacillus cereus* TISTR 687 was chosen as test organisms. Test procedures, including inoculum preparation, suitable pH and salinity levels of incubating solution were determined and optimized. The inhibition percentage (% inhibition) was used as an endpoint of DHA assay. Both elutriate, as well as whole sediment phases were tested.

For this study, 37 sediment samples were collected from Songkhla Lake Basin. Physical and chemical properties, including particle size distribution, pH, organic content, cation exchange capacity, salinity and electrical conductivity, of the sediment samples were evaluated. The results were as follows:

4.1. Method Standardization

Since results of toxicity testing using an enzymatic activity could be influenced by several experimental conditions (Blaise, 1991) a few factors, including suitable density of the test inoculum, appropriate pH and salinity levels were determined.

4.1.1. Test microbial suspension preparation

For the purpose, the relationship of the optical density and the relative viable cell counts of bacteria *Bacillus cereus* over the 24 hr. incubation period in nutrient broth were observed. The results were shown in Figure 4-1

The cell number was found to be increased slowly during the first two hours of incubation. This represented the lag growth phase. After this period, their growth started to increase rapidly until the fifth hours of incubation was reached. The steady growth was observed between the fifth and the twelfth hour of incubation period. Cell suspension with 0.40-0.50 OD at 600 nm was found to contain viable cells in the range between 3.5-4.5x10^7 CFUs/ml. This was observed at early log phase after 2 hour and 30 min incubation period. According to Liß (1997), the cell density was

Copyright by Mahidol University

suitable for preparation of cell suspension for toxicity test procedure. Therefore, the observed OD was applied for all later sediment toxicity assessment experiments.

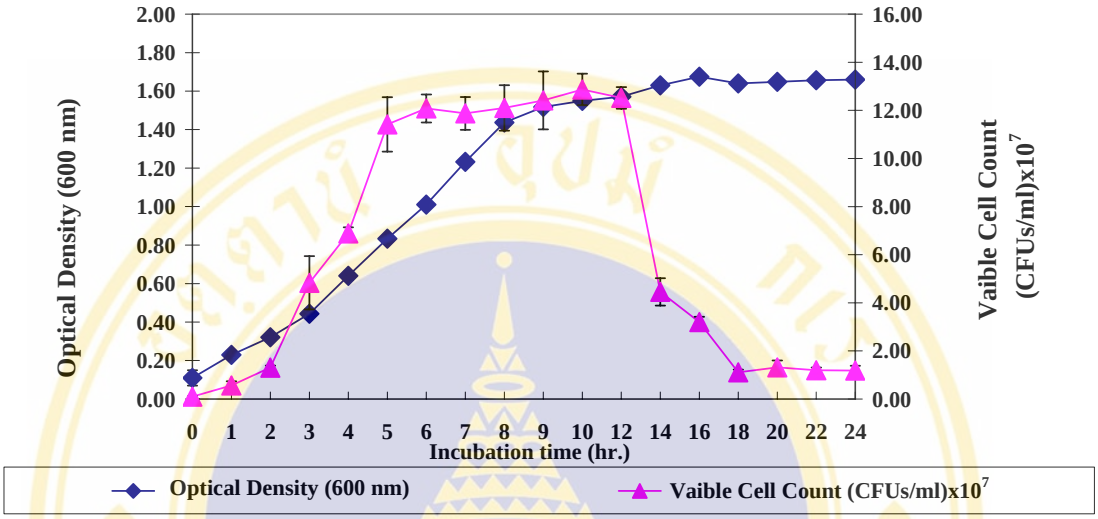


Figure 4-1 Growth curve of *Bacillus cereus* incubated in an incubate shaker at 37 ºC, as a function of optical density (abs), determined at 600 nm wavelength, as well as the relative viable cell counts (CFUs/ml), against incubation time (hr). Bar indicated standard deviation obtained from three separate experiments (n=3).

4.1.2. Suitable pH level

Natural sediment samples, especially sediment from brackish water sources, i.e. Songkhla Lake Basin, prone to have varying pH levels depending on their source (Thamrongwang, *et al.*, 1999). This could influence activity of several enzymes in variety of way. Since most enzymes are sensitive to pH, all of them required a specific ranges pH for optimum activity (Colowick and Kaplan, 1962). McDermid, McKee, and Marsh (1988) suggested that most enzymes function between a pH of 6 - 8; however pepsin in the stomach works best at a pH of 2 and trypsin at a pH of 8 Unsuitable pH levels possibly denature (alter) the three dimensional shape of the enzyme by breaking weak bonds such as ionic, and hydrogen stop enzyme activity resulting to reduction of enzyme activities.

In order to evaluate suitable pH range for application of dehydrogenase assay to these sediment samples, effects of varying pH levels on

Copyright by Mahidol University

activity of dehydrogynase enzyme were observed. The results were shown in Figure 4-2.

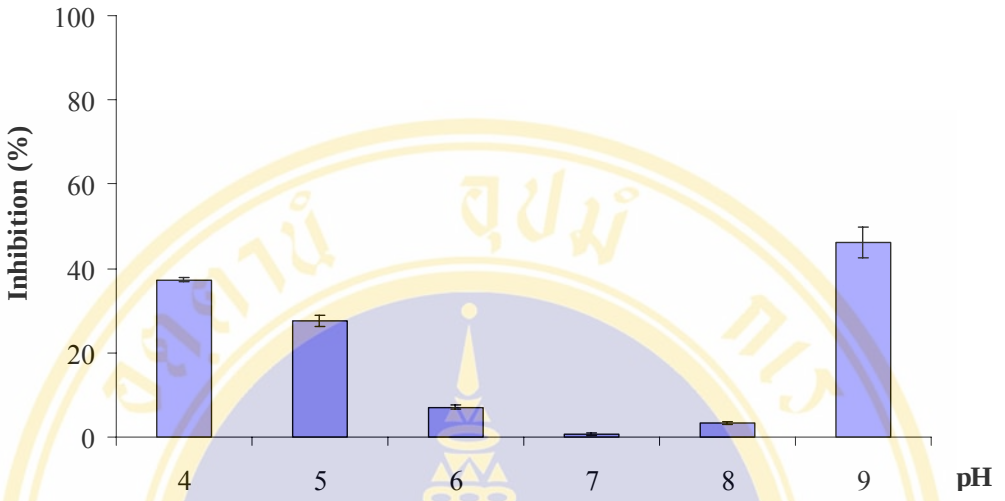


Figure 4-2 Effects of varying pH levels on of dehydrogenase activity. Bar indicate standard deviation obtained from three separate experiments (n=6).

The results indicated that pH level of the growth media had quite strong influence on activity of dehydrogenase enzyme, especially at higher or lower pH range. The most toxic pH level was the pH 9, while the inhibition effects reduced rapidly in order from pH 4, 5 and 6, respectively (Table A-3 in Appendix A). There was no inhibition effects observed at pH 7 and 8. The finding was similar to what was observed by Mosher, *et al*. (2003) and Rönnpagel, *et al*. (1995).

This revealed a specific disadvantage of dehydrogenase assay as a sediment toxicity test. If natural sediment samples have pH lower than 6 or higher than 8, test results not only refer to sediment toxicity but also to adverse effects resulting from unsuitable pH levels. This could complicate the interpretation of the results.

4.1.3 Effects of salinity on Dehydrogenase activity

Since salinity of sediment samples could inhibit activity of dehydrogenase enzyme, suitable salinity range for the test was determined. The results were shown in Figure 4-3.

Copyright by Mahidol University

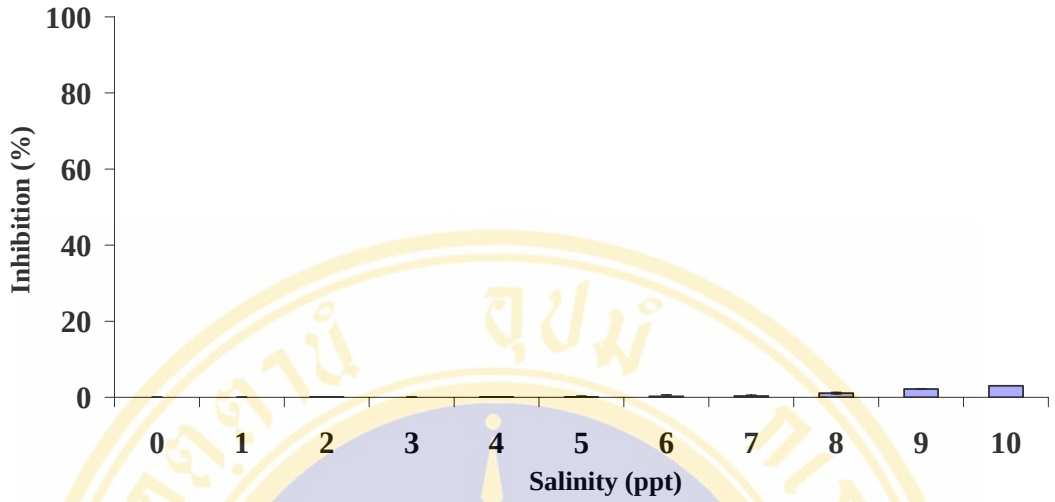


Figure 4-3 Effects of varying salinity levels on dehydrogenase activity of bacteria *Bacillus cereus*. Bar indicate standard deviation obtained from three separate experiments (n=6).

Comparing to the control, i.e. 0 ppt salinity, the range of the tested salinity levels between 1 to 10 ppt seemed to have only minimum effects on the enzyme activities. Statistical analysis (Table A-4 in Appendix A) also revealed no significant differences among these findings. This finding indicated the possibility of applying DHA method for assessing toxicity of sediment samples with salinity up to 10 ppt.

4.2 Toxicity Test

For this study, 37 sediment samples from Songkhla Lake Basin were tested for their quality, as well as toxicity levels. DHA method was employed to assess toxicity levels of the samples. Both elutriate and whole sediment phases were tested. The results were as follows:

4.2.1 Sediment quality

Physical and chemical properties of sediment samples, including particle size distribution, pH, % organic matter (% OM), cation exchange capacity (CEC), sediment moisture content (% MC), salinity and electrical conductivity were examined. The results were reported into three parts depending on localities.

Copyright by Mahidol University

Thale Noi and its tributary

Thale Noi lies to the north of the main lake. Sampling stations in the area included stations SK1, SK, SK3 in Thale Noi, as well as stations PAC1 and PAC2 in its tributary, Khlong Pa Payom.

- Station SK1

Station SK1 (Ban Thale Noi, Kuan Kanoon, Patthalung) was situated in Thale Noi near Ban Thale Noi village. The water was about 1 m deep, greenish, still and was densely covered with several types of aquatic plants, mostly water hyacinth. The area was being dredged during the sampling period.

- Station SK2

Station SK2 was located in open water near the center of Thale Noi, Kuan Kanoon, Patthalung. The water was about 1 m deep, still and clear. Several types of macro algae were observed at the site.

- Station SK3

Station SK3 was located in Khlong Nang Riam, Kuan Kanoon, Patthalung, which drains water from Thale Noi into Thale Luang. At the time of sampling, it was impacted by road construction that would connect Kuan Kanoon, Patthalung and Ranot, Songkhla. The water channel was about 15 m wide and 1 m deep. Strong flow and quite clear water were observed.

- Station PAC1

Station PAC1 was located downstream of Khlong Pa Payom, Pa Payom, Patthalung This site was effected by residential land use in an adjacent area. Quite turbid and dirty water was observed.

- Station PAC2

Station PAC2 was located at the Yothatikan Bridge 2541 upstream of Pa Payom canal adjacent to Wat Khlong Yai School, Pa Pa Yom, Patthalung. Brownish, yet quite clear water was observed.

Physical and chemical properties of sediment samples collected from Thale Noi and its tributary were shown in Table 4-1. The reference sediment reported was topsoil obtained from a quarry at Lam Tei, Hong Kong. This soil was relatively uncontaminated as indicated by the relatively low concentrations of heavy metals (Martin, *et al.*, 2003).

Copyright by Mahidol University

Table 4-1 Physical and chemical properties of sediment samples collected from Thale Noi and its tributaries

Station	Sediment Properties									
	% MC	CEC	Conduct. (ms/cm)	Salinity (ppt)	% OM	pH	% Sand	% Silt	% Clay	Type
SK 1	3.57	24.20	1.23	0.50	6.56	4.55	0.00	75.12	24.88	Silty loam
SK 2	4.39	3.60	2.48	1.20	6.56	4.38	60.68	21.44	17.88	Sandy loam
SK 3	4.25	27.74	1.10	0.50	3.11	6.04	0.00	43.12	56.88	Silty clay
PAC 1	0.28	2.73	0.12	0.00	0.23	7.07	0.00	75.12	24.88	Silty loam
PAC 2	0.88	5.08	0.05	0.00	1.04	5.88	85.96	4.00	10.04	Loamy sand
Ref. Sed.	-	36.1[1]	-	-	1[1]	6.2[1]	30[2]	20[2]	48[2]	Clay

Ref. Sed.: Reference Sediment referred to clean sediment.

[1] from: Plum (1981)

[2] from: Still, et al. (2000)

Sediment sample collected from station PAC1 and PAC2, which was in a tributary of Thale Noi, represented fresh water sediment in nature (Brown, Pezeshki, and DeLaune, 2006). Sediment samples collected from main area of Thale Noi, with salinity in the range between 0.5 to 1.20 ppt was, however, brackish. Organic content of the samples indicated similar trend as salinity of sediment, with higher values found in samples collected from the main area of Thale Noi than in its tributary. The samples with high % OM seemed to have also low pH level indicating active microbial activities (McDermid, McKee, and Marsh, 1988). The highest CEC value, 27.74 was observed in the sample collected from station SK3. This sample also contained the highest clay content. Three samples were consider silty loam or silty clay (SK1, SK3, PAC1), while the rest (SK2 and PAC2) contained mostly sand and was categorized as sandy loam or loamy sand.

Copyright by Mahidol University

Thale Luang and its tributaries

Thale Luang connects to Thale Noi in the north and Songkhla Lake in the south. The properties of its water in the area adjacent to Songkhla lake is, therefore, strongly influenced by ebb-tide rhythm and high salinity contents. Four stations, including stations SK4, SK5, SK6 and SK7 were located in the main reservoir, while the rests were located in its tributaries, including stations RNC1, RNC2, MKC1, MKC2, TKC1, TKC2, NTC1, NTC2, TNC1, TNC2, TCC1 and TCC2.

- Station SK4

Station SK4 was located upstream of water control structure in Khlong Ban Rong, Ranot, Patthalung and represents downstream flow of that particular tributary before going into Thale Luang. The water was about 0.6-0.8 m deep, slightly greenish and covered densely with water mimosa.

- Station SK5

Station SK5 was located around the center of Thale Luang, Ranot, Patthalung. The water was about 2 m deep, quite turbid and was affected by fishing activities in the surrounding area.

- Station SK6

Station SK6 was located in Khlong Lam Pam, Muang, Patthalung. It receives final flow from the watershed before going into Thale Luang. The water channel is about 30 m wide and 2 m deep. The water was turbid and quite greenish.

- Station SK7

Station SK7 was located at Laem Chong Tanon, Khao Chai Son, Patthalung. The water was shallow (0.4 m deep) and quite clear. At the site, some macro algae were observed.

- Station RNC1

Station RNC1 was located at Pak Khlong Ranot, Ranot, Songkhla, where flow from watershed is drained into Thale Luang. The water channel was about 35 m and 1.5 m deep. Low flow, turbid as well as greenish water were observed. The surface was covered partly with water mimosas.

Copyright by Mahidol University

- Station RNC2

Station RNC2 was located upstream in Khlong Ranot at Ban Ton Sumrong, Ranot, Songkhla. The water channel is about 20 m wide and 1.5 m deep. It flows slowly through quite densely populated community. A sewer outlet was observed draining residential waste water into the canal. The water was turbid and greenish. Several types of aquatic plants, including water mimosas, swamp morning glory and water hyacinth were observed on the water surface.

- Station MKC1

Station MKC1 was located upstream of a water control structure at Pak Khlong Ma Ha Kan, Ranot, Songkhla. It receives the final water that drains from the watershed before going into Thale Luang. The site was about 10 m wide and 2 m deep. The water was quite turbid and slightly greenish.

- Station MKC2

Station MKC2 was located upstream of Khlong Ma Ha Kan, at Ban Ma Ha Kan, Ranot, Songkhla. The channel was about 10 m and 2 m deep. The water flow slowly through a community. It was turbid and slightly greenish. Some fishing activities, as well as fruit orchard along the canal were observed.

- Station TKC1

Station TKC1 was located at Pak Khlong Ta Kriea, Ranot, Songkhla. The water channel is about 15 m wide and 1 m deep. The water was very turbid, slightly greenish and partly covered with water mimosa and water hyacinth. Several stationary fishing gears were situated along the bank of the canal.

- Station TKC2

Station TKC2 was located upstream in Khlong Ta Kriea at Wat Kaset Chonlati, Ranot, Songkhla. The water channel is about 15 m wide and 2 m deep. The site was adjacent to several houses and a temple. The water was very turbid, slightly greenish and its surface was densely covered with water mimosa and water hyacinth.

- Station NTC1

Station NTC1 was located in Khlong Na Tom at Na Tom Weir, Muang, Patthalung. The site collects water that drains from the watershed. The water channel was about 15 m wide and 2 m deep. The water was quite clear.

Copyright by Mahidol University

- Station NTC2

Station NTC2 was located upstream in Khlong Na Tom at Ban Tam Nan, Muang, Patthalung. The water channel was only 5 m wide and 0.5 m deep. It flows through a residential area resulting to very turbid and greenish water. Several pieces of floating garbage were observed in the canal.

- Station TNC1

Station TNC1 was located downstream in Khlong Ta Nae at Ban Pak Pra, Kuan Kanoon, Patthalung where it connects to Thale Luang. The flow was quite low with turbid water. The water channel is 50 m wide and 1 m deep. Several evidences relating to agricultural activities, including paddy field, and stationary fishing gears along both side of the canal were observed.

- Station TNC2

Station TNC2 was located upstream in Khlong Ta Nae at Ban Talad Pak Khlong, Kuan Kanoon, Patthalung. The canal flows through a densely populated community. It was about 2 m wide and 0.4 m deep. The flow was blocked by a small weir. The water was found to be turbid and dark greenish.

- Station TCC1

Station TCC1 was located in Khlong Ta Chierd at Sapan Ban Pak Pon, Bangkaew, Patthalung. The canal is about 15 wide and 1 m deep. It flows through community and fish farms. The water was very turbid and greenish.

- Station TCC2

Station TCC2 was located upstream in Khlong Ta Chierd at Kaeng Hoo Rae, Bangkaew, Patthalung. The water channel is about 15 m wide and 0.2 m deep. The water was quite clear and slightly brownish. Several floating restaurants were built over the water channel.

Physical and chemical properties of sediment samples collected from Thale Luang and its tributaries were shown in Table 4-2.

