ความสัมพันธ์ระหว่างสมบัติทางเคมีและความเป็นพิษของน้ำชะจากพื้นฝังกลบมูลฝอยที่ระดับการบำบัดต่างกัน

นางสาวสุธิดา ทีปรักษพันธุ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHEMICAL CHARACTERISTICS AND BIO-TOXICITY RELATIONSHIP IN LEACHATE FROM MUNICIPAL SOLID WASTE LANDFILL AT DIFFERENT DEGREE OF TREATMENT

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Thesis Title	CHEMICAL CHARACTERISTICS AND BIO-TOXICITY RELATIONSHIP IN LEACHATE FROM MUNICIPAL SOLID WASTE LANDFILL AT DIFFERENT DEGREE OF TREATMENT
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งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพการบำบัดน้ำชะมูลฝอยในระดับการบำบัดต่างกัน ของระบบบำบัดน้ำชะมูลฝอยจากพื้นที่ฝังกลบขยะมูลซึ่งประกอบด้วย ระบบตกตะกอนเคมี, ระบบกรองผ่านถังกรองทราย, ระบบกรองผ่านเมมเบรนขนาด 5 ไมโครเมตร และระบบรีเวอร์ส ออสโมซิส (RO) โดยการหาความสัมพันธ์ระหว่างคุณสมบัติทางเคมีของน้ำชะมูลฝอยโดยตรวจพารามิเตอร์ตามมาตรฐานน้ำทิ้งอุตสาหกรรมและการตรวจหาสารอินทรีย์ที่เป็นพิษโดยเครื่องแก๊สโครมาโทรกราฟี แมสสเปกโทรมิเตอร์ (GC-MS) ร่วมกับการตรวจความเป็นพิษต่อสิ่งมีชีวิต ซึ่งใช้วิธีประเมินการตายอย่างเฉียบพลัน (Acute test) เพื่อหาค่า *LC50* ที่ 48 ชั่วโมงของไรแดง *(Moina macrocopa)* และที่ 96 ชั่วโมงสำหรับ ปลานิล *(Oreochromis niloticus)* และปลาไน *(Cypinus carpio)* และตรวจระดับการถูกทำลายของดีเอ็นเอในเซลล์เม็ดเลือดแดงของปลาทั้งสองชนิดโดยวิธีโคเมท (comet assay)

ผลการทดลองพบว่า ประสิทธิภาพของการกำจัดในทุกพารามิเตอร์หลังผ่านระบบรีเวอร์ส ออสโมซิสมีค่าเฉลี่ยร้อยละ 95.1 ในขณะที่กลุ่มสารอินทรีย์ที่เป็นพิษมีค่าเฉลี่ยร้อยละ 99.9 การทดสอบความเป็นพิษฉับพลันพบว่า ความเป็นพิษแนวโน้มลดลงในทุกระดับการบำบัดจนไม่ปรากฏการตายในน้ำที่ผ่านระบบรีเวอร์ส ออสโมซิสในขณะที่การทดสอบระดับการถูกทำลายของ DNA พบว่า ระบบบำบัดสามารถลดระดับการถูกทำลายของ DNA ที่ 7 และ 14 วัน ของปลานิลจากร้อยละ 11.74 และ 24.28 เหลือร้อยละ 1.04 และ 0.95 ในขณะที่ปลาไนจากร้อยละ 11.56 และ 17.18 เหลือ 1.06 และ 1.08 หลังจากผ่านระบบรีเวอร์ส ออสโมซิส

สาขาวิชา.....การจัดการสิ่งแวดล้อม....ลายมือชื่อนิสิต .....สุธิดา ทีปรักษพันธุ์.....

ปีการศึกษา.....2553.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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SUTHIDA THEEPHARAKSAPAN: CHEMICAL CHARACTERISTICS AND BIO-TOXICITY RELATIONSHIP LEACHATE FROM MUNICIPAL LANDFILL WITH DIFFERENT DEGREE OF TREATMENT. THESIS ADVISOR: ASSOCIATE PROFESSOR CHART CHIEMCHAISRI, D.Eng., THESIS CO-ADVISOR: ASSOCIATE PROFESSOR WILAI CHIEMCHAISRI, D. Tech. Sc., 102 pp.

This study aims to evaluate the performance of landfill leachate treatment process which utilized chemical coagulation followed by sand filtration, microfiltration (MF) and reverse osmosis (RO) membrane. The chemical characteristics were analyzed follow industrial effluent standard parameters, and toxic organic compounds which were analyzed using gas chromatography mass spectrometer (GC-MS). Simultaneously, acute toxicity tests were determined by LC_{50} of water flea (*Moina macrocopa*) after 48 hour exposure and after 96 hour exposure of Nile Tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*), and genotoxicity were confirmed using comet assay in erythrocyte of fish.

The result found that average chemical parameters removal efficiency of discharged from RO process was 95.1%, whereas the average toxic organic compounds removal was 99.9%. In acute testing, it was found that acute toxicity tends to decrease along the treatment process, until non-appear the mortality in effluent from RO. Furthermore, this treatment could reduce the level of DNA damage of Nile Tilapia and common carp after 7 and 14 exposures. In case of Nile tilapia, the level of DNA damage was reduced from 11.74% and 24.28% into 1.04% and 0.95%, whereas common carp were reduced from 11.56% and 17.18% into 1.06% and 1.08% respectively

Field of Study : Environmental Management Student's Signature Suthida Theepharaksapan
Academic Year : 2010 Advisor's Signature
Co-Advisor's Signature

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Finally, the author takes this opportunity to dedicate this thesis with due respect to my parents and brother for their enduring patience, constant encouragement, and love throughout my entire study.

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NOMENCLATURES

sBOD	=	soluble biochemical oxygen demand
sCOD	=	soluble chemical oxygen demand
C. carpio	=	*Cypinus carpio*
DNA	=	deoxyribonucleic acid
EDTA	=	ethalenediamine teraacetic acid
EC50	=	50% effective concentration
EROD activity	=	ethoxyresorufin-O-deethylase acitivity
FeCl3	=	ferric chloride
GC-MS	=	gas chromatography mass spectrometer
LC50	=	50% lethal concentration
LD50	=	50% dose concentration
LC10	=	10% lethal concentration
LMA	=	low melting point agarose
MF	=	microfiltration
M. macrocopa	=	*moina macrocopa*
O. niloticus	=	*Oreochromis niloticus*
PBS	=	phosphate buffer saline
RO	=	reverse osmosis
SPE	=	solid phase extraction
SSPE	=	sequential solid phase extraction
SCGE	=	single cell gel electrophoresis
TOC	=	total organic carbon
TUs	=	toxic unit
Vtg	=	vitellogenin
XOCs	=	xenobiotic organic compounds

CHAPTER I
INTRODUCTION

1.1 Background

Municipal solid waste disposal in the landfill is the most common solid waste management practice followed throughout the world. Household waste and non-hazardous industrial waste disposed lead to contain a mixture of many chemical compounds generating from the various discarded products. In landfill site, the infiltration water passes through the solid waste mainly generates landfill leachate leaching soluble components and degradation products. Consequently, leachate is a high strength wastewater that contains substantial amounts of dissolved organics, Xenobiotic Organic Compound (XOCs), inorganic salts, ammonia, heavy metals and other toxicants (*Pivato et al., 2005*). They constitute a potential risk to quality of receiving water bodies when are released untreated into the environment.

In order to minimize risk from those substances, effective leachate treatment system utilizing the integration of treatment processes is required. Usually, conventional treatments are being considered as the most appropriate technologies for manipulation and management of high strength effluents like fresh leachate (*Abbas et al., 2009*). However, with the continuous hardening of the discharge standards in most countries and the ageing of landfill sites with more and more stabilized leachate, the advance treatments are suggested to purpose the level of purification until the effluent standard is satisfied. Therefore, recent years, more effective treatments base on advance treatment has be coupled to conventional treatments to comply water quality regulations in most countries, specially reverse osmosis (RO) membrane which was considered as the ultimate treatment step yielding highest pollutant rejection efficiencies *(Renou et al., 2008).*

However, complex environmental mixture of leachate has be still limited database information about the compounds present in leachate, both with respect to their

identity and to the concentrations in MSW leachates, especially toxicity assessment in leachate. These kinds of information require for the evaluation of fate and effect of leachates, and needs to selections the treatment methods and evaluations the performance of treatments.

In many leachate studies, the information available on traditional chemical parameters, but it would be technically impossible to analyze all the contaminants in a leachate. Furthermore, it would be economically unfeasible to analyze all of these chemicals.

Bioassay is the biological toxicity tests which are used to quantify the amount of a substance an organism can be exposed to before adverse effects are observed. In contrast to chemical analysis, it can provide a direct functional response that relates to the overall toxic properties of the mixture of compounds present in the leachate sample without the need for assumptions and extrapolations made from chemical analysis. Recent year, it has been induced to leachate toxicity assessment by several authors using a number of different organisms representing algae, fish and invertebrates (*Kjeldsen et al., 2001*).

In order to provide a more complete and accurate evaluation of discharged leachate and treated leachate from solid waste landfill treatment, this study combine the chemical analyses and bio-toxicity testing of leachate along treatment unit utilizing chemical coagulation, sand filtration, microfiltration (MF) and reverse osmosis (RO) membrane processes, with physico- chemical parameters, toxic organic compounds, acute toxicity and genotoxicity to living organisms, i.e. Water flea (Moina macrocopa), Nile Tilapia (Oreochromis niloticus) and Common Carp (Cypinus carpio).

1.2 Objectives

The main objective of this study is to correlate the chemical characteristics and bio-toxicity in solid waste landfill leachate before and after treatment by utilizing chemical coagulation, sand filtration, microfiltration (MF) and reverse osmosis (RO) membrane processes. In order to achieve the goal, we have divided the main objective into two sub-objectives as follows:

1. To determine chemical characteristics using chemical analysis in raw leachate and treated leachate at different degree of treatment.
2. To determine bio-toxicity of landfill leachate and treated leachate and correlate with chemical parameters and the presence of toxic compounds different of treatment using local bioindicator organisms.

1.3 Scopes of the Study

1. Organism species for the experiment are Water flea (*Moina Macrocopa)*, Nile tilapia (*Oreochromis niloticus)* and Common Carp (*Cyprinus Carpio)*.
2. Both of fish species are tested for 50% lethal concentration (LC_{50}) after 96 hour exposure, and genotoxicity test was conducted to assess deoxyribonucleic acid (DNA) damage using comet assay in erythrocytes after 14 days.
3. Water flea was tested for 50% immobilization (LC_{50}) after 48 hour exposure.
4. All of water samples were collected from the leachate treatment plant in Nonthaburi dump site Thailand.
5. Organic compounds were extracted using solid phase extraction (SPE) method and analyzed using gas chromatography mass spectrometer (GC-MS).

CHAPTER II
LITERATURE REVIEW

2.1 Landfill Leachate

Landfill leachate is a polluting liquid which accumulates beneath a landfill site resulting from the infiltration and percolation of rainfall and moisture in landfill. It contain large amount of pollutants including organic and inorganic matter, and high potential to pollute ground and surface water (*Kjeldsen et al., 2001*). The quality of leachate depends mainly on these factors, following landfill age, quality and quantity of solid waste, biological and chemical processes occurring in the landfill, amount of precipitation and percolation

2.1.1 Leachate Generation

Depending on the geographical and geological nature of a landfill site, leachate may be composed of surface drainage rainfall and moisture content of solid waste that through solid waste and has extracted contaminants. Basic factors influencing leachate generation are climatology of the area, topography, solids, hydrogeology of site, final and vegetation cover and types of waste material in the landfill.

2.1.2 Solid waste Decomposition in Landfill

Generation of the principal landfill gases through the decomposition of solid waste in landfill is accepted that landfill undergo at least five phases of decomposition as illustrated in Figure 2.1. (*King and Eliassen, 1993*)

Phase I – Initial adjustment phase: This phase, biological decomposition occurs under aerobic condition because a certain amount of air is trapped within the landfill

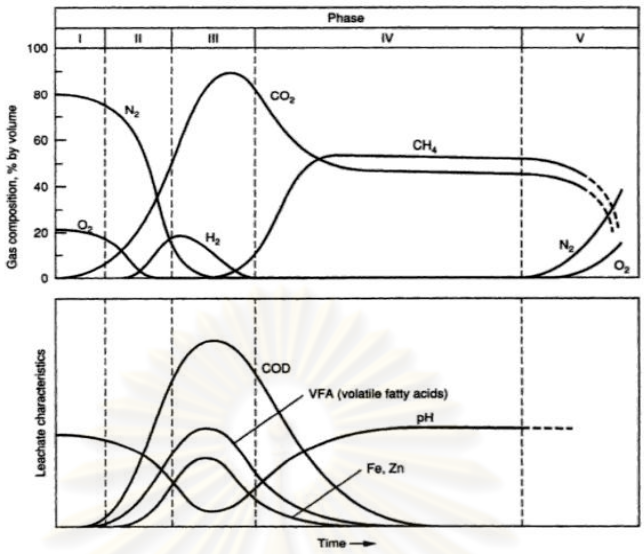

Figure 2.1 generalized phases in the generation of landfill gases

Adopted from Tchobanoglous, 1993

Phase II - Transition phase: Oxygen is depleted and anaerobic conditions begin to develop. As the landfill becomes anaerobic, nitrate and sulfate, which can serve as electron acceptors in biological conversion reactions, are often reduced to nitrogen gas and hydrogen sulfide. In this phase, pH of the leachate, if formed, starts to drop due to the presence of organic acids and the effect of the elevated concentrations of carbon dioxide (CO_2) within the landfill.

Phase III – Acid formation phase: The bacterial activity initiated in Phase II is accelerated with the production of the significant amounts of organic acids and lesser amounts of hydrogen gas. These microorganisms are often identified in the engineering literature as acidulous or acid formers.

Phase IV - Methane Fermentation phase: Methanogenic bacteria convert the acetic acid and hydrogen gas formed by acid formers in the acid phase to methane (CH_4) and CO_2, become more predominant. The microorganisms responsible for this conversion are strict anaerobes and are called methanogenic. Collectively, they are identified in the literature as methadone's or methane formers.

Phase V - Maturation phase: This phase occurs after the readily available biodegradable organic material has been converted to CH_4 and CO_2. During maturation phase, the leachate will often contain humic and fulvic acids, which are difficult to process further biologically.

2.1.3 Leachate Characteristic

During the first few years (< 5 years), the landfill is in acidogenic phase and the leachate is generally referred to as "young" or carbon-based leachate due to the high concentration of organic carbon present. Landfill greater than 10 years old are generally in the methanogenic phase and the leachate generated is referred to as "old" or nitrogen-based leachate (*Mavinic, 1998*). Table 2.1 gives the characteristic of leachate present in acidogenic and methanogenic phases.

The factors affecting the leachate quality is inter-related and affects the overall variance in leachate quality and characterization. The changes in the BOD/COD, COD/TOC, VS/FS and VFA/TOC ratios of leachate are depends greatly on the age of the

Table 2.1 Leachate Characteristic in Acidogenic and Methanogenic Phase in a Landfill

Parameters	**Acidogenic Phase**		**Methanogenic Phase**	
	Range	Average	Range	Average
pH	6.1	4.5 – 7.5	8	7.5 – 9
BOD_5	13000	4000 – 40000	180	20 – 550
COD	22000	6000 – 60000	3000	500 – 4500
BOD_5/COD (ratio)	0.58		0.06	
Sulfate	500	70 – 1750	80	10 – 420
Calcium	1200	10 – 2500	60	20 – 600
Magnesium	470	50 – 1150	180	40 – 350
Iron	780	20 – 2100	15	3 – 280
Manganese	25	0.3 -65	0.7	0.03 - 45

Ehrig, 1983

landfill. During the init stages, the landfill dials aerobic rich in biodegradable organic content. As the landfill age increases, the microorganism present in the landfill tend to degrade these organic compounds into inorganic components. When anaerobic phase begins, the COD starts increasing causing a decrease in BOD/COD ratio. This decrease in BOD/COD ratio observed, suggests the change in biodegradability of the leachate with time. For young landfill, the ratio is around 0.5-0.8 while it reaches almost 0.1 in the old landfill. The reason for low biodegradability in the old landfill could be due to the presence of humic and fluvic acids (*Wichitsathian, 2004*). Table 2.2 presents the leachate characteristic of landfill in various countries.

For other compounds, nitrogen, ammonia, phosphorus and chloride, their concentration are slightly changed during acidogenic and methanogenic phase. As leachate has high nitrogen but low phosphorus, unlike domestic wastewater. Nitrite and nitrate are rapidly use in biodegradation so the grate major of nitrogen is ammonia nitrogen.The ammonium concentration in the leachate also varies with age of the landfill, with young leachate having a high COD (>5,000 mg/L) and low nitrogen content (< 400 mgN/L) and old leachate having a high concentrations of ammonia (> 400 mg N/L) and recalcitrant compounds and a low biodegradable organic fraction (BOD_5/COD = 0.1).

2.1.4 Leachate Composition

The present solid waste landfill leachate composition can be classified the pollutant into four groups (*Kjeldsen et al, 2001*).

I - Dissolved organic matters: They are used to describe the content of dissolved organic matter in leachate; TOC (Total Organic Carbon), COD (Chemical Oxygen Demand), and BOD (Biochemical Oxygen Demand) covering a variety of organic degradation products ranging from small volatile acids to refractory fluvic and humic-like compounds (*Kjeldsen et al., 2001*).

Table 2.2 Comparison of Leachate Characteristics of Landfills Surveyed in Asia, Europe and America

Parameter	Thailand[1]			Malaysia[2]				USA[3]	Europe[4]
	Phitsanuklok	Nakhonpathom	Pathumthani	On-Nutch	Air Hitam	Sungai Sedu	Ampar Tenang		
Years in operation	1	4	9	20	12	9	9	-	-
pH	7.1 – 8.3	8.2 – 8.5	8.1	7.5	7.4	7.57	7.9	3.7 – 8.5	5.3 – 8.5
Chloride	-	655 – 2,200	2350	-	-	-	-	4.7 – 2,467	-
Alkalinity	300 – 4,700	960 – 2,740	6,620	-	8,300	2,860	1,580	0 – 20,850	300 – 11,500
SS	1,950	8.4 – 15.7	12.5	488	-	-	-	10 – 700	-
TS	6700	274 – 1,200	848	11,320	9,780	7,220	6,042	0 – 59,200	-
COD	4,900 – 11,000	800 – 3,575	3,200	1,200	7,600	3,733	1,640	40 – 89,520	150 – 100,000
BOD_5	3,000 – 7,150	100 – 240	280	130	1,228	866	507	18 – 33,360	100 – 90,000
TKN	-	64 – 1,260	1,256	700	-	-	-	-	50 – 5,000
NH_3-N	150 – 1250	-	-	-	957	470	321	0- 1,106	1 – 1,500
Ni	0.02 – 1.56	0.1	0.25	0.035	-	-	-	-	0.02 – 2.05
Cd	0.037	0.001	0.002	-	-	-	-	0.03 – 17	0.14
Pb	0.03 – 0.45	0.05	-	0.52	-	-	-	< 0.1 – 2	1.02
Cr	0.5 – 1.7	0.08 – 2.9	0.07	-	-	-	-	-	0.03 – 1.6
Hg		-	-	0.684	-	-	-	-	0.05

Note: All data with the exception of pH values are in mg/L.

1 Pollution Control Department, 2000

2. Alkassasbeh et al.,2009

3. Chain and Dewalle , 1976

4. Wichitsathian , 2004

II - Inorganic macrocomponents: Calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), ammonium (NH^{4+}), iron (Fe^{2+}), manganese (Mn^{2+}), nitrite (NO^{2-}), nitrate (NO^{3-}), chloride (Cl^{-}), sulfate (SO_4^{2-}) and hydrogen carbonate (HCO_3^{-}). Figure 2.2 shows the inorganic macrocomponents which can be calculated on an Asian landfill leachate *(Denis et al., 2008*)

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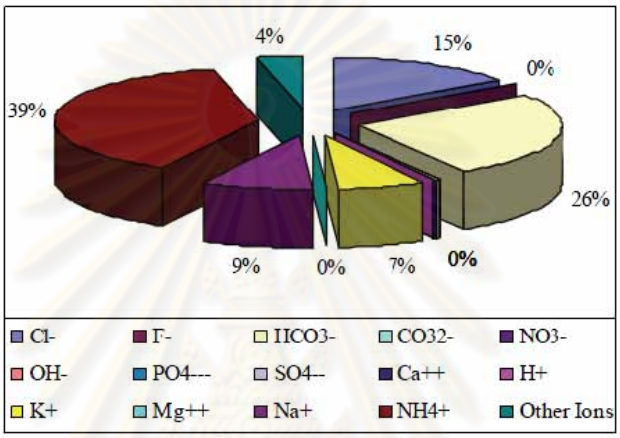


Figure 2.2 Inorganic macrocomponents in Asian landfill leachate.

III - Heavy metals: Cadmium (Cd^{2+}), chromium (Cr^{3+}), copper (Cu^{2+}), lead (Pb^{2+}),nickel (Ni^{2+}) and zinc (Zn^{2+}) . Other compounds may be found in leachate from landfills: for example, borate, sulfide, arsenate, selenate, barium, lithium, mercury, and cobalt. Generally, these compounds are found in very low concentrations. Table 2.3 shows heavy metal concentration range in landfill leachate.

IV - Xenobiotic organic compounds (XOCs): Originating from household or industrial chemicals and present in relatively low concentrations (usually less than 1 mg/l of individual compounds).These compound include plasticizer, phenolics, pesticides, aliphatic and aromatic hydrocarbons, pharmaceuticals, polyaromatichydrocarbons, chlorinated/non-chlorinated hydrocarbons, alkylphenol ethoxylates and alkyl phosphates which are presented in Table 2.4*(Paxéus, 2000, Kjeldsen et al., 2002, Schwarzbauer et al., 2002, Baun et al., 2004).*

Table 2.3 Heavy metal concentration range in leachate

Heavy Metal	Concentration range (mg/l)
Cadmium	0.00001-0.4
Nickel	0.0036
Zinc	0.003-1000
Copper	0.002-10
Lead	0.001-50
Chromium	0-1.62
Mercury	0.00005-0.16
Arsenic	0.01-1
Cobalt	0.005-1.5

Table 2.4 Xenobiotic Organic Compounds (XOCs) Observed in Landfill Leachate

Compound	Range (µg/l)
Aromatic hydrocarbon	
Benzene	0.2-1630
Toluene	1-12300
Xylenes	0.8-3500
Ethvlbenzene	0.22329
Trimethvlbenzenes	0.3-250
n-Propylbenzene	0.3-16
t-Butylbenzene	2.1-21
o-Ethytoluene	0.5-46
m-Ethyltoluene	0.3-21
p-Ethyltoluene	0.2-10
Naphthalene	0.1-260
Fenchone	7.3-83.0
Benzyl succinic acid	0.6-19.3
Halogenated hydrocarbons	
Chlorobenzene	0.1-110
1,2-Dichlorobenzene	0.1-32
1,3-Dichlorobenzene	5.4-19
1,4-Dichlorobenzene	0.1-16

Table 2.4 (Continued)

Compound	Range (µg/l)
1,2,3-Trichlorobenzene	I
1,2,4-Trichlorobenzene	4.3
Hexachlorobenzene	0.025-10
1,1-Dichloroethane	0.6-46
1,2-Dichloroethane	<6
1,1,1-Trichloroethane	0.01-3810
1,1,2-Trichloroethane	2.5-16
1,1,2,2-Tetrachloroethane	I
Trans-1,2-Dichloroethylene	1.6-6582
Cis-1,2-Dichloroethylene	1.4-470
Trichloroethylene	0.05-750
Tetrachloroethylene	0.01-250
Dichloroethane	1.0-827
Trichloromethane	1.0-70
Carbontetrachloride	4.0-9.0
Phenol	
Phenol	0.6-1200
Ethylphenols	<300
Cresols	1-2100
Bisphenol A	200-240
3,5-Dimethylphenol	0.7-27.3
2,5-Dimethylphenol	0.4-4.5
2,4-Dimethylphenol	0.1-12.5
3,4–Dimethylphenol	0.03-10.4
2,6-Dimethylphenol	0.3-1.9
2-Methoxyphenol	I
2/3-Chlorophenol	0.03-1.6
4-Chlorophenol	0.2-1.3
4-Chloro-m-cresol	1.2-10.2
3,5-Di-chlorophenol	0.08-0.63
2,3,4,6-Tetrachlorophenol	0.079-3.0
Alkylphenols	
Nonylphenol	6.3-7

Table 2.4 (Continued)

Compound	Range (µg/l)
Nonylphenolmonocarboxylate	0.5-3
Pesticides	
Ametryn	0.12
amino-3-(5-methyl-3-oxo-1,2-	
oxazol-4-yl)propanoic acid (AMPA)	3.8-4.3
Atazin	0.16
Bentazon	0.3-4.0
Chloridazon	1.6
Chlorpropham	26
Dichlobenil	0.1-0.3
Fenpropimorf	0.1
Glyphosat	1.7-27
Hexazinon	1.3
Hydroxyatrazin	0.7-1.7
Hydroxysimazin	0.6-1.7
Isoproturon	1.2
Lindane	0.025-0.95
Mecoprop	0.38-150
2-methyl-4-chlorophenoxyacetic acid (MCPA)	0.2-9.1
Propoxuron	2.6
Simazine	2.3
Tridimefon	2.1
2-(4-chlorophenoxy) propanoic acid (4CPP)	15-19
2,4-Dichlorophenoxyacetic acid (2,4-D2)	1-0.5
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	I
2,4-Dichlorophenoxyacetic acid (2,4-DP)	0.3-5.2
2,6-Dichlorprophenoxyacetic acid (2,6-DCPP)	0.7-1.3
Phthalates	
Monomethyl phthalate	I
Dimethyl phthalate	0.1-7.7
Diethyl phthalate	0.1-660
Methyl-ethyl phthalate	2-340
Mono-(2-ethylhexyl) phthalate	4-14

Table 2.4 (Continued)

Compound	Range (µg/l)
Di-(2-ethylhexyl) phthalate	0.6-235.9
Mono-butylphthalate	4-16
Di-n-butylphthalate	0.1-70
Di-isobutylphthalate	3-6
Mono-benzylphthalate	6-16
Butylbenzyl phthalate	0.2-8
Dioctylphthalate	1-6
Phthalic acid	2-14,000
Aromatic sulfonates	
Naphtlene-1-sulfonate	506-616
Naphtalene-2-sulfonate	1143-1188
Naphtalene-1,5-disulfonate	<2.5-5.1
Naphtalene-1,6-disulfonate	366-397
Naphtalene-2,7-disulfonate	129-145
2-aminonapphtalene-4,8-disulfonate	73-109
p-toluenesulfonate	704-1084
Phosphonates	
Tri-n-butylphosphate	1.2-360
Triethylphosphate	15
Miscellaneus	
Acetone	6-4400
2(3H)-Benzothiazolone	10-50
Camphor	20.6-255.2
Cumen	0.3-7.4
Fenchone	7.3-83
Tetrahydrofuran	9-430
Indane	0.2-20
Methylethylketone	110-6600
Methyle-iso-butylketone	1.1-176
Dimethoxymethane	1.1
Methyl tert-butyl ether (MTBE)	0.8-35
Styrene	0.5-1.6

2.1.5 Leachate Toxicity

Risk assessment of landfill leachate is traditionally base on chemical analysis of specific compounds present in leachate. However, risk assessment is not sufficiently developed to take into account interactions among chemical or toxic degradation products for constituents in a complex mixture (*Kjeldsen et al., 2001*). Testing of landfill leachates in biological toxicity tests (biotests) provides a direct functional response that relates to the overall toxic properties of the mixture of compounds present in the leachate sample. Table 2.4 shows the direct biotesting of landfill leachates has been carried out previously.