Copyright by Mahidol University

Table 4-2 Physical and chemical properties of sediment samples collected from Thale Luang and its tributaries

Station	Sediment Properties									
	% MC	CEC	Conduct. (ms/cm)	Salinity (ppt)	% OM	pH	% Sand	% Silt	% Clay	Type
SK4	3.03	27.77	1.78	0.80	3.14	5.97	0.00	50.12	49.88	Silty clay
SK5	2.67	12.39	2.34	1.10	2.83	6.00	0.00	82.12	17.88	Silt
SK6	0.87	4.40	0.63	0.23	1.78	5.20	79.12	9.00	11.88	Sandy loam
SK7	0.39	33.73	2.05	1.00	1.33	6.94	75.12	14.88	10.00	Sandy loam
RNC1	3.20	28.06	1.93	0.80	3.60	5.67	0.00	4.00	96.00	Clay
RNC2	4.55	25.81	2.43	1.10	6.43	5.89	85.96	4.00	10.04	Loamy sand
MKC1	1.78	12.00	1.33	0.60	2.08	6.56	28.40	55.00	16.60	Silty loam
MKC2	4.25	33.80	1.24	0.50	2.61	7.60	0.00	26.12	73.88	Clay
TKC1	3.44	33.60	1.68	0.70	5.66	6.91	11.96	45.72	42.32	Silty clay
TKC2	3.24	16.20	1.45	0.60	3.95	5.26	10.00	66.96	23.04	Silty loam
NTC1	1.85	13.20	0.09	0.00	0.89	6.95	15.40	40.00	44.60	Silty clay
NTC2	1.95	10.60	0.10	0.00	3.57	5.23	43.68	37.72	18.60	Loam
TNC1	5.11	58.00	2.21	1.10	6.68	5.60	22.68	32.72	44.60	Clay
TNC2	1.20	12.80	0.31	0.10	2.85	6.96	54.68	26.00	19.32	Sandy loam
TCC1	3.89	22.40	4.54	2.20	3.31	5.86	0.00	62.12	37.88	Silty clay loam
TCC2	0.14	0.40	0.02	0.00	6.66	5.69	86.40	0.00	13.60	Loamy sand
Ref. Sed.	-	36.10[1]	-	-	1.00[1]	6.20[1]	30[2]	20[2]	48[2]	Clay

Ref. Sed.: Reference Sediment referred to clean sediment.

[1] from: Plum (1981)
[2] from: Still et al. (2000)

Most of sediment sample collected from sampling stations in tributaries of Thale Luang, except stations RNC2, TNC1 and TCC1, indicated low salinity values below 1.0 ppt. Salinity of sediment samples collected from Thale Luang, was also relatively low and fell in the range between 0.2 to 1.10 ppt. Organic content of the samples collected from the stations located in the tributaries of Thale Luang seemed to

Copyright by Mahidol University

be quite variable and fell in the range between 0.89 – 6.68 %. Only samples from station RNC2, TKC1, TNC1 and TCC2, however, contained organic content in the high range above 5.0 %. The rest of the samples contained less than 4.0% OM, which was in agreement with % OM contained in samples collected from stations located in the main area of Thale Luang. The pH levels of all the sediment samples fell in the range between 5.2-7.6. Except seven samples that were considered sandy loam, silt, silty loam or loamy sand (SK5, SK6, SK7, RNC2, TKC2, TNC2, TCC2), the rest of the sediment samples contained quite high clay content. This possibly was the reason that very high CEC value was detected at several stations (De La Rosa, 1979). The highest CEC value 58.0 was observed in the sample collected from station TNC1.

Sediment properties of Songkhla Lake area

The Songkhla Lake area is located at the southernmost of all Songkhla Lake Basin area. It has outlet that connects to open ocean area in the south. This leads to large fluctuation of water salinity, as well as other physical and chemical properties depending on period of the year in the area. The sampling stations in the main lake included stations SK8, SK9, SK10, SK11, SK12, SK13, SK14 and SK15, while stations PBC1, PBC2, PRC1, PRC2, PMC1, PMC2, UTC1 and UTC2 were located in its tributaries.

- Station SK8

Station SK8 was located at Ban Pak Payun, Pak Payun, Patthalung. This station was adjacent to a small pier and a market. A sewer outlet was observed draining residential wastewater into the area. Several evidences related to fishery activities, including stationary fishing gears and cage culture scattered around the area. The water was dark green with several floating refuge.

- Station SK9

Station SK9 was located at Ban Pak Ja, Khuan Niang, Patthalung. It was the channel that connects Thale Luang and Songkhla Lake. The site was about 150 m wide. Strong flow with turbid water was observed. The bank of the channel was covered mostly with mangrove trees.

Copyright by Mahidol University

- Station SK10

Station SK10 was located at Pak Klong U-Ta Pao, Hat Yai, Songkhla. The canal flows into Songkhla Lake and is about 30 m wide and about 1 m deep. The water was very turbid.

- Station SK11

Station SK11 was located nearby Praem Tinasulanon Park at Pak Klong Pa Wong, Muang, Songkhla. The canel was 15 m wide and 1.0 -1.5 cm deep.

- Station SK12

Station SK12 was located under Tinnasulanonda Bridge at Kho Yor, Muang, Songkhla. Several stationary fishing gears and cage culture were observed in the surrounding area. The water was quite turbid.

- Station SK13

Station SK13 was located at Pak Khlong Sum Rong, Muang, Songkhla. There were a fish market and squid and fish processing factories nearby. The water was very dark and dirty with very strong hydrogen sulfide odor. The water surface was covered with oil film and lots of refuge. The canal was about 6 m wide and 1.0 m deep.

- Station SK14

Station SK14 was located at Ban Leam Son Pier at Wat Suwankiri, Muang, Songkhla. Several fishing activities in the area were observed. A fishing pier was situated nearby. The water was quite still, low in turbidity and slightly greenish.

- Station SK15

Station SK15 was located near the outlet of Songkhla Lake, Muang, Songkhla. The water channel was about 200 m wide and 10 m deep. A seaport was situated at the left bank of the canal, while the park was situated at the right. Several boats frequently passed through the sites. The water is quite clear.

- Station PRC1

Station PRC1 was located downstream in Khlong Pru Por at Wat Khuan Peng, Pa Bon, Patthalung before going into Songkhla Lake. The water was quite still and very turbid. The water channel was about 4 m wide and 0.4 m deep. The water surface was densely covered with water hyacinth.

Copyright by Mahidol University

- Station PRC2

Station PRC2 was located upstream in Khlong Pru Por, Pha Bon, Patthalung. The water channel was about 10 m wide and about 1.5 m deep. The flow was blocked by a small weir resulting to still water condition. The water was quite clear. The area was established as a fish conservation area by the Fishery Department.

- Station PBC1

Station PBC1 was located at Pak Khlong Pa Bon Bridge, Pak Payun, Patthalung. The water channel was about 15 m wide and 1.0-1.5 m deep. Low flow, turbid as well as greenish water were observed. The bottom of the canal was densely covered with several types of macro algae. The water was covered partly with lotus and water mimosas. Several fish farms were observed in this area.

- Station PBC2

Station PBC2 was located upstream in Khlong Pa Bon at Yothathikan Bridge, Pak Payun, Patthalung. The water channel was about 20 m wide and 2 m deep. Slow flow was observed. The water was slightly turbid and brownish. Both sides on the bank of the canal were covered densely with several types of vegetation.

- Station PMC1

Station PMC1 was located downstream in Khlong Poom at Yotha Thikan Bridge, Khuan Piang, Patthalung. The water channel was about 10 m wide and 0.3 m deep. The water was quite clear and its surface was covered partly with water mimosas. The water flows through community and agricultural areas, including coconut orchards and corn fields.

- Station PMC2

Station PMC2 was located upstream in Khlong Poom at Klong Poom Bridge, Rat Ta Poom, Patthalung. The site was about 5 m wide and 0.50 m deep. Low flow and quite clear water was observed.

- Station UTC1

Station UTC1 was located downstream of Khlong U-Tha Pao at Wat Sa Pan Koo Tao, Hat Yai, Songkhla. The site was about 15 m wide and 1 m deep. The water was quite turbid. Several types of aquatic plants, including water mimosas, swamp morning glory and water hyacinth were observed on the water surface.

Copyright by Mahidol University

- Station UTC2

Station UTC 2 was located upstream in Khlong U-Tha Pao at Wat Hat Yai Nai pier, Hat Yai, Songkhla. The water channel was about 15 m wide and 2 m deep. The water current slowly flowed through a densely populated community. The water was very turbid and brownish. The surface of water was covered partly with water mimosa and water hyacinth.

Physical, chemical properties of sediment samples collected from Songkhla Lake were shown in Table 4-3.

Copyright by Mahidol University

Table 4-3 Physical and chemical properties of sediment samples collected from Songkhla Lake area and its tributaries

Station	Sediment Properties									
	% MC	CEC	Conduct. (ms/cm)	Salinity (ppt)	% OM	pH	% Sand	% Silt	% Clay	Type
SK8	3.61	0.30	0.71	7.07	3.50	6.36	0.00	58.40	41.60	Silty clay
SK9	3.71	20.12	0.65	6.47	3.21	6.00	25.68	45.72	28.60	Clay loam
SK10	2.39	0.30	0.06	0.60	2.05	6.27	22.68	38.00	39.32	Clay loam
SK11	2.97	0.30	0.40	3.97	3.22	7.24	40.12	29.00	30.88	Clay loam
SK12	2.10	11.20	0.45	4.53	1.68	7.79	65.12	1.00	33.88	Sandy clay loam
SK13	3.35	18.40	0.40	4.03	6.44	7.33	67.12	5.00	27.88	Sandy clay loam
SK14	2.04	10.60	0.40	4.03	2.25	7.65	50.68	23.72	25.60	Sandy clay loam
SK15	2.15	9.78	0.50	5.00	2.08	8.03	31.68	29.72	38.60	Sandy clay loam
PBC1	2.28	16.00	0.00	0.00	2.52	4.60	28.68	33.44	37.88	Clay loam
PBC2	0.98	5.38	0.00	0.00	1.22	4.79	28.68	33.44	37.88	Clay loam
PRC1	0.23	0.60	0.00	0.00	0.17	5.52	69.96	16.00	14.04	Sandy loam
PRC2	0.24	34.34	0.00	0.00	0.3	5.49	69.96	16.00	14.04	Sandy loam
PMC1	1.80	9.34	0.14	1.40	2.34	7.44	33.12	36.00	30.88	Clay loam
PMC2	0.53	13.29	0.00	0.03	4.03	5.98	53.12	19.00	27.88	Sandy clay loam
UTC1	2.84	25.2	0.10	1.00	2.83	6.55	48.68	26.44	24.88	Loam
UTC2	1.30	12.40	0.01	0.10	1.23	6.51	48.68	26.44	24.88	Loam
Ref. Sed.	-	36.10[1]	-	-	1.00[1]	6.20[1]	30[2]	20[2]	48[2]	Clay

Ref. Sed.: Reference Sediment referred to clean sediment.

[1] from: Plum (1981)

[2] from: Still et al. (2000)

Copyright by Mahidol University

Most of sediment samples collected in Songkhla Lake area, except SK8 SK9, SK11, SK13 and PMC2 contained relatively low organic content (< 3.0 %). The highest organic content, 6.44% OM was observed from Station SK13. The pH levels of all the sediment samples fell in the range between 4.60 to 8.03. Only samples from station, which were PBC1, PBC2, PRC1 and PRC2, showed lower pH levels than optimized pH level (6-8). The CEC of sediment samples in Songkhla Lake was found to be relatively low comparing to that of the reference sediment (Table 4-3). The range was between 0.30-34.34 meq/100g. The salinity of sediment samples in the Songkhla lake area seemed to be relatively high (between 4.03 to 7.07 ppt.), especially at stations that were located nearby the outlet of the lake (stations SK8, SK9, SK11, SK12, SK13, SK14, SK15) that connected to the open sea.

4.2.2. Toxicity Testing

The most important issue in sediment toxicity testing protocols is the question, which test phase (solid or liquid) should be used. Whole-sediment exposure protocols is usually considered the most realistic scenario to simulate *in situ* exposure conditions in the laboratory (Feiler, 2006). Benthic organisms, in particular, have direct contact with sediment, and the contaminant level in the sediment may have greater impact on their survival than do aqueous concentrations. Ahlf (2006) suggested that whole sediment assessment (contact assay) is considerably more complex than elutriate assay due to the many site-specific parameters that need to be considered, which do not factors for water.

For the present study, thus, toxicity assessment of 37 natural sediment samples from Songkhla Lake Basin using both exposure phases were conducted. The results were shown in Figure 4-4 to Figure 4-12.

Copyright by Mahidol University

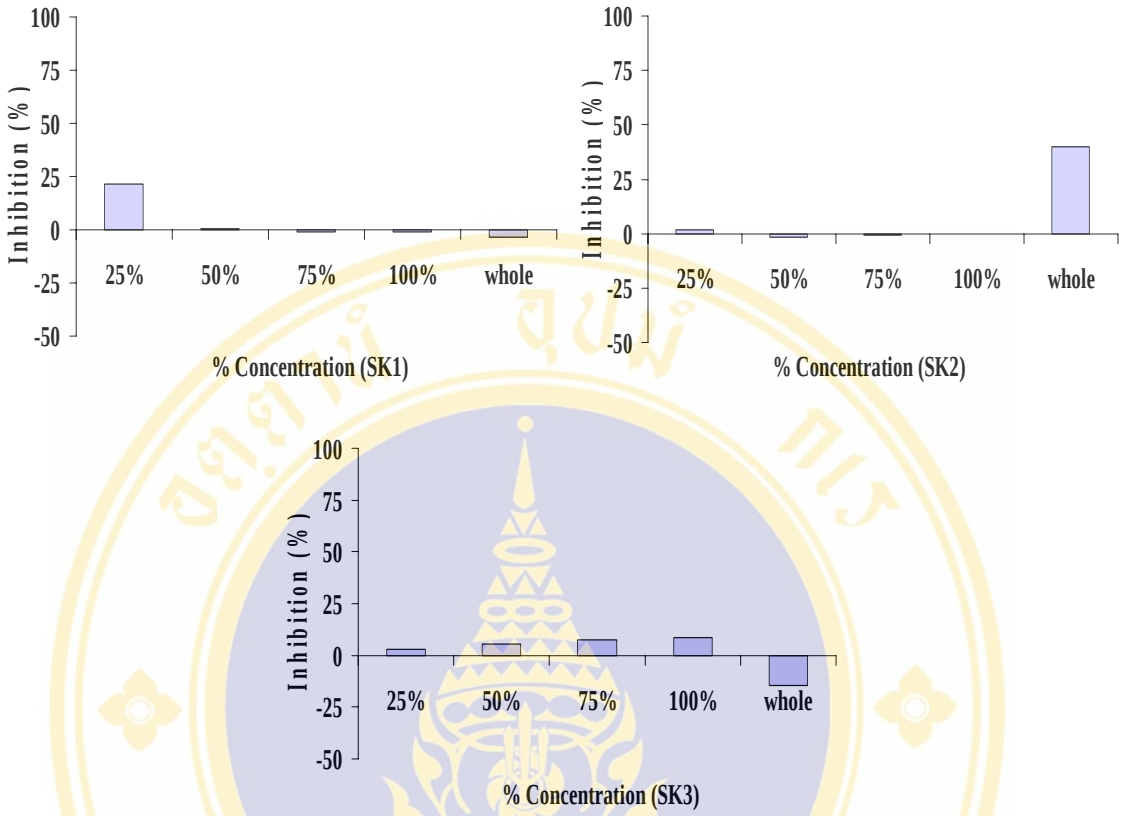


Figure 4-4 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Noi (SK1, SK2 and SK3)

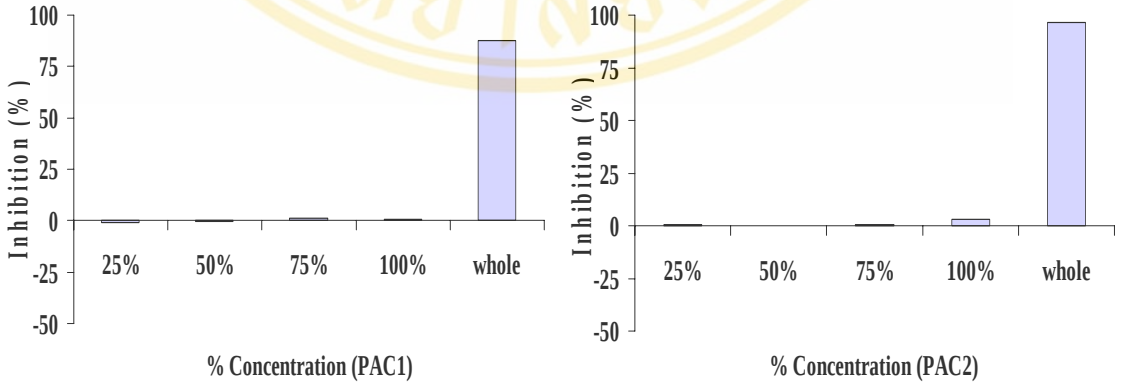


Figure 4-5 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Noi tributaries (PAC1 and PAC2)

Copyright by Mahidol University

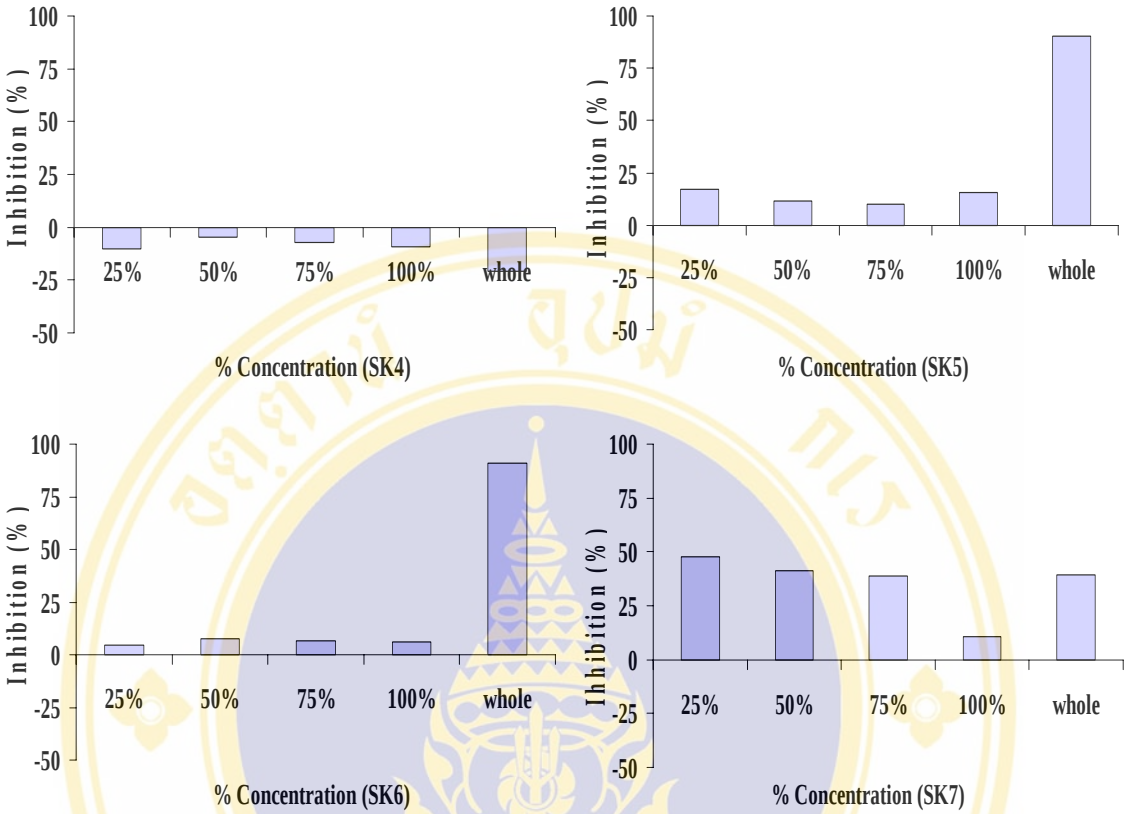


Figure 4-6 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang (SK4, SK5, SK6 and SK7)

Copyright by Mahidol University

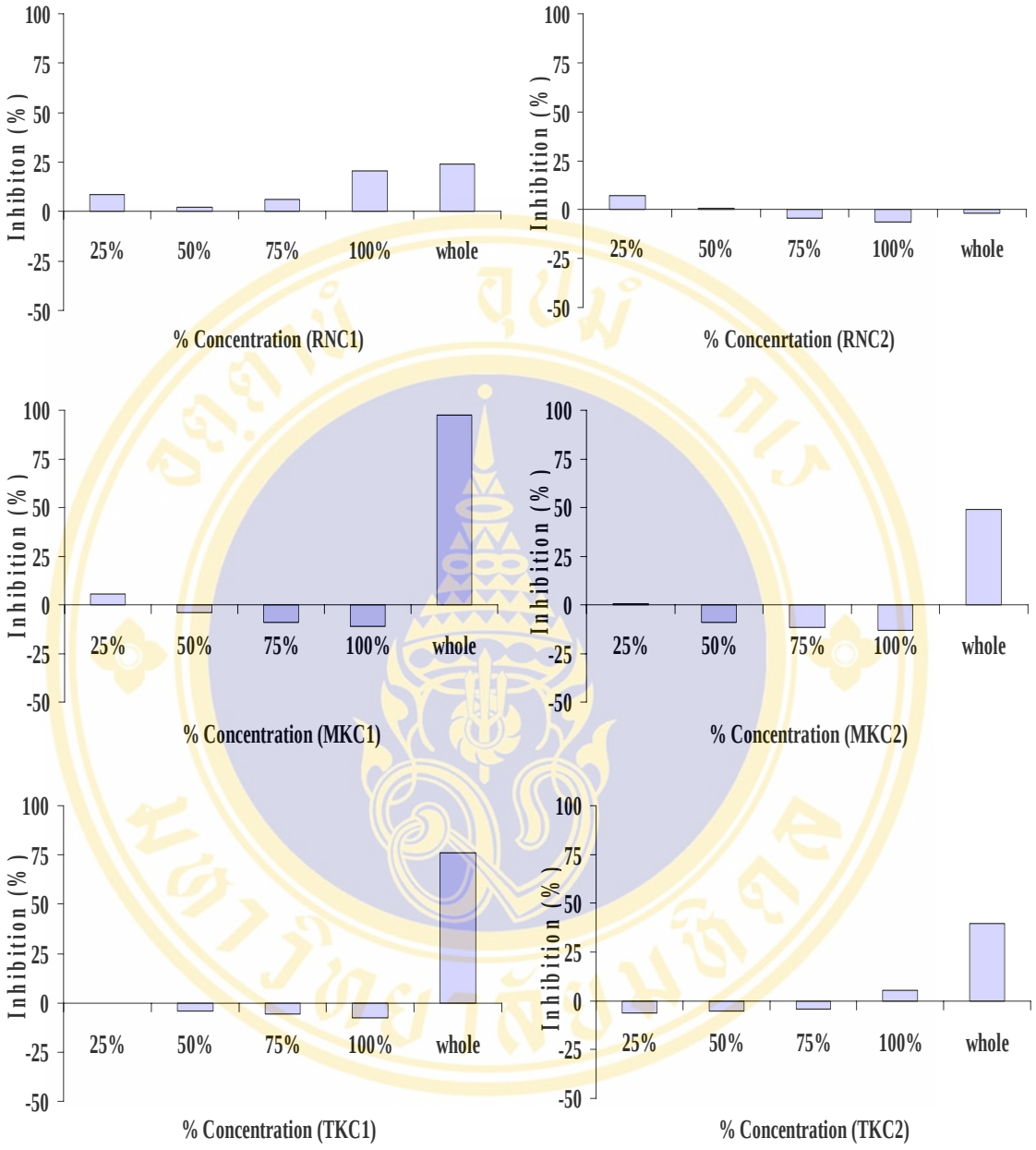


Figure 4-7 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang tributaries (RNC1, RNC2, MKC1, MKC2, TKC1 and TKC2)

Copyright by Mahidol University

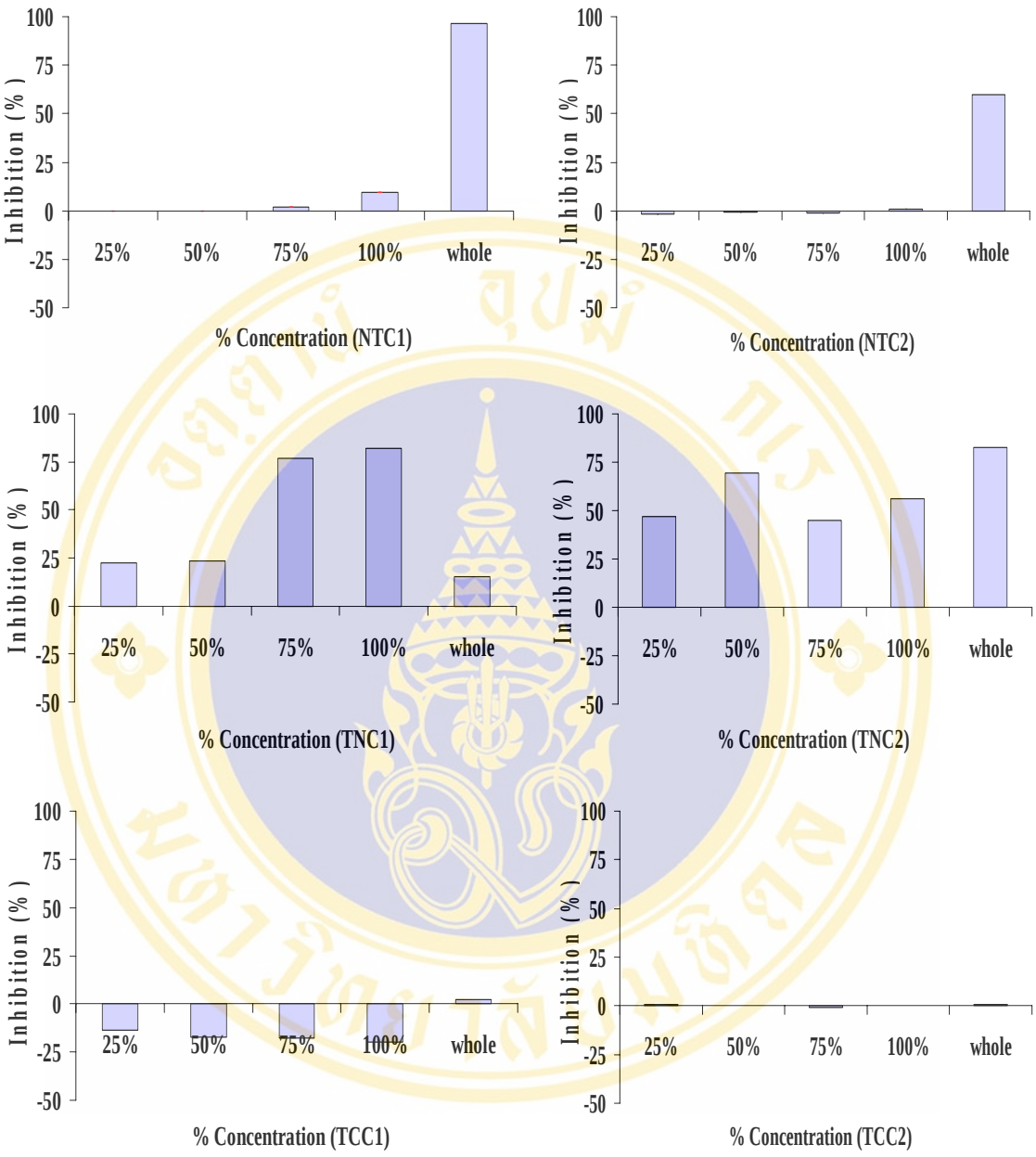


Figure 4-8 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Thale Luang tributaries (NTC1, NTC2, TNC1, TNC2, TCC1 and TCC2)

Copyright by Mahidol University

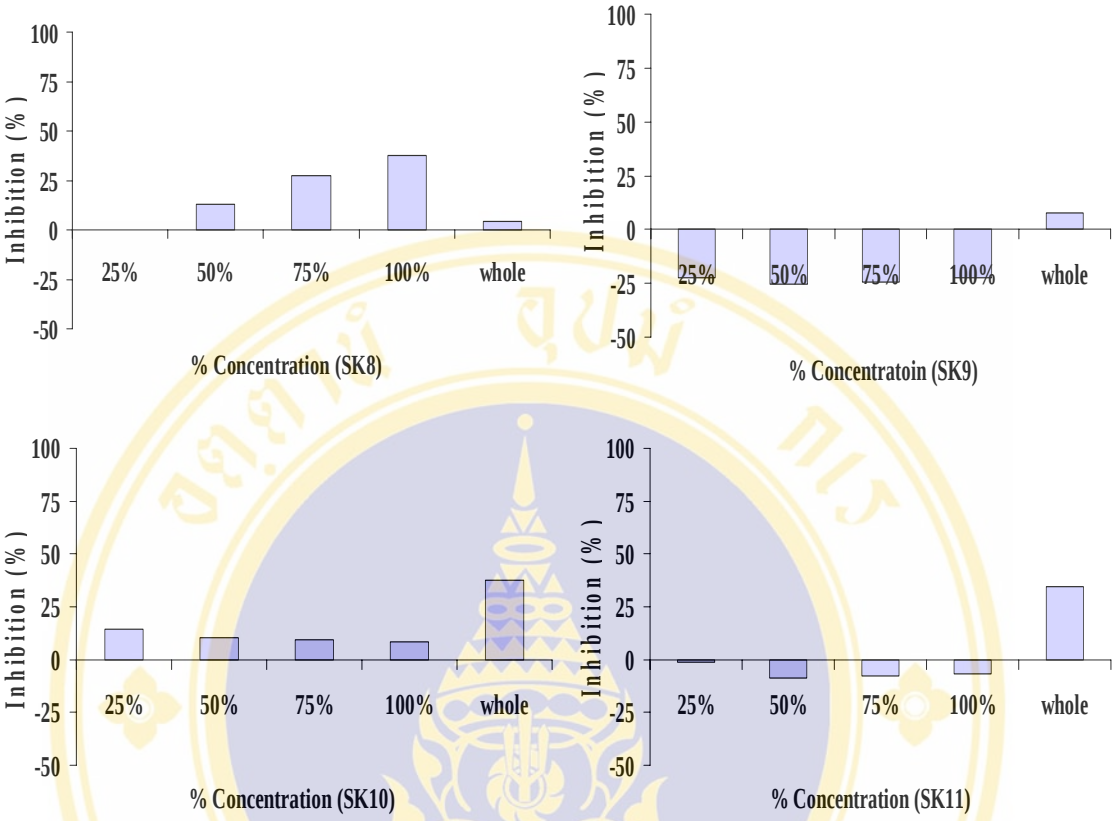


Figure 4-9 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Songkhla Lake (SK8, SK9, SK10 and SK11)

Copyright by Mahidol University

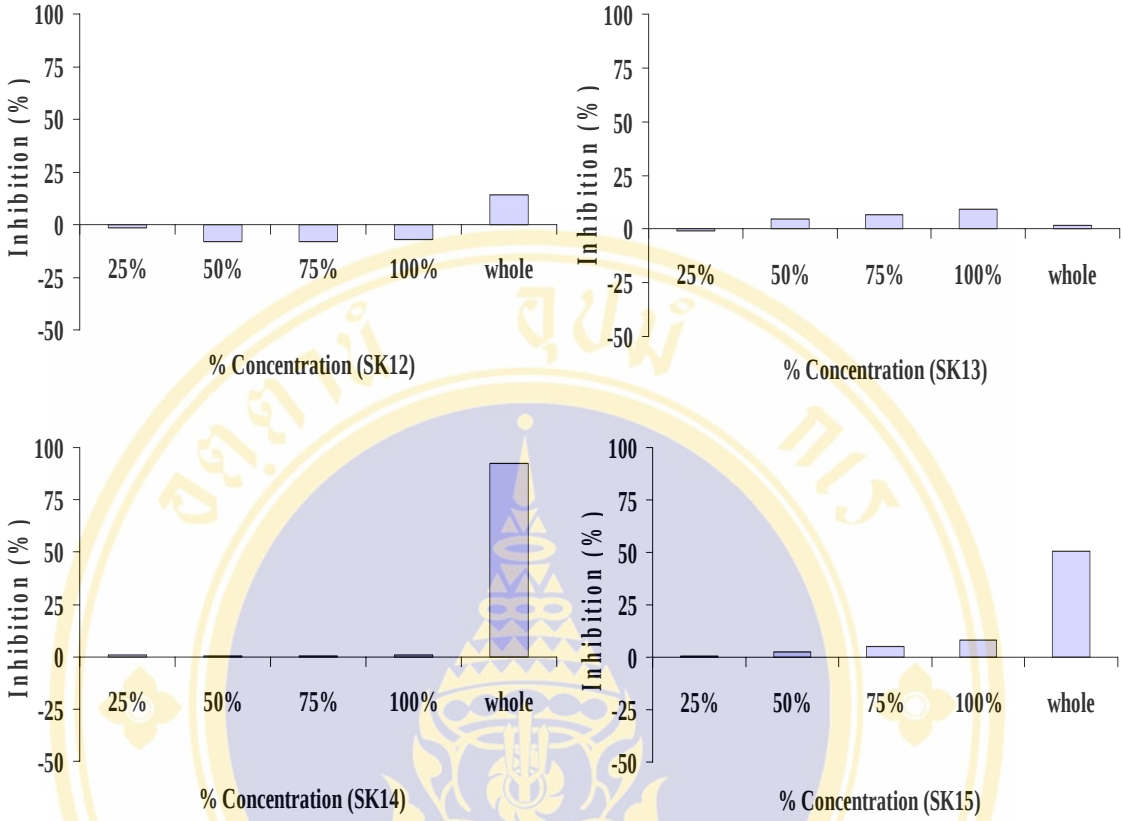


Figure 4-10 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Songkhla Lake (SK12, SK13, SK14 and SK15)

Copyright by Mahidol University

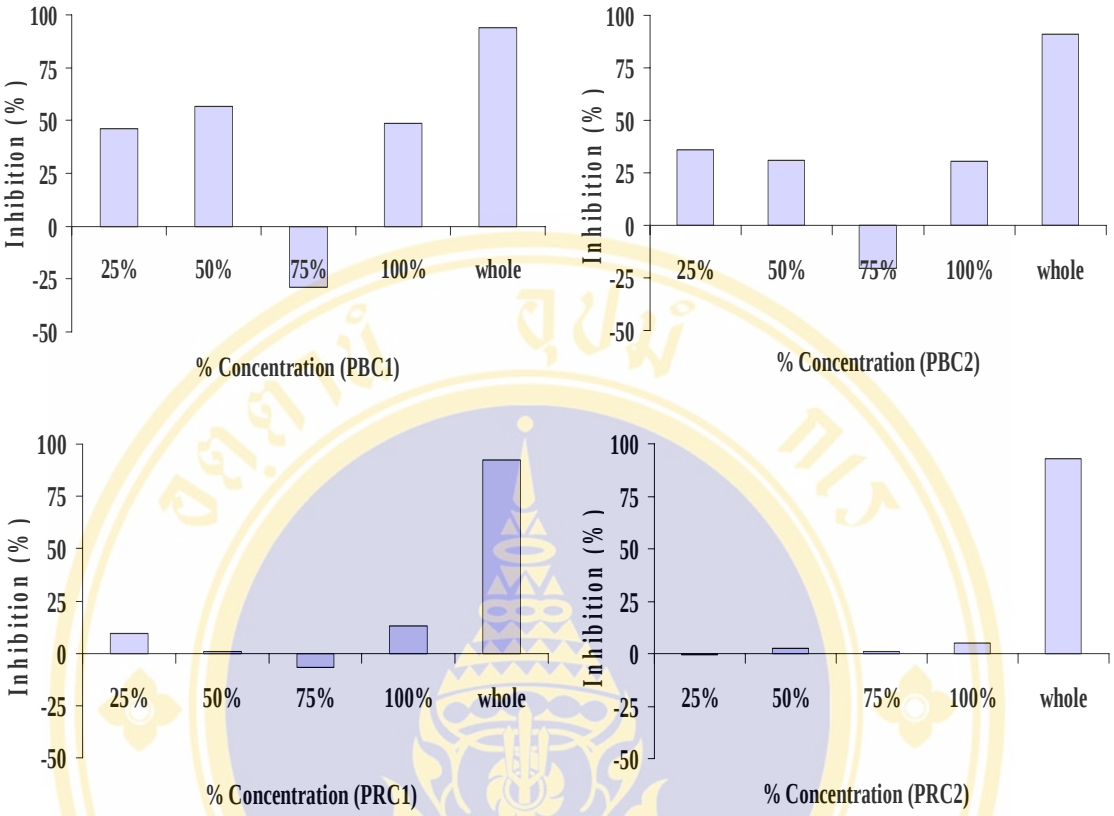


Figure 4-11 Toxicity levels of sediment samples, both elutriate and whole sediment phases, collected from Songkhla Lake tributaries (PBC1, PBC2, PRC1 and PRC2)

Copyright by Mahidol University

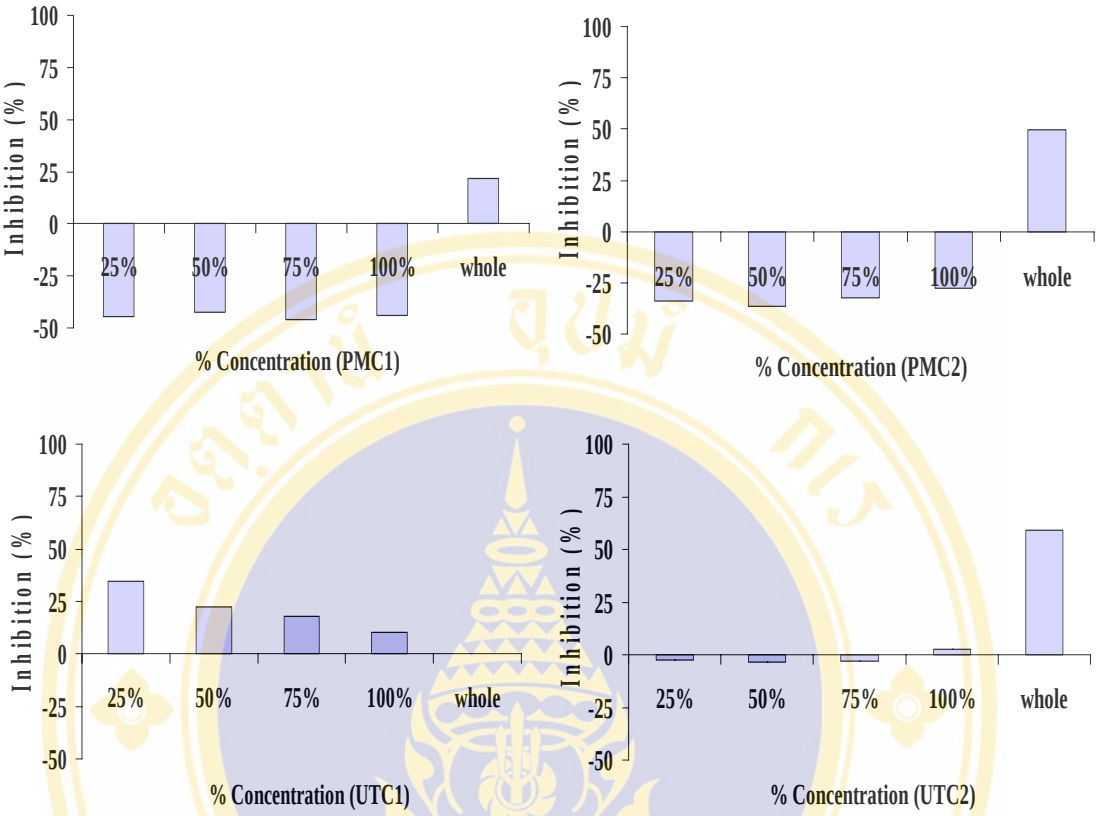


Figure 4-12 Toxicity levels of sediment samples, both elutriate (25%, 50%, 75%, 100%) and whole sediment phases, collected from Songkhla Lake tributaries (PMC1, PMC2, UTC1 and UTC2)

Copyright by Mahidol University

Toxicity testing of sediment elutriates is important because dissolved forms of pollutants are more bioavailable to aquatic biota for uptake and are the primary cause of adverse impacts in aquatic ecosystems (Ludwig *et al.*, 1989). Elutriate fractions were originally used to mimic the open water disposal of dredged material; they have also been used to determine the toxicity of contaminated sediment (Liβ and Ahlf, 1997). Although whole sediment exposures seem to be most realistic laboratory simulation of *in situ* exposure, elutriate have been often used to circumvent problems which occur, when sediment has to be diluted for the characterization of toxicity (Liß, 1997).