Ernst et al. (1994) concluded that ammonia was the primary cause of acute toxicity ofmunicipal landfill leachate, whereas the chronic effects of the range of XOCs identified in the leachate could not be determined. In the bioassay studies by Alkassasbeh et al. (2009) and Svensson et al. (2005), it was also concluded that ammonia was the main cause of the toxicity measured in the biotests. Other studies have indicated that factors like pH, conductivity, and the concentrations of chloride, copper, or zinc may also be of major importance to aquatic toxicity assessed by aquatic bioassays (*Cameron and Koch, 1980; Atwater et al., 1983; Kross and Cherryholmes, 1993; Assmuth and Penttilae, 1995; Clément and Merlin, 1995*).

Recent years, the chronic effect of landfill leachate have assessed in some detail of the different long-term effects mutagenicity/genotoxicity (*Kjeldsen et al., 2001*). Omura et al. (1992) covered leachates collected from eight MSW landfills. It was found that the leachates were mutagenic after preconcentration, and the authors suggested that organic compounds in the leachate caused the mutagenic activity. Through the application of several methods, it was confirmed that these leachates can induce genetic damage in biological species (Table2.5).

A number of studies reported that analytical measurements of XOCs did not correlate well with the toxicity observed in bioassays (*Kjeldsen et al., 2001*). This may

be due to the fact that toxicity caused by the sample matrix (e.g., ammonia, alkalinity, and salts) masks the toxic effect of XOCs (*Baun et al., 2000*).

Table 2.5 Toxicity results [LC (EC) $_{50}$-t] for MSW landfill leachates using different types of tests.

Biological Species	**LC(EC)$_{50}$ –t [%]**	**Reference**
Luminescent bacteria		
Vibrio fisheri	EC50-48h = 1.3 – 6.1	*Baun et al., 2004*
	EC50-48h =11.3 – 15	*Silva et al., 2004*
	6.1<EC50-15 min > 90.0	*Ward et. al.,2002*
Crustaceans		
Daphnia similis	LC50-48h = 4.8 – 89	*Atawater et.al., 1983*
	LC50-96h = 3.8 – 86	*Atawater et.al., 1983*
	LC50-48h = 62 – 66	*Plotkin and Ram, 1984*
	EC50-48h = 2.0 - 2.3	*Silva et al., 2004*
Artemia salina	LC50-48 h = 11.9 – 25.6	*Silva et al., 2004*
	LC50-24 h = 3.20	*Olivero-Verbel et al., 2008*
	LC50-48 h = 39.93	*Olivero-Verbel et al., 2008*
Fish		
Zebra fish *(Brachydanio rerio)*	LC50-48 h= 2.2-5.7	*Sisinno et al. 2000*
	LC50-48h = 2.2	*Silva et al., 2004*
Tilapia *(Sarotherodon mossambicus)*	LC50-96h = 1.4 - 12	*Wong 1988*
Medaka fish *(Oryzias latipes)*	LC50-48h = 19.2 (larvae)	*Kashiwada et al. 2006*
	LC50-48h = 53 (adult)	*Kashiwada et al. 2006*
Common Carp *(Cyprinus Carpio)*	LC50-96h = 1.13-3.82	*Alkassasbeh et al.,2009*
Clarias Gariepinus	LC50-96h = 36.6	*Oshode et al., 2008*
Plant		
Algea (*Scenedesmus quadricauda)* Note: growth inhibition test	EC50-72h = 3.0 – 14.8	*Słomczyńska and Słomczyński, 2001*

Table 2.6 Genotoxicity testing for MSW landfill leachate using different types of tests.

Species	Observation	Effect	Reference
Acacia confuse *Leucaena leucocephala*	The growth and induction of physiological change of plant	Started wilting within one month	*Chan et al., 1999*
Allium cepa	Comet assay on Meristematic cells from the roots	Increase DNA damage	*Bortolotto et al., 2009*
G. Brasiliensis	Comet assay on Erythrocytes from peripheral blood Micronucleus test on Erythrocytes from peripheral blood	Increase DNA damage	
Salmonella Bacillus subtilis Aspergillus nidulans	Mutagenicity bioassay (Ames assay) DNA repair bioassay Chromosome damage bioassay	Increase DNA damage Increase DNA damage Increase DNA damage	*Schrab. Et al., 1993*
goldfish (*Carassius auratus*)	Comet assay on Erythrocytes from peripheral blood and gill cells	Increase DNA damage	*Deguchi et al.,2007*
Hordeum vulgare	Chromosomal abnormalities in cells rom rat bone marrow	Increase DNA damage	*Sang et al., 2006*
Mice	Oxidative damage in cells from the heart, kidney, spleen, brain and liver	Increase oxidation damage	*Li et al., 2006*
Vicia faba	Micronucleus test on Meristematic cells from the roots	Increase DNA damage	*Sang et al., 2004*

2.1.6 Physico-Chemical Treatment of Leachate

The propose of physico-Chemical treatments are used in addition at the treatment line (pre-treatment or last purification) or to treat a specific pollutant (stripping for ammonia).

I – Neutralization

Neutralization is the simplest and most common treatment method for inorganic contaminants. It involves the addition of acid and base to adjust the pH to an acceptable level. This level is usually between pH 6-9. Some common bases are lime, calcium hydroxide, caustic, soda ash, and ammonium hydroxide. Common acids include sulfuric, hydrochloride, and nitric acid. Neutralization reactions produce soluble and insoluble salts.

II- Precipitation/Flocculation/Sedimentation

This treatment is effective on leachate with high molecular weight organic material such as fulvic and humic acid. In precipitation reactions, chemicals are added to transform dissolved constituents to from insoluble precipitates. Metals are precipitated as hydroxides, sulfides, and carbonates by adding appropriate precipitant and adjusting the pH to favor insolubility. Chian and DeWalle (1977) and Ho, *et al*. (1974) reported that precipitation using lime could remove organic matter with molecular weight greater than 50,000 Da. This particular fraction is present in a low concentration in young landfills and absent in older landfills. Therefore, lime treatment is most effective in medium-age landfills.

In a flocculation reaction, alum, lime, ferric chloride, or polyelectrolyte are added to the inflow to reduce the repulsive forces between the precipitated particles. These particles aggregate, forming large flocs of material, which can be settled out in a sedimentation tank.

III - Chemical oxidation

Chemical oxidation technologies are useful in the oxidative degradation or transformation of a wide range of pollutants present in drinking water, groundwater and wastewater treatment (*Venkatadri and Peter, 1993*). The reaction involves the addition of chemical oxidizing under controlled pH. Generally, chemical oxidation processes are incorporated in to treatment sequences to treat constituents of wastewater that are resistant to biodegradation or create toxicity in biological reactors. Chemical oxidation processes are widely used in leachate treatment. A variety of chemical oxidants are used for leachate treatment, which includes hydrogen peroxide, ozone, chlorine, chlorine dioxide, hypochlorite, UV radiation and wet oxidation. Since, oxidation processes are energy intensive and expensive, their application is limited. Moreover, as oxidation processes are dependent on the stoichiometry, a large amount of oxygen is required for higher organic concentrations (*Webber and Smith, 1986*)

IV – Membrane filtration

Membrane filtration can be defined as the separation of solid immiscible particles from a liquid or gaseous stream primarily based on size differences. The classifications of membrane separation processes are base on particle and molecular size. The processes such as Reverse Osmosis (RO), Nanofiltration (NF), Ultrafiltration (UF) and Microfiltration (MF) do not generally require aggressive chemicals and can be operated at ambient temperature making these processes both an environmentally friendly and economically attractive to conventional operating units.

In case of leachate treatment, membrane filtration is less effective in treating young or acidogenic leachate. The efficiency of different membrane technology in treating methanogenic leachate is presented in **Table 2.7**. Although NF and RO are quite effective in leachate treatment in terms of organic, inorganic, nitrogen and AOX removal, the disadvantage is its susceptibility to fouling and short lifetime

Table 2.7 Effectiveness of treatment vs. leachate characteristics

Process	Character of leachate			Average of % removal		
	Young	Medium	Old	BOD	COD	TKN
Physico/Chemical						
Coagulation/flocculation	Poor	Fair	Fair	-	40 - 60	< 30
Chemical precipitation	Poor	Fair	Poor	-	< 30	< 30
Adsorption	Poor	Fair	Good	>80	70 – 90	-
Oxidation	Poor	Fair	Fair	-	30 – 90	-
Stripping	Poor	Fair	Fair	-	< 30	> 80
Biological						
Aerobic processes	Good	Fair	Poor	>80	60 - 90	>80
Anaerobic processes	Good	Fair	Poor	>80	60 – 80	>80
Membrane bioreactor	Good	Fair	Fair	>80	>85	>80
Membrane filtration						
Ultrafiltration	Poor – Fair	-	-	-	50	60 – 80
Nanofiltration	Good	Good	Good	80	60 – 80	60 – 80
Reverse Osmosis	Good	Good	Good	> 90	>90	>90

(*Renou et al., 2007*)

2.2 Biological Toxicity Testing

2.2.1 Effect of toxicity

I - Acute effects

Effects occur rapidly as a result of short-term exposure to a chemical within a few hours, days, or weeks. The most commonly measured acute effect in aquatic organisms is death. Acute Toxicity Tests determine whether some concentration of test material or effluent will produce an adverse effect on a group of test organisms during a short-term exposure under controlled conditions. A chemical is considered acutely toxic if by its direct action it kills 50% (LC_{50} and LD_{50}) or more of the exposed population of test organisms in a relatively short period of time, such as 24 to 96 h (EPA 821-R-02-012, 2002).

II - Chronic effects

Effect may occur when the chemical produces deleterious effects as a result of a single exposure (e.g., to a strong acid), but more often they are a consequence of repeated or long-term exposures to low levels of persistent chemicals, alone or in combination. Chronic effects also may be lethal or sublethal (such as abnormal growth and/or reproduction). Chronic Toxicity Test used to determine the concentration of a substance that produces an adverse effect from prolonged exposure of an organism to that substance. In this test, mortality, number of young per female, and growth are used as measures of chronic toxicity (EPA 712–C–96–120, 1996).

2.2.2 Biomarker

Biomarkers are tools, which enable to measure the true exposure in the response of the organisms to chemical and its potential susceptibility to toxic effects. There are three types of biomarkers: biomarkers of exposure of the organism to the toxic substance, biomarkers of response of the organism to that exposure, and biomarkers of susceptibility of the organism to the chemical.

I - Biomarkers of Exposure

Measurement of the dose is determination of the amount of chemical administered or the amount to which the animal or human is exposed (such as in air or water). However, it cannot be assumed that all of the dose will be absorbed, even in the case of a drug given to a patient. Therefore, a more precise estimate of exposure is often needed.

II - Biomarkers of Response

Biomarker of response is measurement of the adverse response on living organisms to chemical exposure, ranging from biochemical or physiological to pathological. However, all biomarkers of response must be validated in relation to certain criteria. It cannot be assumed, because a gene is switched on or off, a protein is increased or decreased, or a metabolic pathway is influenced by a chemical, that the measurement is a usable biomarker, which reflects toxicity.

III - Biomarkers of Susceptibility

Biomarkers, which indicate variation in the susceptibility of the organism, can be determined, and again, these cover a range of types from deficiency in metabolic enzymes to variation in repair systems. These would typically be measured in individual members of a population. A less common type of susceptibility marker is that reflecting increased responsiveness of a receptor or resulting from a metabolic disorder, such as glucose 6-phosphate dehydrogenase deficiency, leading to increased susceptibility to toxicity.

2.2.3 Dose Response Relationship

Acute toxicity of a chemical is quantified by its dose-response curve. This relationship between dose of chemical administered and the resulting response is established by exposing groups of organisms to various concentrations of the chemical. Ideally doses are selected that will elicit > 0% effect but < 100% effect during the course of the experiment. At defined time periods following dosing, effects (e.g., mortality) are recorded. Results are plotted in order to define the dose –response curve that shows in Figure 2.3.

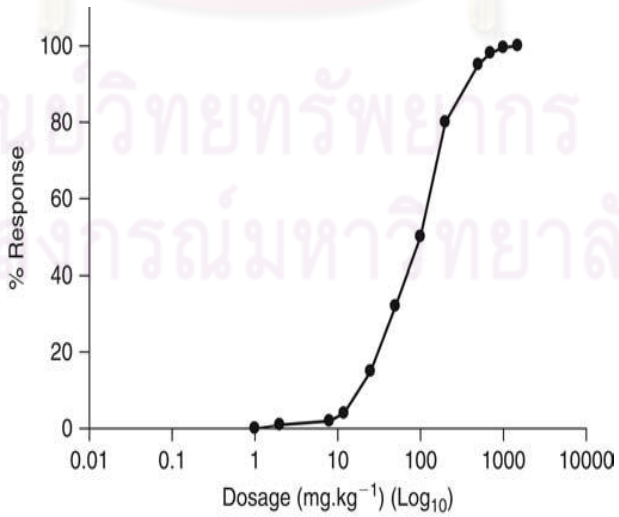


Figure 2.3 A tropical dose-response curve

2.2.4 Analysis of toxicity

The sample concentration factor that caused a 50% decrease in bacteria Based on the empirical toxicity scale (expressed in TU) adopted in Belgium and approved by the European Community Commission (*Persoone et al., 1993*), the toxicity judgment shows in Table2.8 brightness(EC_{50}) are thus converted into toxic unit (TUs) which are proportional to toxicity:

Table 2.8 The judgment of toxicity

Observation	Level of Toxic Unit
Extremely toxic	TU > 100
Very toxic	10 < TU < 100
Toxic	1 < TU < 10
Weakly toxic	TU < 1
Not toxic	TU = 0

2.3 Biological of test species

2.3.1 Nile tilapia (*Oreochromis niloticus)*

General classification of *Oreochromis niloticus* is Kingdom Animalia, Phylum Chordat, Subphylum Vertebrata (Craniata), Class Osteichthyes, Order Perciformes, Family Cichlidae, Genus Oreochromis, Species Oreochromis niloticus. Common name is Nile tilapia, Nile mouth-brooder.

The original distribution of *O. nilloticus* is the Africa continent. They occur innatural waters throughout the tropic, even in Australia (*Philipart and Ruwet, 1982*) and become to fishes of economic importance in tropic and subtropics countries. It is also used in aquaculture for human food production. The species is distinguished from other perch-like fishes in having one nostril on each side of the snout. Its body is fairly

elongate, moderately deep and greatly compressed. Dorsal and ventral profiles are about equally convex. All the tilapias, in the broad sense, have in common a mainly omnivorous diet, also behaves as detritivorous diet (*Cesar K. et al., 2009*). Structural adaptations to this diet are the long, coiled intestine, the bicuspid and tricuspid teeth of the jaws and the small, sharp pharyngeal teeth (*Trewavas, 1982*). Adult of Nile tilapia is shown in Figure 2.4 (A). Nile tilapia is recommended by international institution (Organization for Economic Co-operation and Development; OECD) and the national regulation (cf. Materials and Methods section) as standard organism.

2.3.2 Common Carp *Cypinus carpio*

General classification of *Cypinus carpio* is Kingdom Animalia, Phylum Chordata, Subphylum Vertebrata (Craniata), Class Osteichthyes, Order Perciformes, Family Cichlidae, Genus Cyprinus, Species Cyprinus carpio. Common name is Common Carp, mirror carp, leather carp, koi, and Israeli carp.

Common carp is one of the largest members of the minnow family. This species is one of the most widely distributed fish species in North America. In Indiana, common carp are found throughout the rivers and streams of the state, many natural lakes and impoundments, and some farm ponds. Carp hatch from tiny eggs less than 0.4 inches (1 mm) in diameter and grow to a weight of 33 pounds (15 kg) and a length of 40 inches (1 meter) in 5 to 6 years. Carp have stocky bodies, large scales, and range in color from dark olive bronze on the top of the back to lighter silvery yellow on the belly. Adult of Common carp is shown in Figure 2.4 (B).

Carp are omnivorous (eating both plants and animals). They have sensitive smell/taste organs in and around the snout that assist in feeding. They are sight and smell feeders, eating insects, seeds, and other small organisms and plants in clear water, and relying on their sensitive sense of smell to locate food in turbid waters. The mouth and lips are adapted to extend like a short tube for sucking up food.

2.3.3 Water flea *Moina macrocopa*

General classification of *Moina macrocopa* is Kingdom Animalia, Phylum Arthropoda, Subphylum Crustacea, Class Branchiopoda, Order Cladocera, Family Moinidae, Genus Moina, Species Moina macrocopa. Common name is water fleas

Moina macrocopa are small freshwater cladoceran crustaceans. Structurally, *Moina macrocopa* appear similar to *D. magna* and *D. pulex.* There are approximately half the maximum lengths of *Daphnia*. Adult *Moina* are 700-1000 μm, and young *Moina* are less than 500 μm. *Moina* have a body consisting of a head and a trunk. The antennae are the main means of locomotion. Large compound eyes lie under the skin on the sides of the head. Adult of water flea is shown in Figure 2.4 (C).

Moina appear in high concentrations in pools, ponds, lakes, ditches, slow-moving streams and swamps where organic material is decomposing. Species of *Moina* have been reported to play an important role in the stabilization of sewage in oxidation lagoons.The reproductive cycle of *Moina* has both a sexual and asexual phase. Normally, the population consists of all females (95%) that are reproducing asexually. Under optimum conditions, *Moina* reproduce at only 4–7 days of age, with a brood size of 4–22 per female. *Moina* has proven to be a useful test species for the study of sensitivity to environmental toxicants (Sujata and Lakshmipathi, 1991), because it demonstrates high susceptibility to toxic substances and in particular, metals and is generally short-lived (2–3 weeks) (*Nandini and Sarma 2000*).

(A) (B) (C)

Figure 2.4 Adult of tested species: A) *Oreochromis niloticus* (Nile tilapia) B) *Cypinus carpio* (Common carp) *C*) *Moina macrocopa* (Water flea*)*

2.4 Water quality for aquatics

The complete list of contaminants and the criteria for the protection of aquatic life are shown in Table 2.9 and Table 2.10.

Table 2.9 The criteria for the protection of aquatic life

Water variants	Acceptable levels	Lethal concentration levels
Oxygen	> 6 ppm, up to 100%	< 3 ppm, >100% sat.
Carbon dioxide	1.5 – 3.0 ppm	> 15 ppm
pH	6.7-8.6	< 4-5, > 9-10
Ammonia (unionized)	< 0.02 ppm	> 0.2 – 1.0 ppm
Nitrate	< 1.0 ppm	> 100 ppm
Nitrite	< 0.1 ppm	> 2.0 ppm (fresh)
		>20 ppm (salt)
Total Hardness	20 – 200 ppm	>200 ppm (CO2 excess)
Salinity		>800 ppm (all causes)
Total suspended solids	< 80 ppm	>5000-100,000 ppm
Total dissolve solids	<400 ppm	>5000-20,000 ppm
Hydrogen disulphide	<0.002 ppm	> 0.5-1.0 ppm

The department of fisheries Government of Western Australia (2008)

Table 2.10 Water quality of heavy metal for finfish

Water variants	Acceptable levels	Lethal concentration levels
Heavy metal		
Aluminum	-	> 0.1-5 ppm
Cadmium	< 0.005 ppm soft water	> 3 ppm
	< 0.003 ppm hard water	
Copper	< 0.006 ppm	> 0.5 ppm
Mercury	< 0.0002 ppm	> 0.15 ppm
Lead	< 0.02 ppm	1-5 ppm
Zinc	< 0.005 ppm	>0.5-1 ppm

The department of fisheries Government of Western Australia (2008)

2.4.1 Effect of ammonia to aquatics

Total ammonia nitrogen (TAN) is composed of toxic (un-ionized) ammonia(NH_3) and nontoxic (ionized) ammonia (NH^+_4). The acute criterion for un-ionized ammonia is dependent on pH and species and the chronic criterion are dependent on pH and temperature (U.S. EPA, 1999) is shown in Table 2.11.

Table 2.11 Effect of un-ionized ammonia to aquatics

Range (ppm)	Effect to aquatic organisms.
0.020 to 0.049	Tolerate but will cause long term harm to its growth, immune system, health, etc. especially to eggs or very young animals.
0.050 to 0.199	Perhaps to tolerate for only a few days and is very harmful
0.200 to 0.499	Perhaps tolerate for a day or two and will probably kill
0.500 +	Deadly and will probably kill within a day

(Individual species of fish, amphibians, invertebrates etc. vary enormously on their tolerances of low levels of ammonia and the issue is made further complicated as young are far more susceptible to ammonia than older animals).

2.5 Comet assay (single cell gel electrophoresis assay)

2.5.1 Applications of the Comet assay

The Comet Assay, or single cell gel electrophoresis (SCGE), is the result Of studies undertaken by *Östling* and *Johanson*, who developed the methodology of DNA electrophoresis in micro-gel, and those by *Singh et al.* (1988), who improved the technique, which led to a sensitive version of the assay that could assess both double- and single-strand DNA breaks as well as the alkali labile sites expressed as frank strand break in the DNA. The result present as comet that is divided into two part, head comet and tail comet. The DNA damage appears in the tail of comet. Figure 2.5 shows the comet picture of undamaged and damaged DNA.

The advantages of the Comet assay include its demonstrated sensitivity for detecting low levels of DNA damage (one break per 1010 Da of DNA; *Gedik et al. 1992*), requirement for small number of cells (~10,000) per sample, flexibility to use proliferating as well as nonproliferating cells, low cost, ease of application, and the short time needed to complete a study.

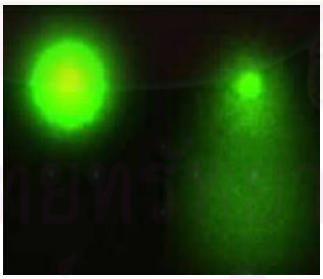


Figure 2.5 Nuclei after comet assay. (Right) Nucleus with undamaged DNA. (Left) Nucleus with damaged DNA (www.massey.ac.nz)

2.5.2 Comet assay statistical analysis

The comets can be successfully evaluated by the tail moment, defined as the product of tail length and percentage of the fluorescence intensity in the tail. Figure 2.6 shows head and tail area after comet assay. 50 to 100 cells were measured in one experiment and the distribution of tail moments within one sample evaluated. Considering the individuality of each cell, histograms are prepared for the interpretation of result (*Bauer et al., 1998*)

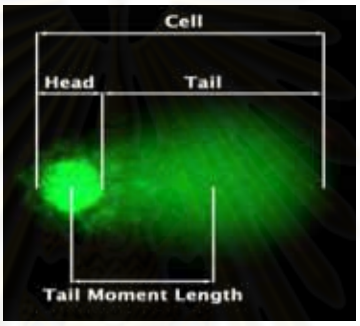


Figure 2.6 Head area and Tail area after comet assay

To quantify level of DNA damage, the following formula (Sriussadaporn *et al*., 2003)

$$\text{Level of DNA damage} = \frac{\sum_{i=1}^{n}\left(\frac{T}{T+H}\right)_i}{\sum_{i=1}^{n}\left(\frac{T}{T+H}\right)_{i,\max}}$$

By

T = Tail area of comet cell

H = Head area of comet cell

i = Sequence of images

n = amount of total comet cells

$\sum_{i=1}^{n}\left(\frac{T}{T+H}\right)_i$ = Summation of fractional DNA damage from comet cells, amount n

$\sum_{i=1}^{n}\left(\frac{T}{T+H}\right)_{i,\max}$ = Summation of maximum fractional DNA damage from comet cells, amount n

In case of more tail area due to more DNA damage (T>>H)

$\frac{\sum_{i=1}^{n}\left(\frac{T}{T+H}\right)_i}{n}$ = Level of DNA damage

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

This experiment conducted using aquatic organisms to assess the acute toxic and genotoxic potential of raw leachate and treated leachate with different degree of treatment along the treatment train. The toxic level was confirmed by chemical characterization of toxic compounds. Acute toxicity tests were conducted using 3 different species: *Moina macrocopa*, *Oreochromis niloticus* and *Cyprinus carpio* to determine the LC_{50} of water samples whereas genotoxic effects were studied using single cell gel electrophoresis (comet) assay in fish species. Degree of toxic level reduction was evaluated by determining the removal of toxic chemicals, LD_{50} and degree of gene damage.

3.1.1 Leachate Treatment Plant

Nonthaburi solid waste disposal site or "Sainoi dumpsite" is one of solid waste disposal sites in Thailand. It has been in operation for more than 20 years and currently receiving approximately 850-900 tons of municipal solid wastes daily from Nonthaburi province. At the present, the waste disposal activities have generated and accumulated up to 300,000 m^3 of leachate which was not discharged off the site but being stored in a stabilization pond (Figure A-1) because it is highly contaminated with organic and colored substances. Table 3.1 shows characteristics of leachates which one was collected from garbage truck (fresh leachate) , and leachate which was storaged in stabilization pond (stabilized leachate). In order to solve leachate problem, full-scale leachate treatment plant with intake capacity of 1,000 m^3/d utilizing coagulation followed by sedimentation, sand/carbon filtration, Microfiltration and reverse osmosis (RO) units respectively.

Table 3.1 Raw leachate characteristics

Parameters	Raw Leachate Characteristics			
	Fresh leachate	Avg.Inf	Stabilized leachate	Avg.Inf
pH	3.72 – 4.55	3.97 ± 0.3	8.17 – 8.65	8.44 ± 0.16
Chloride	2,084 – 3,330	2,815 ± 505	4960 – 7750	6,800± 1100
sBOD	30,400 – 54,700	47,274± 8,300	200 – 560	400 ± 130
sCOD	32,000 – 67,200	52,650± 9,980	2400 – 2880	2,700 ± 300
TOC	13,900 – 40,300	25,320 ± 7,510	420 – 770	650 ± 160
TKN	280 – 672	430 ± 120	90 – 340	210 ± 92
NH_3-N	120 – 280	240 ± 68	80 – 140	112± 25
TDS	17,900 – 42,000	32,500± 7,900	11,700 – 20,400	14,600± 2,600
SS	13,500 – 32,000	27,500± 6,150	220 – 570	290± 110
Cr	-	0.24± 0.14	-	0.17± 0.10
Cu	-	0.53± 0.26	-	0.50± 0.44
Ni	-	0.76± 0.52	-	0.32± 0.20
Pb	-	ND	-	ND
Cd	-	0.056± 0.040	-	0.050± 0.024

In the system, stabilized leachate was pumped to open jet clarifier in which coagulation, flocculation and clarification take place using ferric chloride ($FeCl_3$) of 2.0 g/l and polymer 0.01 g/l as coagulant. The supernatant was then pumped into pressurized sand filter to remove further suspended solids before feeding to RO unit. At the RO system, microfiltration membrane (MF) of 5 μm pore size was used as pre-treatment. The RO system consists of 6 pressurized vessels and 42 membrane elements (LFC3 LD spiral wound, Nitto Denko Corp, Japan). The percent recovery and operating pressure in RO unit was maintained at 50% and 15-25 atm respectively. Schematic of the leachate treatment system is shown in Figure 3.1

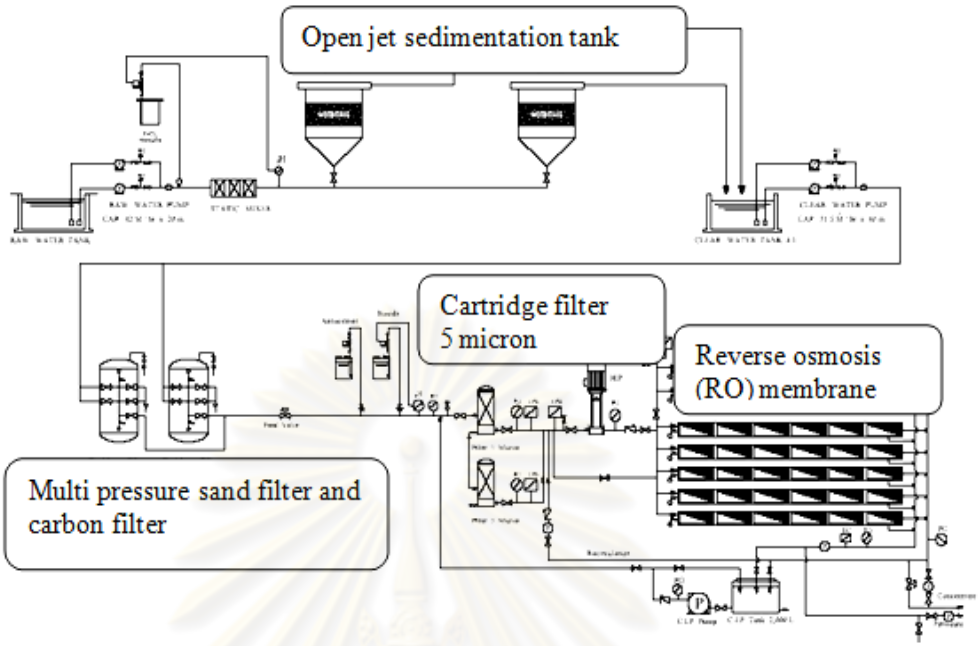


Figure 3.1 Schematic of advanced leachate treatment system

3.1.2 Material for toxic organic compounds determination

Material for extraction

a. Solid phase extraction (SPE) sorbent tubes
   - C18 VertiPak^{TM} SPE tube, silica-base sorbent, 6 ml, 500 ml
   - HBP VertiPak^{TM} SPE tube, polystyrene divinylbenzene copolymer (PS-DVB) sorbent, 6 ml, 200 ml

b. Chemical substance for extraction
   - Hexane, Ar grade
   - Dichloromethane, Ar grade
   - Methanol, Ar grade
   - Acetonitrile, HPLC grade

Material for GC-MS analysis

c. Gas Chromatrography mass spectrometer (GC-MS); Shimadzu GC-MS model 2010 Plus

d. GC-MS Column ; RTX -35MS, ID 0.25 mm, 30 m length

3.1.3 Test Species Preparation

There were three different test species including: Nile tilapia (*Oreochromis niloticus*)and Common Carp (*Cyprinus carpio*) and Water flea (*Moina macrocopa*).