When elutriate was chosen as an exposure path in this study, most of the sediment samples did not show inhibition on dehydrogenase activities of *Bacillus cereus*. This could probably be explained by the low contamination levels in the sediment extract (Leppänen and Kukkonen, 2000), which might not be high enough to trigger adverse effects on dehydrogenase activities of *B. cereus*. For this study, the 2 hrs required for sediment extraction was relatively short. In other studies (Rönnpagel *et al.*, 1995; Beg *et al.* 2001), the extraction period lasted 24 hrs. This could enhance bioavailability of some contaminants (Prasad, 2001). Theoretically, even an overnight period might not be long enough the purpose. This depends largely on properties of the tested sediments, as well as the contaminant species (Liβ, and Ahlf, 1997). In a study by Beg *et al.*, 2001), no adverse effects of elutriate was observed, even the 24 hrs extraction period was employed. Only trace amount of total petroleum hydrocarbon and trace elements could be extracted during the preparation of elutriate samples.

In a few sediment samples, including SK1, SK7, MKC1, PRC1 and UTC1, the observed toxicity levels of elutriate samples at low elutriate concentrations were greater than that of the high elutriate concentrations. Natural sediments usually contain a mixture of chemicals with stimulating, as well as inhibiting effects (Schüürmann and Markert, 1998). As dilution factor increases, amount of stimulants might be diluted enough that it could not cover toxicity effects of the toxicants resulting to apparent adverse effects of the more diluted elutriate samples than the less diluted ones.

Copyright by Mahidol University

Toxicity tests of elutriate, however, represent only a part of hydrophilic contaminants, due to the fact that water was used as a carrier in the extraction process. Hence, underestimation of the type and quantity of present bioavailability contaminants could occur. In a study by Rönnpagel *et al.* (1995), an extracted efficiency of copper less than 1 % of the soil loading washed through elutriation was observed. Elutriates from the soil sample spikes with 900 mg Cu/kg contained only 0.2 mg Cu/l.

As suggested by Zimmer and Ahlf (1994), the nearest test phase to that of natural condition is whole sediment phase. Consequently, this could indicate the true bioavailability of contaminants in the sediment (Kwan and Dutka, 1995). Therefore, it should receive priority over other exposure phases, including pore water, as well as elutriate phase.

The results obtained from exposure with whole sediment phase strongly indicated higher toxicity levels ($\alpha < 0.05$) of several sediment samples than that from exposure with elutriate phase (Table B-2 in Appendix B). Similar results were observed by Rönnpagel *et al.* (1998). They reported that in a contact assay (whole sediment), the stimulating effects of benzalkonium-chloride on dehydrogenase activity of *B. cereus* could be observed, while the elutriate testing failed to give any response.

Accumulation of both polar and nonpolar contaminations occurs often in whole sediment (Peper, *et al.*, 2006). The organic matter, which is less polar than water, provides a more favorable environment for nonpolar organic contaminant. Hydrophilic substances, however, are better available in the whole sediment phase than any other exposure routes. Though, exceptions exist for highly lipophilic substances. Highly lipophilic substances that strongly adsorb like naphthalene are neither toxic in elutriate test nor in the whole sediment test (Mc Donald, 2005).

When compared sediment properties with results from whole sediment phase, negative correlations ($p<0.01$) could be observed (Table B-3 in Appendix B). These sediment properties included % Organic Matter, % Moisture Content, electrical conductivity and salinity. There was no correlation, however between sediment properties and sediment toxicity levels when elutriate phase were tested.

The negative correlation between toxicity levels and % organic matter possibly could be explained by the nature of organic matter in the sediments. It usually

Copyright by Mahidol University

prone to complex with contaminants, both polar contaminant such as metals (Hodgson et al., 1965 and Jansen et al., 2003) and nonpolar organic chemicals such as pyrene (Irma, 2003). Thus, high organic contents usually indicate low bioavailability of the contaminants resulting to low toxicity being observed.

In the case of moisture contents, the direct explanation for the negative correlation with the toxicity levels of sediment samples might not exist. Correlation between % organic matter and moisture content in soils or sediment is normally not uniform (Brouwer, Murphy and McArdle, 1990). In the sediment layer surrounding plant roots or benthos, it may differ from that in the bulk sediment. This difference can result in an increase or decrease in the concentrations of toxic elements. At low moisture content dissolved organic matter concentration varies as a function of the sediment or sediment: solution ratio (You et al., 1999). In field conditions at low moisture content, the dissolved organic matter concentration can be much higher than in suspensions and its influence on metal mobility and toxicity can be very significant (Pedersen and Van Gestel, 2001 and Kramer et al., 2004).

In the present study, it was found that salinity up to 10 ppt did not significantly affect dehydrogenase activities. The negative correlation with toxicity levels, thus, probably was a consequence of other causes. In some cases, high salinity was related to low toxicity of contaminants, especially heavy metals such as cadmium, chromium, copper, mercury, nickel, and zinc, as reported in the study by Hall and Anderson (1995). Salinity affects the availability of metals, because high salinity causes formation of metal-chloride complex, which are hard for plants and other organisms to take up. This is likely related to the greater bioavailability of the free metal ion (toxic form) at lower salinity conditions. There was generally no consistent trend for the toxicity of most organic chemicals with salinity. The one exception to this was reported with organophosphate insecticides, the toxicity of which appeared to increase with increasing salinity. In the case of conductivity, the correlation with toxicity levels of sediment may vary with ionic compositions and therefore may not be the best predictor of toxicity (Plum, 1981). It was, however, positively correlate ($p>0.01$) with salinity (Prasad, 2001), therefore it was also found to be negatively correlated to the toxicity levels.

Copyright by Mahidol University

From information on 9 different heavy metal contents i.e. Cu, Hg, Pb, Cd, Zn, Cr, Fe, Mn and Ni, of all 37 sediment samples obtained from Pollution Control Department (2006) (Table A-6), correlation between contents of heavy metals, and sediment toxicity were evaluated.

Table 4-4 Concentration range of 9 different heavy metals in sediment samples collected from 3 different areas, Thale Noi, Thale Luang, Songkhla lake, as well as their tributaries, in Songkhla Lake Basin (Pollution Control Department, 2006)

Concentration (mg/kg sediment)	Standard * (mg/kg sediment)	Concentration range (mg/kg sediment)		
		Thale Noi & tributaries	Thale Luang & tributaries	Songkhla Lake & tributaries
Cu	390	0.05 – 13.00	0.28 - 88.00	0.04 - 99.00
Hg	0.41	0.03 – 0.21	0.02 – 0.71	0.02 – 0.28
Pb	450	4.00 – 32.00	2.00 - 176.00	3.00 - 74.00
Cd	5.1	0.07 – 0.62	0.05 – 1.30	0.05 – 1.30
Zn	410	19.00 – 65.00	3.00 - 323.00	6.14 - 248.00
Cr	260	21.00 – 71.00	3.00 - 75.00	7.00 - 61.00
Fe	-	7,979 – 2,820	1,241 – 29,650	1,168 – 25,185
Mn	-	68.00 – 83.00	14.00 – 1,109	15.00 – 1,037.00
Ni	-	5.00 – 19.00	< 0.750 - 46.00	< 0.750 - 28.00

** From: www.epa.gov, 2548*

The results indicated no significant correlations ($p<0.05$) between heavy metal contents, i.e. Cu, Ni, Pb, Fe, Mn, Zn, Cd, Cr, Zn in each sediment samples and its respective toxicity levels obtained from toxicity testing with elutriate phase. Concentrations of heavy metals in the observed sediment samples, except Hg observed at station SK6, were less than the American standard concentration of heavy metal in sediment (Table A-5, A-6 and A-7 in Appendix A). These relatively low contamination levels of the individual metal species in the samples might be the reason for the finding.

Different results were obtained from toxicity test with whole sediment phase. Negative correlations were observed between microbial test results

Copyright by Mahidol University

and Nickel and iron ($p<0.05$), as well as zinc and chromium ($p<0.01$) (Table B-4 in Appendix B). Since nickel, iron, zinc and chromium are all essential elements, insufficient amount of such metals could lead to inhibiting effects on growth and reproduction, as well as on activity of several enzymes (Walker, 2006, Mertz, 1969 and Jaouen, 2006).

4.2.3 Toxicity ranking

In order to categorize study sites in relation to their toxicity levels, centroid hierarchical cluster analysis were applied. The results indicated that sediment samples collected from Songkhla Lake Basin could be separated into four different toxicity groups. These were non toxic, slightly toxic, moderately toxic and highly toxic as shown in Table 4-4.

Table 4-5 Toxicity levels of sediment samples as categorized by their inhibition effects on dehydrogynase activities of *Bacillus cereus*

Toxicity levels	Inhibition percentage
non toxic	< 25
slightly toxic	25 -59
moderately toxic	60-74
highly toxic	75-100

Sediment samples that showed inhibition effects on dehydrogynase activities below 25 percent could refer as non toxic. For the lightly and moderate toxic groups, the range was between 25 - 59 % and 60 - 74 %, respectively. Above 75 % inhibition indicated highly toxic group.

From the toxicity test results obtained from elutriate phase, most of all the 15 sediment samples from the main lake, except from station SK7 in Thale Luang was found to be in the non toxic group (Figure 4-9). Sediment obtained from the tributaries, especially those flows into Thale Luang showed different findings (Figure 4-10). Sample from stations TNC1 and TNC2 in Klong Ta Nae were found to be in the highly toxic and slightly toxic group, respectively. The station PBC1 and PBC2 were both in the moderately toxic group.

Copyright by Mahidol University

Results obtained from whole sediment phase indicated several stations both in the main area of Songkhla Lake Basin, as well as in its tributaries to be in different toxic level group rather than mostly in the non toxic group as was specified by elutriate phase (Figure 4-11 and 4-12). Samples obtained from station SK5 and SK6 in Thale Luang, as well as SK14 in Songkhla Lake were considered to belong to the highly toxic group, while from stations SK2 in Thale Noi, SK7 in Thale Luang, as well as SK10, SK11 and SK15 in Songkhla Lake were categorized as slightly toxic. The results indicated that several stations in the tributaries, including PRC1, PRC2, PBC1, PBC2, NTC1, RNC1, MKC1 and TNC2, as well as PAC1 and PAC2, that drain water into Thale Luang, and Thale Noi, respectively belong to the highly toxic group. The slightly toxic group included stations MKC2, TKC1, NTC2, PMC2 and UTC2. The first three stations were locate d in the tributaries of Thale Luang, the rest were in Klong U-Ta Pao that drains into Songkhla Lake.

Copyright by Mahidol University

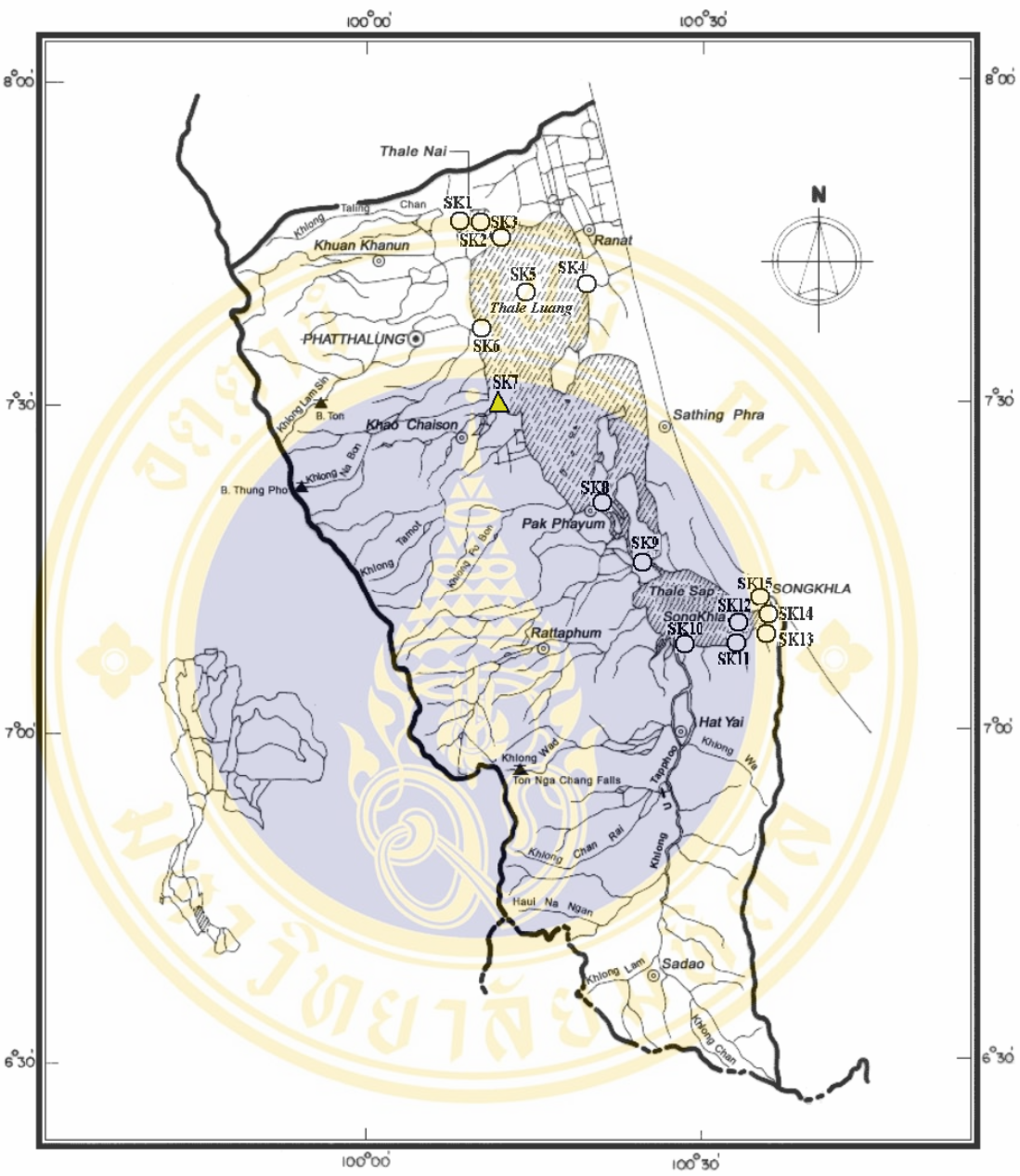


Figure 4-13 Toxicity levels of sediment samples collected from main Songkhla Lake resulted from elutriate phase. The toxicity was classified into four categories as:

○ = non toxic △ = slightly toxic

■ = moderately toxic ● = highly toxic

Copyright by Mahidol University

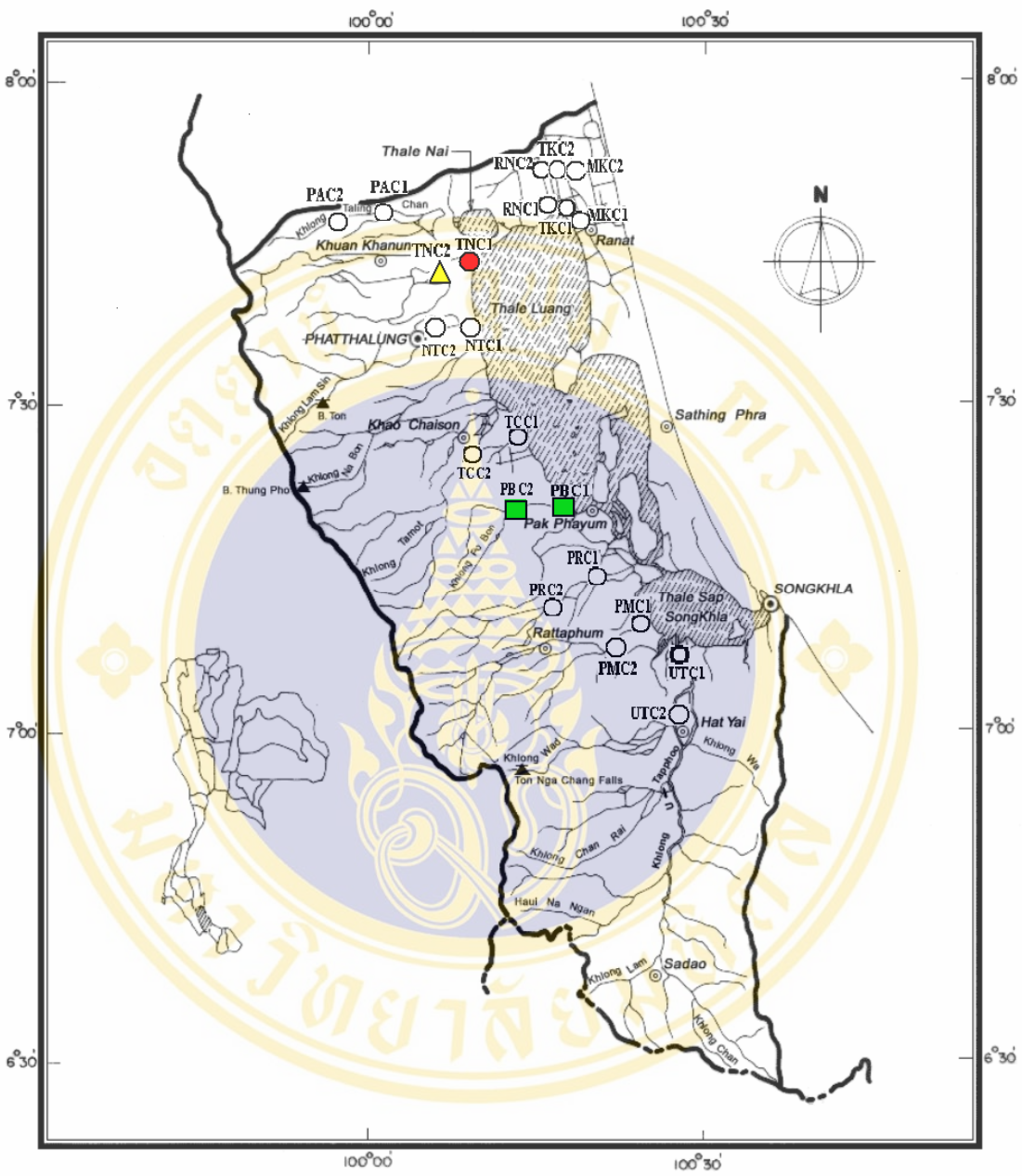


Figure 4-14 Toxicity levels of sediment samples collected from Songkhla Lake tributaries resulted from elutriate phase. The toxicity was classified into four categories as:

○ = non toxic △ = slightly toxic

■ = moderately toxic ● = highly toxic

Copyright by Mahidol University

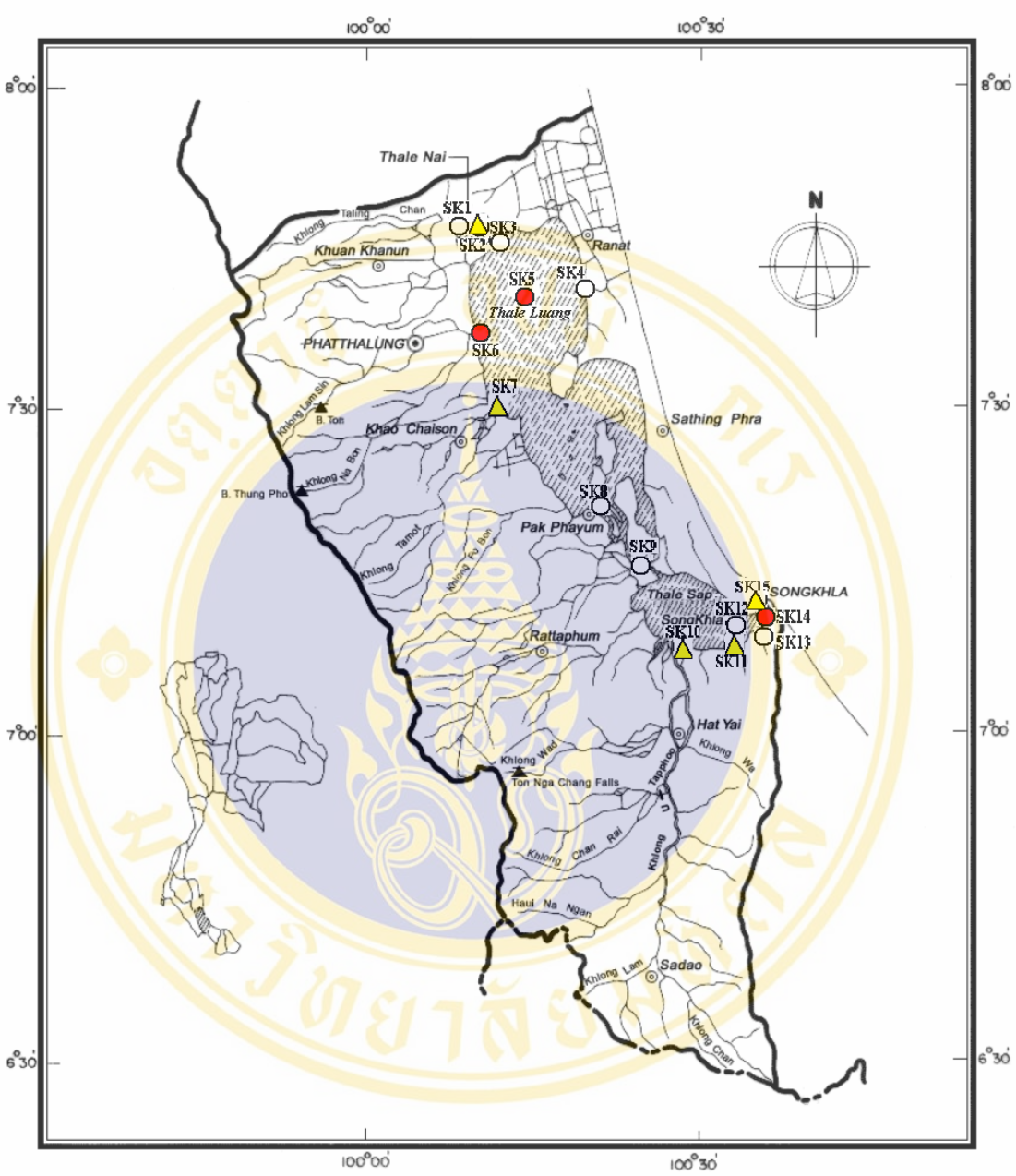


Figure 4-15 Toxicity levels of sediment samples collected from main Songkhla Lake resulted from whole sediment phase. The toxicity was classified into four categories as:

○ = non toxic △ = slightly toxic

■ = moderately toxic ● = highly toxic

Copyright by Mahidol University

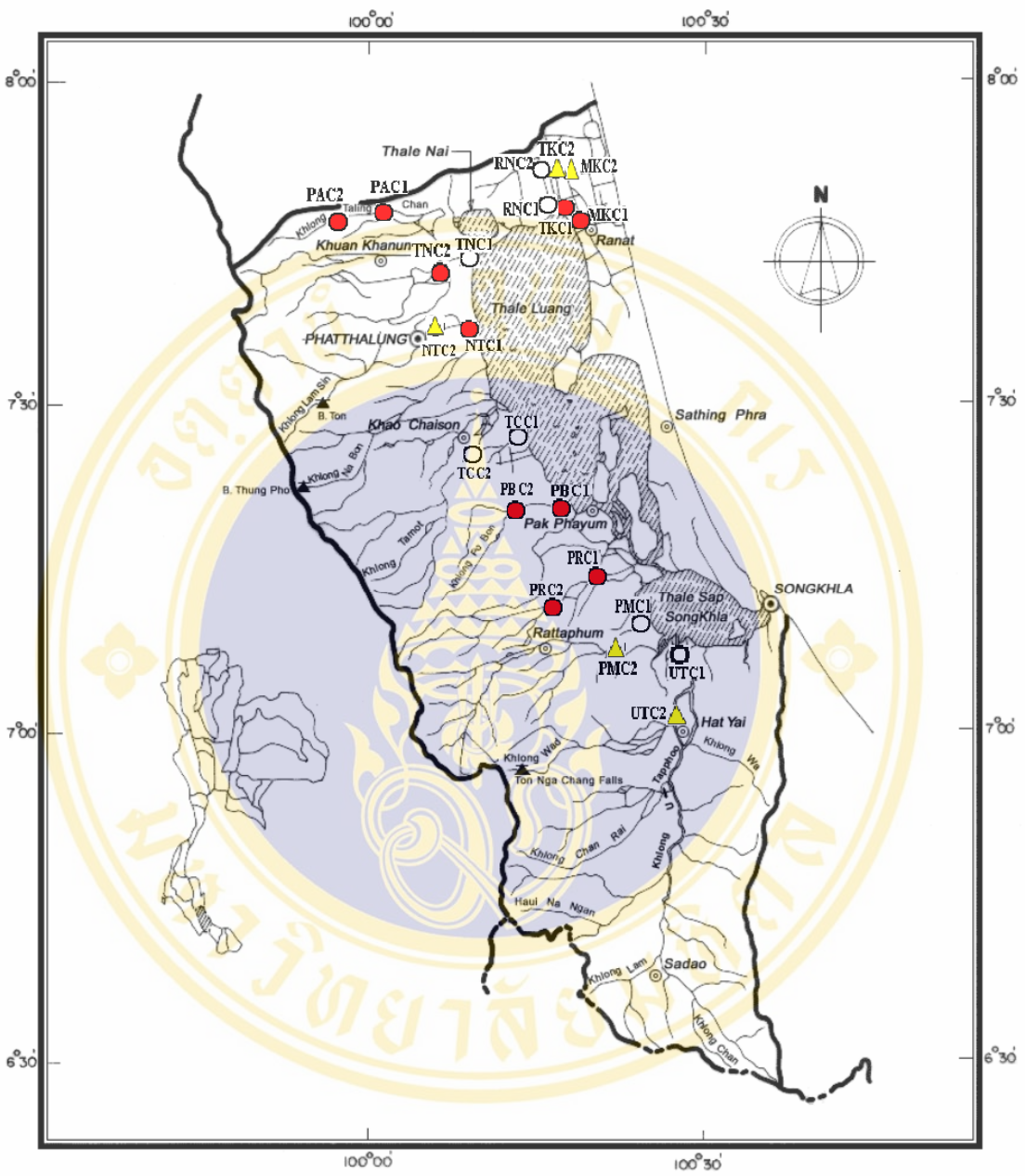


Figure 4-16 Toxicity levels of sediment samples collected from Songkhla Lake tributaries resulted from whole sediment phase. The toxicity was classified into four categories as:

○ = non toxic △ = slightly toxic

■ = moderately toxic ● = highly toxic

Copyright by Mahidol University

4.2.4 Toxicity comparison

Three different sediment toxicity assessment procedures, i.e. toxicity testing with elutriate and whole sediment phases, as well as benthos diversity index were compared. The results were shown in Table 4-5.

Table 4-6 Comparison of sediment toxicity levels resulting from three different toxicity assessment procedures observed from 37 sampling stations in Songkhla Lake Basin

Sampling station	Elutriate phase	Whole sediment phase	Benthos diversity index*
SK 1	1	1	3
SK 2	1	1	2
SK 3	1	1	2
PAC 1	1	2	2
PAC 2	1	3	2
SK4	1	1	2
SK5	1	1	-
SK6	1	1	2
SK7	2	2	3
RNC1	1	1	3
RNC2	1	1	3
MKC1	1	4	2
MKC2	1	2	2
TKC1	1	4	2
TKC2	1	2	2
NTC1	1	4	2
NTC2	1	2	2
TNC1	4	1	2
TNC2	3	4	2
TCC1	1	1	2
TCC2	1	1	2
SK8	1	1	3
SK9	1	1	3

1 = non toxic, 2 = slightly toxic, 3 = moderately toxic, 4 = highly toxic

* Modified from Pollution Control Department (2005)

Copyright by Mahidol University

Table 4-6 Comparison of sediment toxicity levels resulting from three different assessment procedures observed from 37 sampling stations in Songkhla Lake Basin (continued)

Sampling station	Elutriate phase	Whole sediment phase	Benthos diversity index*
SK10	1	2	2
SK11	1	2	3
SK12	1	1	2
SK13	1	1	4
SK14	1	4	3
SK15	1	2	2
PBC1	3	4	2
PBC2	3	4	2
PRC1	1	4	2
PRC2	1	4	2
PMC1	1	1	3
PMC2	1	2	2
UTC1	1	1	3
UTC2	2	1	3

1 = non toxic, 2 = slightly toxic, 3 = moderately toxic, 4 = highly toxic

* Modified from Pollution Control Department (2005)

The results indicated that results obtained from benthos diversity index seemed to be closely related to that found by whole sediment phase than elutriate phase. Benthos diversity index was estimated from number of different benthic species, as well as their abundant in the sediment (Still, Rabke and Candler, 2000). It represented direct effects of contaminants to those organisms in the sediment under field conditions (Landrum and Robbins, 1990). Results from toxicity testing with whole sediment phase, which is the closest exposure phase to the real condition, thus indicated similar results to that obtained from Benthos diversity index. In addition, it could even identify several possible highly toxic sites, which required prompt action. This strongly suggested the suitability of dehydrogenase assay using *Bacillus cereus* for assessing toxicity levels of natural sediment samples.

Copyright by Mahidol University

CHAPTER V
CONCLUSION AND RECOMENDATION

5.1 Conclusion

Dehydrogenase (DHA) Assay using bacteria *Bacillus cereus* TISTR 687 was applied for assessing toxicity level of 37 sediment samples collected from Songkhla Lake Basin. For the purpose, test procedures, including suitable density of the test inoculum, appropriate pH and salinity levels were tested. The toxicity assessment of the sediment samples were performed with both elutriate, as well as whole sediment phases. In addition, physical and chemical properties of sediment samples were evaluated. The results of the study could be concluded as follows:

1) For DHA method, the bacteria culture reached middle log phase (exponential phase) after 2 hours 30 minute incubation. The suspension contained about 3.5-4.5x10^7 CFUs/ml. This represented suitable density of inoculum for the toxicity test method.

2) The pH levels of toxicity test conditions influence strongly to enzyme activities and metabolism of bacterial cells. The pH levels 7 and 8 were found to be the most suitable pH range of the condition for the DHA method.

3) Salinity of the test media up to 10 ppt did not show inhibition effects on enzyme activities observed in DHA method using *Bacillus cereus.*

4) Physical and chemical properties of sediment samples from Songkhla Lake Basin varied depending on locations. Sediment types ranged from sandy loam to clay. Most of these sediment samples were high in organic matter. The highest content, i.e. 6.68 %, was found in silty clay sample from Station TNC1 around Pak Klong Taa Nae that connected to Thale Luang. The salinity of the samples ranged between 0.00-7.07. The high salinity values, i.e. 4.03 – 7.07, were observed mostly in sediment samples collected from Songkhla Lake (SK8-SK15). The pH of the sediment samples was quite variable, but fell mostly in the range between pH 6 to 8.

Copyright by Mahidol University

5) The toxicity testing results indicated that most of elutriate samples was not toxic. The exception was observed from station SK7 and TNC2; PBC 1 and PBC2; and TNC1, which were considered as slightly toxic; moderately toxic; and highly toxic, respectively.

6) From toxicity test results obtained from whole sediment phase, fourteen sediment samples, including SK1, SK3, SK4, SK8, SK9, SK12, SK13, TCC1, TCC2, TNC1, RNC1, RNC2, PMC1, UTC1 and UTC2 were found to be non toxic. Ten samples, i.e. SK2, SK7, SK11, SK15, NTC2, MKC2, TKC2 SK10 and PMC2 were considered slightly toxic, while for the sediment samples SK5, SK6, SK14, PAC1, PAC2, NTC1, MKC1, TNC2, TKC1 PBC1, PBC2, PRC1 and PRC2 the results indicated high toxicity.

7) Results obtained from whole sediment phase seemed to indicated higher toxicity among sediment samples from Songkhla Lake Basin than those obtained from elutriate phase.

8) The overall relatively higher toxicity of sediment samples in the canals, which flowed into Songkhla Lake than that of samples in the main lake area, including Thale Noi, Thale Luang and Songkhla Lake implied the possibility of excessive human influence in such area.

5.2 Recommendation

1) Effects of different group of chemicals on dehydrogenase assay using *Bacillus cereus* should be tested as baseline information on sensitivity of the method against toxicity of single, as well as multiple chemicals.

2) A series of different extraction duration for preparation of elutriate should be conducted.

3) Although results from this study revealed that DHA method was suitable for utilizing as sediment toxicity screening test, “battery of test”, including other toxicity test systems should still be simultaneously conducted to estimate accurate potential risk of natural sediment samples.

Copyright by Mahidol University

REFERENCE

Ahlf, W. (2006). *Sediment Toxicity - A Result of Distribution Processes Outside and Inside of Organisms*. Institute of Environmental Technology and Energy Economics, Hamburg, Germany.

Ahtiainen, J. (2002). *Microbiological tests and measurements in the assessment of harmful substances and pollution*. Finish Environment Institute, Helsinki, Finland.

Ansar Ahmed, S.A., Gogal, R.M. and Walsh, J.E. (1994). *A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: and alternative to the ^{3}H thymidine incorporation assay*, J. Immunol. Methods 170: 211–224.

American Public Health Association. (1989). Water Pollution Control Federation and American Water Works Association. Standard Methods for the Examination of Water and Wastewater, 18th ed., APHA, Washington, D. C.

American Society for Testing and Materials (ASTM). (1992). Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods**.** American Society for Testing and Materials, Philadelphia.

Ames, B.N., McCann, J. and Yakasai, E. (1975). *Methods for detecting carcinogens and mutagens with Salmonella typhimurium minisome test*, Mutation. Res., 31 347-364.

Beg, M.U., Saeed, T., Al-Muzaaini, S., Beg, K. R., Al-Obaid, T. and Kurin, T. (2001). *Extraction, Fractionation, and Toxicity Determination of Organic Contaminants in Sediment from Coastal Area Receiving Industrial Effluents in Kuwait*. Bull. Environ. Contam. Toxicol.67:881-888.

Bitton, G., and Koopman, B. (1986). *Biochemical tests for toxicity screening*. p. 27-55. In: Bitton, G. and Dutka, B. J. (1986). Toxicity Testing Using Microorganisms. Boca Raton, CRC Press, Inc. Boca Raton, FL.

Copyright by Mahidol University

Bitton, G., Campbell, M. and Koopman, B. (1992) *MetPAD: A bioassay kit for the specific determination of heavy metal toxicity in sediments from hazardous waste sites*. Environ Toxicol Water Qual 7:323–328.

Bitton, G., Jung, K. and Koopman, B., 1994. *Evaluation of a microplate assay specific for heavy metal toxicity*. Arch Environ Contam Toxicol 27, pp. 25–28.

Blaise, C. (1991*). Microbiotest in Aquatic Ecotoxicology: Characteristics, Utility and Prospect. Environ*. Toxicol. Water. Qual. Int. 6: 145-156.

Blinova, I. (2000). *Use of bioassays for toxicity assessment of polluted water*. Proceedings of the Symposium dedicated to the 40th Anniversary of Institute of Environmental Engineering at Tallinn Technical University. 23-24 September, Tallinn. Tallinn, pp. 149-154.

Borgert, C. J., Gross, T.S., Guiney, P.D., Osimitz, T. G., Price, B. and Wells, C. (2002). Interactive effects of DDE and methoxychlor on hormone production in bass gonadal cultures. *The Toxicologist* 56 (1): 180.

Botsford, J. L., Hillaker, T., Robertson, B., Gonzales, M, Benavidez, M., Jones, B., Baker, R., Steen, W., Pacheco, F., Homer, V., Lucero, O., Matthews, M. and Koehler, V. (1996). A Simple, Rapid, Inexpensive Assay for Toxic Chemicals Using a Bacterial Indicator. Department of Biology, New Mexico State University, Las Cruces.

Brouwer, H., Murphy, T. and McArdle, L. (1990). *A sediment- contact bioassay with Photobacterium phosphoreum*. Env. Toxicol. and Chemistry 9: 1353–1358.

Brown, C.E., Pezeshki, S.R. and DeLaune, R.D. (2006). *The effects of salinity and soil drying on nutrient uptake and growth of Spartina alterniflora in a simulated tidal system*. Environ. Exp. Bot. 58 (1-3): 140-148.

Bulich, A. A. (1982). *A practical and reliable method for monitoring the toxicity of aquatic samples*. Process Biochemistry 17: 45-47.

Burton, G. A., Jr. (1991). *Assessing freshwater sediment toxicity*. Environ. Toxicol. Chem. 10: 1585–1627.

Burton G. A., Jr., Baudo, R., Beltrami M., and Rowland, C. (2001). Assessing Sediment Contamination Using Six Toxicity Assays. Institute for Environmental Quality, Wright State University, Dayton, Ohio, USA.

Copyright by Mahidol University

Burton G. A., Jr. and Landrum, P. F. (2003). Toxicity of Sediments. Encyclopedia of Sediments and Sedimentary Rocks. Kluwer Academic Publisher, Dordrecht.

Carlin, F., Girardin, H., Peck, M. W., Stringer, S. C., Barker, G. C., Martinez, A., Fernandez, A., Fernandez, P., Waites, W. M., Movahedi, S., Leusden, V., Nauta, F., M., Moezelaar, R., Torre, M. D. and Litman, S. (2000). *Research on factors allowing a risk assessment of spore-forming pathogenic bacteria in cooked chilled foods containing vegetables: a FAIR collaborative project*. Int. J. Food Microbiol. 60:117-135.

Choma, C., Guinebretiere, M. H., Carlin, F., Schmitt, P., Velge, P., Granum, P. E. and Nguyen-The, C. (2000). *The Prevalence, characterization and growth of Bacillus cereus in commercial cooked chilled foods containing vegetables*. J. Appl. Microbiol. 88: 617-625.

Christofi, N., Hoffmann, C. and Tosh, L. (2002). *Hormesis responses in free and immobilised light-emitting bacteria*. Ecotoxicol Environ Saf. 52: 227–231.

Collins, Y. E. and Stotzky, G. (1992). *Heavy metals alter the electrokinetic properties of bacteria, yeasts, and clay minerals*. Appl. Environ. Microb. 58: 1592-1600.

Colowick, S. P. and Kaplan, N. O. (1962) Methods in Enzymology, vol. V. Academic Press, New York.

Conn, H. J. (1961). Biological Strains. The William & Wilkins Company. Baltimore.

Crossley, D.A. (1970). *Roles of microflora and fauna in soil systems*. In Pesticides in the Soil: Ecology, Degradation and Movement. East Lansing: Michigan State Univ., pp. 30-35.

Curtis, C., Lima, A., Lorano, S. J. and Veith, G. D. (1982). *Evaluation of a bacterial bioluminescence bioassay as a method for predicting acute toxicity of organic chemicals to fish*. In: Pearson, J.G., Foster, R.B. and Bishop, W.E. (eds.). Aquatic Toxicity and Hazard Assessment, STP 766, American Society for Testing and Materials, Philadelphia, PA., pp. 170-178.