Nile tilapia (*Oreochromis niloticus)* and Common Carp (*Cyprinus carpio)* at the age 3 weeks were purchased from the Aomnoi Breeding farm. Fish were transported to glass aquarium in laboratory. For acclimatization purposes, they held in 120-L glass aquarium with well aerated and dechlorinated water for 14 days and the water renewed every 3 days. The fish is fed with commercial fish food once daily. Feeding is terminated 48 h prior the initiating of the experiment to reduce metabolic wastes.

Water flea (*Moina macrocopa)* was obtain from commercial breeding and placed into 1-litre-beakers .Then select five healthy adult into 10 ml-glass tubes for cultivation. They were fed regularly with green algae *Chlorella Pyrenoidosa*. Collect little cladoceran exceed 24 h. old from the preliminary cultivation into 100 ml beaker, where they are located before the test start without feeding. Figure 3.2 shows the cultivation of water flea (*Moina macrocopa)*

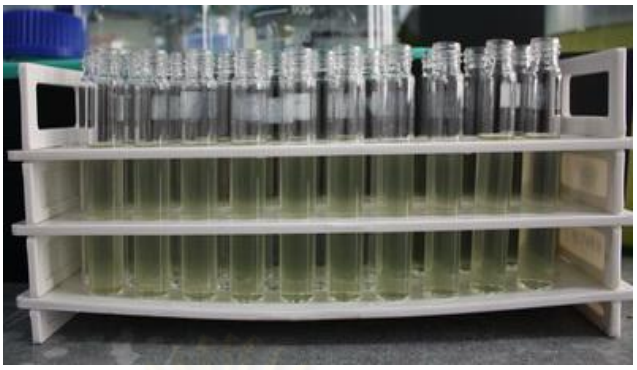


Figure 3.2 the cultivation of water flea (*Moina macrocopa)*

3.1.4 Material for Comet assay

Equibments for comet assay

a. Electrophoresis Chamber and power supply
b. Fluorescence microscope (OLYMPUS)
c. 2 trip Comet™ Slide (Travigen), cover slip
d. Autoclave (Yamato) and Hotplate
e. 1 ml syringe and No. 27 hypodermic needle

Chemical substance for comet assay

a. Low melting point Agarose (LMA)
b. Dimethylsulfoxide (DMSO)
c. Ethelenediamine tetraacetic acid (EDTA) disodium salt
d. Syber safe green
e. Phosphate Buffer Saline (PBS)
f. Tris (Tris [hydroxymethyl]aminomethane)
g. Triton X-100

3.2 Method

3.2.1 Physico-chemical Characteristics Determination

In order to investigate water qualities, physicochemical parameters were measured one time per month from May 2009 through January 2010 by using the procedures presented in the Standard Methods for the Examination of water and Wastewater (APHA 1998) that is showed in Table 3.2 In laboratory, water samples were filtrated through GF/C filter and storage at 4^{o}C in the dark until use.

Table 3.2 Physicochemical parameters and Frequency for water quality analysis

Parameters	**Method/instrument**	**Frequency**
pH	pH meter	Once a month
Alkalinity	Standard method 2320 B: Titratric Method	Once a month
Conductivity, Salinity	Conductivity meter	Once a month
sBOD (mg/l)	Standard method 5210 B:5-day BOD test	Once a month
sCOD (mg/l)	Standard method 5210 B: Closed dichromate-reflux	Once a month
TOC (mg/l)	TOC-V 5000 A analyzer	Once a month
NH_4-N	Standard method 4500 B: Distillation method	Once a month
TKN	Standard method 4500 B: Macro Kjedahl method	Once a month
Total Solids	Standard method 2540 B	Once a month
Total Dissolved Solids	Standard method 2540 C	Once a month
Total Suspended Solids	Standard method 2540 D	Once a month
Cl^-	Standard method 4500 C: Mercuric Nitrate	Once a month
Heavy Metal (Zn,Cr^{3+},Cd,Cu,Ni,Pb)	Atomic Absorption Spectroscopy	Once a month

(APHA 1998)

3.2.2 Toxic Organic Compounds Determination

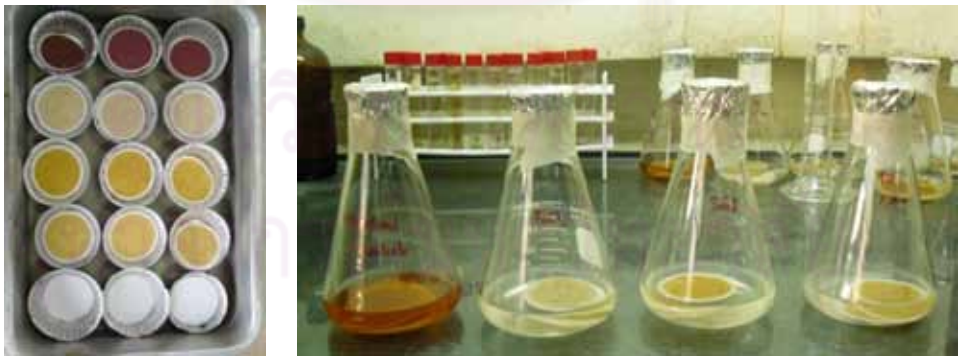
To considering the hazardous organic compounds, water samples were submitted to an extraction procedure based on sequential solid phase extraction (SSPE) methodology involving fractionation of the sample according to the polarity of the

organic content, followed by gas chromatography-mass spectrometry (GC-MS) techniques with electron impact (EI) ionization.

3.2.2.1 Sampling and Preparation

Raw leachate (fresh and stabilized leachate) were collected in August and October2010. Fresh leachates were collected from eight different garbage trucks whereas stabilized leachates were collected from eight different location of stabilization pond (Figure A-2). Treated leachates were collected in September and November 2010 from effluent along treatment process (Figure A-3 to A-6).

All samples were collected in glass bottles, and were filtrated using GF/C filter to separate organic compounds determination in suspended solid phase and soluble phase. The GF/C filter with suspended solid were added MeOH/CH_2Cl (8:2 v/v) in 250 ml, Erlenmeyer flask. Figure 3.3 shows preparation of suspended solid for extraction. Then the mixtures were homogenized and extracted for 1 hour in an ultrasonic bath, and then shaken at 200 rpm for 30 minutes. Extracts were concentrated to about 0.5 ml and drawn on the top of SPE tubes.



(A) (B)

Figure 3.3 the suspended solid phase preparation for organic compounds extraction (A) The GF/C with suspended solid (B) The mixtures of suspended solid and solvent

3.2.2.2 Extraction procedure

All SSPE based experiments were applied from M. Castillo et al. (2001) and XU (2008) to preconcentration of leachate and treated leachate samples. Two different sorbents were used: a Vertipak ™ C18 (500 mg, 6 ml) and Vertipak ™ HBP (200 mg, 6 ml). The same conditioning step was used for both tubes consisting on applying 7 ml of methanol followed by 3 ml of water. Loading sample step, 200 ml of each water sample was loaded to C18 tubes and eluted as follows: 2x5 ml of hexane allows obtaining fraction A; fraction B was eluted with 2x5 ml of dichloromethane and 2x5 ml of methanol/dichloromethane (9:1, v/v) was lead to fraction C. For HBP tubes, water samples were first acidified to pH 3 using 6 mol/L of Hydrochloric acid (HCl), and then the sorbent was eluted with 2x5 ml of dichloromethane/acetonitrile mixture (1:1, v/v) to obtain fraction D. All the elution was evaporated to dryness with anhydrous Na_2SO_4 and reconcenstituted to a final volume of 0.5 ml. Figure 3.4 shows the extraction step.

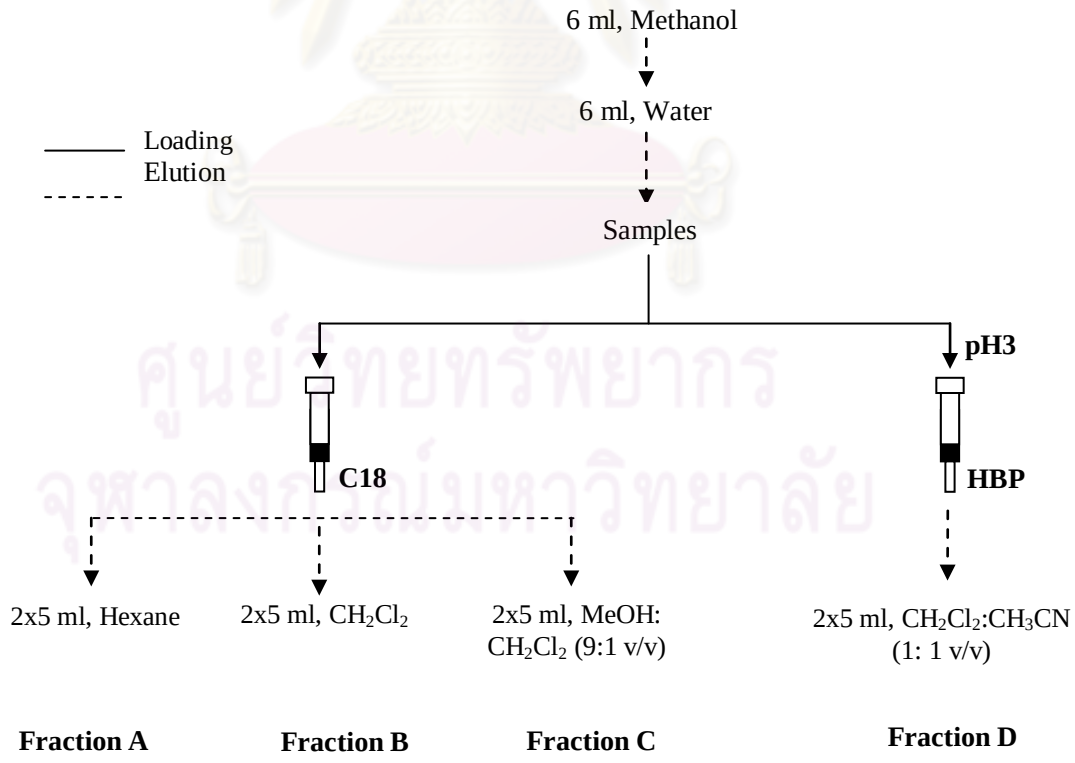


Figure 3.4 SPE phases, their elution and the compound classes found in each fraction

3.2.2.1 Gas chromatography analysis

The instrumental analysis was performed by gas chromatography with mass spectrometer detector (GC/MS) with model of Shimadzu GC-MS model 2010. Helium was used as the carrier gas with an inlet pressure of 15 psi. A 1 µl sample was injected. The gas chromatograph temperature program started at 60^{o}C increasing to 175^{o}C at 6^{o}C/min, and then increased to 270^{o}C at 3^{o}C/min. The EI conditions were as follows: ionization energy 70 eV, source temperature set at 200^{o}C and interface temperature at 350^{o}C. The compounds were identified by the GC/MSD library (Wiley), and compared peak areas to determine the relative peak areas of organic compounds contain in raw leachate and treated leachate.

3.2.2 Acute Toxicity Testing

3.2.2.1 *Oreochromis niloticus* and *Cypinus carpio* acute toxicity test

In triplicate, 10 adult healthy fish of similar size (about 35-40 mm length, Figure A-10) were randomly sampled and transferred from the acclimation tank into test chambers that were glass aquarium of 25 liters capacity. Each aquarium was stocked with fish with 20 liters of water sample that were diluted using filtered tap water as dilution water. Fish were exposed to five different water sample concentrations and carried out at temperature room of 28 ±1 ^{o}C for 96 h under conditions of 12:12 h light: dark and aerated all time. In triplicate, nonexposed fish were observed in dilution water only under same conditions as mentioned above to be control experiment. The number of dead fish was recorded every 24 h, and removed from tested tanks. The 96 h LC_{50} for fish and its 95% confidence limits are calculated using a program based on Probit Analysis Method using SPSS version 16 for Window (Statical Package for the Science/Personal Computer Plus for Window)

3.2.3.2 Moina macrocopa acute toxicity test

For preparation of dilution water, stock solution of salt was made with composition in following Table 3.3. Cultivation medium was prepared by addition of 5 ml of each stock solution into 0.5 liter of dilution water and afterwards adjust to total volume of 1 liter, pH is adjusted to 8.2 $\pm 1^{o}$C. The dilution water was aerated through night at least a day before its use for oxygen saturation and perfect salts dissolving and homogenization. Collect new born cladoceran exceed 24 h. old from the preliminary cultivation into 250 ml beaker, where they are located before the test start without feeding.

Table3. 3 Stock solutions of salts for Water flea test

Stock solution	Chemical	Concentration (mg/l)
ZR1	$CaSO_4.2H_2O$	120
ZR2	$MgSO_4.7H_2O$	120
ZR3	$NaHCO_3$	174
ZR4	KCL	8
ZR5	CaCO3	170

In triplicate, use dilution medium for the preparation the water samples in 100 ml beaker. Put the newborn organisms (one day) 10 individuals in one beaker. Every the established period 24 h, immobilized cladoceran were recorded. In triplicate, controls (only dilution medium) were applied within the test. The 48 h LD_{50} for cladoceran and its 95% confidence limits were calculated using a program based on Probit Analysis Method using SPSS version 16 for Window (Statistical Package for the Science/Personal Computer Plus for Window)

3.2.4 Genotoxicity and mutagenesis assay

3.2.4.1 The analysis of *O. niloticus* and *C.carpio* DNA strand breaks

In triplicate, 10 adult healthy fish of similar size (about 35-40 mm length, Figure (A-10) were randomly sampled and transferred from the acclimation tank into test chambers that were glass aquarium of 25 liters capacity. Each aquarium was stocked with fish with 20 liters of water sample that were diluted using filtered tap water as dilution water to the ten percentage of lethal concentration (LC_{10}) for 14 days under conditions of 12:12 h light: dark, and aerated all time. In triplicate, nonexposed fish were observed in fresh water under same conditions to be control experiment. Blood of fish were collected at 0, 7, 14days all of the experiment.

3.2.4.2 Sampling and Testing of comet assay

The alkaline comet assay was performed by a small modification the method of *Y. Deguchi et al.* in 2007 and *Praditta in 2007*. Peripheral blood of fish was collected from a caudal vein using 1 ml heparinized syringe. 15 microliters of blood is diluted with 1 ml of PBS pH 7.5 (135 mM NaCl, 2.5 mM KCl, 1.75 mM K_2HPO_4, 8 mM Na_2HPO_4). Slide preparation, 2.5 μL of diluted sample were mixed with 50 μL of PBS and 50 μL of 0.5% LMP agarose in microcentrifuge tube at 37-40 ^oC and layered on comet slide (Figure 3.5), and after this layer will be solidified at 4 ^oC . The slides were immersed in the alkalilysis buffer (1% sodium sarcosinate, 2.5M NaCl, 100mM Na2EDTA, 10M Tris HCl, pH 10, 10% DMSO, 1% Triton X-100) for 3-24 h. at 4 ^oC in the dark. And then, slides were placed in alkaline electrophoresis buffer (10 N NaOH, 200 mM EDTA 5 ml, pH>13) for 10 min. Electrophoresis was performed at 15 V, 250 mA for 25 min at 4^oC. The slides were then neutralized with neutralization buffer (Tris-hydroxymethyl-aminomethane 48.5 g, pH 7.5) for 20 min. Slides are immerged then in Ethyl alcohol for 5 min and pure water for 10 min. It's dried by placement on hot plate 50 ^oC.

Figure 3.5 Comet slide

Finally, the cells were stained with 50 μL of SYBER safe ^{TM} Green. Comet images are analyzed using a fluorescence microscope, 515 nm and Barrier filter, 590 nm (magnification 10×) to determine the sufficient cell dispersed. It's equipped with a CCD camera (charge-coupled device, CCD), transmit picture signal to monitor.

3.2.4.3 Analysis of comet assay

One hundred cells were examined per fish by using Tritek Comet [Score] Freeware Version 1.5 by head area (H) and tail area (T) will be measured from cell comet picture (Sriussadaporn et al.) , were calculated percent of DNA damage using SPSS for Window (Statical Package for the Science/Personal Computer Plus for Window)

$$\% \text{ of DNG damage } = \frac{\sum_{i=1}^{n} (T/(T+H)}{n} X100$$

T is tail area, H is head area, and n is amount of cell

3.2.4.2 Correlation analysis

For all cases, bivariate relationships were conducted using Pearson correlation and the association between physicochemical variables, mortality level of DNA damage was performed using multiple linear regressions.

In order to analyze multiple interrelationships among all the variables, including physicochemical and toxicological ones, factor analysis was employed. Frequently used in a variety of environmental and toxicological studies (*Ren et al., 2004; Zeng and Rasmussen, 2005; Olivero-Verbel et al.2008*). Variances extracted by the factors are called the correlation values, which presented the linear relationship between two variables in strength and magnitude. For all statistical analysis, significance was set at $P<0.05$. Figure 3.6 shows all of experimental steps in this research.

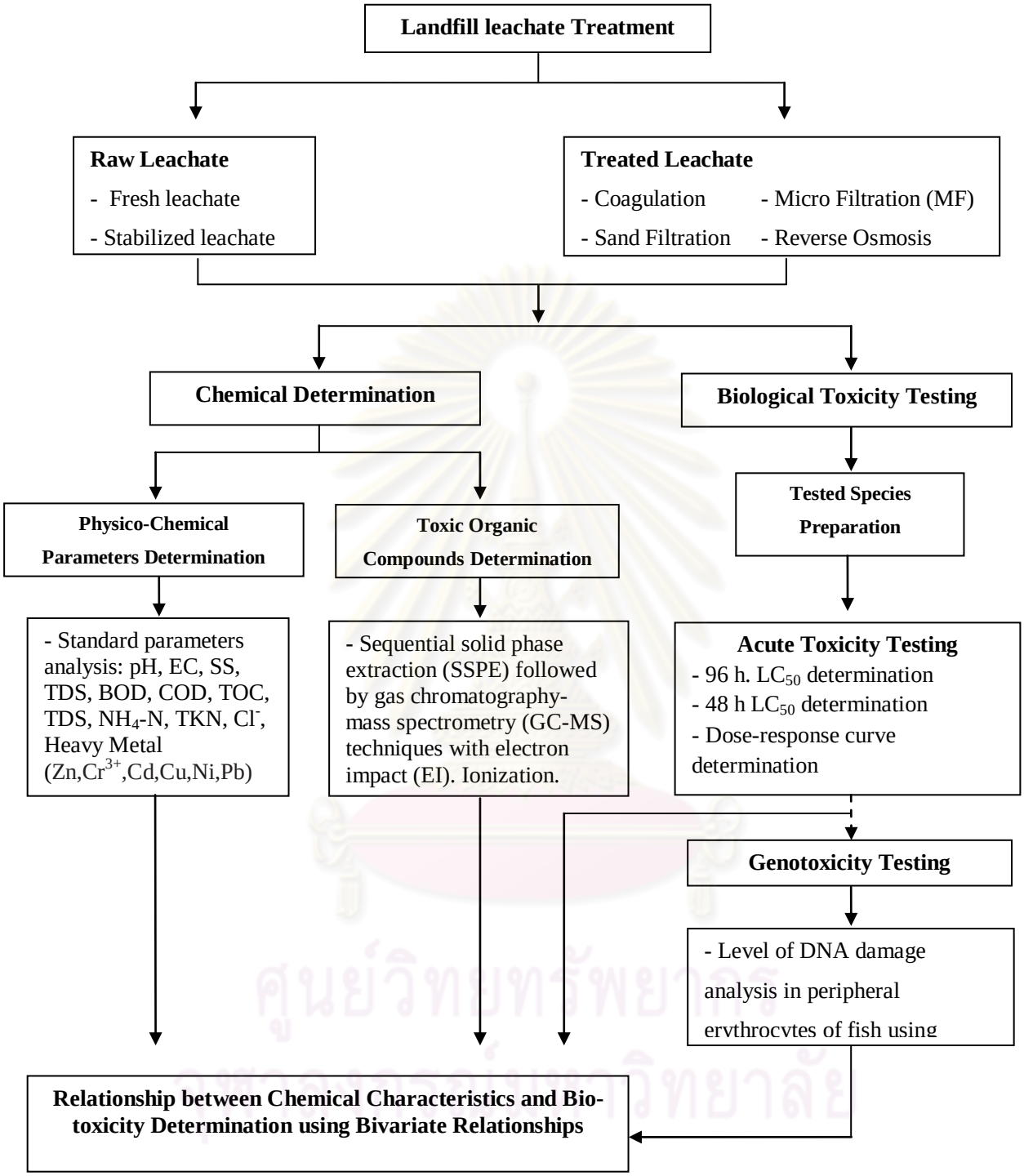


Figure 3.6 Experimental step of research

CHAPTER IV
RESULTS AND DICUSSIONS

This experiment correlated the chemical characteristic and biotoxicity of leachate and treated leachate along treatment systems. Standard chemical parameters, toxic organic compounds, acute toxicity and genotoxicity to living organisms, i.e. Water flea (Moina macrocopa), Nile Tilapia (Oreochromis niloticus) and common carp (Cyprinus carpio) of leachate samples obtain along the treatment processes were studied and compared in this work.

4.1 Leachate characteristics

The central difference between fresh and stabilized leachate characteristics were found that fresh leachate was acidic in range of 3.72 – 4.55 and stabilized leachate was alkaline in range of 8.17 – 8.65, which was in agreement of the postulate that the pH of leachate increases with landfill age (*Silva et al., 2004*). Fresh leachate contained much higher organic concentrations in terms of sBOD, sCOD and TOC as 30,400–54,700 mg/l, 32,000-67,200 mg/l, and 13,900-40,300 mg/l respectively by a factor of 20-90 compared to stabilize leachate as 200-560 mg/l, 2,400-2,880 mg/l, 420-770 mg/l respectively, and their concentrations were found within the reported range (*Kjeldsen and Christophersen, 2001, D. Kulikowska, E. Klimiuk, 2008, S. Renou et al.)*. A measure of biodegradability is sBOD/sCOD ratio. The results suggested that stabilized leachate was much less biodegradable than fresh leachate, with the average values of 0.91 for fresh leachate and 0.15 for stabilized leachate. Similar result was reviewed by *Kjeldsen et al. (2002),* a sBOD/sCOD ratio greater than 0.5 indicates a young landfill, when the ratio is less than 0.1, the landfill can be considered old and stable, whereas the ratio 0.1-0.5 indicates partially stable leachate. Nitrogen concentration in fresh leachate was about 4.5 times higher than stabilized leachate as 320-660 mg/l, 95-175 mg/l respectively in TKN, and 240-652 mg/l, 72-128 mg/l respectively in NH_3 concentration. The heavy

metals (Cr, Cu, Ni, Pb, and Cd) were found at low concentration and were mostly below the standard limit. The values are in agreement with the literature data. For example, a review of 106 Danish landfills showed that metal concentrations for all landfills were low as 0.006 mg Cd/l, 0.13 mg Ni/l, 0.07 mg Cu/l, 0.07 mg Pb/l and 0.08 mg Cr/l (*Kjeldsen and Christophersen, 2001*). **Table 4.1** shows the characteristics of treated leachate along the treatment process and the industrial effluent standards by Ministry of Industry, Thailand.

Table 4.1 Raw and treated leachate characteristics*

Parameters	**Treatment System**				**Standard****
	Coagulation	Sand Filtration	MF	RO	
pH	4.7± 0.4	5.1± 0.4	4.96± 0.1	6.33 ± 0.5	5.5-9
EC	23,800±2650	17,800±1900	6,300±420	3.09± 0.99	-
Chloride	5,500± 820	4,900± 800	5,660± 1410	2,300± 884	20
sBOD	47± 10	24± 12	11± 1	5± 0.4	120
sCOD	850 ± 90	510 ± 155	120 ± 57	15± 0.7	-
TOC	164 ± 15	152 ± 19	68 ± 15	7.5± 1.4	-
TKN	77 ± 12	59 ± 12	36± 13	4.3± 1.2	100
NH_3-N	66± 10	50± 13	29± 10	3.3± 0.8	-
TDS	14,200 ±3000	12,700 ±2200	9,900± 4000	1,870± 413	3,000
SS	240 ± 52	190 ± 42	ND	ND	50
Cr	NA	NA	NA	0.069± 0.012	0.25
Cu	NA	NA	NA	0.002± 0.001	2.0
Ni	NA	NA	NA	0.003± 0.002	1.0
Pb	NA	NA	NA	ND	0.2
Cd	NA	NA	NA	0.002± 0.001	0.03

** Industrial effluent standard, Ministry of Industry, Thailand

In coagulation process, sCOD was reduced from the influent value of 2,400-2,880 mg/l to 720 – 960 mg/l in the effluent, meaning 68% of total sCOD. In the literature, old leachate, coagulation and flocculation can be expected to remove between 40% and 90% of COD (*S. Renou et al., 2008*). The degree of ammonia removal was 40.9% of total ammonia while suspended solid was low level of removal as 17% of total suspended

solid from the influent. After coagulation process, the leachate underwent sand filtration, 80% of COD, 72.3% TKN, 56 % NH_3, and 34% suspended solid (SS) were removed. The result indicated that for stabilized leachate, chemical coagulation using $FeCl_3$ followed by sand filtration could effectively reduced organic (both biodegradable and recalcitrant) substances and partially removed nitrogenous compounds in leachate

Further treatment by MF and RO membranes reduced most pollutant concentrations to below the standard limit. Most of suspended solid (SS) were remove at MF process up to 40% from the effluent of sand filtration. In literature, MF was used as a pre-treatment for another membrane process (UF, NF or RO) for separating colloidal and suspended particles (*S. Renou et al., 2008*). Consequently, this treatment processes could reduce total dissolved solid (TDS), suspended solid (SS), ammonia nitrogen, heavy metal, and COD by >90.5%, >99%, >96.7, >90%and >99% respectively. The result suggests that COD was majorly removed by coagulation as whereas NH_3 concentration was largely eliminated at the membrane processes, and most of ionic pollutants were removed at RO process, the final treatment stage.