D'Eustachio, A. J. and Johnson, D. R. (1968). Instrumental Approach to Rapid Microbiology. Internal Publish, E.I. Dupont Nemours and Co., Wilmington, DE.

Copyright by Mahidol University

David, D. B, Kirkby, G. R. and Noble, B. A. (1994). *Bacillus cereus endophthalmitis*. Br J Ophthalmol. 78: 577–580.

De La Rosa, D. (1979). *Relation of Several Pedological Characteristics to Engineering Qualities of Soil.* Eur J of Soil Science. 30(4): 793-799.

DeBen, W. A., Lee (II), H. and Ozertich, R. J. (1994). *Sediment toxicity, Contaminant and amphipod abundance at a DDT and Dieldrin – contaminated site in San Francisco Bay*. Environ. Toxicol. Chem. 13: 949-962.

Design, T. (1983). Luminescence Rev. Bull. #204. Turner Design, Mountain View, CA.

Doherty, F. G. (1983). *Interspecies Correlations of Acute Aquatic Median Lethal Concentration for Four Standard Testing Species*. Environ Science. Technol. 17: 661 – 665.

Duncker, S. (1994). Der Pollen Tube Growth Test und der Algewiderergrünungstest als mögliche Alternativen zum Draize Augenirritationstest bei der Bestimmung der Toxizität von Tensiden. [M.S. Thesis]. Hamburg Universität.

Dutka, B. J. and Gorrie, J. F. (1989) *Assessment of toxicant activity in sediments by the ECHA Biocide Monitor*. Environ Pollut 57: 1-7.

Dutton, R.J., Bitton, G., Koopman, B. and Agami, O. (1990). *Effect of environmental toxicants on enzyme biosynthesis: a comparison of β-galactosidase, α-glucosidase and tryptophanase*. Arch. Environ. Contam. Toxicol. 19: 395-398.

Feiler, U., Ahlf, W. , Fahnenstich, C., Gilberg, Hammers-Wirtz, D. M., Höss, S. H., Meller, H. M., Melbye, K., Neumann-Hense, H., Ratte, H.-T., Seiler, T. B., Weber, J. and Heininger, P. (2006). The SeKT Joint Research Project: Definition of Reference Conditions, Control Sediments and Toxicity Thresholds for Limnic Sediment Contact Tests. Institute of Environmental Technology and Energy Economics, Hamburg, Germany.

Finney, D.J. (1978). Statistical Method in Biological Assay, 3rd ed. Charles Griffin and Company, Ltd., London.

Copyright by Mahidol University

Franklin, T. J. and Snow, G. A. (1989). Biochemistry of Antimicrobial Action, 4th ed., Chapman and Hall, USA.

Genc, S. and Özturk, D. (1998). *Determination of structure-toxicity relationship of amphiprotic compounds by means of the inhibition of the dehydrogenase activity of Pseudomonas putida*. Turk J Chem 22: 341-349.

Grabrielson, J. (2004). Assessing the Toxic Impact of Chemicals Using Bacteria. Karolinska University Press, Stockholm, Sweden.

Granum, P. E. (1994). *Bacillus cereus and its toxins*. Soc. Appl. Bacteriol. Symp. Ser. 23: 61S-66S.

Guinebretiere, M. H., Girardin, H., Dargaignaratz, C., Carlin, F. and Nguyen, C. (2003). *The Contamination flows of Bacillus cereus and spore-forming aerobic bacteria in a cooked, pasteurized and chilled zucchini puree processing line*. Int. J. Food Microbiol. 82: 223-232.

Gunkel, J., Rönnpagel, K. and Ahlf, W. (1993). *Suitability of microbiological bioassays for bound contaminants.* Acta Hydrochim. Hydrobiol. 12: 1-6.

Hall, L. W., Jr. and Anderson, R. D. (1995). *The influence of salinity on the toxicity of various classes of chemicals to aquatic biota*. Crit. Rev. Toxicol., 25 (4): 281-346.

Haig, S. and Rennie, A. (1994). *Rapid methods to assess the effects of chemicals on microbial activity in soil*. Environ. Toxicol. Wat. Qual. 9: 347–354.

Hermans, J., Busser, F., Leevwangh, P. and Musch, A. (1985). *Quantitative structureactivity relationships and mixture toxicity of organic chemicals in Photobacterium phosphoreum*. *The Microtox test.* Ecotoxicol. Environ. Safety. 9: 17-25.

Hodgson, J. F., Geering, H. R. and Norvel**,** W. A. (1965). *Micronutrient cation complexes in soil solutions: partitioning between complexed and uncomplexed forms by solvent extraction*. Soil Sci. Soc. Am. Proc. 29: 665–669.

Hoffman, D. J., Rattner, B. A., Burton, G. A., Jr. and Carlin J., Jr. (1994). Handbook of Ecotoxicology. Lewis Publishers, USA.

Holme-Hansen, O. (1973). *Determination of total microbial biomass by measurements of 90 adenosine triphosphate*. In: Stevenson, L.H. and

Copyright by Mahidol University

Lowell, R.R. (eds.). Estuarine Microbial Ecology. University of South Carolina Press, Columbia, SC.

Irha, N., Slet, J. and Petersell, V. (2003). *Effect of heavy metals and PAH on soil assessed via dehydrogenase assay.* Environ. Intern. 28: 779-782.

Janice, M. S. (2005). In Vitro Toxicity of Cadmium Oxide Particles in BRL 3A Rat Liver Cells. Air Force INST of Tech Wright-Patterson AFB OH School of Engineering and Management, UK.

Jansen, B., Nierop, K. G. J. and Verstraten, J. M. (2003). *Mobility of Fe(II), Fe (III) and Al in acidic forest soils mediated by dissolved organic matter: influence of solution pH and metal/organic carbon ratios*. Geoderma 113: 323–340.

Jaouen, G. (2006). Bioorganometallics: Biomolecules, Labeling, Medicine; Wiley-VCH: Weinheim.

Johnson, S. L. (2006). Risk Assessment Guidance for Superfund, Human Health Evaluation Manual. Office of Emergency and Remedial Response, U. S. Environmental Protection Agency, Washington DC.

Kaiser, K. L. E. (1993). *Qualitative and quantitative relationships of Microtox data with toxicity data for other aquatic species*. In Richardson, M. L. (ed.). Ecotoxicology Monitoring. Weinheim: VCH Publishers, pp. 197–211.

Kenaga, E. E. (1979). *Aquatic test organisms and methods useful for assessment ofchronic toxicity of chemicals*. In: Dickson, K. L., Maki, A. W., and Cairns, J., Jr. (eds.). Analyzing the Hazard Evaluation Process. American Fisheries Society, Washington, DC., pp. 101-111.

Kilroy, A. and Gray, N. F. (1995). *Treatability toxicity and biodegradability test methods*. Biol. Rev 70, 243 – 275.

Kozlova, O., Zwinderman, M. and Christofi, N. (2005). *A new short-term toxicity assay using Aspergillus awamori with recombinant aequorin gene.* BMC Microbiol. 5: 40.

Kramer, K. J. M., Jak, R. G., Hattum, B. V, Hooftman, R. N. and Zwolsman, J. G. (2004). *Copper toxicity in relation to surface water-dissolved organic matter: biological effects on Daphnia magna*, Environ. Toxicol. Chem. 23: 2971–2980.

Copyright by Mahidol University

Kwan, K.K. (1993). *Direct toxicity assessment of solid-phase samples using the toxi-chromotest kit*. Environ. Toxicol. Water Qual. 8: 223–230.

Kwan, K.K. and Dutka , B.J. (1995). *Comparative assessment of two solid-phase toxicity bioassays: the direct sediment toxicity testing procedure (DSTTP) and the Microtox solid-phase test (SPT)*. Bull. Environ. Contam. Toxicol. 55: 338–346.

Landis, W. G. and M-H Yu. (1995). *Introduction to environment toxicology: impacts of chemicals upon ecological systems*. CRC Press Inc. Florida, USA.

Landrum, P. F. and Robbins, J. A. (1990). *Bioavailability of sediment-associated contaminants to benthic invertebrates*. In: Baudo, R., Giesy, J.P. and Muntau, H. (etds). Sediments: Chemistry and Toxicity of In-place Pollutants, Chelsea, Michigan (1990), pp. 237–263.

LeBlanc, G.A. (1984). *Interspecies relationships in acute toxicity of chemicals to aquatic organisms*. Environ. Toxi. Chem. 3: 47-60.

Lennette, E. H., Balows, A., Housler, W. J. and Shadomy, H. J., Jr. (1985). Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, DC.

Leppänen, M. T. and Kukkonen, J. V. K. (2000). *Effect of sediment–chemical contact time on availability of sediment-associated pyrene and benzo[a]pyrene to oligochaete worms and semi-permeable membrane devices*. Aqua. Toxicol. 49 (4): 227-241.

Liβ, W. (1997). Vergleich mikrobieller Biotests zur ökotoxikologischen Bewertung kontaminierter Sedimente unter Berücksichtigung der Wirkpfade von Schadstoffen. Fortschr. – Ber. VDI Reihe 15, Nr. 176. Düsseldorf.

Liβ, W. and Ahlf, W. (1997). *Evidence from Whole-Sediment, Porewater, and Elitriate Testing in Toxicity Assessment of Contaminanted Sediments*. Eco. Env. Saf. 36: 140-147.

Liu, D. (1983). *Resazurin reduction method for activated sludge process control*. Env. Sci.Tech.17: 407-411.

Liu, D. and Dutka, B.J. (1984). Toxicity Screening Procedures Using Bacterial Systems. Marcel Dekker Inc, New York, NY.

Copyright by Mahidol University

Liu, D. and Strachan, W. M. J. (1981). *Biodegradation of carbaryl in simulated aquatic environment*. Bull. Environ. Contam. Toxicol. 27: 412–417.

Luoma, S. N., and Bryan, G. W. (1978). *Factors controlling availability of sediment-bound lead to the estuarine bivalve Scorbicularia plana*. J. Mar. Biol. Ass. UK 58: 793-802.

Ludwig, D. D., Sherrard, J. H. and Amende, R. A. (1989). *Evaluation of the standard elutriate test as an estimator of contamination release at dredging site*. Res J Wat Pollut Control Fed 61: 619-631.

Marshall, H.G. (1984). Seasonal Phytoplankton Composition and Concentrations in the Lower Chesapeake Bay and Vicinity. Old Dominion University Research Foundation Grant DACW65-81-C-0051.

Materne, M. (2000). The Plant Guide. The U.S. Department of Agriculture (USDA), NRCS 2000, NRCS, Louisiana State Office, Plant Materials, Baton Rouge, Louisiana .

Maier, R. M., Pepper, L. and Gerba, C. P. (2000). Environmental Microbiology. Academic Press. San Diego, CA.

Maxam, G., Rila, J.-P., Dott, W. and Eisentraeger, A. (2000). *Use of bioassays for assessment of water-extractable ecotoxic potential of soils*. Ecotoxico. Environ. Saf. 45: 240-246.

McBride, M., Martinez, C.E. and Sauvé, S. (1998) *Copper (II) activity in aged suspensions of goethite and organic matter*. Soil Sci. Soc. Am. J. 62: 1542–1548.

McCarty, J. P. and Secord, A. L. (1999). *Reproductive ecology of tree swallows (Tachycineta aicolor) with high levels of polychlorinated biphenyl contamination*. Environ. Toxi. Chem 18(7): 1433–1439.

McDermid, A. S., McKee, A. S., and Marsh, P. D. (1988). *Effect of environmental pH on enzyme activity and growth of Bacteroides gingivalis W50*. Infect. Imm 56: 1096-1100.

Mertz W., 1969. *Chromium occurrence and function in biological systems*. Physiol Rev. 49: 163-239.

Copyright by Mahidol University

Mosher, J. J, Levison, B.S. and Johnson, C. G. (2003*). A simplifield dehydrogenase enzyme assay in contaminated sediment using 2-(p-iophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride*. J. Microbiol. Meth 53: 411-415.

Nachaingmai, N. (1991). *Water quality of outer Songkhla Lake Basin Songkhlanakarin*. Songklanakarin J Sci Technol 3-4: 189-94.

Obst, U., Holzapfel-Pschorn, A. and Wiegand-Rosinus, M. (1988). *Application of enzyme assays for toxicological water testing*. Toxicity Assess. 3: 81-91.

Office of the National Environment Board. (1990). Environmental Monitoring in Songkhla Lake Basin. Bangkok, Thailand, pp. 266.

Ongley, E. D., Birkholz, D. A., Caray, J. H. and Samoiloff, M. G. (1988). *Is water a relevant sampling medium for toxicity chemicals? An alternative environmental sensing strategies*. J. Environ. Qual. 17: 391-401.

Organisation for Economic Co-operation and Development. (1989). Report of the OECD Workshop on Ecological Effects Assessment. OECD Environment Monograph No. 26. Organisation for Economic Co-Operation and Development, Paris.

Peltier, W. H. and Weber, C.I. (1985). Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. 3rd ed. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Penry, J. J. and Saley, J. T. (1997). Microbiology: Dynamic and Diversity. Sander College Publising, USA.

Pepper, I. L., Gerba, C. P., . Brusseau, M. L., Josephson, K. L. and Loya, E. R. (2006). Environmental & Pollution Science. 2nd ed. Elsevier/ Academic Press, Boston.

Pedersen, M.B. and Van Gestel, C. A. M. (2001). *Toxicity of copper to the collembolan Folsomia fimetaria in relation to the age of soil contamination*, Ecotoxicol. Environ. Saf. 49: 54–59.

Piper, C. S. (1942). Soil and Plant Analysis. Inter Science Publishers, New York.

Plumb, R. H., Jr. (1981). Procedures for Handling and Chemical Analysis of Sediment and Water Samples, Technical Report EPA/CE-18-1. US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Copyright by Mahidol University

Pollution and Control Department. (2005). Water Quality Monitoring and Assessment Program for Songkhla Lake Basin Project. Ministery of Resources and Environment. Bangkok, Thailand.

Prasad, M. N. V. (2001). Metal in the Environment Analysis by Biodiversity. Marcel Dekker, Inc. Switzerland.

Raymont, G. E. and Higginson, F. R. (1992). Australian Laboratory Handbook of Soil and Water Chemical Methods, Melbourne, Inkata Press, Australia.

Reinhartz, A., Lampert, I., Herzberg, M. and Fish, F. (1987). *A new, short-term, sensitive, bacterial assay kit for the detection of toxicants*. Toxicity Asses. 2: 193-206.

Rhoades, J.D. (1982). Cation exchange capacity. In: Page, A.L., Miller, R.H. and Keeney, D.R. (eds.) Methods of soil analysis. Part 2. Agron. Monogr. 9, Am. Soc. Agron., Madison, WI. p. 149-157.

Ronald, M. A. and Richard, B. (1981). Microbial Ecology Fundamentals and Applications. Addison – Wesley Publishing Company, Inc., Philippine.

Rönnpagel, K., JanBen, E. and Ahlf, W. (1998). *Asking for the Indicator Function of Bioassays Evaluating Soil Contamination: are Bioassay Results Reasonable Surrogates of Effects on Soil Microflora?* Chemosphere 36(6): 1291-1304.

Rönnpagel, K., JanBen, E. and Ahlf, W. (1995). *Microbial bioassays to assess the toxicity of solid-associated contaminants*. Ecotox. Environ. Saf. 31: 99-103.

Rossa, C. D. (2005). *Tools and Data Needed to Assess Multiple Chemical Exposures. National Capital Area Chapter Society of Toxicology*. US Agency for Toxic Substances and Disease Registry, Atlanta, GA

Schüürmann, G. and Markert, B. (1998). Ecotoxicology: Ecological Fundamentals, Chemical Exposure and Biological Effect. John Wiley & Sons, Inc., New York, USA.

Sims, J. T. (1996). *Lime requirement*. In: Sparks, D.L. (ed.), Methods of Soil Analysis. Part 3. SSSA Book Series No. 5. SSSA, Madison, WI., pp. 491-515.

Slooff, W., Van Oers, J.A.M. and De Zwart, D. (1986). *Margins of uncertainly in ecoloxicological hazard assessment*. Env. Toxi Chem. 5: 841-852.

Copyright by Mahidol University

Still, I., Rabke, S. and Candler, J. (2000). *Development of a standardized reference sediment to improve the usefulness of marine benthic toxicity testing as a regulatory*. Environ.Toxicol.15(5): 406-416

Strotmann, U. J., Butz, B. and Bias, W. R. (1993). *The dehydrogenase assay with resazurin: practical performance as a monitoring system and pH-dependent toxicity of phenolic compounds*. Ecotoxic. Environ.Safety 25: 79-89.

Suedel, B. C., Deaver, E. and Rodgers, J. H., Jr. (1995). Formulated Sediment as a Reference and Dilution Sediment in Definitive Toxicity Tests. University of Mississippi, Biological Field Station, Department of Biology, University, Mississippi, USA.

Sunahara, G. I., Renoux, A. Y., Thellen, C., Gaudet, C. L. and Pilon, A. (1998). Environment Analysis of Contaminated Sites. John Wiley & Sons. Inc., Canada.

Suter, G. W. (1996). Risk Characterization for Ecological Risk Assessment of Contaminated Sites. The Environmental Restoration Risk assessment Program Lockheed Martin Energy Systems, Inc., Oak Ridge, Tennessee.

Thamrongwang, P, Juntiwong, S, Jirasuktaveekul, W. and Krisornpornson, B. (1999). Some Physical and Chemical Qualities of Water at Songkhla Lake Basin. Department of Technology & Industry, Prince of Songkhla University, Songkhla, Thailand.

Torslove, J. (1993). *Comparison of bacteria toxicity tests base on dehydrogenase activity, and esterase activity of Pseudomonas fluorescence*. Ecotoxico Environ Saf. 25: 35-40.

Tsui, M. T. K., and Chu, L. M. (2004). Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures. Arch. Environ. Contam. Toxicol. 46: 316–323.

United States Department of Agriculture (1996). Soil Survey Laboratory Method Manual. Soil Survey Investigations. Report No. 42 Version 3.0 693.

US Environmental Protection Agency. (1990). Technical Support Document for Water Quality-Based Toxics Control. Office of Water. Washington, DC.

Copyright by Mahidol University

US Environmental Protection Agency. (1994). Catalogue of Standard Toxicity Tests for Ecological Risk Assessment. Office of Solid Waste and Emergency Response. Washington, DC.

Walkley, A. (1935). *An examination of methods for determining organic carbon and nitrogen in soils*. J. Agric. Sci. 25:598-609

Walkley, A. and Blank, I.A. (1947). *Chromic acid titration method for determination of soil organic matter*. Soil Sci. Amer. Proc. 6: 63-257

Wilemsen, A., Vaal, M. A. and De Zwart, D. (1995). Microbiotest as tools for Environmental Monitoring. National Institute of Public Health and Environmental Planning (RIVM), The Netherlands Report No. 9, 607042005.

Wlodarczyk, T., Stepniewski, W., Brzezinska, M. (2005) *Nitrous Oxide Consumption and Dehydrogenase Activity in Calcaric regosols***,** Pol J Soil Sci. 38(2): 97-110.

Calmano, W., Ahlf, W. and Förstner, U. (1988). *Study of metal sorption/adsorption processes on competing sediment phases with a multichamber device*. Environ. Geol. Water Sci. 11: 77-84.

Wright, D. A. (2002). Environmental Toxicology. Cambridge University Press, ……., United Kingdom.

Yadong, Y., Impellitteri, C. A., You, S.-J. and Allen, H. E. (2002). *The importance of organic matter distribution and extract soil: solution ratio on the desorption of heavy metals from soils*. Sci. Total Environ. 287: 107–119.

You, S.-J., Yadong, Y. and Allen, H. E. (1999). *Partitioning of dissolved organic matter in soils: effects of pH and water*: *soil ratio*. Sci. Total Environ. 227: 155-160.

Zimmer, M and Ahlf, W. (1994). Erarbeitung von Kriterien zur Ableitung von Qualitatszielen fur Sediment und Schwebstoffe. Literaturstudie UBA Texte 69/94, Umweltbundessamt, Berlin, Germany.

http:// www.epa.gov, 2548

http://www.uoguelph.ca/cntc/publicat/toxtalk/toxtalk_fall99.shtml, 2548

Copyright by Mahidol University

APPENDIX

Copyright by Mahidol University

APPENDIX A

Table A-1 Growth of bacteria *Bacillus cereus* incubated in nutrient broth at 37°C, based on its optical density observed at 600 nm wavelength over 24 hour period (n = 3)

Incubation duration (hr)	Optical Density (OD)			Mean	SD	%CV
	1	2	3			
0	0.10	0.12	0.11	0.11	0.04	36.36
1	0.24	0.21	0.24	0.23	0.01	3.12
2	0.45	0.42	0.46	0.44	0.01	2.75
3	0.64	0.64	0.64	0.64	0.01	2.08
4	0.84	0.84	0.82	0.83	0.02	2.68
5	1.02	1.00	1.01	1.01	0.01	0.66
6	1.25	1.21	1.24	1.23	0.01	1.17
7	1.45	1.44	1.42	1.44	0.02	1.30
8	1.52	1.51	1.52	1.52	0.01	0.80
9	1.55	1.52	1.57	1.55	0.02	1.00
10	1.58	1.56	1.58	1.57	0.01	0.73
12	1.63	1.63	1.63	1.63	0.02	1.32
14	1.68	1.66	1.68	1.67	0.01	0.64
16	1.63	1.65	1.64	1.64	0.01	0.77
18	1.63	1.67	1.64	1.65	0.01	0.47
20	1.64	1.67	1.65	1.66	0.01	0.74
22	1.65	1.66	1.66	1.66	0.02	0.92
24	1.64	1.65	1.66	1.65	0.01	0.83

Copyright by Mahidol University

Table A-2 Growth of bacteria *Bacillus cereus* incubated in nutrient broth at 37°C, based on its viable cell counts (CFUs/ml) over 24 hour period (n = 3)

Incubation duration (hr.)	Bacteria concentration (cell/ml) x 10^7			Mean	SD	%CV
	1	2	3			
0	0.09	0.08	0.12	0.10	0.02	22.31
1	0.53	0.75	0.40	0.56	0.18	31.48
2	1.21	1.36	1.36	1.31	0.08	6.50
3	4.15	4.23	6.11	4.83	1.11	22.93
4	6.63	7.13	6.90	6.89	0.25	3.63
5	11.74	12.35	10.15	11.41	1.13	9.92
6	11.41	12.41	12.42	12.08	0.58	4.79
7	11.75	12.61	11.26	11.87	0.68	5.74
8	12.11	13.04	11.15	12.10	0.95	7.81
9	12.54	13.55	11.15	12.42	1.20	9.70
10	12.45	13.62	12.54	12.87	0.65	5.08
12	12.45	13.00	12.10	12.52	0.45	3.62
14	4.15	5.11	4.10	4.45	0.57	12.75
16	3.22	3.41	2.98	3.20	0.22	6.78
18	1.05	1.24	1.05	1.11	0.11	9.93
20	1.01	1.35	1.59	1.32	0.29	22.13
22	1.15	1.10	1.33	1.19	0.12	10.07
24	1.10	1.41	1.04	1.18	0.20	16.82

Copyright by Mahidol University

Table A-3 Effects of varying pH levels on dehydrogenase activity of *Bacillus cereus*

Exp.	Replicate	inhibit 0 %	pH						inhibit 100 %
			4	5	6	7	8	9	
1	1	0.2558	0.8630	0.7560	0.4050	0.2744	0.3420	1.1882	2.1161
	2	0.2864	0.9980	0.7714	0.3371	0.2453	0.2821	1.0851	2.1157
	3	0.2904	0.9910	0.8618	0.4308	0.2759	0.3516	1.2351	2.1160
	4	0.2464	0.8789	0.8674	0.3897	0.2417	0.3297	1.0714	2.1151
	5	0.2367	0.9974	0.7670	0.3977	0.2518	0.2962	1.1445	2.1155
	6	0.2378	0.9785	0.7694	0.4341	0.2840	0.2930	1.1481	2.1162
	average	0.2589	0.9511	0.7988	0.3991	0.2622	0.3158	1.1454	2.1158
	SD	0.0239	0.0627	0.0513	0.0352	0.0180	0.0290	0.0616	0.0004
	%CV	9.2249	6.5928	6.4157	8.8233	6.8777	9.1796	5.3816	0.0198
		1.8569	1.1646	1.3169	1.7167	1.8536	1.8000	0.9704	
	%inhibit		**37.2790**	**29.0770**	**7.5477**	**0.1760**	**3.0616**	**47.7400**	
2	1	0.3011	0.9375	0.7888	0.3879	0.3305	0.3744	1.0265	2.1632
	2	0.2804	0.9947	0.7716	0.4411	0.3426	0.3453	1.0799	2.1158
	3	0.3655	1.0006	0.8198	0.4042	0.3467	0.3759	1.0647	2.1420
	4	0.3167	0.9375	0.8076	0.3480	0.3411	0.4117	1.0619	2.1294
	5	0.2892	1.1176	0.7883	0.4417	0.2910	0.3518	1.1310	2.1027
	6	0.3112	0.9039	0.8140	0.5949	0.2975	0.4084	1.1001	2.1294
	average	0.3107	0.9820	0.7984	0.4363	0.3249	0.3779	1.0774	2.1304
	SD	0.0300	0.0761	0.0184	0.0853	0.0244	0.0277	0.0357	0.0209
	%CV	9.6688	7.7496	2.3085	19.5496	7.5169	7.3263	3.3174	0.9830
		1.8197	1.1485	1.3321	1.6941	1.8055	1.7525	1.0531	
	% inhibit		**36.8890**	**26.7990**	**6.903**	**0.781**	**3.6947**	**42.1300**	
3	1	0.2914	1.0026	0.7375	0.4533	0.3488	0.3479	1.1299	2.0811
	2	0.3037	1.0014	0.6975	0.4169	0.2915	0.3377	1.1907	2.0941
	3	0.2579	0.9001	0.8864	0.3537	0.3180	0.3031	1.2417	2.0970
	4	0.2772	1.0540	0.7189	0.3869	0.2779	0.4064	1.2487	2.0971
	5	0.3124	0.9010	0.7874	0.4006	0.2923	0.3549	1.0174	2.0861
	6	0.2965	0.9730	0.7983	0.4488	0.2985	0.3674	1.1874	2.0977
	average	0.2899	0.9720	0.7710	0.4100	0.3045	0.3529	1.1693	2.0922
	SD	0.0196	0.0612	0.0687	0.0380	0.0253	0.0341	0.0860	0.0069
	%CV	6.7744	6.2977	8.9052	9.2673	8.3161	9.6616	7.3526	0.3321
		1.8023	1.1202	1.3212	1.6822	1.7877	1.7393	0.9229	
	% inhibit		**37.84908**	**26.69595**	**6.668208**	**0.81284**	**3.498243**	**48.7951**	

Copyright by Mahidol University

Table A-4 Effect of varying salinity levels on dehydrogenase activity of *Bacillus cereus*

Exp.	Rep.	pH									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	**1**	0.2573	0.2345	0.2457	0.2415	0.2464	0.2443	0.2451	0.2664	0.2935	0.3097
	2	0.2354	0.2457	0.2557	0.2457	0.2548	0.2547	0.2501	0.2577	0.2971	0.2987
	3	0.2377	0.2556	0.2458	0.2780	0.2469	0.2474	0.2478	0.2678	0.2641	0.3163
	4	0.2470	0.2587	0.2540	0.2585	0.2489	0.2571	0.2591	0.2693	0.2910	0.3099
	5	0.2458	0.2456	0.2554	0.2157	0.2444	0.2420	0.2540	0.2601	0.3023	0.2841
	6	0.2687	0.2563	0.2356	0.2547	0.2561	0.2540	0.2463	0.2733	0.2784	0.3089
	$\overline{X}$	0.2487	0.2494	0.2487	0.2490	0.2496	0.2499	0.2504	0.2658	0.2877	0.3046
	SD	0.0092	0.0125	0.0079	0.0207	0.0048	0.0053	0.0062	0.0058	0.0141	0.0115
	%CV	3.6977	5.0181	3.1718	8.2998	1.9161	2.1203	2.4729	2.2002	4.8891	3.7848
	% Inhibit	**0.0098**	**0.0499**	**0.0123**	**0.0299**	**0.0598**	**0.0776**	**0.1035**	**0.9264**	**2.1028**	**2.9965**
2	**1**	0.2945	0.2973	0.3457	0.3076	0.3178	0.3150	0.3151	0.3179	0.3451	0.3497
	2	0.3057	0.3054	0.2957	0.3190	0.3189	0.3118	0.3001	0.3298	0.3421	0.3854
	3	0.3356	0.2977	0.3178	0.3258	0.3020	0.3246	0.3438	0.3268	0.3584	0.3493
	4	0.3080	0.3137	0.2940	0.3010	0.3176	0.3150	0.2991	0.3605	0.3478	0.3994
	5	0.3051	0.3458	0.2954	0.3054	0.3157	0.3142	0.2941	0.3280	0.3378	0.3539
	6	0.3160	0.3087	0.3156	0.3120	0.2984	0.2940	0.3263	0.3454	0.3547	0.3597
	$\overline{X}$	0.3108	0.3114	0.3107	0.3118	0.3117	0.3124	0.3131	0.3347	0.3477	0.3662
	SD	0.0180	0.0140	0.0202	0.0092	0.0091	0.0192	0.0100	0.0155	0.0077	0.0211
	%CV	5.7884	4.4818	6.4970	2.9503	2.9078	6.1512	3.2084	4.6159	2.2267	5.7565
	% inhibit	**0.0073**	**0.0412**	**0.0009**	**0.0613**	**0.0577**	**0.0961**	**0.1319**	**1.3216**	**2.0314**	**3.0455**
3	**1**	0.2945	0.2973	0.3457	0.3076	0.3178	0.3150	0.3151	0.3179	0.3451	0.3497
	2	0.3057	0.3054	0.2957	0.3190	0.3189	0.3118	0.3001	0.3298	0.3421	0.3854
	3	0.3356	0.2977	0.3178	0.3258	0.3020	0.3246	0.3438	0.3268	0.3584	0.3493
	4	0.3080	0.3137	0.2940	0.3010	0.3176	0.3150	0.2991	0.3605	0.3478	0.3994
	5	0.3051	0.3458	0.2954	0.3054	0.3157	0.3142	0.2941	0.3280	0.3378	0.3539
	6	0.3160	0.3087	0.3156	0.3120	0.2984	0.2940	0.3263	0.3454	0.3547	0.3597
	$\overline{X}$	0.3108	0.3114	0.3107	0.3118	0.3117	0.3124	0.3131	0.3347	0.3477	0.3662
	SD	0.0180	0.0140	0.0202	0.0092	0.0091	0.0192	0.0100	0.0155	0.0077	0.0211
	%CV	5.7884	4.4818	6.4970	2.9503	2.9078	6.1512	3.2084	4.6159	2.2267	5.7565
	% inhibit	**0.0073**	**0.0412**	**0.0009**	**0.0613**	**0.0577**	**0.0961**	**0.1319**	**1.3216**	**2.0314**	**3.0455**

Copyright by Mahidol University

Table A-5 Heavy metal contents of sediment samples collected from Thale Noi and its tributaries (Pollution Control Department, 2006)

Sampling station	Concentration (mg/kg)								
	Cu	Ni	Pb	Fe	Mn	Zn	Cd	Cr	Hg
SK 1	0.05	12.20	20.86	17,514	463.00	65.30	0.59	46.03	0.045
SK 2	0.04	18.30	20.61	32,820	463.00	60.68	0.62	70.72	0.044
SK 3	7.60	11.20	23.10	13,938	133.00	35.8	0.45	46.10	0.094
PAC 1	3.35	1.65	7.88	7,979	68.80	22.3	0.54	20.90	0.036
PAC 2	7.05	6.53	14.50	12,000	651.00	29.01	0.36	28.00	0.078
Reference Sediment.[1]	*2.00*	*0.36*	*18.40*	-	-	*16.70*	*0.24*	*<0.10*	-
Sediment Standard.[2]	*390*	-	*450*	-	-	*410*	*5.1*	*260*	*0.41*

[1] *Martin, et al. (2003)*

[2] *www.epa.gov (2548)*

Copyright by Mahidol University

Table A-6 Heavy metal contents of sediment samples collected from Thale Luang and its tributaries (Pollution Control Department, 2006)

Sampling station	Concentration (mg/kg)								
	Cu	Ni	Pb	Fe	Mn	Zn	Cd	Cr	Hg
SK4	9.12	0.15	25.60	17,628	149.00	48.10	0.51	59.60	0.05
SK5	5.56	8.72	25.80	19,353	474.00	36.50	0.56	40.52	0.08
SK6	2.91	1.66	11.40	4,327	84.20	19.00	0.51	16.50	0.71
SK7	0.57	< 0.75	3.21	1,708	10.37	5.54	0.54	13.00	0.04
RNC1	9.53	14.70	21.80	17,967	281.00	71.60	0.51	53.60	0.09
RNC2	87.9	45.80	176.00	29,650	417.00	323.00	1.26	75.40	0.26
MKC1	3.48	7.42	13.20	13,038	1,109.00	27.50	0.56	34.10	0.05
MKC2	8.75	18.80	24.30	25,990	859.00	60.42	0.46	63.90	0.06
TKC1	15.3	15.30	21.30	17,936	280.10	64.70	0.54	54.20	0.11
TKC2	8.88	12.10	15.90	14,216	128.00	49.20	0.51	43.10	0.08
NTC1	8.49	6.13	28.20	20,299	302.00	31.40	0.63	39.10	0.12
NTC2	7.81	5.68	24.01	13,916	166.00	38.50	0.61	30.27	0.08
TNC1	13.5	9.98	24.70	12,918	396.00	30.56	0.73	39.30	0.02
TNC2	9.56	4.82	18.50	15,281	3,587.00	49.40	0.63	42.02	0.08
TCC1	4.57	10.57	28.20	13,776	40.93	25.50	0.44	49.10	0.08
TCC2	0.28	< 0.750	2.03	1,241	17.50	4.05	0.54	10.32	0.02
Reference Sediment.[1]	*2.00*	*0.36*	*18.40*	-	-	*16.70*	*0.24*	*<0.10*	-
Sediment Standard.[2]	*390*	-	*450*	-	-	*410*	*5.1*	*260*	*0.41*

[1] *Martin, et al. (2003)*

[2] *www.epa.gov (2548)*

Copyright by Mahidol University

Table A-7 Heavy metal contents of sediment samples collected from Songkhla Lake and its tributaries (Pollution Control Department, 2006)

Sampling station	Concentration (mg/kg)								
	Cu	**Ni**	**Pb**	**Fe**	**Mn**	**Zn**	**Cd**	**Cr**	**Hg**
SK8	24.03	13.80	41.60	22,856	378.00	63.90	0.67	60.90	0.09
SK9	15.90	10.16	24.50	21,592	159.00	51.64	0.62	50.01	0.05
SK10	15.40	8.84	23.10	19,546	77.60	40.62	1.04	47.80	0.15
SK11	23.50	17.40	21.50	24,044	848.00	46.70	0.67	50.50	0.07
SK12	20.49	9.39	25.40	17,796	316.00	40.74	0.80	37.90	0.09
SK13	91.50	11.40	67.03	18,624	132.00	248.00	1.87	48.80	0.21
SK14	42.40	8.54	40.38	17,510	317.00	87.20	0.55	42.70	0.06
SK15	9.14	9.20	19.90	17,212	327.00	40.65	0.55	40.62	0.15
PBC1	5.22	5.39	30.95	7,019	60.76	27.90	0.63	34.80	0.01
PBC2	1.36	1.83	8.67	2,984	26.30	11.30	0.54	21.20	0.08
PRC1	0.76	< 0.75	3.40	1,358	15.40	6.82	0.51	11.80	0.04
PRC2	0.41	< 0.75	4.94	1,168	14.60	6.27	0.57	10.98	0.03
PMC1	5.32	3.02	20.00	7,666	99.70	28.60	0.52	21.60	0.07
PMC2	9.26	0.97	9.97	5,113	124.00	22.07	0.53	14.90	0.05
UTC1	11.20	7.37	15.10	13,842	145.00	65.30	0.73	34.20	0.09
UTC2	8.35	6.60	12.60	13,321	144.00	64.80	0.64	38.20	0.09
***Reference Sediment.*[1]**	*2.00*	*0.36*	*18.40*	-	-	*16.70*	*0.24*	*<0.10*	-
***Std.*[2]**	*390*	-	*450*	-	*410*	*5.1*	*260*	-	*0.41*

[1] *Martin, et al. (2003)*

[2] *www.epa.gov (2548)*

Copyright by Mahidol University

APPENDIX B

Statistical analysis

Table B-1 Statistical comparison on effects of varying pH levels on dehydrogynase activity of *Bacillus cereus* using ANOVA analysis at α = 0.05

One way

Descriptives

INHIBIT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
4	3	37.3391	.48279	.27874	36.1398	38.5384	36.89	37.85
5	3	27.5239	1.34601	.77712	24.1803	30.8676	26.70	29.08
6	3	7.0397	.45540	.26293	5.9084	8.1709	6.67	7.55
7	3	.5900	.35895	.20724	-.3017	1.4817	.18	.81
8	3	3.4182	.32403	.18708	2.6133	4.2231	3.06	3.69
9	3	46.2223	3.58242	2.06831	37.3231	55.1216	42.13	48.80
Total	18	20.3555	18.18530	4.28632	11.3122	29.3989	.18	48.80

ANOVA

INHIBIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5591.345	5	1118.269	437.971	.000
Within Groups	30.640	12	2.553		
Total	5621.984	17			

Copyright by Mahidol University

Homogeneous Subsets

INHIBIT

			Subset for alpha = .05				
	PH	N	1	2	3	4	5
Duncan[a]	7	3	.590003				
	8	3	3.418187				
	6	3		7.039654			
	5	3			27.523923		
	4	3				37.339091	
	9	3					46.222347
	Sig.		.051	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Copyright by Mahidol University

Post Hoc Tests

Multiple Comparisons

Dependent Variable: INHIBIT

LSD

(I) PH	(J) PH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4	5	9.815168*	1.3046830	.000	6.972508	12.657828
	6	30.299437*	1.3046830	.000	27.456777	33.142097
	7	36.749088*	1.3046830	.000	33.906427	39.591748
	8	33.920904*	1.3046830	.000	31.078244	36.763564
	9	-8.883256*	1.3046830	.000	-11.725916	-6.040596
5	4	-9.815168*	1.3046830	.000	-12.657828	-6.972508
	6	20.484269*	1.3046830	.000	17.641609	23.326929
	7	26.933920*	1.3046830	.000	24.091259	29.776580
	8	24.105736*	1.3046830	.000	21.263076	26.948396
	9	-18.698424*	1.3046830	.000	-21.541084	-15.855764
6	4	-30.299437*	1.3046830	.000	-33.142097	-27.456777
	5	-20.484269*	1.3046830	.000	-23.326929	-17.641609
	7	6.449651*	1.3046830	.000	3.606991	9.292311
	8	3.621467*	1.3046830	.017	.778807	6.464128
	9	-39.182693*	1.3046830	.000	-42.025353	-36.340033
7	4	-36.749088*	1.3046830	.000	-39.591748	-33.906427
	5	-26.933920*	1.3046830	.000	-29.776580	-24.091259
	6	-6.449651*	1.3046830	.000	-9.292311	-3.606991
	8	-2.828183	1.3046830	.051	-5.670843	.014477
	9	-45.632344*	1.3046830	.000	-48.475004	-42.789684
8	4	-33.920904*	1.3046830	.000	-36.763564	-31.078244
	5	-24.105736*	1.3046830	.000	-26.948396	-21.263076
	6	-3.621467*	1.3046830	.017	-6.464128	-.778807
	7	2.828183	1.3046830	.051	-.014477	5.670843
	9	-42.804161*	1.3046830	.000	-45.646821	-39.961500
9	4	8.883256*	1.3046830	.000	6.040596	11.725916
	5	18.698424*	1.3046830	.000	15.855764	21.541084
	6	39.182693*	1.3046830	.000	36.340033	42.025353
	7	45.632344*	1.3046830	.000	42.789684	48.475004
	8	42.804161*	1.3046830	.000	39.961500	45.646821

*. The mean difference is significant at the .05 level.