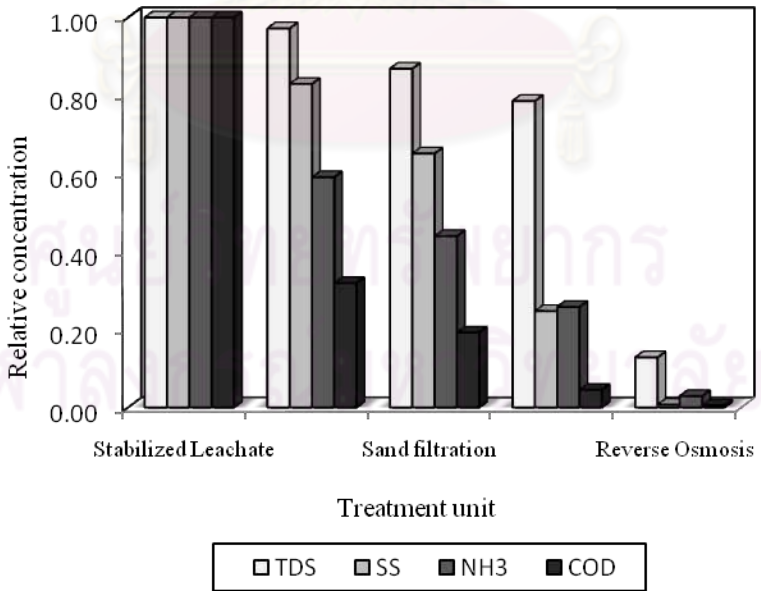


Figure 4.1 Relative concentration of TDS, SS, NH_3, and COD in raw (stabilized) and treated leachate along the treatment process

4.2 Toxic organic compounds determination

4.2.1 Occurrence of Organic Compounds

Table 4.2 shows organic compounds found in raw leachate (fresh and stabilized leachate) and treated leachate along the treatment process. These compounds were categorized to 10 groups of organic compounds, e.g. aliphatic hydrocarbon, aromatic hydrocarbons, aldehydes, acids and esters, alcohols and ethers, phthalates, phenolics, nitrogen-containing, silica –containing, and pharmaceuticals. From 69 identified organic compounds 9 are classified as Priority Pollutants (*USEPA, 2005*); including Xylene, Di-ethylphthalate (DEP), Di-butyl phthalate (DBP), Di-(2-ethylhexyl) phthalate (DEHP), Di-N-octyl-phthalate (DOP), Cresols, Bisphenol A, Naphthalene, and Naphthalene, 1-methyl-. From the literature data, these compounds appear to be commonly xenobiotic organic compounds (XOCs) for landfill leachate, which they were reported in previous studies, including aromatic, phenols, and phthalates (*Paxéus, 2000, Schwarzbauer et al., 2002; Baun et al., 2003, 2004*). These compounds originate from disposed many different types of waste from household such as cosmetics, paints, solvents, oils, cleaning compounds, pesticides, plasticizers degreasing compounds as well as plasticizers and pharmaceutical materials routinely disposed in landfill (*Paxéus, 2000, Slack et al., 2005*).

The relative concentrations of organic compounds were compared using peak areas of GC-MS chromatograms that were shown in Appendix C. The behavior of toxic contaminates as vary in different treatment were considered using solid and soluble fractionation. In raw leachate, the result shows that fresh leachate contained different organic compound groups with stabilized leachate as some those compounds were eliminated or leached from solid wastes after long term storage in landfill and storage pond. A large groups of compounds found in fresh leachate were acids and esters, as fat, oil, and wax originate from food scraps and natural products, whereas stabilized leachate, organic compounds mainly contain higher molecular weight compounds, as phthalates, aromatic hydrocarbons, phenols, and nitrogen containing compounds.

4.2.2 Removal of organic compounds

The organic compounds removals along treatment process were also presented as Table 4.2. The result suggests that most of organic compounds were eliminated more than 80 % at sand filtration process. The average of organic removal each groups were 89.57%, 100%, 100%, 94.20%, 50.79, 74.67%, 97.36%, 99.44%, 93.98%, 100% for aliphatic hydrocarbon, aromatic hydrocarbon, acids and esters, alcohols and ethers, phenols, phthalates, substituted benzenes, nitrogen-containing, silica –containing, and pharmaceuticals respectively. The result of fractionation showed that phenols and phthalates mainly detected in soluble form, so their removal through sand filtration process were lower. For Phthalates, DEHP and DBP were mainly detected in solid bounded form but their removals through sand filtration process were lower also. Furthermore, their removals through microfiltration process were much different. One possible reason is that DEHP and DBP may be small colloid particle which could penetrate through the sand filtration, and DBP may be smaller colloid particle attach onto soluble phase which could penetrate through the micro filtration. Meanwhile Bisphenol A and DBP were predominated in soluble form and thus not highly removed. Subsequent treatment by RO process effectively removed those remaining toxic compounds resulting in total elimination efficiencies of 100%, 100%, 100%, 100%, 99.44%, 99.85, 100%, 100%, 99.84%, and 100% respectively.

Table 4.2 Categories of organic compounds found in raw leachate and treated leachate

Compounds	Raw leachate				Relative concentration along treatment process			
	Fresh leachate		Stabilized leachate		Stabilized leachate	Sand filtration	Micro Filtration	Reverse Osmosis
	Solid	Soluble	Solid	Soluble				
Aliphatic hydrocarbons								
n-Octadecane	0.00	1.00	0.81	0.19	1.00	0.00	0.00	0.00
n-Nonadecane	0.40	0.60	0.96	0.04	1.00	0.13	0.01	0.00
n-Eicosane	0.11	0.89	-	-	-	-	-	-
n-Docosane	1.00	0.00	-	-	-	-	-	-
n-Tetracosane	0.00	1.00	0.43	0.57	1.00	0.26	0.07	0.00
n-Hexacosane	0.00	1.00	-	-	-	-	-	-
n-Octacosane	-	-	0.00	1.00	1.00	0.00	0.00	0.00
n-Nonacosane	0.00	1.00	0.00	1.00	1.00	0.00	0.00	0.00
Aromatic hydrocarbons								
p-Xylene *	-	-	1.00	0.00	1.00	0.00	0.00	0.00
Naphthalene	-	-	1.00	0.00	1.00	0.00	0.00	0.00
Naphthalene, 1-methyl-*	-	-	1.00	0.00	1.00	0.00	0.00	0.00
1,1'Biphenyl, 2-methyl-	-	-	1.00	0.00	1.00	0.00	0.00	0.00
1,1'Biphenyl, 4-methyl-	-	-	1.00	0.00	1.00	0.00	0.00	0.00
Aldehydes								
Benzaldehyde	-	-	1.00	0.00	1.00	0.15	0.00	0.00
Benzeneacetaldehyde	0.72	0.28	-	-	-	-	-	-
Acids and Esters								
Benzoic acid	0.00	1.00	-	-	-	-	-	-
Phthalic acid	0.00	1.00	-	-	-	-	-	-
Oceanic acid (Caprylic acid)	0.25	0.75	-	-	-	-	-	-
Decanoic acid (Capric acid)	0.37	0.63	-	-	-	-	-	-
Dodecanoic acid (Lauric acid)	0.88	0.12	-	-	-	-	-	-
Tetradecanoic acid (Myristic acid)	1.00	0.00	-	-	-	-	-	-
Pentadecannoic acid	0.61	0.39	1.00	0.00	1.00	0.00	0.00	0.00

N of Raw leachate = 8, N of treated leachate = 2

Table 4.2 (Continued)

Compounds	Raw leachate				Relative concentration along treatment process			
	Fresh leachate		Stabilized leachate		Stabilized leachate	Sand filtration	Micro filtration	Reverse Osmosis
	Solid	Soluble	Solid	Soluble				
Hexadecanoic acid (Palmitic acid)		0.00	-	-	-	-	-	-
Heptadecanoic acid (Margaric acid)	1.00	0.09	-	-	-	-	-	-
Octadecanoic acid (Stearic acid)	0.91	0.00	1.00	0.00	1.00	0.00	0.00	0.00
Stearic acid, methyl ester	1.00	0.72	1.00	0.00	1.00	0.00	0.00	0.00
Linoleic acid, methyl	0.28	0.74	0.30	0.70	1.00	0.00	0.00	0.00
Octadecanoic acid, ethyl ester	0.26	-	1.00	0.00	1.00	0.00	0.00	0.00
Palmitoleic acid, ethyl ester	-	0.00	1.00	0.00	1.00	0.00	0.00	0.00
Oleic acid, methyl ester	1.00	-	1.00	0.00	1.00	0.00	0.00	0.00
Palmitic acid, methyl ester	-	0.00	1.00	0.00	1.00	0.00	0.00	0.00
Margaric acid, methyl ester	1.00	-	1.00	0.00	1.00	0.00	0.00	0.00
Palmitoleic ester	-	0.00	1.00	0.00	1.00	0.00	0.00	0.00
Oleic acid, ethyl ester	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00
Stearic acid, ethyl ester	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00
Linoleic acid, ethyl ester	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00
	1.00							
Alcohols and Ethers								
Benzyl alcohol		-	1.00	0.00	1.00	0.04	0.00	0.00
2-Propanol,1-(2-methoxypropoxy)-	-	-	0.00	1.00	1.00	0.17	0.00	0.00
Ethanol, 2-butoxy-	-	-	0.98	0.02	1.00	0.00	0.00	0.00
Phytol	-	0.50	1.00	0.00	1.00	0.00	0.00	0.00
Thiophene, 2-tert-butoxy-	0.50	-	0.00	1.00	1.00	0.00	0.00	0.00
	-							
Phenols								
Phenol, methyl (Cresols)*	-	-	0.00	1.00	1.00	0.00	0.00	0.00
BHT-aldehyde (2,6-di(t-butyl)-4-hydroxybenzaldehyde)	-	-	0.00	1.00	1.00	0.27	0.00	0.00
Phenol,2,4-bis-(tert-butyl)-	0.00	1	0.00	1.00	1.00	0.43	0.06	0.00
Phenol,2,6-bis-(tert-butyl)-	-	-	0.00	1.00	1.00	0.41	0.00	0.00
Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl-	0.00	1	-	-	-	-	-	-
Bisphenol A*	0.00	1	0.00	1.00	1.00	0.85	0.18	0.02

N of Raw leachate = 8, N of treated leachate = 2

Table 4.2 (Continued)

Compounds	Raw leachate				Relative concentration along treatment process			
	Fresh leachate		Stabilized leachate		Stabilized leachate	Sand filtration	Micro filtration	Reverse Osmosis
	Solid	Soluble	Solid	Soluble				
Phthalates								
Di-ethylphthalate *	0.66	0.34	-	-	-	-	-	-
Di-butyl phthalate *	-	-	0.75	0.25	1.00	0.32	0.28	0.004
Di-(2-ethylhexyl) phthalate *	0.71	0.29	0.82	0.18	1.00	0.44	0.06	0.00
Di-N-octyl-phthalate	1.00	0.00	-	-	-	-	-	-
Nitrogen-containing								
2-Pyrrolidinone,1-butyl-	0.35	0.65	1.00	0.00	1.00	0.03	0.02	0.00
Formamide, N,N-dibythyl-	-	-	0.00	1.00	1.00	0.00	0.00	0.00
Acetamide, N,N-dibythyl-	-	-	0.89	0.11	1.00	0.00	0.00	0.00
Benzamide, N,N-diethyl-3-methyl-	0.00	1.00	-	-	-	-	-	-
Benzenesulfonamide, N-ethyl-4-methyl-	-	-	0.00	1.00	1.00	0.00	0.00	0.00
N,N-diethyl-m-toluamide	-	-	0.00	1.00	1.00	0.00	0.00	0.00
N-Acetylpiperidine	-	-	0.00	1.00	1.00	0.00	0.00	0.00
Pyrazine, tetramethyl-	-	-	0.00	1.00	1.00	0.00	0.00	0.00
Pyrazine, 2-ethyl-3,5-dimethyl-	-	-	0.00	1.00	1.00	0.00	0.00	0.00
1,2-Benzisothiazole	-	-	0.00	1.00	1.00	0.00	0.00	0.00
Silica –containing								
Silane, tri-methoxy-methyl-	0.36	0.64	0.00	1.00	1.00	0.12	0.02	0.003
Cyclododeccasiloxane, tetracosanmethyl-	1.00	0.00	-	-	-	-	-	-
Cyclononasilioxane, octadecamethyl-	1.00	0.00	-	-	-	-	-	-
Cyclononasilioxane, decamethyl-	-	-	1.00	0.00	1.00	0.00	0.00	0.00
Pharmaceuticals								
Caffeine	0.00	1.00	-	-	-	-	-	-
Ibuprofen	-	-	0.00	1.00	1.00	0.00	0.00	0.00
Nicotine	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00

N of Raw leachate = 8, N of treated leachate = 2

4.3 Acute Toxicity Determinations

4.3.1 LC_{50} value determination

Dose-response relationships of fresh and stabilized leachate on tested organisms are shown in Figure 4.2. The results found that the lowest level which response begin of *Moina macrocopa, Oreochromis niloticus, and Cypinus carpio* in fresh leachate were 1.145% (v/v), 0.597% (v/v), and 1.237 % (v/v) respectively, whereas stabilized leachate were higher than, with the threshold of 2.273% (v/v), 1.215% (v/v), and 5.709 % (v/v) respectively. Furthermore, the sensitivity of tested species could be measured from slope of dose-response curve. For fresh leachate, it found that *Oreochromis niloticus* were most sensitive organism as compared to *Cypinus carpio* and *Moina macrocopa*, with the slope of 1.737, 1.346, and 0.871, whereas stabilized leachate, the most sensitive organism was *Cypinus carpio* as 0.351, with compared to *Oreochromis niloticus* and *Moina macrocopa* as 0.1572 and 0.081 respectively

The Determination of LC_{50} and its 95% confident limit during acute toxicity test on living organisms are presented in Table 4.3. Based on Probit Analysis method, the mean LC_{50} values of fresh and stabilized leachate using *O. niloticus*, *C. Carpio* and *M. Macrocopa* were found to be 1.81 % (v/v), 1.91 % (v/v) and 0.98 % (v/v) concentration on volumetric basis, whereas stabilized leachate, they were 7.80%, 8.05% and 4.22% respectively. The results suggested that fresh leachate was more toxic than stabilized leachate on all tested organisms, and it affect *O. niloticus* more than other organisms.

Figure 4.3 shows the comparisons of dose-response relationships between stabilized leachate and treated leachate along treatment process. They founds that lowest level at which response begin tend to increase along treatment process from stabilized leachate, coagulation unit, sand filtration unit, and microfiltration unit as range of 1.2-5.7% (v/v), 10.7-10.9% (v/v), 14.3-20.5% (v/v), and 22.0-23.3% (v/v) respectively.

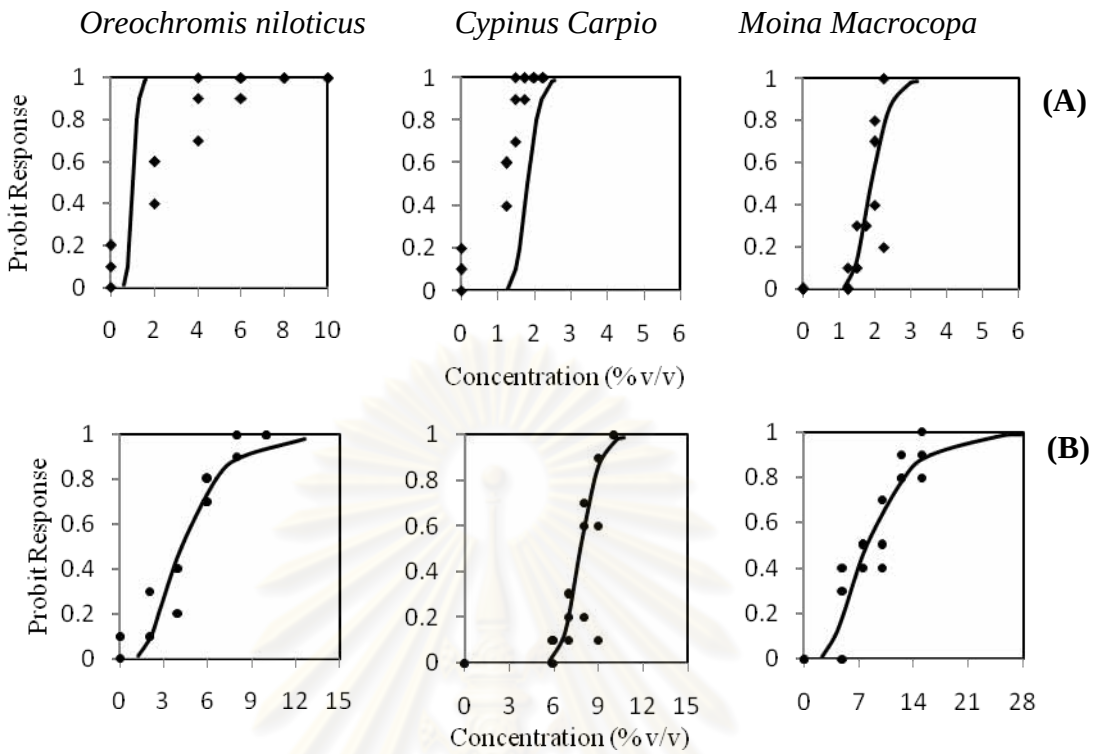


Figure 4.2 Dose-response curves of raw leachate on tested species

(A) Fresh leachate **(B)** Stabilized leachate

Table 4.3 Average 96-hours LC_{50} values with 95% confidence limit of raw and treated leachate on test species.

Water Samples	LC 50 (% v/v) and 95% Confident Limits					
	Oreochromis niloticus		*Cypinus carpio*		*Moina macrocopa*	
Raw Leachate						
Fresh Leachate	0.98	0.81 – 1.74	1.81	1.32 – 1.83	1.91	1.63 – 2.32
Stabilized Leachate	4.22	2.74 – 6.08	7.80	7.32 – 8.32	8.05	5.29 – 7.75
Treated Leachate						
Coagulation	21.69	18.93 – 24.63	18.44	16.99 – 19.95	17.49	15.91 – 18.99
Sand Filtration	28.37	25.08 – 31.99	26.63	25.29 – 28.00	24.03	22.81 – 25.33
Micro Filtration	38.42	31.13 – 41.92	29.04	27.91 -30.23	34.85	32.01 – 37.94
Reverse Osmosis	100	nd	100	nd	100	nd

nd: not detected

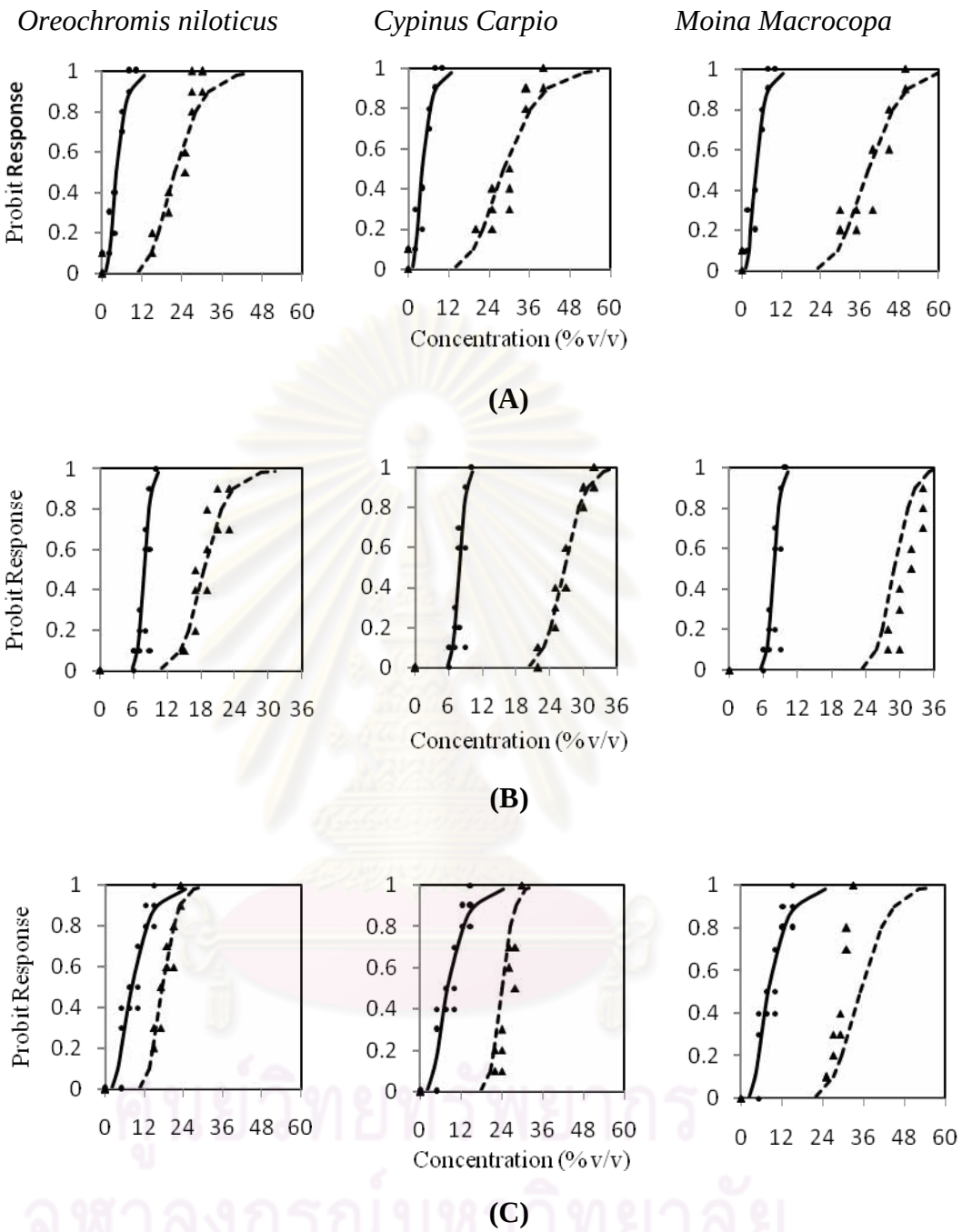


Figure 4.3 Comparisons of dose-response relationships between stabilized leachate and treated leachate along treatment process **(A)** Coagulation unit (B) Sand Filtration unit **(C)** Microfiltration unit

The LC_{50} and 95% confident limit during acute toxicity of different degree of treated leachate is also presented in Table 4.3. The results show that the effluent from each of treatment unit respectively increase the 50% lethal concentration value (LC_{50}), with the range of 17.49-21.69% (v/v), 24.03-28.37 % (v/v), 29.04-38.42% (v/v), and 100% (v/v) respectively. It indicate that the effluent from along treatment processes were less toxic to all tested species as range of. Treatment process could effectively reduce acute toxicity, especially reverse osmosis membrane which could eliminate acute toxic to be non apparent mortality on tested species.

4.3.2 Correlation between acute toxicity and chemical pollutant

Bivariate correlation analyses between mortality and physicochemical parameters are show in Table. 4.4 They indicated the correlation coefficient and significant differences between mortality of tested organisms and physicochemical parameters which were un-ionized ammonia, COD, conductivity, pH, and chloride. The correlation coefficient between mortality of *O. niloticus, C. carpio and M. macrocopa* and COD concentration values were 0.580, 0.275, and 0.197 respectively, whereas un-ionized ammonia concentration values were 0.417, 0.611, and 0.722 respectively. The significant levels of *O. niloticus, C. carpio and M. macrocopa* and COD concentration values were 0.000, 0.008, and 0.058 respectively, whereas un-ionized ammonia concentration values were 0.000, 0.000, and 0.000 respectively. The result indicated that mortality of *C. carpio and M. macrocopa* was significant positive correlated ($P < 0.01$) with ammonia and sCOD. Further parameter, conductivity was correlated with mortality of *O. niloticus, C. carpio and M. macrocopa* as 0.354, 0.479, and 0.697respectively. The significant levels were 0.001, 0.000, and 0.000 respectively. It means that as *M. macrocopa* were most significant positive correlated ($P<0.05$) with ionized compounds in leachate, compared with *C. carpio* and *O. Niloticus* respectively. Chloride lowly was correlated with tested organisms as positive correlation; 0.212, 0.396, and 0.344. The significant levels were 0.041, 0.000, and 0.001 respectively. A negative correlation were found as mortality of *O. niloticus, C. carpio and M. macrocopa* and pH values, with the value of -0.378(0.000), -0.382(0.000), -0.131(0.000).

Table 4.4 Correlation between acute toxicity and physicochemical parameters.

	Mortality			un-ionized ammonia	COD	Conductivity	pH	Cl-
	O. Niloticus	*C. Carpio*	*M. Macrocopa*					
Mortality of *O. Niloticus*	1 (0.000)							
Mortality of *C. Carpio*	0.796** (0.000)	1 (0.000)						
Mortality of *M. Macrocopa*	0.853* (0.000)	0.845* (0.000)	1 (0.000)					
un-ionized ammonia	**0.417**** (0.000)	**0.611**** (0.000)	**0.722**** (0.000)	1 (0.000)				
COD	**0.580**** (0.000)	**0.275**** (0.008)	**0.197*** (0.058)	-0.222) (0.333)	1 (0.000)			
Conductivity	**0.354*** (0.001)	**0.479*** (0.000)	**0.605*** (0.000)	0.765 (0.000)	0.714 (0.000)	1 (0.000)		
pH	**-0.378*** (0.000)	**-0.382*** (0.000)	**-0.131*** (0.211)	0.765 (0.000)	0.213 0.426	0.053 (0.932)	1 (0.000)	
Cl^-	**0.212*** (0.041)	**0.396*** (0.000)	**0.344*** (0.001)	0.143) 0.308)	0.375 (0.095)	0.719 (0.000)	0.765 (0.407)	1 (0.000)

N= 93

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

The result suggests that un-ionized ammonia have a direct relationship to toxicity, it is increase the sensitivity of *M. macrocopa, and C. carpio,* and O. *niloticus* respectively. The result also suggests that organic matters, which were presented in COD concentration, have direct relationship toxicity, it is increase the sensitivity of *O. niloticus* .This difference can be caused by nature of organisms tested. However, ammonia was main cause of mortality.

Based on pollutant concentration, Clement *et al*. (1993) concluded that ammonia was the main cause of the toxicity measured in the bio-tests, whereas several studies based on genotoxicity test found that organic compounds in leachate may cause the mutagenic activity (*Kjeldsen et al., 2002*). However, toxicity in leachate from Taiwan was not dependent on ammonia content and a significant degree of variation was detected on several factors that may influence leachate toxicity (*Fan et al., 2006*).

4.4 Genotoxicity determination

4.4.1 Level of DNA damage

This study, the comet assay was utilized as biomarker of the genotoxic potential of the raw and treated leachate, which was diluted using degree of acute toxic level as ten percent of lethal concentration (LC_{10}), on fish species. Figure 4.4 and Figure 4.5 shows DNA damage appearances of comet in peripheral erythrocytes of *O. Niloticus* and *C. Carpio* after exposure in raw and treated leachate. Level of DNA damage was analyzed using image analysis on 100 cells per sample wheat are summarized in Table 4.5. The results show that both of raw leachate induced damage to the DNA of cells from the peripheral blood of *O. niloticus* and *C. Carpio,* with a significant differences ($P<0.05$) of percentage of DNA damage at fresh and stabilized leachate compared to the control.