Copyright by Mahidol University

Table B-2 Statistical comparison on effects of varying elutriate dilution, as well as whole sediment on dehydrogynase activity of *Bacillus cereus* using ANOVA analysis at $\alpha = 0.05$

One way

ANOVA

INHIBIT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51895.73	4	12973.932	17.261	.000
Within Groups	135296.9	180	751.649		
Total	187192.6	184			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: INHIBIT

	(I) DILUTION	(J) DILUTION	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	25	50	1.1246	6.37414	.860	-11.4531	13.7023
		75	4.7243	6.37414	.460	-7.8533	17.3020
		100	1.6549	6.37414	.795	-10.9228	14.2325
		whole	-39.8125*	6.37414	.000	-52.3902	-27.2348
	50	25	-1.1246	6.37414	.860	-13.7023	11.4531
		75	3.5997	6.37414	.573	-8.9779	16.1774
		100	.5303	6.37414	.934	-12.0474	13.1079
		whole	-40.9371*	6.37414	.000	-53.5148	-28.3594
	75	25	-4.7243	6.37414	.460	-17.3020	7.8533
		50	-3.5997	6.37414	.573	-16.1774	8.9779
		100	-3.0695	6.37414	.631	-15.6471	9.5082
		whole	-44.5368*	6.37414	.000	-57.1145	-31.9592
	100	25	-1.6549	6.37414	.795	-14.2325	10.9228
		50	-.5303	6.37414	.934	-13.1079	12.0474
		75	3.0695	6.37414	.631	-9.5082	15.6471
		whole	-41.4674*	6.37414	.000	-54.0450	-28.8897
	whole	25	39.8125*	6.37414	.000	27.2348	52.3902
		50	40.9371*	6.37414	.000	28.3594	53.5148
		75	44.5368*	6.37414	.000	31.9592	57.1145
		100	41.4674*	6.37414	.000	28.8897	54.0450

*. The mean difference is significant at the .05 level.

Copyright by Mahidol University

Homogeneous Subsets

INHIBIT

	DILUTION	N	Subset for alpha = .05	
			1	2
Duncan[a]	75	37	-.3746	
	100	37	2.6949	
	50	37	3.2251	
	25	37	4.3497	
	whole	37		44.1622
	Sig.		.507	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 37.000.

Copyright by Mahidol University

Table B-3 Spearman rank correlation results presenting possible association between toxic effects on dehydrogynase activity of *Bacillus cereus* of varying elutriate dilution, as well as whole sediment and chemical and physical properties in each respective sediment sample

Nonparametric Correlations

Correlations

			TOX25	TOX50	TOX75	TOX100	TOXWHOLE	MC	CEC	CONDUCT	SALINITY	OM	PH
Spearman's rho	TOX25	Correlation Coefficient	1.000	.803**	.446**	.594**	.204	-.020	.111	-.199	-.179	-.055	-.239
		Sig. (1-tailed)	.	.000	.003	.000	.113	.453	.256	.119	.145	.373	.077
		N	37	37	37	37	37	37	37	37	37	37	37
	TOX50	Correlation Coefficient	.803**	1.000	.694**	.851**	.158	-.100	.058	-.153	-.135	-.118	-.192
		Sig. (1-tailed)	.000	.	.000	.000	.176	.277	.367	.182	.213	.243	.127
		N	37	37	37	37	37	37	37	37	37	37	37
	TOX75	Correlation Coefficient	.446**	.694**	1.000	.665**	-.016	.004	.075	.069	.068	.013	.077
		Sig. (1-tailed)	.003	.000	.	.000	.463	.491	.329	.343	.345	.469	.326
		N	37	37	37	37	37	37	37	37	37	37	37
	TOX10	Correlation Coefficient	.594**	.851**	.665**	1.000	.271	-.137	-.042	-.251	-.234	-.169	-.231
		Sig. (1-tailed)	.000	.000	.000	.	.053	.209	.403	.067	.082	.159	.084
		N	37	37	37	37	37	37	37	37	37	37	37
	TOXW	Correlation Coefficient	.204	.158	-.016	.271	1.000	-.514**	-.248	-.425**	-.432**	-.560**	-.032
		Sig. (1-tailed)	.113	.176	.463	.053	.	.001	.070	.004	.004	.000	.426
		N	37	37	37	37	37	37	37	37	37	37	37
	MC	Correlation Coefficient	-.020	-.100	.004	-.137	-.514**	1.000	.397**	.600**	.585**	.636**	-.040
		Sig. (1-tailed)	.453	.277	.491	.209	.001	.	.008	.000	.000	.000	.406
		N	37	37	37	37	37	37	37	37	37	37	37
	CEC	Correlation Coefficient	.111	.058	.075	-.042	-.248	.397**	1.000	.054	.057	.253	-.047
		Sig. (1-tailed)	.256	.367	.329	.403	.070	.008	.	.376	.369	.065	.392
		N	37	37	37	37	37	37	37	37	37	37	37
	COND	Correlation Coefficient	-.199	-.153	.069	-.251	-.425**	.600**	.054	1.000	.990**	.353**	.457**
		Sig. (1-tailed)	.119	.182	.343	.067	.004	.000	.376	.	.000	.016	.002
		N	37	37	37	37	37	37	37	37	37	37	37
	SALIN	Correlation Coefficient	-.179	-.135	.068	-.234	-.432**	.585**	.057	.990**	1.000	.353**	.426**
		Sig. (1-tailed)	.145	.213	.345	.082	.004	.000	.369	.000	.	.016	.004
		N	37	37	37	37	37	37	37	37	37	37	37
	OM	Correlation Coefficient	-.055	-.118	.013	-.169	-.560**	.636**	.253	.353**	.353**	1.000	-.248
		Sig. (1-tailed)	.373	.243	.469	.159	.000	.000	.065	.016	.016	.	.069
		N	37	37	37	37	37	37	37	37	37	37	37
	PH	Correlation Coefficient	-.239	-.192	.077	-.231	-.032	-.040	-.047	.457**	.426**	-.248	1.000
		Sig. (1-tailed)	.077	.127	.326	.084	.426	.406	.392	.002	.004	.069	.
		N	37	37	37	37	37	37	37	37	37	37	37

**. Correlation is significant at the .01 level (1-tailed).

*. Correlation is significant at the .05 level (1-tailed).

Copyright by Mahidol University

Table B-4 Spearman rank correlation results presenting possible association between toxic effects on dehydrogynase activity of *Bacillus cereus* of varying elutriate dilution, as well as whole sediment and texture characteristics in each respective sediment sample

Nonparametric Correlations

Correlations

			TOX25	TOX50	TOX75	TOX100	TOXWHOLE	SAND	SILT	CLAY
Spearman's rho	TOX25	Correlation Coefficien	1.000	.803**	.446**	.594**	.204	.115	-.177	-.182
		Sig. (1-tailed)	.	.000	.003	.000	.113	.250	.148	.140
		N	37	37	37	37	37	37	37	37
	TOX50	Correlation Coefficien	.803**	1.000	.694**	.851**	.158	.142	-.157	-.121
		Sig. (1-tailed)	.000	.	.000	.000	.176	.201	.177	.238
		N	37	37	37	37	37	37	37	37
	TOX75	Correlation Coefficien	.446**	.694**	1.000	.665**	-.016	.030	-.111	-.094
		Sig. (1-tailed)	.003	.000	.	.000	.463	.430	.256	.291
		N	37	37	37	37	37	37	37	37
	TOX10	Correlation Coefficien	.594**	.851**	.665**	1.000	.271	.011	-.079	.001
		Sig. (1-tailed)	.000	.000	.000	.	.053	.474	.320	.498
		N	37	37	37	37	37	37	37	37
	TOXW HOLE	Correlation Coefficien	.204	.158	-.016	.271	1.000	.174	-.028	-.246
		Sig. (1-tailed)	.113	.176	.463	.053	.	.152	.434	.071
		N	37	37	37	37	37	37	37	37
	SAND	Correlation Coefficien	.115	.142	.030	.011	.174	1.000	-.807**	-.700*
		Sig. (1-tailed)	.250	.201	.430	.474	.152	.	.000	.000
		N	37	37	37	37	37	37	37	37
	SILT	Correlation Coefficien	-.177	-.157	-.111	-.079	-.028	-.807**	1.000	.329*
		Sig. (1-tailed)	.148	.177	.256	.320	.434	.000	.	.023
		N	37	37	37	37	37	37	37	37
	CLAY	Correlation Coefficien	-.182	-.121	-.094	.001	-.246	-.700**	.329*	1.000
		Sig. (1-tailed)	.140	.238	.291	.498	.071	.000	.023	.
		N	37	37	37	37	37	37	37	37

**. Correlation is significant at the .01 level (1-tailed).

*. Correlation is significant at the .05 level (1-tailed).

Copyright by Mahidol University

Table B-5 Spearman rank correlation results presenting possible association between toxic effects on dehydrogynase activity of *Bacillus cereus* of varying elutriate dilution, as well as whole sediment and heavy metal contents in each respective sediment sample

Nonparametric Correlations

Correlations

			TOX25	TOX50	TOX75	TOX100	TOXWHOLE	CU	NI	PB	FE	MN	ZN	CD	CR	HG
Spearman's rho	TOX25	Correlation Coefficient	1.000	.803*	.446*	.594*	.204	-.223	.084	-.114	-.122	.112	-.040	.153	-.106	-.111
		Sig. (1-tailed)	.	.000	.003	.000	.113	.093	.322	.252	.236	.254	.408	.183	.265	.256
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	TOX50	Correlation Coefficient	.803*	1.000	.694*	.851*	.158	-.068	-.092	-.015	-.201	-.091	-.070	.250	-.176	.068
		Sig. (1-tailed)	.000	.	.000	.000	.176	.345	.305	.465	.116	.296	.341	.068	.149	.345
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	TOX75	Correlation Coefficient	.446*	.694*	1.000	.665*	-.016	.077	.088	.006	.033	.125	.101	.168	-.014	.239
		Sig. (1-tailed)	.003	.000	.	.000	.463	.325	.313	.486	.423	.231	.276	.161	.467	.077
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	TOX10	Correlation Coefficient	.594*	.851*	.665*	1.000	.271	-.087	-.080	-.010	-.185	-.076	-.125	.139	-.175	.054
		Sig. (1-tailed)	.000	.000	.000	.	.053	.304	.329	.476	.137	.328	.231	.206	.150	.375
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	TOXW	Correlation Coefficient	.204	.158	-.016	.271	1.000	-.266	-.383*	-.259	-.315*	.034	-.409*	-.154	-.432*	-.156
		Sig. (1-tailed)	.113	.176	.463	.053	.	.056	.014	.061	.029	.421	.006	.181	.004	.178
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	CU	Correlation Coefficient	-.223	-.068	.077	-.087	-.266	1.000	.353*	.612*	.573*	.377*	.648*	.479*	.538*	.521*
		Sig. (1-tailed)	.093	.345	.325	.304	.056	.	.022	.000	.000	.011	.000	.001	.000	.000
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	NI	Correlation Coefficient	.084	-.092	.088	-.080	-.383*	.353*	1.000	.405*	.713*	.425*	.619*	.197	.791*	.176
		Sig. (1-tailed)	.322	.305	.313	.329	.014	.022	.	.010	.000	.007	.000	.135	.000	.164
		N	33	33	33	33	33	33	33	33	33	33	33	33	33	33
	PB	Correlation Coefficient	-.114	-.015	.006	-.010	-.259	.612*	.405*	1.000	.687*	.391*	.567*	.378*	.696*	.309*
		Sig. (1-tailed)	.252	.465	.486	.476	.061	.000	.010	.	.000	.008	.000	.010	.000	.032
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	FE	Correlation Coefficient	-.122	-.201	.033	-.185	-.315*	.573*	.713*	.687*	1.000	.588*	.750*	.414*	.877*	.403*
		Sig. (1-tailed)	.236	.116	.423	.137	.029	.000	.000	.000	.	.000	.000	.005	.000	.007
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	MN	Correlation Coefficient	.112	-.091	.125	-.076	.034	.377*	.425*	.391*	.588*	1.000	.563*	.236	.537*	.183
		Sig. (1-tailed)	.254	.296	.231	.328	.421	.011	.007	.008	.000	.	.000	.080	.000	.140
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	ZN	Correlation Coefficient	-.040	-.070	.101	-.125	-.409*	.648*	.619*	.567*	.750*	.563*	1.000	.405*	.771*	.451*
		Sig. (1-tailed)	.408	.341	.276	.231	.006	.000	.000	.000	.000	.000	.	.006	.000	.003
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	CD	Correlation Coefficient	.153	.250	.168	.139	-.154	.479*	.197	.378*	.414*	.236	.405*	1.000	.190	.191
		Sig. (1-tailed)	.183	.068	.161	.206	.181	.001	.135	.010	.005	.080	.006	.	.130	.128
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	CR	Correlation Coefficient	-.106	-.176	-.014	-.175	-.432*	.538*	.791*	.696*	.877*	.537*	.771*	.190	1.000	.338*
		Sig. (1-tailed)	.265	.149	.467	.150	.004	.000	.000	.000	.000	.000	.000	.130	.	.020
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37
	HG	Correlation Coefficient	-.111	.068	.239	.054	-.156	.521*	.176	.309*	.403*	.183	.451*	.191	.338*	1.000
		Sig. (1-tailed)	.256	.345	.077	.375	.178	.000	.164	.032	.007	.140	.003	.128	.020	.
		N	37	37	37	37	37	37	33	37	37	37	37	37	37	37

**. Correlation is significant at the .01 level (1-tailed).

*. Correlation is significant at the .05 level (1-tailed).

Copyright by Mahidol University

Table B-6 Toxicity ranking of 37 sampling stations in Songkhla Lake Basin resulting from centroid hierarchical cluster analysis

Cluster

Case Processing Summary[a,b]

Cases					
Valid		Missing		Total	
N	Percent	N	Percent	N	Percent
37	100.0	0	.0	37	100.0

a. Squared Euclidean Distance used

b. Centroid Linkage

Copyright by Mahidol University

Centroid Linkage

Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	11	12	.065	0	0	11
2	10	14	.348	0	0	3
3	10	19	1.634	2	0	4
4	10	36	4.579	3	0	5
5	10	37	5.131	4	0	6
6	10	17	6.692	5	0	7
7	2	10	6.677	0	6	17
8	13	15	9.792	0	0	10
9	3	6	15.353	0	0	14
10	13	29	23.571	8	0	12
11	11	32	25.582	1	0	15
12	13	16	26.653	10	0	14
13	33	35	27.280	0	0	17
14	3	13	44.841	9	12	18
15	11	23	59.907	11	0	16
16	11	22	57.389	15	0	19
17	2	33	61.111	7	13	18
18	2	3	88.020	17	14	24
19	4	11	125.450	0	16	20
20	4	31	146.240	19	0	25
21	1	34	148.548	0	0	26
22	5	30	191.010	0	0	26
23	9	18	200.708	0	0	30
24	2	28	240.719	18	0	25
25	2	4	285.973	24	20	27
26	1	5	557.127	21	22	27
27	1	2	451.356	26	25	31
28	20	21	612.432	0	0	30
29	24	25	1159.717	0	0	34
30	9	20	1333.609	23	28	33
31	1	8	2290.641	27	0	33
32	7	27	2874.942	0	0	34
33	1	9	3825.585	31	30	35
34	7	24	4650.121	32	29	35
35	1	7	6398.551	33	34	36
36	1	26	13293.782	35	0	0

Copyright by Mahidol University

Cluster analyze

Study site	Elutriate				Whole sediment	Cluster (elutriate)	Cluster (whole)
	25%	50%	75%	100%			
SK1	21.46	0.27	-1.18	-1.19	-3.4161	1	1
SK2	1.57	-1.55	-0.82	-0.02	39.9844	1	2
SK3	2.94	5.24	7.44	8.38	-14.666	1	1
SK4	-10.32	-4.86	-7.06	-9.46	-21.046	1	1
SK5	17.05	11.28	9.97	15.42	90.2744	1	4
SK6	4.61	7.49	6.48	5.81	90.9809	1	4
SK7	47.6	41.42	38.78	10.54	39.1339	2	2
SK8	0.13	12.79	27.57	37.77	3.1181	1	1
SK9	-22.52	-25.32	-24.3	-22.25	7.6841	1	1
SK10	1.1	0.72	0.62	0.54	37.663	1	2
SK11	-1.34	-8.53	-7.95	-6.92	34.4271	1	2
SK12	-1.34	-8.32	-7.84	-7.01	14.0819	1	1
SK13	-0.94	4.18	5.81	5.86	1.8934	1	1
SK14	0.76	0.31	0.41	0.67	92.394	1	4
SK15	0.4	2.71	5.3	8.22	50.3156	1	2
NTC1	-0.4	-0.26	2.03	9.69	96.5303	1	4
NTC2	-1.59	-0.74	-1.06	0.87	59.6187	1	2
TCC1	-13.63	-16.99	-17.55	-19.61	2.2744	1	1
TCC2	0.72	0.01	-0.62	0.39	0.7752	1	1
PMC1	-44.5	-42.57	-46.12	-43.99	21.8139	1	1
PMC2	-33.76	-36.37	-32.11	-27.8	49.7669	1	2
MKC1	5.66	-4.14	-8.98	-10.99	97.2719	1	4
MKC2	0.45	-9.29	-11.46	-12.98	49.017	1	2
PBC1	46.04	56.48	-29.08	48.52	93.8044	3	4
PBC2	36.07	30.77	-20.5	30.47	90.9317	3	4
TNC1	22.65	23.52	76.78	82.21	15.0102	4	1
TNC2	47.06	69.26	44.83	55.96	82.88	2	4
PRC1	9.63	1.03	-6.57	12.98	92.24	1	4
PRC2	-0.4	2.58	1.16	5.17	92.76	1	4
RNC1	8.65	2.21	6.37	20.44	23.98	1	1
RNC2	7.17	0.9	-4.39	-6.57	-1.72	1	1
TKC1	-0.34	-3.97	-5.78	-7.53	76.05	1	4
TKC2	-6.21	-5.24	-4.00	5.58	39.80	1	2

Copyright by Mahidol University

Cluster analyze (continued)

Study site	Elutriate				Whole	Cluster elutriate	Cluster whole
	25%	50%	75%	100%			
UTC1	23.97	10.2	4.88	-3.82	2.83	1	1
UTC2	-2.46	-3.39	-3.19	2.57	59.25	1	2
PAC1	-0.99	-0.32	0.96	0.76	87.62	1	4
PAC2	0.31	0.13	0.32	2.85	96.66	1	4

Copyright by Mahidol University

BIOGRAPHY

NAME : Miss Bongkoch Tongsadayu

DATE OF BIRTH : September 23rd, 1982

PLACE OF BIRTH : Chonburi, Thailand

INSTITUTIONS ATTENDED : Burapha University, 2002:
Bachelor of Science (Aquatic Science)

Mahidol University, 2007:
Master of Science
(Technology of Environmental Management)

HOME ADDRESS : 103/11 Moo 2 Prayasajja Road, Samed, Meung, Chonburi 20000, Thailand.
E-mail: b_aquatic@hotmail.com

Copyright by Mahidol University