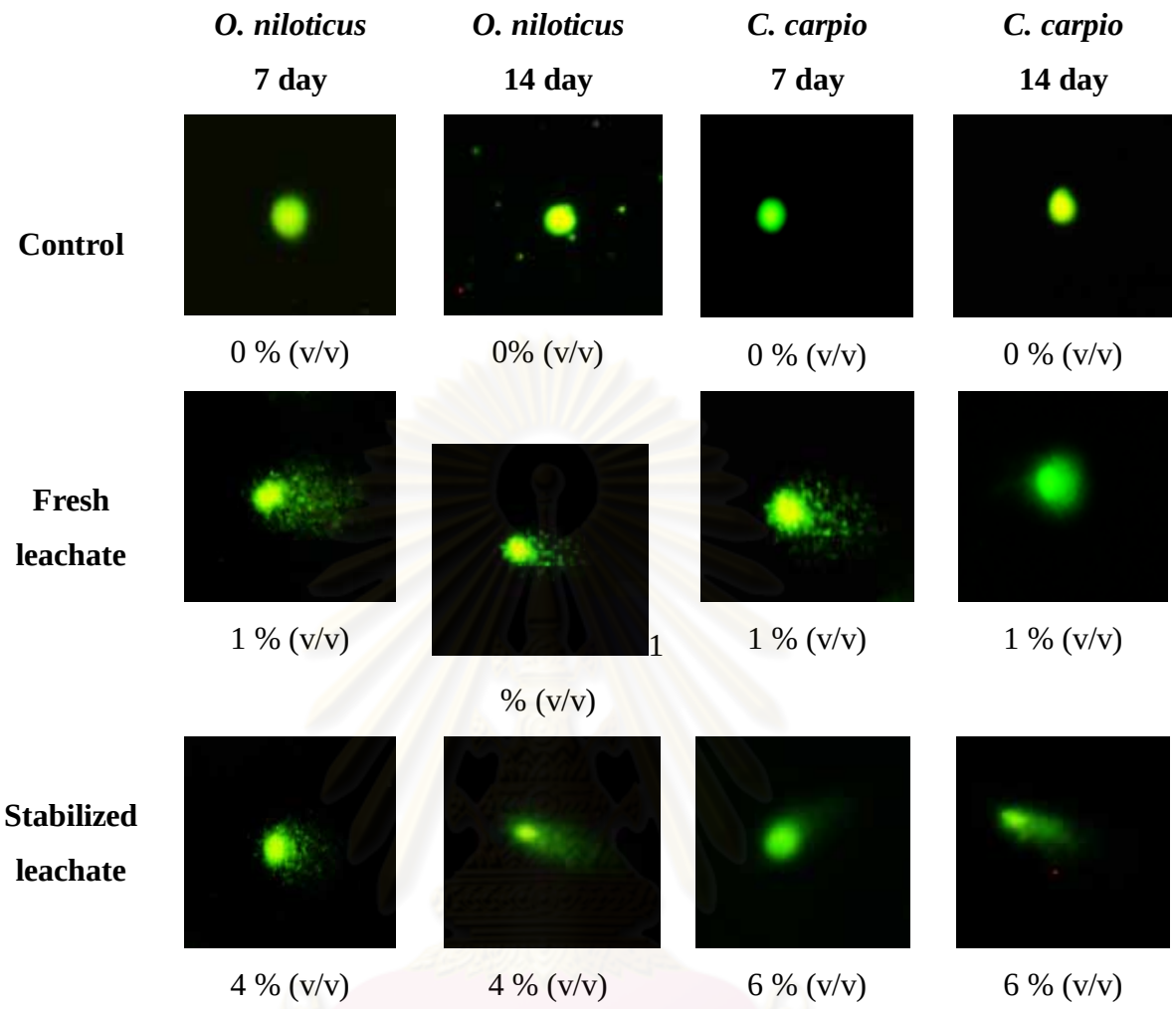


Figure 4.4 DNA damage appearances of comet in peripheral erythrocytes of fish species (*O. niloticus* and *C.carpio*) as a result of fresh leachate and stabilized leachate at LC_{10}

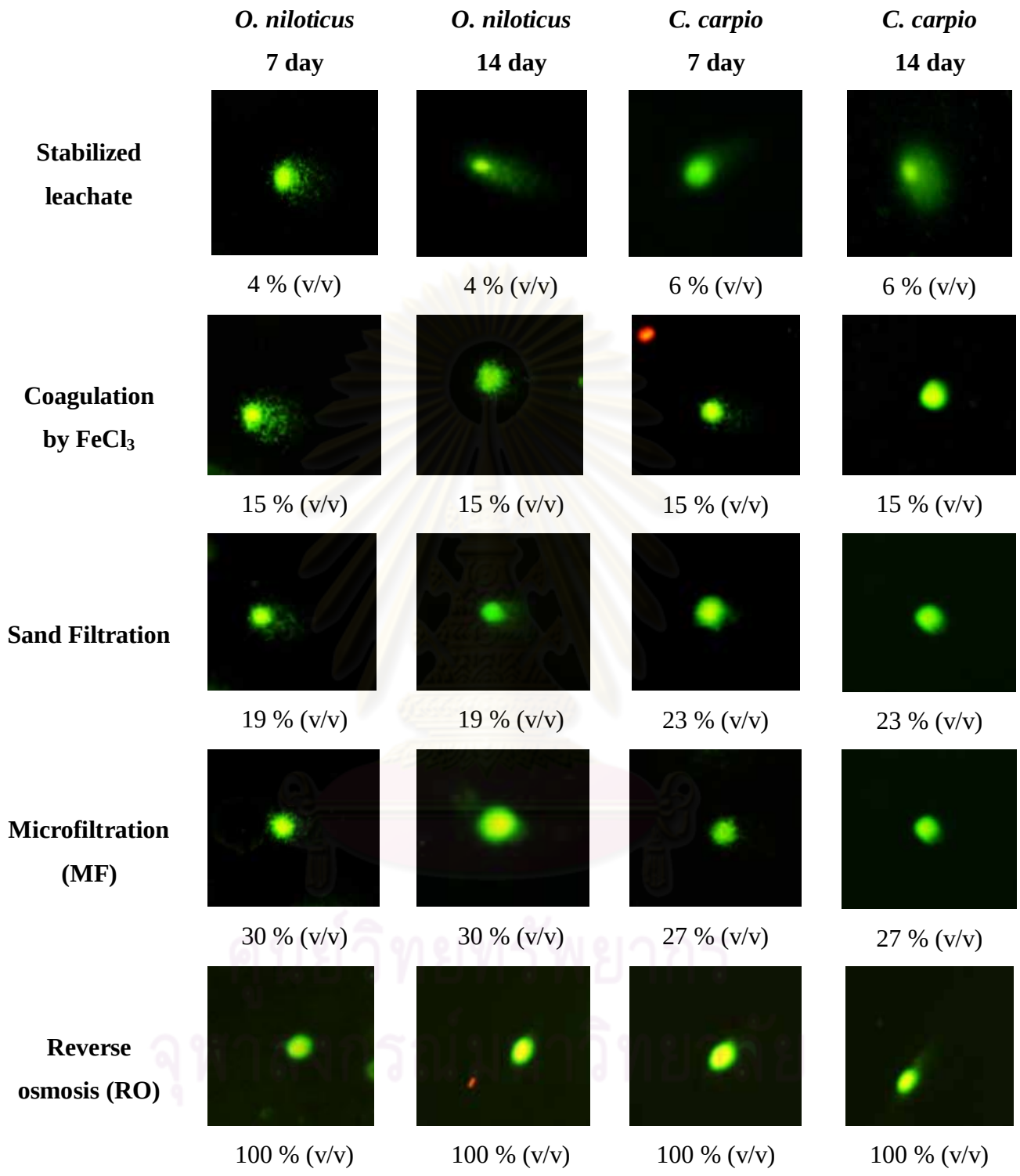


Figure 4.5 DNA damage appearances of comet in peripheral erythrocytes of fish (*O. niloticus* and *C.carpio*) as a result of treated leachate along treatment process at LC_{10}.

Table 4.5 Level of DNA damage of raw and treated leachate at 7 day and 14 day.

Water Sample	Level of DNA Damage (%)			
	7 day		14 day	
	O. Niloticus	*C. Carpio*	*O. Niloticus*	*C. Carpio*
Control	0.36661 ±0.0111	0.4419 ±0.0254	0.7869 ±0.0208	0.7719 ±0.0507
Raw Leachate				
Fresh leachate	15.4412 ±0.2233	12.5369 ±0.1484	9.4711 ±0.1924	8.8859 ±0.1028
Stabilized leachate	11.7434 ±0.1978	11.5574 ±0.1453	24.2768 ±0.2629	17.175 ±0.2296
Treated Leachate				
Coagulation	14.4424 ±0.1768	11.6117 ±0.1535	11.6037 ±0.1793	8.71036 ±0.1528
Sand filtration	12.6771 ±0.2152	9.4962 ±0.2517	9.9473 ±0.1984	7.1424 ±0.1760
Micro filtration	10.2011 ±0.1825	6.8536 ±0.1611	6.7278 ±0.1192	3.1689 ±0.1006
Reverse Osmosis	1.0389 ±0.0290	1.0630 ±0.0356	0.9543 ±0.0314	1.2774 ±0.0418

Mann-Whitney, $p < 0.05$

Analysis of percentage of DNA damage in blood cells of demonstrated that these was the significant differences ($P>0.05$) between fish in stabilized leachate and fish in treated leachate by coagulation, sand filtration, and microfiltration whereas these was the significant differences ($P<0.05$) with fish in reverse osmosis. These result indicate that genotoxicity was not reduced by the pre-treatment of the leachate treatment process, but can be reduced by reverse osmosis process.

After period exposure, DNA damage in blood cells showed the reversible, with a reduction of percentage of DNA damage compared 7^{th} and 14^{th} exposure days (Table 4.5). In literature, this type of damage is possibly reversible, which has been observed in environmental monitoring studies by other (*Nacci et al., 1996, Pandrangi et al., 1995, N.G. Lemos et al., 2005)* that after a recuperation period under non-polluted conditions in the laboratory, reflecting the reversibility and non-persistence of such damage. Michelmore and Chipman (1998) commented that DNA strand breaks, particularly as measured by the comet assay, act as a biomarker of mutagenicity in fish and other aquatic species. They also emphasized that this approach should be combined with the use of other biomarkers.

The sensitivity of tested species, the result shows that the % DNA damage values of *O. Niloticus* were higher than % DNA damage of *C. Carpi*, demonstrating that *O. Niloticus* was considerably more sensitive. This difference can be caused by different food web of tested fish. In previous study, *Grisolia et al. (2009)* evaluated genotoxic in several fish species by using the micronucleus (MN) test, the comet assay and nuclear abnormality assessment in peripheral erythrocytes. They found that *O. niloticus* (omnivorous/detritivorous) presented higher DNA damage than *C. Carpi* (algivorous), and suggested that food web should be consider for biomonitoring aquatic genotoxic under field conditions.

4.4.2 Correlation between DNA damage and chemical pollutants.

Bivariate correlation analyses between genotoxicity and pollutant concentrations are shown in **Table 4.6**. It is indicated the significant differences between DNA damage at 7^{th} and 14^{th} days with chemical pollutant concentration including, COD, unionized-ammonia, EC, pH, and chloride. The result indicates that these chemical pollutants do not have a direct relationship to DNA damage ($P>0.01$ and $P>0.05$). It is possible that DNA damage and these parameter may not correlate at this level of significant or this size of sample (n=700).Considering found organic compounds, their compounds were identified to xenobiotic compounds (XOCs), which can induce long term effects mutagenicity/genotoxicity such as aromatic hydrocarbons, phthalates, and Bisphenol A. Comparisons with *Baun* (2004) covered leachate collected from ten Danish landfill. It was found that the leachates were mutagenic after preconcentration, and the authors suggested that XOCs in leachate caused the mutagenic activity. Base on multiple genotoxicity tests of leachate from MSW landfills, *Kashiwada* (2005) founds that Ethoxyresorufin-O-deethylase (EROD) activity and vitellogenin (Vtg) induction were observed in response to exposure in leachate. However, both studies reported that analytical measurements of XOCs did not correlate with the toxicity observed in bioassay. It is suggested that landfill leachate may contain a large variety of organic compounds that are acutely and chronically toxic, and these leachate toxicity remains largely unknown.

Table 4.6 Correlation between DNA damage and physicochemical parameters.

	DNA Damage								
	O. Niloticus 7 days	*O. Niloticus* 14 days	*C. Carpio* 7 days	*C. Carpio* 14 days	COD	UIA	EC	pH	Cl-
DNA Damage									
O. Niloticus 7 day	1								
	(0.000)								
O. Niloticus 14 day	0.881**	1							
	(0.009)	(0.000)							
C. Carpio 7 day	0.938**	0.977**	1						
	(0.002)	(0.000)	(0.000)						
C. Carpio 14 day	0.966**	0.809	0.910**	1					
	(0.000)	(0.028)	(0.004)	(0.000)					
COD	**0.382***	**0.621***	**0.483***	**0.643***	1				
	(0.397)	(0.136)	(0.272)	(0.119)	(0.000)				
UIA	**0.746***	**0.392***	**0.731***	**0.418***	-0.013	1			
	(0.054)	(0.384)	(0.062)	(0.319)	(0.927)	(0.000)			
EC	**0.460**	**0.357**	**0.378**	**0.199**	-0.245	0.165	1		
	(0.359)	(0.478)	(0.460)	(0.706)	(0.643)	(0744)	(0.000)		
pH	**0.068**	**0.437**	**0.258**	**0.689**	0.264	0.852	-0.561	1	
	(0.898)	(0.386)	(0.622)	(0.130)	(0.614)	(0.028)	(0.250)	(0.000)	
Cl-	**0.222**	**0.063**	**0.103**	**-0.163**	-0.425	-0.029	0.637	-0.413	1
	(0.672)	(0.906)	(0.846)	(0.758)	(0.401)	(0.684)	(0.174)	(0.196)	(0.000)

UIA = unionized-ammonia; N = 700; ** Correlation is significant at the 0.01 level, * Correlation significant at the 0.05 level

CHAPTER V

CONCLUSIONS AND RECOMMENTATION

5.1 Conclusions

Base on the results obtained from this investigation, following conclusion can be made:

1. Raw leachate from solid waste disposal site including fresh leachate and stabilized leachate contained high pollutant concentration, with sCOD concentration in range of 32,000-67,200 mg/l and 2400-2880 mg/l, and TKN in range of 280-672 mg/l and 90-340 mg/l. Furthermore, 69 individual organic compounds were detected from both of raw leachate, fresh leachate mostly contained acids and esters, as fat, oil, wax originate from food scraps, whereas stabilized leachate simply remained high macular weight compounds because of long term elimination and leaching in storage and landfill.

2. The treatment processes was effective for chemical substance removal, could effectively reduced organic (both biodegradable a recalcitrant) substances and partially removed nitrogenous compounds in leachate after coagulation followed sand filtration, with 80% of COD, 72.3% TKN, and more than 80% of toxic organic compounds. While treatment by MF and RO membranes reduced most physico-chemical concentrations to below the standard limits. Furthermore, the remaining toxic organic compounds from sand filtration was eliminated nearly 100% after RO process.

3. Both of raw leachate could induce toxic effect to tested species. The level of 50% lethal concentration (LC_{50}) shows that fresh leachate was higher acute toxic than stabilized leachate with range of 0.98-1.91% (v/v), whereas stabilized leachate was range of 4.22 – 8.05 % (v/v). Furthermore, the genotoxicity testing by comet assay demonstrates that at low concentrations which not acute effect, both of raw leachate

could induce the damaging of DNA damage on erythrocytes of tested species, with percentage of DNA damage in range of 8.9-15.4% in fresh leachate exposure, and 11.5-24.3% in stabilized leachate exposure.

4. Advance treatment process can reduce acute toxicity and genotoxicity along treatment process to be non-mortality level and level of DNA damage similar non-exposure with raw leachate at effluent from RO process.

5.2 Recommendations

1. The correlation of chemical characteristic and biotoxicity should have batch scale test for measurement the effect in each compounds on tested species, to be complete and accurate relationship analysis.

2. In toxic organic compounds determination, the concentration of individual compounds should be analysis for comparisons and explanation the effect in each of compounds on tested species with literature data.

3. Comet assay should analysis combine with other methods because when the individuals were exposed to the treated leachate, which presents low toxicity, the capacity for DNA repair was unaffected, with non detected effect.

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APPENDICES

APPENDICE A
Picture of Experiments

Figure A-1 Stabilized pond

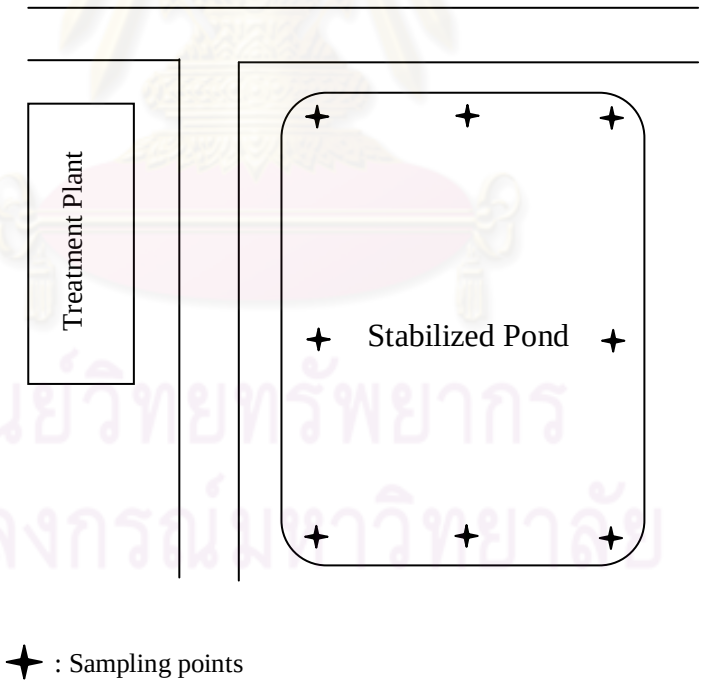


Figure A-2 Sampling points in stabilized pond

Figure A-3 Coagulation unit

Figure A-4 Sand Filtration Unit

Figure A-5 Microfiltration (5 µm) unit

Figure A-6 Reverse Osmosis (RO) unit

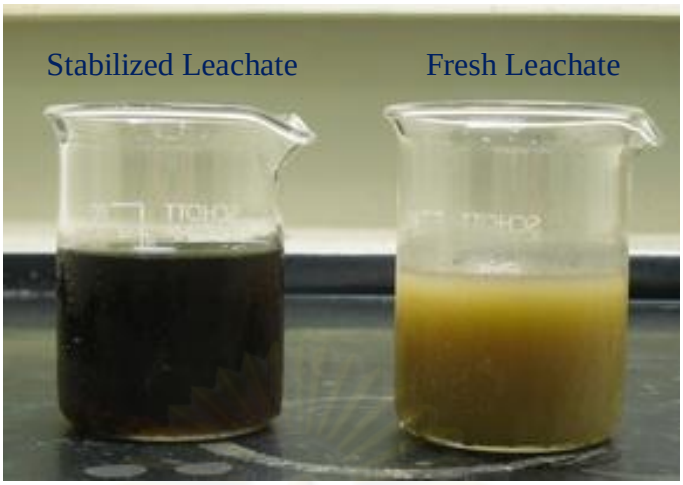


Figure A-7 Color Comparisons of Raw Leachate

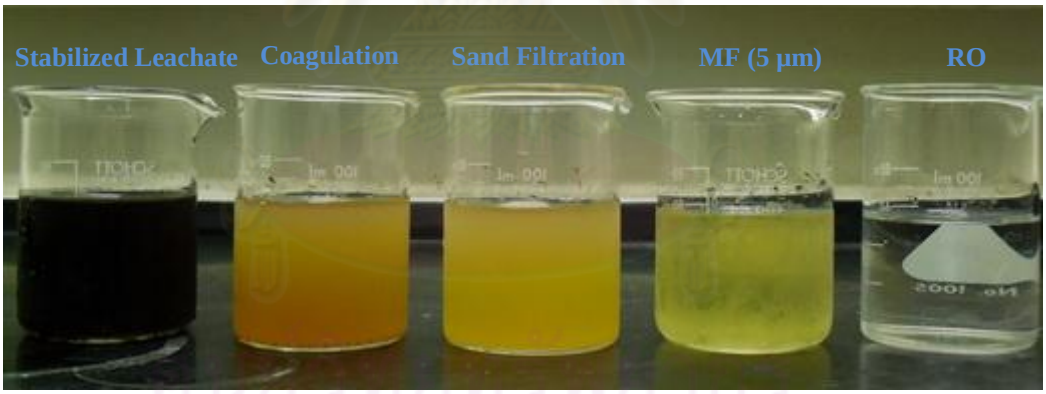


Figure A-8 Color Comparisons of Stabilized Leachate with Treated Leachate

(A) (B)

Figure A-9 Glasses Aquarium using Biotoxicity Experiments A) Glass Aquarium for Fish Preparation B) Glass Aquarium for testing

(A) (B)

Figure A-10 Tested Species using Biotoxicity Experiments A) *Oreochromis niloticus* (Nile Tilapia) B) *Cyprinus carpio* (Common Carp)

APPENDICE B
Leachate and Treated Leachate Characteristics

Table B-1 Characterization of fresh leachate

Month	Parameters									
	pH	EC (µS)	Chloride (mg/l)	BOD (mg/l)	COD (mg/l)	TOC (mg/l)	TKN (mg/l)	NH3-N (mg/l)	TDS (mg/l)	SS (mg/l)
1	3.93	55,400	2,920	54,700	55,400	13,860	310	220	34,200	13,500
2	4.02	65,400	3750	30,400	32,000	26,300	680	250	42,000	28,200
3	3.72	55,400	2500	54,400	50,250	40,300	480	360	37,800	32,000
4	4.55	56,900	2750	54,700	67,200	NA	370	245	40,700	29,800
5	4.20	55,600	2330	46,700	57,600	28,800	420	280	31,800	27,900
6	3.81	53,300	2750	51,400	57,200	23,700	375	190	31,500	34,400
7	3.87	32,300	2950	40,000	51,600	24,250	280	140	17,900	31,800
8	3.66	55,600	2100	42,400	44,400	20,560	560	280	22,800	25,500
9	4.01	51,800	3400	51,000	58,300	24,900	420	170	33,900	24,500
Average	3.97	53,500	2820	47,300	52,650	25,300	430	240	32.500	27,500
SD	0.27	8,800	505	8320	9990	7,520	125	68	7900	6150

NA: not analysis

Table B-2 Characterization of stabilized leachate

Month	Parameters									
	pH	EC (µS)	Chloride (mg/l)	BOD (mg/l)	COD (mg/l)	TOC (mg/l)	TKN (mg/l)	NH3-N (mg/l)	TDS (mg/l)	SS (mg/l)
1	8.64	15,400	6250	400	2,400	415	90	80	14,800	570
2	8.65	25,250	7600	500	2,880	630	115	84	11,700	300
3	8.17	27,600	5200	480	2,800	460	180	130	17,200	340
4	8.34	36,950	4960	560	2,500	NA	110	85	20,400	220
5	8.56	20,800	7420	310	2,800	770	280	140	13,200	250
6	8.41	20,650	7250	300	3,080	590	330	125	13,700	250
7	8.40	21,000	7550	530	2,680	750	300	130	13,500	230
8	8.34	21,150	7750	280	2,060	880	230	100	14,000	270
9	8.43	21,300	7750	200	2,680	720	280	140	13,000	230
Average	8.44	23,350	6850	400	2,650	650	210	115	14,600	290
SD	0.16	6110	1110	130	300	160	92	26	2,600	110

NA: not analysis

Table B-3 Characterization of treated leachate with coagulation process

Month	Parameters									
	pH	EC (µS)	Chloride (mg/l)	BOD (mg/l)	COD (mg/l)	TOC (mg/l)	TKN (mg/l)	NH3-N (mg/l)	TDS (mg/l)	SS (mg/l)
1	4.50	23,200	5,400	52	930	155	60	56	12,600	190
2	4.73	21,900	7,100	46	720	200	60	50	16,100	170
3	4.14	21,100	5,000	35	960	165	90	80	14,800	290
4	5.02	29,900	4,700	30	900	155	60	56	17,400	190
5	5.12	22,300	5,400	45	960	160	85	70	10,400	250
6	4.83	24,500	5,800	54	830	180	80	68	12,500	300
7	5.00	22,200	5,400	62	800	150	84	70	19,500	220
8	4.18	23,400	4,200	46	720	150	86	77	12,400	270
9	4.67	25,500	5,800	56	800	170	84	70	11,700	290
Average	4.69	23,800	5,400	47	850	165	78	67	14,200	240
SD	0.36	2,650	820	10	95	16	12	10	3,000	52

NA: not analysis

Table B-4 Characterization of treated leachate with sand filtration process

Month	Parameters									
	pH	EC (µS)	Chloride (mg/l)	BOD (mg/l)	COD (mg/l)	TOC (mg/l)	TKN (mg/l)	NH3-N (mg/l)	TDS (mg/l)	SS (mg/l)
1	5.05	15,900	4,750	12	560	159	50	30	12,500	130
2	4.67	18,200	6,600	35	560	162	48	40	11,200	120
3	4.68	15,100	4,750	15	600	154	60	54	16,900	220
4	5.83	17,900	4,250	14	440	176	49	42	10,900	160
5	5.02	17,900	4,900	16	360	172	56	48	11,800	210
6	5.75	18,600	5,300	38	760	152	77	68	14,200	200
7	4.96	21,800	4,750	42	680	136	56	48	11,000	190
8	5.06	18,000	3,900	23	360	144	56	48	22,900	230
9	5.14	17,300	4,400	16	360	112	77	70	24,400	240
Average	5.13	17,800	4,850	24	510	152	59	50	12,700	190
SD	0.41	1900	790	12	155	19	13	13	2,200	42

NA: not analysis

Table B-5 Characterization of treated leachate with Micro filtration (MF) process

Month	Parameters									
	pH	EC (µS)	Chloride (mg/l)	BOD (mg/l)	COD (mg/l)	TOC (mg/l)	TKN (mg/l)	NH3-N (mg/l)	TDS (mg/l)	SS (mg/l)
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	4.91	6,100	6,700	10.5	80	60	44.8	36.4	15,800	94.0
3	5.01	6,200	4,700	10.5	98	78	26.5	21.5	7,200	50.0
4	5.06	6,900	3,400	12.0	160	100	44.8	25.0	9,600	60.0
5	6.14	5,900	3,000	10.5	80	70	42.0	25.0	7,200	52.0
6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	5.46	6,700	2,900	12	80	65	44.8	21.0	10,600	110
Average	5.32	6,400	4,100	11.0	100	73	40.6	25.8	9,900	72
SD	0.50	430	1,600	0.75	35	17	8.0	6.2	4,100	25

NA: not analysis

Table B-6 Characterization of treated leachate with reverse osmosis (RO) process

Month	Parameters									
	pH	EC (µS)	Chloride (mg/l)	BOD (mg/l)	COD (mg/l)	TOC (mg/l)	TKN (mg/l)	NH3-N (mg/l)	TDS (mg/l)	SS (mg/l)
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	6.01	2.86	1,700	4.8	15	8.5	24.0	18.5	1,600	1.6
3	6.64	2.34	2,900	5.3	14	6.5	36.0	27.0	2,200	2.7
4	6.26	2.60	1,050	4.7	16	5.4	49.0	17.0	2,500	3.8
5	6.33	4.54	1,250	5.3	15	5.7	49.0	11.5	2,300	5.2
6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Average	6.31	3.09	1,700	5.03	15	6.7	39.5	39.5	2,200	3.3
SD	0.26	0.99	850	0.4	0.8	1.3	12.0	6.5	382	1.5

NA: not analysis

APPENDICE C
Chromatograms

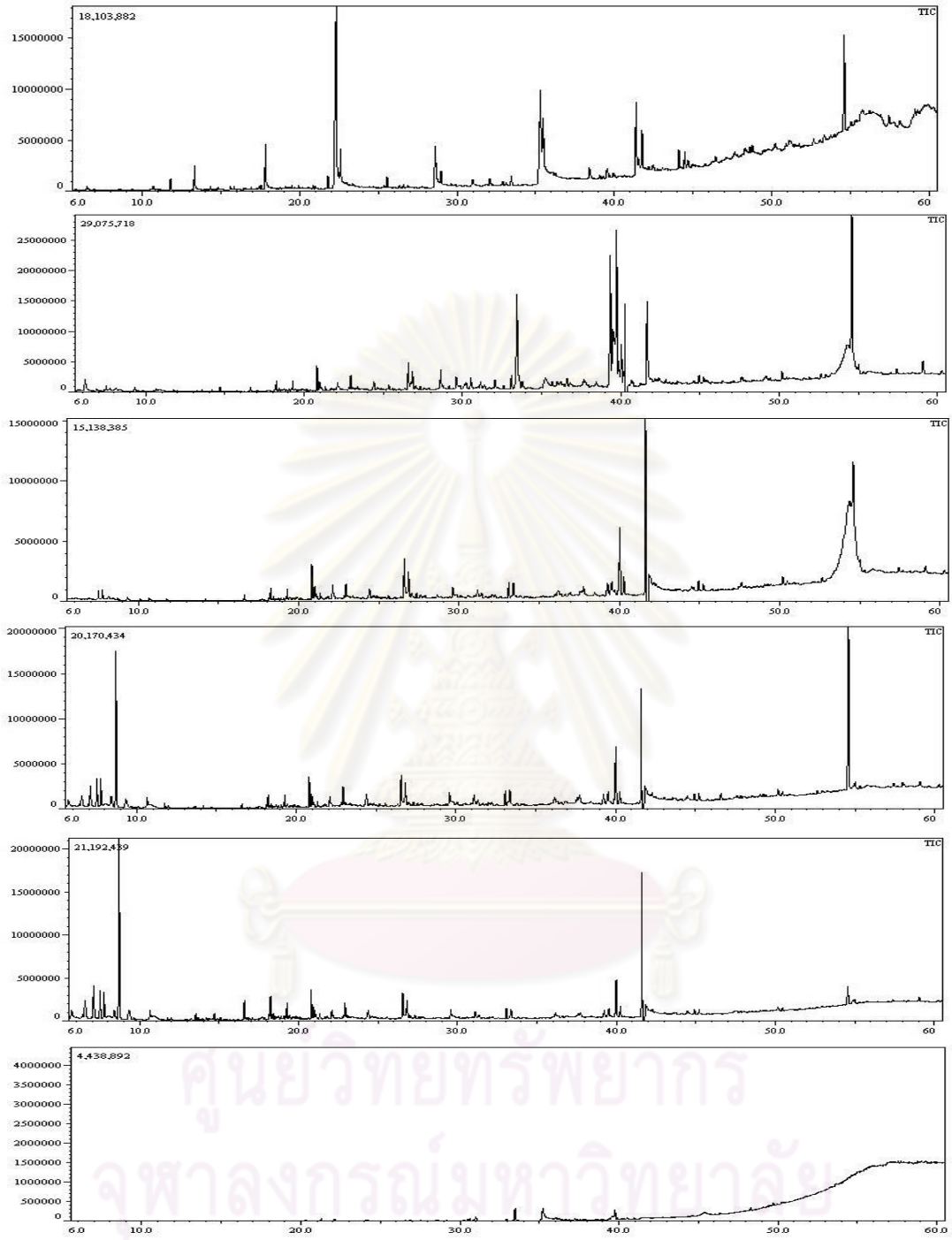


Figure C-1 Chromatograms of organic compounds containing in solid phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through C18 sorbent tube, and eluted by hexane (fraction A).

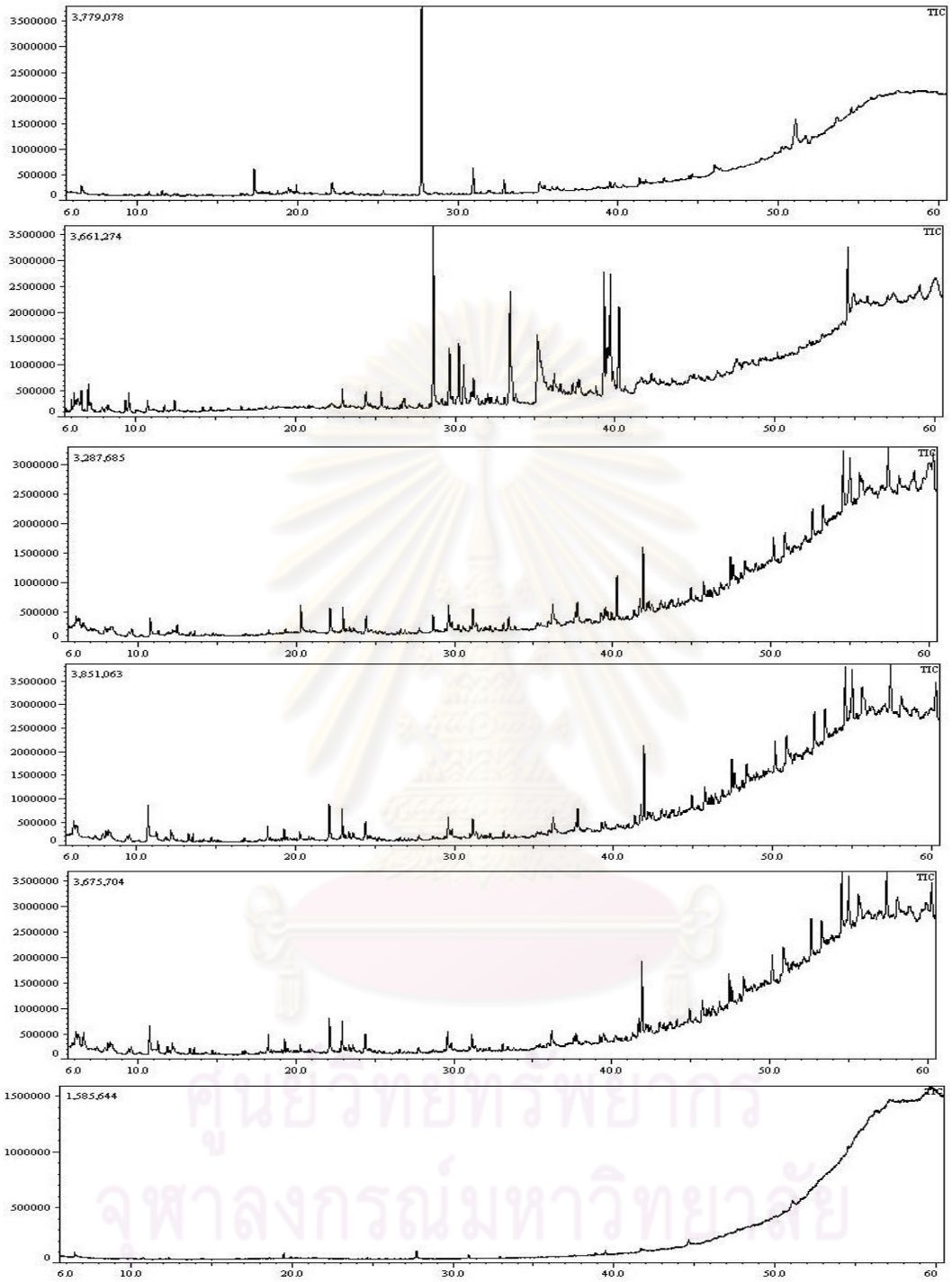


Figure C-2 Chromatograms of organic compounds containing in solid phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through C18 sorbent tube, and eluted by dichloromethane (fraction B).

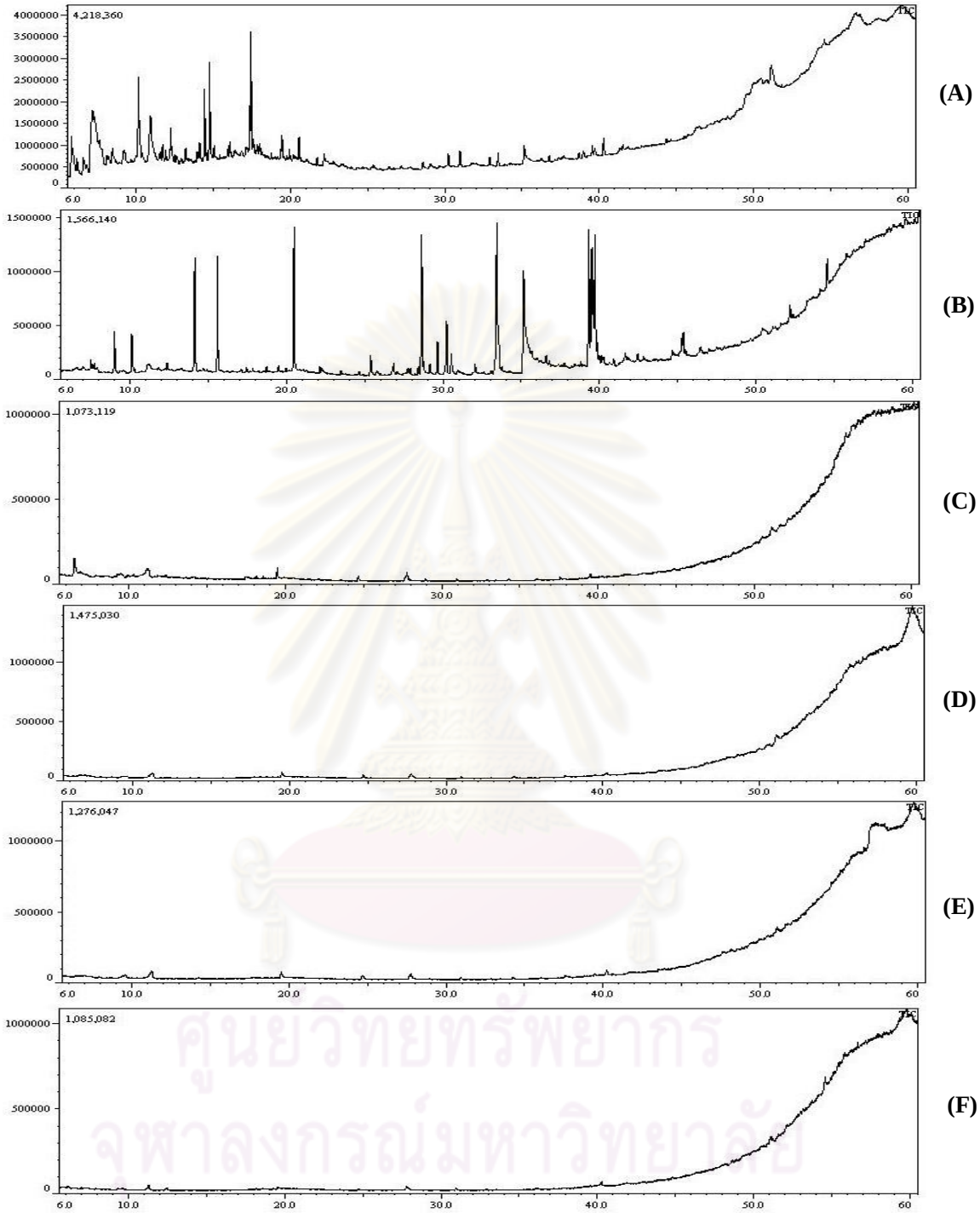


Figure C-3 Chromatograms of organic compounds containing in solid phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through C18 sorbent tube, and eluted by mixture methanol and dichloromethane at 9:1 ratio(fraction C).

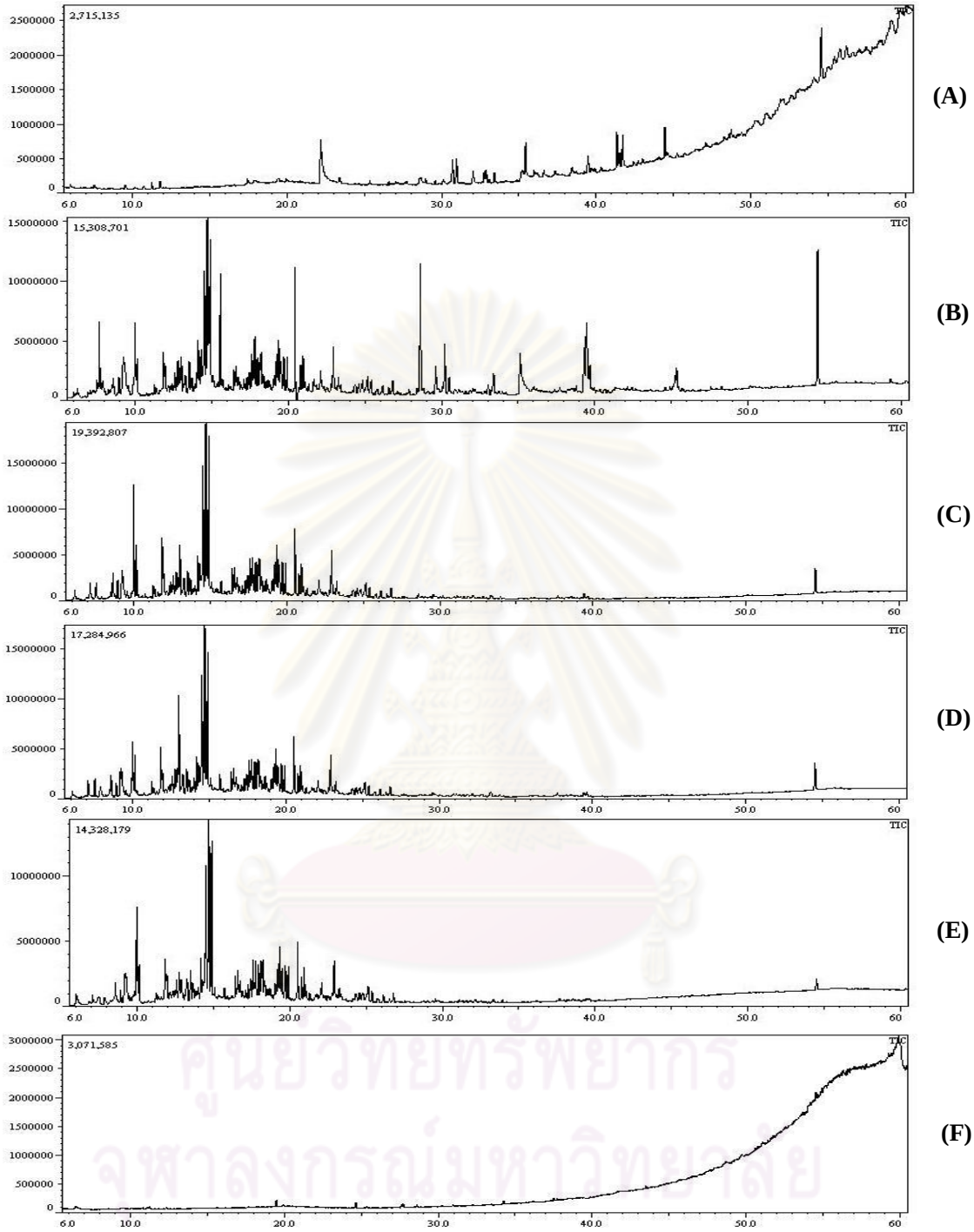


Figure C-4 Chromatograms of organic compounds containing in solid phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through HBP sorbent tube, and eluted by mixture acetonitrile and dichloromethane at 9:1 ratio(fraction D).

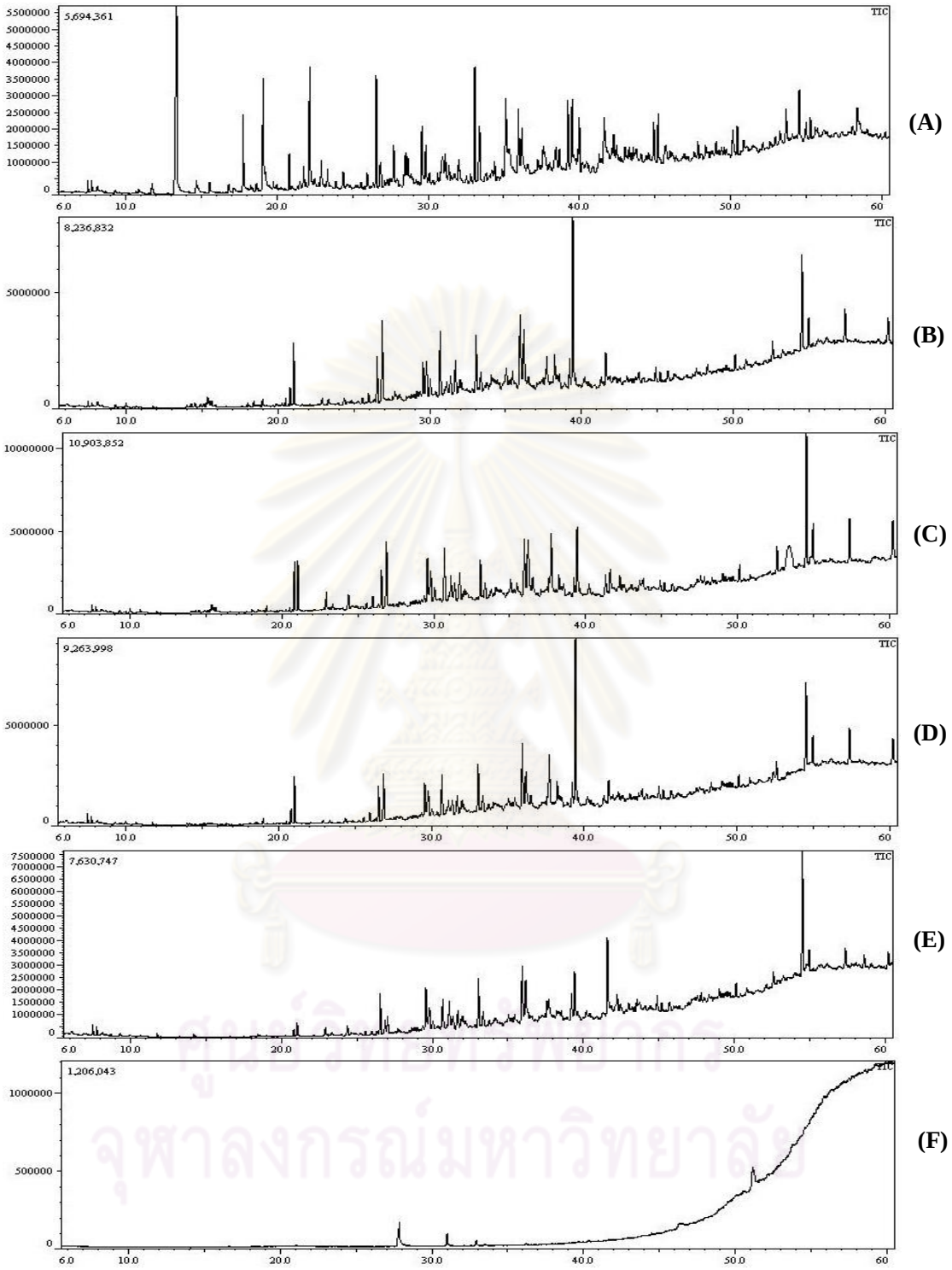


Figure C-5 Chromatograms of organic compounds containing in soluble phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through C18 sorbent tube, and eluted by hexane (fraction A).

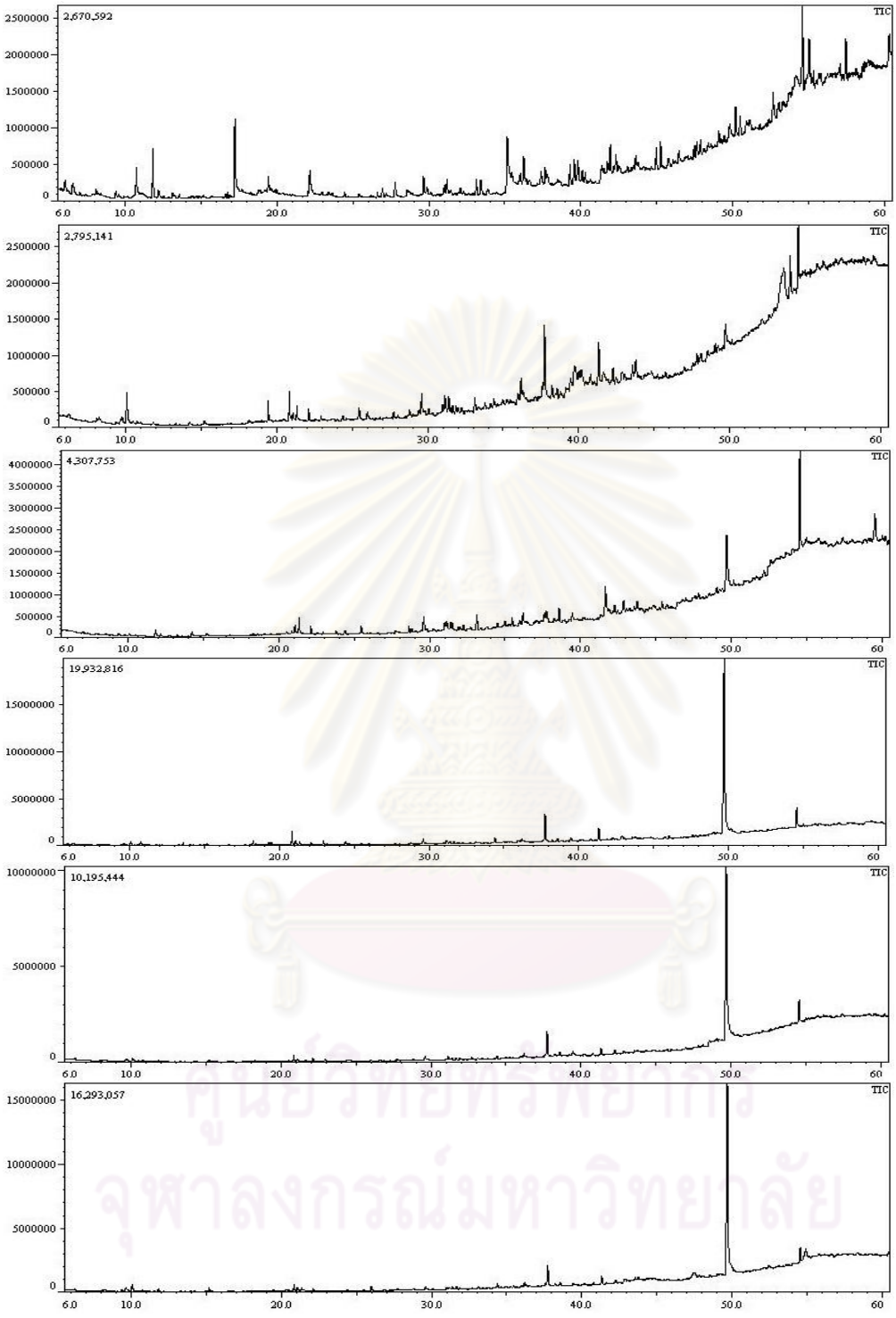


Figure C-6 Chromatograms of organic compounds containing in soluble phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through C18 sorbent tube, and eluted by dichloromethane (fraction B).

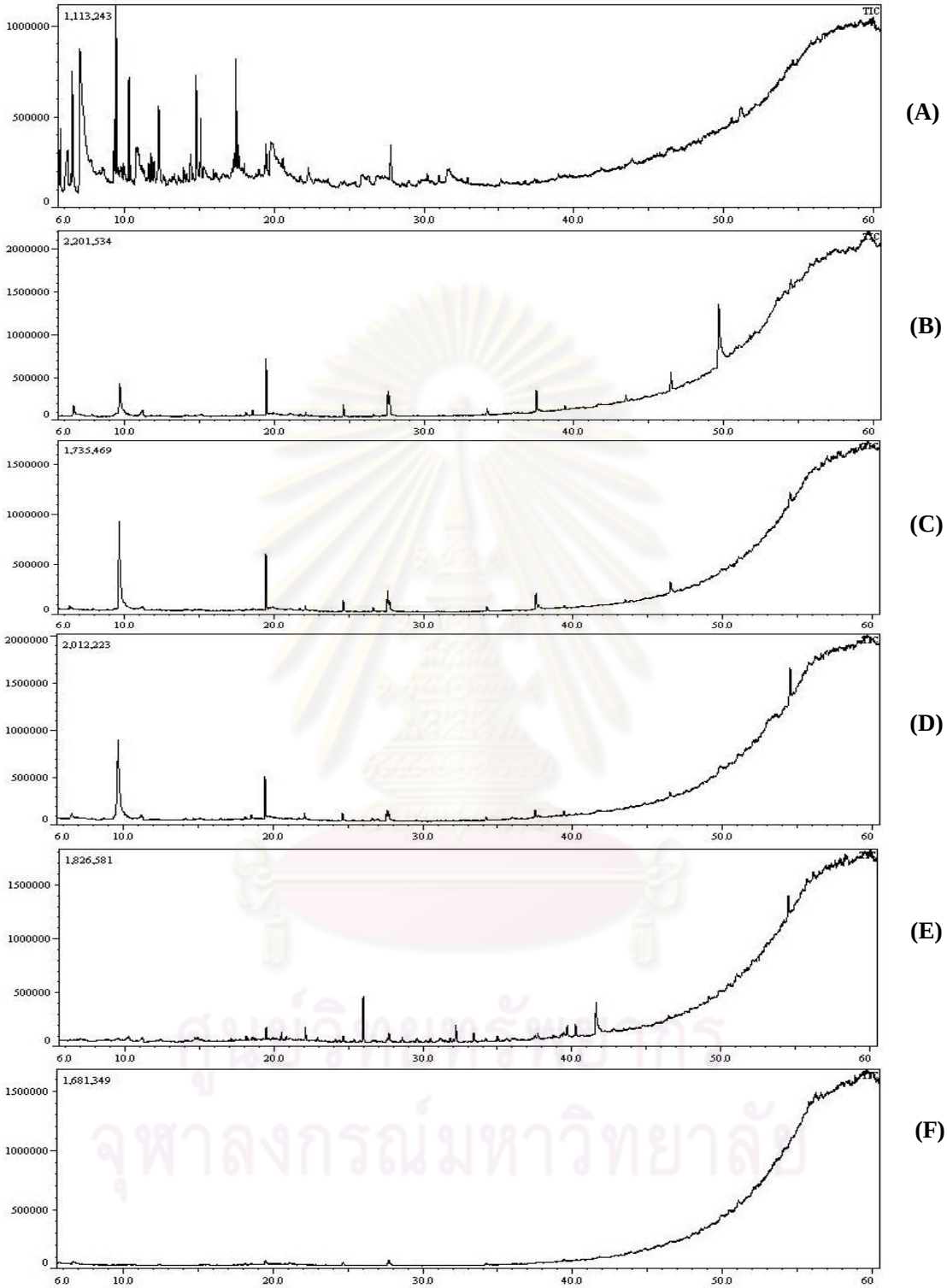


Figure C-7 Chromatograms of organic compounds containing in soluble phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through C18 sorbent tube, and eluted by mixture methanol and dichloromethane at 9:1 ratio(fraction C).

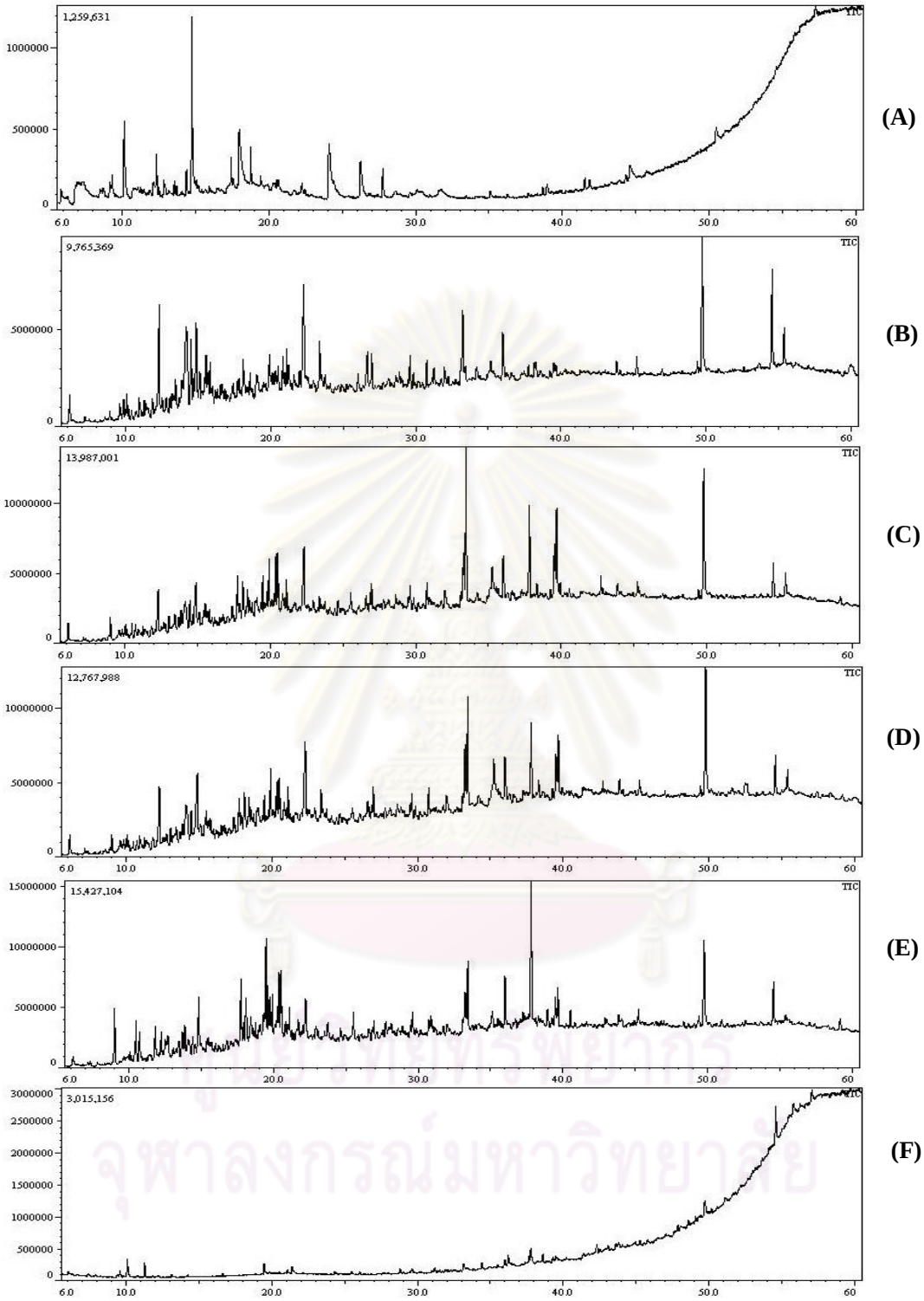
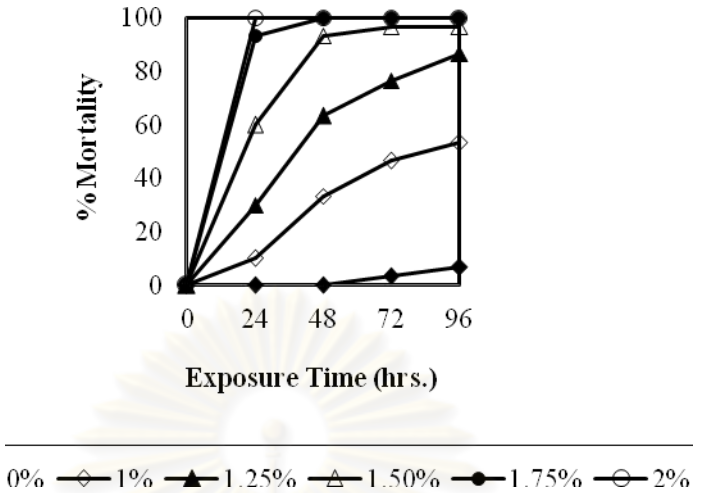


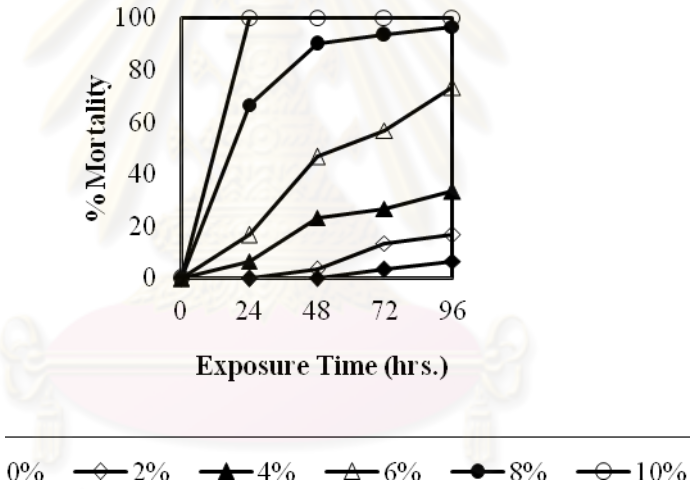
Figure C-8 Chromatograms of organic compounds containing in solid phase of water samples; **(A)** fresh leachate **(B)** stabilized leachate **(C)** coagulation unit **(D)** sand filtration unit **(E)** MF unit **(F)** RO unit. They were extracted through HBP sorbent tube, and eluted by mixture acetonitrile and dichloromethane at 9:1 ratio(fraction D).

APPENDICE D

Effect of acute toxicity testing

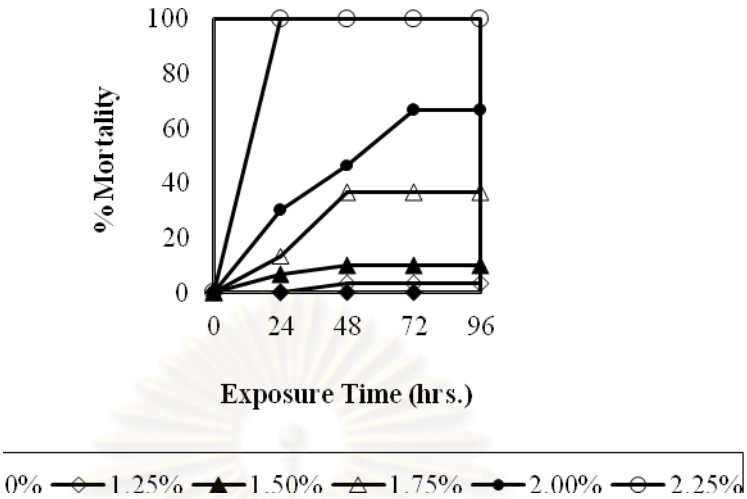


(A)

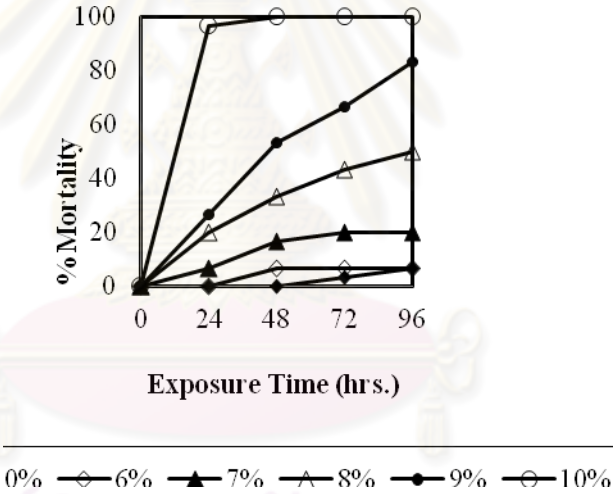


(B)

Figure D-1 Effect of Raw on *Oreochromis Niloticus* in Acute Testing
(A) Fresh Leachate (B) Stabilized Leachate

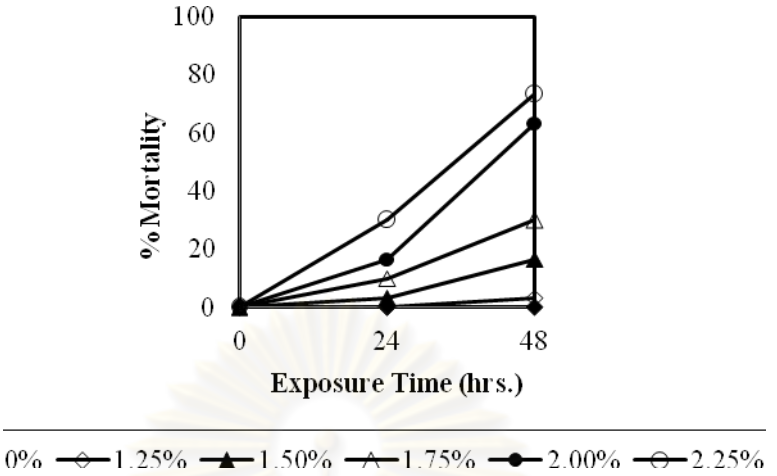


(A)

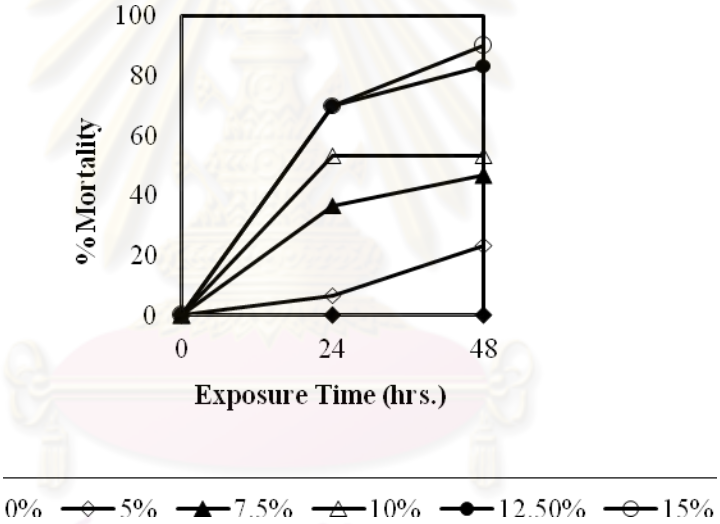


(B)

Figure D-2 Effect of Raw leachate on *Cypinus Carpio*
(A) Fresh Leachate (B) Stabilized Leachate

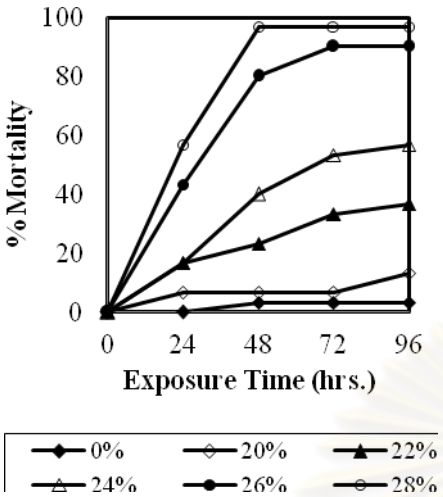


(A)



(B)

Figure D-3 Effect of raw leachate on *Moina Macrocopa* in Acute Testing (A) Fresh Leachate (B) Stabilized Leachate



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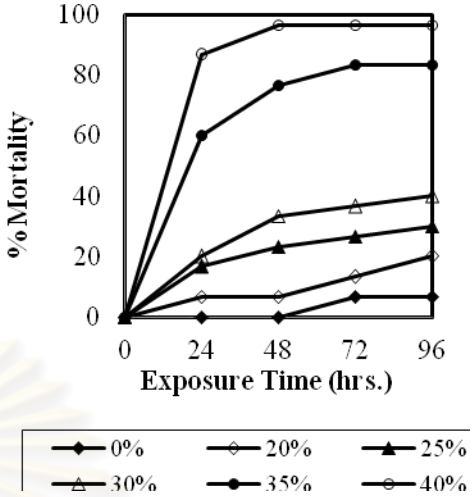

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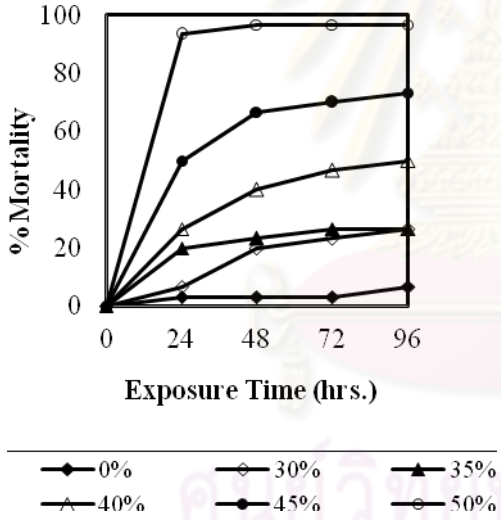
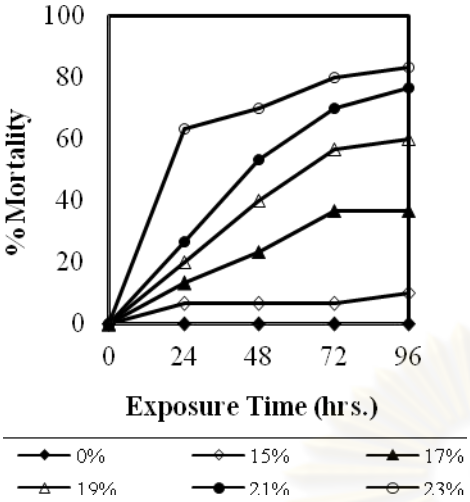


(C)

Figure D-4 Effect of Treated Leachate on *Oreochromis Niloticus* in Acute Testing
(A) Coagulation (B) Sand Filtration (C) Microfiltration



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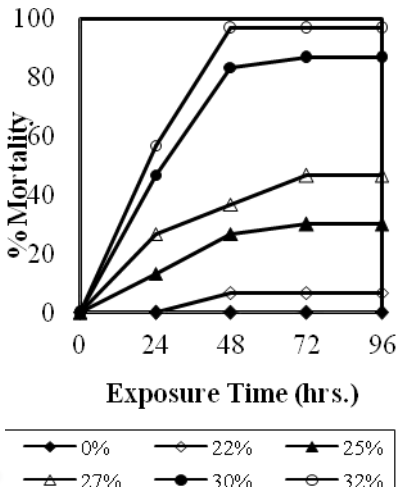

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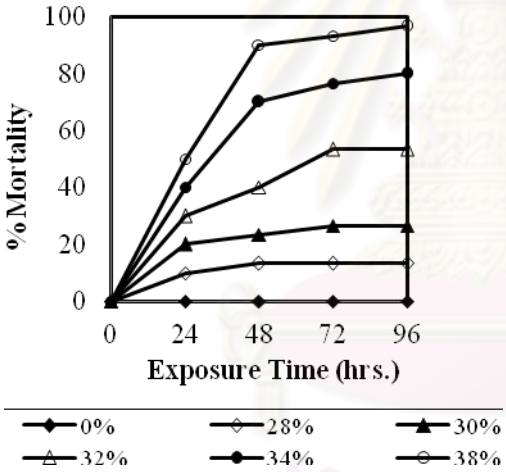
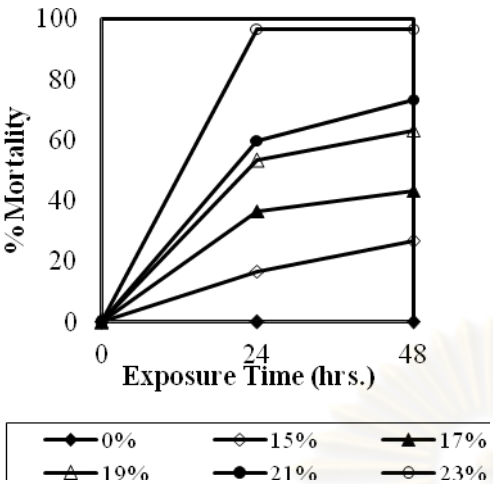
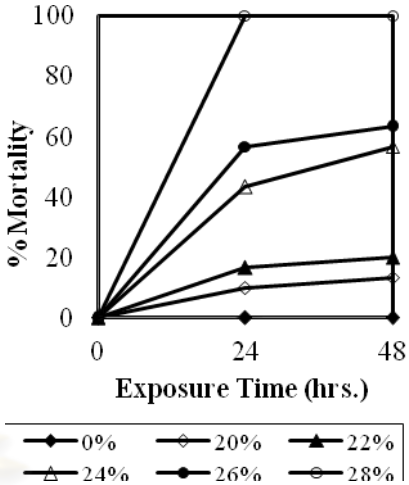


(C)

Figure D-5 Effect of Treated Leachate on *Cypinus Carpio* in Acute Testing
(A) Coagulation (B) Sand Filtration (C) Microfiltration



(A)



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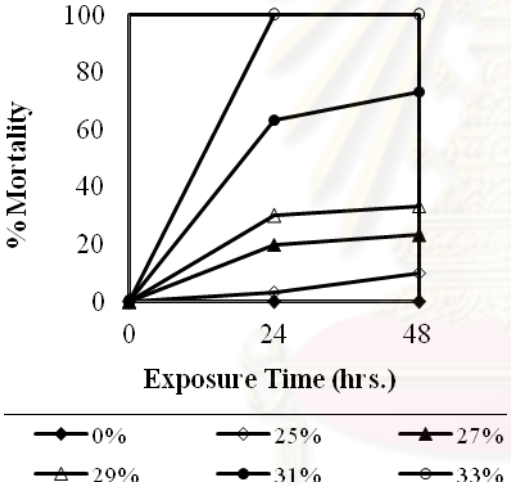

(C)

Figure D-6 Effect of Treated Leachate on *Moina Macrocopa* in Acute Testing
(A) Coagulation (B) Sand Filtration (C) Microfiltration

BIOGRAPHY

Miss Suthida Theepharaksapan was born on March 26, 1986 in Phachuabkirikun, Thailand. She graduated primary school in 1997 from Arunwitaya Phachuabkirikun, and secondary school in 2003 from Prommanusorn Petchaburi. She received her Bachelor's Degree in Environmental Engineering from Faculty of Engineering, Kasetsart University in 2007. She pursued her Master Degree studies in International Postgraduate Programs in Environmental Management, Inter-Department of Environmental Management, Chulalongkorn University in May 2007.

Chemical Characterization and Bio-toxicity Testing of Leachate from Municipal Solid Waste Landfill with Different Degree of Treatment

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Abstract

Advanced leachate treatment system was applied to the treatment of municipal solid waste landfill leachate in Thailand. The system utilizes chemical coagulation using ferric chloride as coagulant followed by sand filtration, microfiltration (MF) and reverse osmosis (RO) membrane. This study is conducted to assess the toxicity of leachate along the treatment process. Acute toxicity tests were conducted using different living organisms, i.e. water flea (*Moina macrocopa*), Nile Tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*). The presence of toxic compounds was confirmed by chemical characterization of leachate using GC-MS analysis. Toxicity reduction was determined from the removal of toxic chemicals and LC_{50} evaluation. The experimental results suggest that ammonia was the main toxic compound in leachate. Chemical coagulation followed by sand filtration, MF and RO were required for effective removal of toxic chemicals and ammonia nitrogen. Toxic organic compounds such as bisphenol A, phthalate compounds were removed by 84.8-100%.

Keywords

Acute toxicity; chemical characterization; landfill leachate; RO; toxic organic compounds

INTRODUCTION

Leachate contains a complex variety of material and organic compounds (humic substances, fatty acids, and aromatic compounds), heavy metals and many other hazardous chemicals (Schrab et al., 1993). It may pose serious risks to ecosystems and human health through its discharge to the environment without proper treatment. In order to minimize those risks, effective leachate treatment system utilizing the integration of treatment processes is required.

Several conventional as well as advanced treatment processes have been applied to the treatment of leachate (Abdulhussain et al., 2009). Usually, conventional processes are effective for the removal of organic substances, suspended solids and served as pre-treatment of subsequent advanced treatment units. For the removal of recalcitrant compounds remaining after conventional treatment, advanced treatment processes such as activated carbon adsorption and membrane technologies are required. Among several membrane processes, reverse osmosis (RO) was considered as the ultimate treatment step yielding highest pollutant rejection efficiencies (Renoua et al., 2008). Integration of conventional and advanced treatment processes usually yielded excellent standard water qualities such as COD, ammonia nitrogen, heavy metals but those chemical parameters alone do not allow evaluation of toxic effects of all compounds present in treated water (Kjeldsen et al., 2002).

Bioassays can be used to characterize the toxicity of landfill leachate to integrate the biological effect of all its constituents. The toxicity of landfill leachate has been assessed by several researchers using a number of different living organisms, including luminescent bacteria Vibrio

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fischeri (Silva et al., 2004), aquatic vertebrates (fishes) (Alkassasbeh et al., 2009). The most popular bioassays are with aquatic invertebrates (especially crustaceans) (Žaltauskaitė et al., 2008). Nevertheless, considerable differences in the sensitivities of different test organisms have been observed in most studies (Kjeldsen et al., 2002). Despite of its potential hazard, the use of bio-toxicity for evaluation of environmental safety from discharging leachate from municipal solid waste disposal site together with its chemical characterization for identifying potential toxic compounds still limited especially in developing countries.

The main aim of the present work is to correlate the chemical characteristics and bio-toxicity of leachate along treatment unit utilizing chemical coagulation, sand filtration, microfiltration (MF) and reverse osmosis (RO) membrane processes. Standard chemical parameters, toxic organic compounds and acute toxicity to living organisms, i.e. water flea (*Moina macrocopa*), Nile Tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) of leachate samples obtain with different degree of treatment along the treatment processes are studied and compared.

MATERIALS AND METHODS

Leachate treatment system

The leachate treatment system installed at a municipal solid waste disposal site in Thailand was used as the study site. This landfill site has been operated for more than 20 years and currently receiving approximately 900 tons of municipal solid wastes daily. The leachate treatment system with a capacity of 1,000 m^3/d utilizes coagulation unit using ferric chloride ($FeCl_3$) as coagulant followed by sedimentation, sand/carbon filtration, MF (5 µm pore size) and RO filtration units respectively. Schematic of the leachate treatment system is shown in **Figure 1**.

Fresh and stabilized leachate samples used for chemical characterization and bio-toxicity testing were obtained directly from the site. Fresh leachate were collected from garbage truck whereas stabilized leachate were collected from a leachate storage pond locating near the treatment system. Treated leachate samples were collected along the treatment system after chemical coagultation, sand/carbon filter, MF and RO units repectively. The samples were collected on monthly basis during May to November 2009 period. The samples were stored under 4^{o}C in dark until analysis.

Leachate characterization

Characterization of the raw and treated leachate samples was carried out for the following parameters: pH, electrical conductivity (EC), chemical oxygen demand (COD), total organic carbon (TOC), suspended solids (SS), total dissolved solids (TDS), ammonia nitrogen (NH_3-N), total kjeldahl nitrogen (TKN), and chloride (Cl^-). All analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

For characterization of toxic organic compounds in leachate samples, solid phase extraction (SPE) and gas chromatography-mass spectrometry (Shimadzu GC-MS model 2010) analysis was used. The sorbent tubes (C_{18}, 500 mg, 6 ml) were pre-conditioned with 6 ml methanol and 6 ml pure water then loaded with 200 water sample and eluted with 2x5 ml of hexane, 2x5 ml of dichloromethane and 2x5 ml of methanol/dichloromethane (9:1, v/v). All the elution was evaporated to dryness with anhydrous Na_2SO_4 and re-adjusted to a final volume of 0.5 ml. The suspended solids were sonicated for 60 min with 50 ml of MeOH/DCM (2:8 v/v). Extracts were concentrated to about 0.5 ml and loaded into C18 tubes. GC-MS operating conditions were: temperature program from 60 to 175^{o}C at 6^{o}C/min, and then increased to 270^{o}C at 3^{o}C/min, electron impact (EI) ionization of 70 eV, source temperature of 200^{o}C and interface temperature of 250^{o}C.

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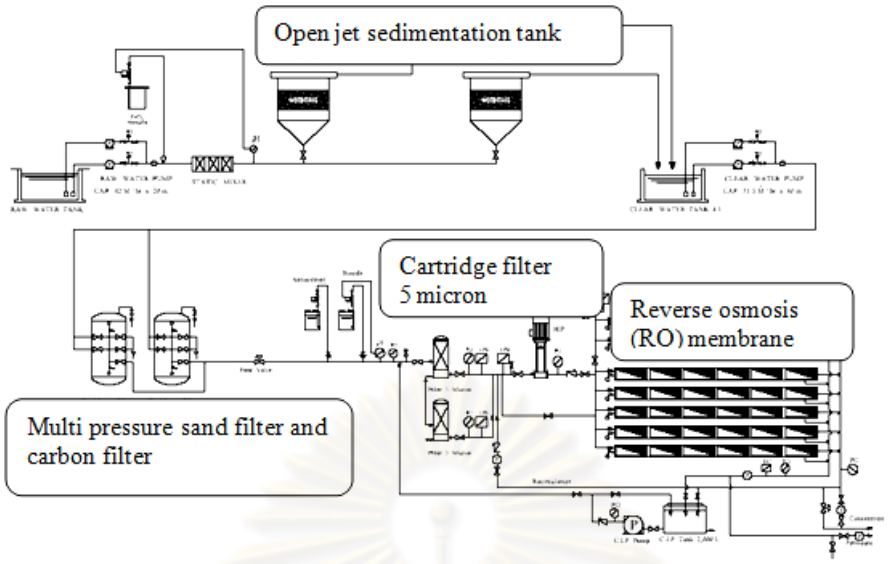

Figure 1 Schematic of advanced leachate treatment system

Bio-toxicity testing

For water flea (*Moina macrocopa*) bio-toxicity test, EPA culture medium used for zooplankton maintenance was prepared by dissolving 0.9 g of $NaHCO_3$, 0.6 g of $CaSO_4$, 0.6 g of $MgSO_4$ and 0.002 g of KCl in one liter of distilled water. In triplicate, water sample was placed into 100 ml beaker, using EPA medium for serial dilution (US.EPA, 2002). Ten newborn of water flea (one day old) were added to the beaker. The numbers of immobilized cladoceran were recorded at 24 and 48 h. In triplicate, controls (only dilution medium) are applied for the test.

For bio-toxicity testing of fish species, Nile Tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) was obtained from a local breeder and transported immediately to the laboratory in appropriately aerated plastic bags. In the laboratory, each fish species was kept separately in 120 liter glass aquaria (0.40 m width*0.75 m length*0.45 m depth) containing de-chlorinated tap water. They were acclimated for 14 days with continuous aeration and the water was renewed every 3 days. The photoperiod was set at 12 h light and 12 h dark condition during the entire experiment. Care is taken in order to keep the mortality rate less than 5% in the last 5 days before the experiments was started In triplicate, 10 adult fishes were placed in water sample that are diluted to five dilution, corresponding to 50-2000 mgCOD/l and 2-12 mg NH_3/l. The exposure test was carried out at temperature room of 28±1^oC for 96 h under 12:12 h light: dark condition. The number of dead fish was recorded every 24 h. In triplicate, non-exposed fish were observed in fresh water under same conditions as mentioned above as control experiment.

The 48 h lethal concentration (LC_{50}) for cladoceran and 96 h LC_{50} for fish species and its 95% confident limits are calculated using a program based on Finneys Probit Analysis method using SPSS for Windows.

RESULTS AND DISCUSSION

Leachate characteristics

The results of raw leachate characteristics are presented in **Table 1**. Fresh leachate was acidic and stabilized leachate was alkaline in nature, in agreement with the postulate that the pH of leachate increases with landfill age (Silva et al., 2004). Fresh leachate contained much higher organic concentrations in terms of BOD, COD and TOC by a factor of 20-90 compared to stabilized

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leachate and their concentrations are found within the reported range (Kjeldsen et al., 2002). The BOD/COD ratio suggested that stabilized leachate was much less biodegradable than fresh leachate whereas NH_3 concentration in fresh leachate was about 4.5 times higher than stabilized leachate. The heavy metals (Cr, Cu, Ni, Pb, and Cd) were found at low concentration and were mostly below the standard limit. Considering among these chemical parameters, ammonia was identified as major toxic compound present in leachate, because of its acute toxicity to aquatic species (Clement et al.,1993). The mechanism by which ammonia concentration can be reduced during solid waste decomposition is leaching (Burton and Watson-Craik, 1998) so their concentrations did not significantly change over time.

Table 1 Raw and treated leachate characteristics*

Parameters	Fresh leachate	Stabilized leachate	After treatment			Standard**
			$FeCl_3$ coagulation & sand filtration	MF	RO	
pH	4.04 (0.4)	8.40 (0.1)	4.68 (0.01)	4.96 (0.1)	6.33 (0.5)	5.5-9
Chloride	2,980 (542)	5,900(1460)	5,700(1350)	5,660 (1410)	2,300 (884)	-
BOD	45,000 (11,800)	514 (40)	25(15)	11(1)	5(0.4)	20
COD	57,800 (21,400)	2,730 (200)	580 (28)	120 (57)	15(0.7)	120
TOC	25,300 (1400)	548 (120)	158 (5)	68 (15)	7.5 (1.4)	-
TKN	450 (170)	135 (40)	54 (9)	36(13)	4.3(1.2)	100
NH_3-N	446 (206)	100 (28)	46(10)	29(10)	3.3(0.8)	-
TDS	32,100 (16,200)	19,700 (2,330)	16,100 (1220)	11,470(6120)	1,870(413)	3,000
SS	15,120 (17,165)	280 (60)	165 (71)	ND	ND	50
Fe	16.29(12.48)	2.95(1.62)	NA	NA	0.24(0.10)	-
Cr	0.24(0.14)	0.17(0.10)	NA	NA	0.069(0.012)	0.25
Cu	0.53(0.26)	0.50(0.44)	NA	NA	0.002(0.001)	2.0
Ni	0.76(0.52)	0.32(0.20)	NA	NA	0.003(0.002)	1.0
Pb	ND	ND	NA	NA	ND	0.2
Cd	0.056(0.040)	0.050(0.024)	NA	NA	0.002(0.001)	0.03

All the values are mg/l except pH (no unit), NA: Not available, ND: Not detected
* Average (SD) values, No. of samples = 7
** Industrial effluent standard, Ministry of Industry, Thailand

Table 1 also shows the characteristics of treated leachate along the treatment process. It was found that chemical coagulation using $FeCl_3$ followed by sand filtration could effectively reduced organic (both biodegradable and recalcitrant) substances and partially removed nitrogenous compounds in leachate. Further treatment by MF and RO membranes reduced most pollutant concentrations to below the standard limit. Most of ionic pollutants were removed at RO process, the final treatment stage. **Figure 2** shows relative concentrations of COD, ammonia nitrogen, and heavy metals along the treatment process. COD was majorly removed by coagulation whereas NH_3 concentration was largely eliminated at the membrane processes. This treatment processes can reduce COD, ammonia nitrogen and heavy metals by >99%, 96.7% and >90% respectively.

Chemical characterization in leachate

Figure 3 shows the chromatograms of raw (fresh and stabilized) leachate. It was found that primary toxic organic compounds detected in raw leachate were di (2-ethylhexyl) phthalate (DEHP), di-butyl phthalate (DBP), bisphenol A and silane. It was found that fresh leachate contained more toxic compounds than stabilized leachate as some of those compounds were eliminated after long term storage in landfill and storage pond. Some of these toxic organic compounds were used as plasticizers in the manufacture of consumer goods such as plastic materials. Other compounds found in leachate include diethyl phthalate (DEP), di-isononyl phthalate (DINP), pesticide and

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herbicide (*N, N*-Diethyltoluamide, DEET), naphthalene, phenolic compounds (cresols, BHT, BHT-aldehyde). These chemicals is originated from chemicals used in household, such as cosmetics, paints, solvents, oils, cleaning compounds, pesticides and degreasing compounds as well as plasticizers and pharmaceutical materials routinely disposed in landfill (Slack et al., 2005).

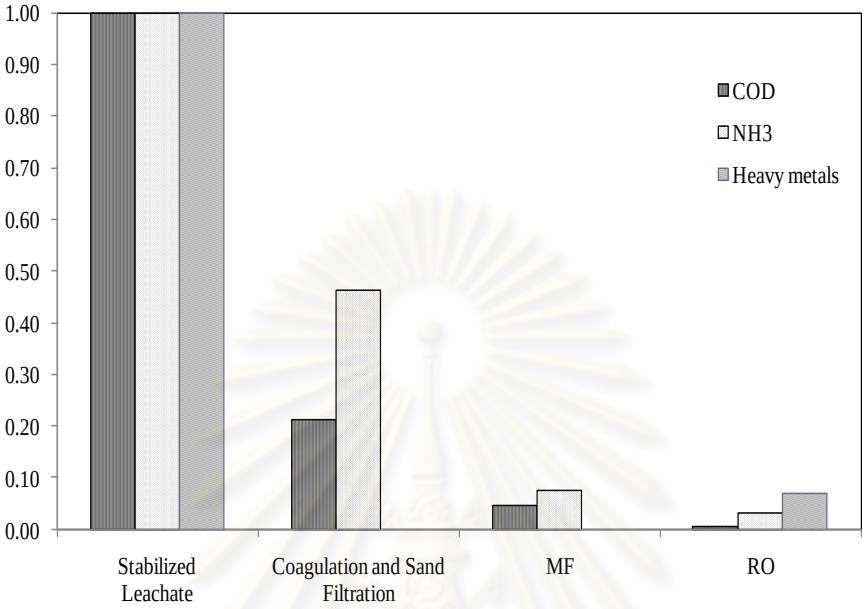

Figure 2 Relative concentration of COD, NH_3 and heavy metal in raw (stabilized) and treated leachate along the treatment process

Relative concentrations of toxic organic compounds in raw and treated leachate along the treatment process were compared using peak areas of GC-MS chromatograms (**Figure 4).** The results suggest that chemical coagulation using $FeCl_3$ followed by sand filtration can remove DEHP, DBP, biophenol A and silane by 35%, 93%, 33% and 50% respectively. It was noted that DEHP and DBP was mainly detected in solid bounded form but their removal through coagulation process were much different. One possible reason is that DEHP may mainly attach onto small colloidal particles which could penetrate through the sand filter. Meanwhile bisphenol A and silane were predominated in soluble form and thus not highly removed. Subsequent treatment by RO process effectively removed those remaining toxic compounds resulting in total elimination efficiencies of 100%, 99.85%, 84.8% and 98.5% respectively.

Bio-toxicity determination

Determination of LC_{50} and its confident limit during acute toxicity test on living organisms are presented in **Table 2.** Based on Finneys Probit Analysis Method, the mean LC_{50} values of fresh and stabilized leachate using Nile Tilapia, common carp and water flea were found to be 4.22%, 7.80% and 8.05% dilution on volumetric basis. For stabilized leachate, they were 0.98%, 1.81% and 1.91% respectively. The corresponding NH_3 concentrations to those LC_{50} values were 4.4-8.5 mg/l for fresh leachate and 4.2-7.8 mg/l for stabilized leachate whereas COD concentrations were 566– 1104 mg/l and 115-213 mg/l for fresh and stabilized leachate respectively. Comparing among the tested species, Nile Tilapia had LC_{50} at NH_3 and COD concentrations of 3.8 – 4.5 mg/l and 104– 578 mg/l whereas those of common carp and water flea were in 6.6–8.1 mg/l, 199– 925 mg/l and 5.7-9.4 mg/l, 155– 1214 mg/l respectively. The results suggested that fresh leachate was more toxic than stabilized leachate on all tested organisms and Nile Tilapia are most sensitive organism as compared to common carp and water flea. The pollutant concentration and mortality percentage curve of tested organisms are shown in **Figure 5.**

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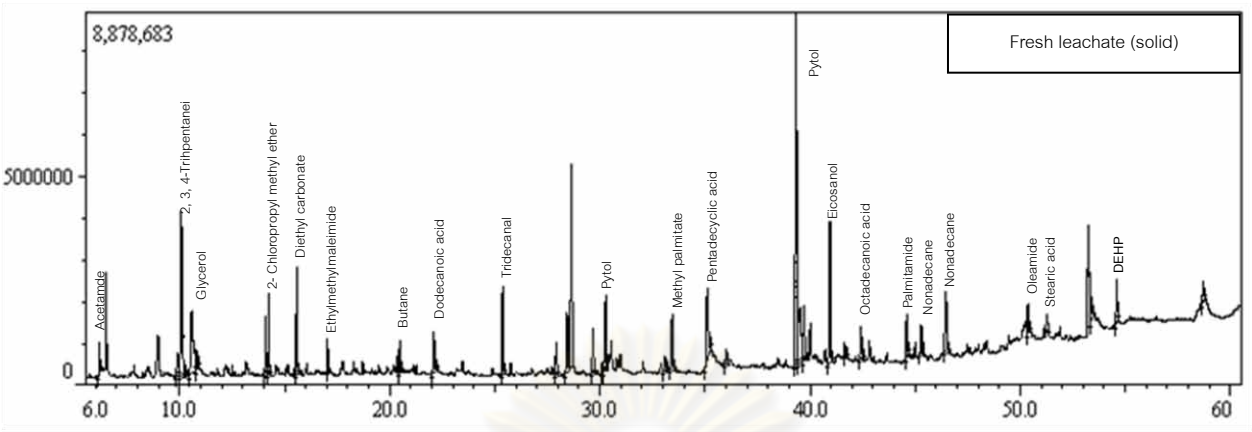

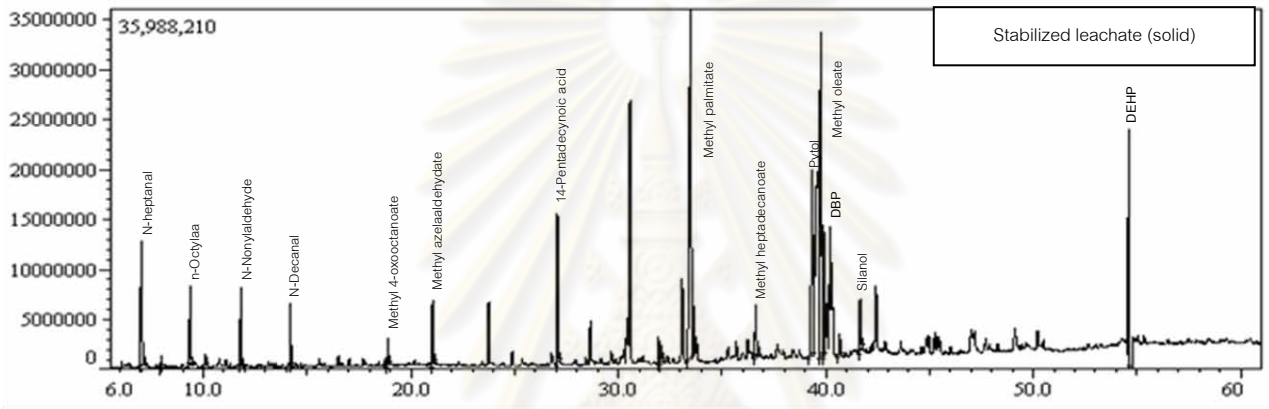

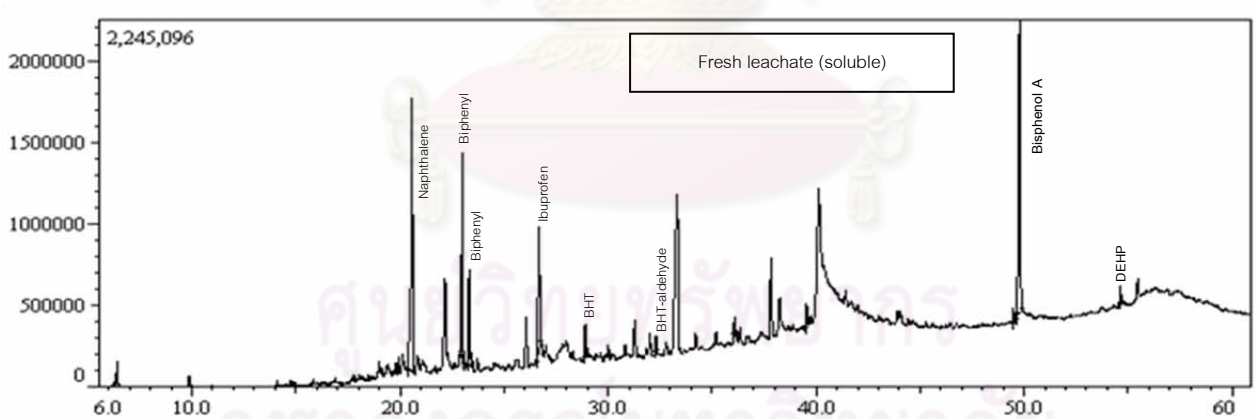

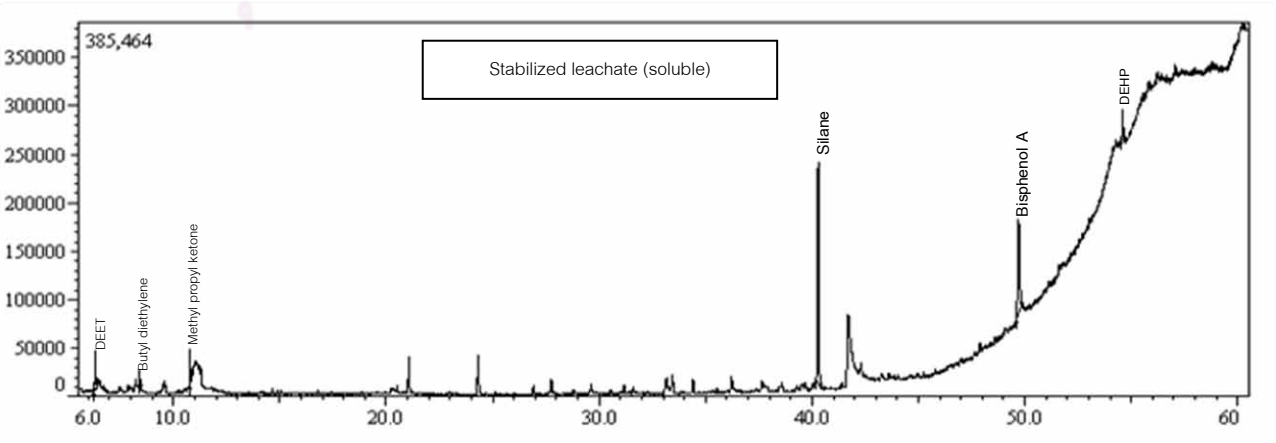

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Figure 3 GC-MS chromatogram of organic compounds containing in fresh and stabilized leachate (solid and soluble forms)

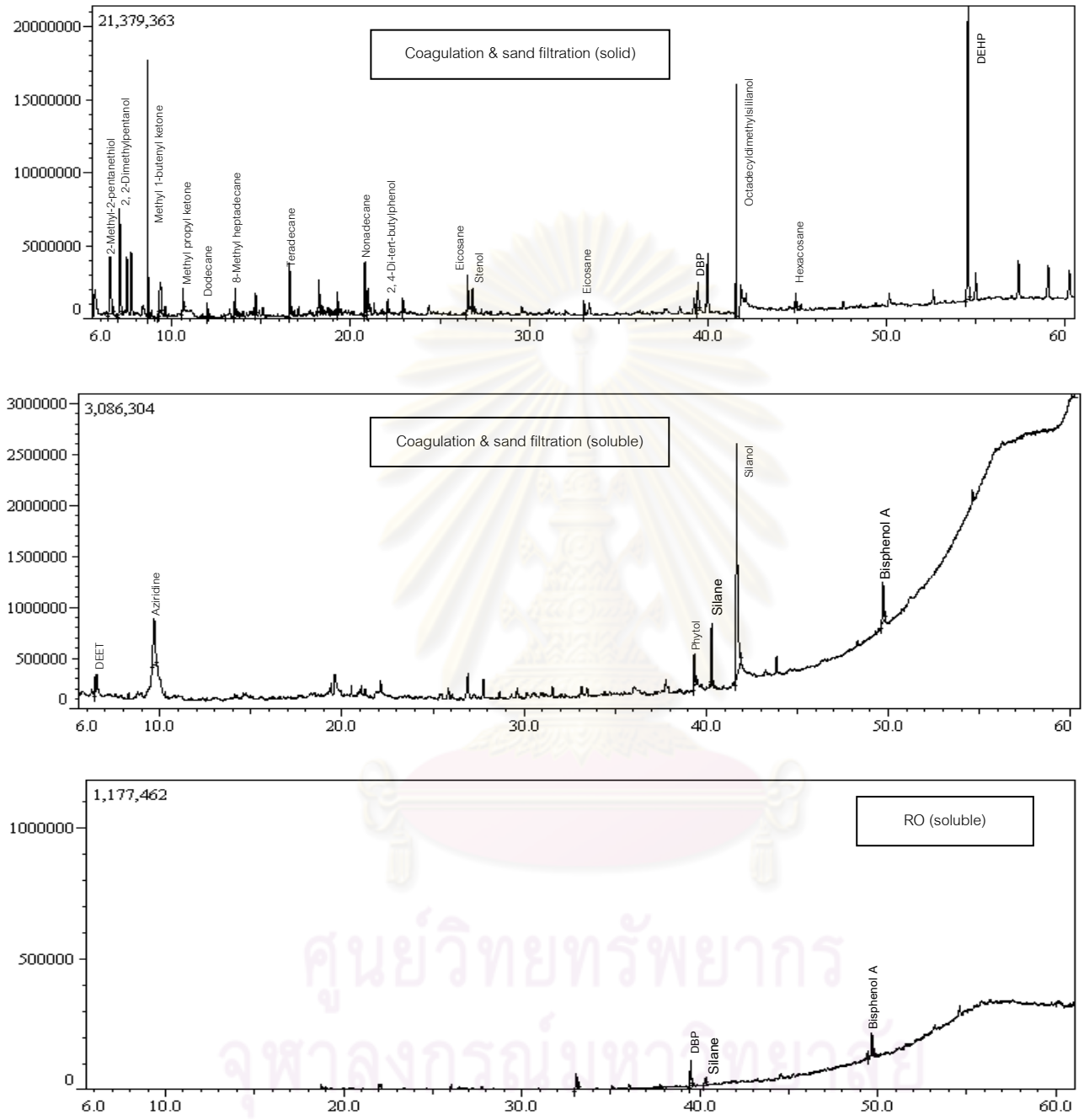

Figure 4 GC-MS chromatogram of organic compounds containing in treated leachate

Because of pollutant concentrations in leachate varied widely from one landfill site to another, direct comparison of LC_{50} values (as dilution percentage) from this study to those reported values in the literatures is not possible. Also, the tested living organisms are different among the studies reported. For instance, Jaffar et al.(2009) evaluated acute toxicity of landfill leachate from three different landfill in Malaysia to common carp (*Cyprinus carpio)* and reported 96 h LC_{50} values of 1.1–3.82 % (v/v). The 96 h LC_{50} for municipal landfill on fingerlings of *Clarias Gariepinus* was 36.6% (v/v) (*Oshode et al., 2008*). The 48 h LC_{50} for leachates of ten sampling from municipal solid wastes landfill on *Artemia franciscana* were 3.2% and 39.3% (Olivero-Verbel et al., 2008).

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Based on pollutant concentration, Clement *et al*. (1993) concluded that ammonia was the main cause of the toxicity measured in the bio-tests, whereas several studies based on genotoxicity test found that organic compounds in leachate may cause the mutagenic activity (Kjeldsen et al., 2002). The 96 LC_{50} values of ammonia acute toxicity test on *C. carpio* fry were reported to be 0.43-2.1 mg/l, 1.009±0.02 mg/l on *O. niloticus* larvae and fingerlings, and 0.47 – 0.48 mg/l on *Moina macrocopa* which is considered more sensitive. These reported values are comparatively lower than the LC_{50} values obtained in this study. This difference can be caused by different size of fish tested, cultivation environment etc.

Table 2 LC_{50} values of fresh and stabilized leachate on tested species

Species	LC_{50} (%dilution v/v)		corresponding pollutant concentrations			
			COD (mg/l)		NH_3 (mg/l)	
Fresh Leachate						
Oreochromis niloticus						
Replicate I	0.96	(0.79 – 1.10)	555	(457 – 633)	4.3	(3.5 – 4.9)
Replicate II	0.98	(0.81 – 1.11)	566	(468 – 641)	4.4	(3.6 – 5.0)
Replicate III	1.00	(0.84 – 1.13)	578	(486 – 653)	4.5	(3.8 – 5.0)
Average	0.98	(0.81 – 1.11)	566	(470 - 642)	4.4	(3.6 – 5.0)
Cyprinus carpio						
Replicate I	1.48	(1.25 – 1.74)	855	(722 – 1004)	6.6	(5.6 – 7.8)
Replicate II	1.60	(1.36 – 1.90)	925	(787 – 1096)	7.1	(6.1 – 8.5)
Replicate III	1.58	(1.36 1.86)	913	(784 –1076)	7.1	(6.1 – 8.3)
Average	1.81	(1.32 – 1.83)	898	(765 – 1059)	6.9	(5.9 – 8.2)
Moina macrocopa						
Replicate I	2.10	(1.80 – 2.62)	1214	(1042 – 1513)	9.3	(8.0 – 11.7)
Replicate II	1.77	(1.50 – 2.11)	1023	(866 – 1222)	7.9	(6.7 – 9.4)
Replicate III	1.86	(1.59 – 2.24)	1075	(918 – 1295)	8.3	(7.1 – 10.0)
Average	1.91	(1.63 – 2.32)	1104	(942 – 1343)	8.5	(7.3 – 10.4)
Stabilized Leachate						
Oreochromis niloticus						
Replicate I	4.47	(3.03 – 6.39)	122	(83 – 174)	4.5	(3.0 – 6.4)
Replicate II	4.37	(2.87 – 6.29)	119	(78 – 172)	4.4	(2.9 -6.3)
Replicate III	3.81	(2.32 – 5.55)	104	(63 –152)	3.8	(2.3 -5.6)
Average	4.22	(2.74 - 6.08)	115	(75 –166)	4.2	(2.7 -6.1)
Cyprinus carpio						
Replicate I	7.30	(6.83 – 7.79)	199	(186- 213)	7.3	(6.8 – 7.8)
Replicate II	8.00	(7.52 – 8.51)	218	(205 – 232)	8.0	(7.5 – 8.5)
Replicate III	8.10	(7.61 – 8.65)	221	(208 – 236)	8.1	(7.6 – 8.7)
Average	7.80	(7.32 - 8.31)	213	(200 – 227)	7.8	(7.3 – 8.3)
Moina macrocopa						
Replicate I	7.06	(5.96 – 9.01)	193	(163 – 246)	7.1	(6.0 – 9.0)
Replicate II	5.67	(4.70 - 6.85)	155	(128 – 187)	5.7	(4.7 – 6.9)
Replicate III	6.01	(5.09 – 7.40)	164	(139 – 202)	6.0	(5.1 – 7.4)
Average	8.05	(5.25 – 7.75)	171	(143 – 212)	6.3	(5.3 – 7.8)

Note: Value in bracket denotes dilution and corresponding concentration ranges for 95% confidence limit

Based on the pollutant removal efficiencies of leachate treatment system (**Table 1**), it is found that the application of chemical coagulation, sand filtration and RO process could reduce NH_3 and COD concentrations to below LC_{50} values thus diminish acute bio-toxicity effect of leachate to living organisms. Even though toxic organic compounds at low concentrations did not have direct contribution on acute toxicity in leachate, it can pose genotoxicity effect to living organisms in long term. The use of RO process helped eliminate these micro-pollutants effectively during the treatment comparing to conventional treatment processes.

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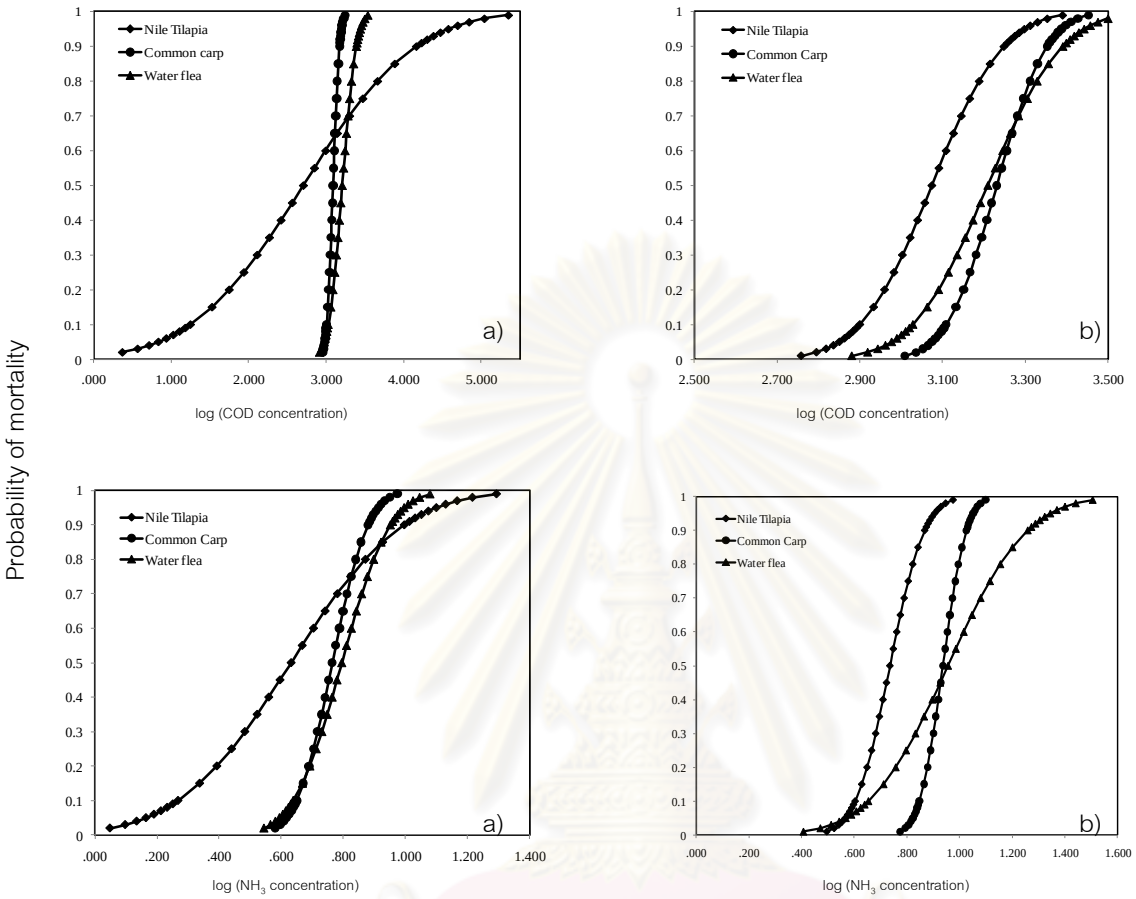

Figure 5 Effect of COD and NH_3 concentration in leachate on mortality of living organisms for a) fresh leachate b) stabilized leachate

CONCLUSIONS

Raw leachate from solid waste disposal site investigated in present work contains high pollutant concentrations that pose acute bio-toxicity effect to the living organisms. Ammonia was main toxic compounds resulting in mortality of fishes and water flea, whereas several toxic organic compounds like bisphenol A, phthalates and silane were also present. These pollutants can be effectively removed (84.8-100% removal efficiencies) by advanced leachate treatment system utilizing chemical coagulation, sand filtration, MF and RO membranes. The utilization of bio-toxicity tests complimenting standard chemical analyses helps improving the evaluation of treatment processes performance and ecological impact of effluent discharged into aquatic environment.

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