THE EFFECTS OF THE EFFLUENTS FROM BATTERY INDUSTRIES ON CLASTOGENICITY OF ROOT TIP CELLS OF SHALLOT (*Allium ascalonicum* L.)

PATUMRAT JAIPET

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(ENVIRONMENTAL TECHNOLOGY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2008

Thesis

Entitled

THE EFFECTS OF THE EFFLUENTS FROM BATTERY INDUSTRIES ON CLASTOGENICITY OF ROOT TIP CELLS OF SHALLOT (*Allium ascalonicum* L.)

……..…...
Miss Patumrat Jaipet
Candidate

……
Assoc. Prof. Poranee Pataranawat,
Ph.D. (Env. Toxicol., Tech.& Mgt.)
Major-Advisor

……
Assoc. Prof. Dechavudh Nityasuddhi,
Ph.D. (Statistics)
Co-Advisor

……
Lact. Duangta Kitkaew,
M.Sc. (Tech. Env. Mgt.)
Co-Advisor

……
Lec. Sopa Chinwetkitvanich,
Ph.D. (Environmental Engineering)
Co-Advisor

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

……
Assoc. Prof. Prayoon Fongsatitkul,
Ph.D. (Environmental Engineering)
Chair
Master of Science Programme in
Environmental Technology
Faculty of Public Health

Thesis

Entitled

THE EFFECTS OF THE EFFLUENTS FROM BATTERY INDUSTRIES ON CLASTOGENICITY OF ROOT TIP CELLS OF SHALLOT(*Allium ascalonicum* L.)

was submitted to the Faculty of Graduate Studies, Mahidol University

for the degree of Master of Science (Environmental Technology)

on

28 April, 2008

……..…...
Miss Patumrat Jaipet
Candidate

……
Assoc. Prof. Komol Sivaborvorn,
Dr. P.H. (Environmental Health)
Chair

……
Assoc. Prof. Poranee Pataranawat,
Ph.D. (Env. Toxicol., Tech.& Mgt.)
Member

……
Lact. Duangta Kitkaew,
M.Sc. (Tech. Env. Mgt.)
Member

……
Assoc. Prof. Dechavudh Nityasuddhi,
Ph.D. (Statistics)
Member

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

……
Assoc. Prof. Phitaya Charupoonphol,
M.D., Dip. Thai Board of epidemiology
Dean
Faculty of Public Health
Mahidol University

ACKNOWLEDGEMENT

I wish to express my sincere appreciation and gratitude to my advisor Assoc. Prof. Poranee Pataranawat and my co-advisor, Assoc.Prof. Dechavudh Nityasuddhi, Lect. Sopa Chinwetkitvanich and Lect. Duangta Kitkaew for their valuable advice helpful guidance and comments in this research.

I wish to thank Assoc. Prof. Komol Sivaborvorn, the external examiner of the thesis defense, for her kindness in examining the research and providing suggestions for improvement.

I wish to express my gratitude to staff of the Department of Sanitary Engineering, Faculty of Public Health, and Department for their kindly support.

I wish to thanks are also extended to my friends who are always nice and friendly. I would like to thank Miss Sasilak Teetong for her suggestion and some materials.

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

Finally, I wish to dedicate my success to my family for their encouragement and support that have enabled me to succeed in my graduate study.

Patumrat Jaipet

April 2008

THE EFFECTS OF EFFLUENTS FROM BATTERY INDUSTRIES ON CLASTOGENICITY OF ROOT TIP CELLS OF SHALLOT (*Allium ascalonicum* L.)

PATUMRAT JAIPET 4637952 PHET/M

M.Sc. (ENVIRONMENTAL TECHNOLOGY)

THESIS ADVISORS: PORANEE PATARANAWAT, Ph.D. (Env. Toxicol., Tech. & Mgt.), DECHAVUDH NITYASUDDHI, Ph.D. (Statistics), SOPA CHINWETKITVANICH, Ph.D. (Environmental Engineering), DUANGTA KITKAEW, M.Sc. (Tech. Env. Mgt.)

ABSTRACT

The objective of this study was to determine the effects of the effluents from two battery industries (factory 1 and factory 2) on the clastogenicity of root tip cells of shallot (*Allium ascalonicum* L.).

The toxicity of the effluents from battery industries was determined by root growth inhibition of shallot at three concentrations of 50, 70 and 100 %v/v for 96 hours of exposure.

In factory 1, results showed that all effluent concentrations inhibited the root growth of shallot at a statistically significant level (p-value<0.05) when compared with control group. The IC_{30}, IC_{50} and IC_{70} values of effluents were 47, 74 and 100 %v/v, respectively. The genotoxicity of effluent from this factory was evaluated by the percentage of the mitotic index and chromosome aberrations, which indicate chromosome damages (clastogenicity) in root meristem cells of shallot. These shallots were exposed to the effluent at three toxicity levels (IC_{30} with total lead 0.19 mg/L, IC_{50} with total lead of 0.30 mg/L and IC_{70} with total lead of 0.41 mg/L) for 48 hours. The mitotic index of treated groups was significantly different from the control group (p-value<0.05). In addition, the toxicity of effluent at various toxicity levels of $IC_{30,}$ IC_{50} and IC_{70} caused chromosome abnormalities of statistical significance when compared with the control group (p-value<0.05). Fragments, laggards and bridges types of chromosome aberration were most frequently found. These abnormalities indicated the clastogenic potential of the effluents from the battery industries. These showed that the effluent from factory 1 inhibited cell division and also induced chromosome aberrations.

In factory 2, the effluents were spiked with Pb in order to obtain the total Pb concentration of 0.28 mg/L. This concentration was compared with the effluent from factory 1 at the concentration of total lead of 0.30 mg/l (or the toxicity level of IC_{50}). The mitotic index of both test solutions was not different whereas the chromosome aberration was significantly different (p-value<0.05).

In conclusion, the effluents from the battery industries with a high Pb concentration exceeding the standard showed the adverse effects on clastogenicity of cell division.

KEY WORDS: EFFLUENTS FROM BATERRY INDUSTRIES / ALLIUM TEST / MITOTIC INDEX / CHROMOSOME ABERRATION / ROOT GROWTH INHIBITION

130 pp.

ผลของน้ำทิ้งจากโรงงานผลิตแบตเตอรี่ต่อความผิดปกติในเซลล์ของปลายรากหอมแดง (*Allium ascalonicum* L.)
(THE EFFECTS OF EFFLUENTS FROM BATTERY INDUSTRIES ON CLASTOGENICITY OF ROOT TIP CELLS OF SHALLOT (*Allium ascalonicum* L.)

ปทุมรัตน์ ใจเพชร 4637952 PHET/M

วท.ม. (เทคโนโลยีสิ่งแวดล้อม)

คณะกรรมการควบคุมวิทยานิพนธ์ : ภรณี ภัทรานวัช, Ph.D. (Env. Toxicol., Tech. & Mgt.), เดชาวุช นิตยสุทธิ, Ph.D. (Statistics), โสภา ชินเวชกิจวานิชย์, Ph.D. (Environmental Engineering), ดวงตา กิจแก้ว, M.Sc. (Tech. Env. Mgt.)

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของน้ำทิ้งจากโรงงานผลิตแบตเตอรี่ 2 โรงงาน (โรงงาน 1 และโรงงาน 2) ต่อความผิดปกติของโครโมโซมในเซลล์ของปลายรากหอมแดง (*Allium ascalonicum* L.)

ความเป็นพิษของน้ำทิ้งจากโรงงานผลิตแบตเตอรี่วัดได้จากการยับยั้งการเจริญเติบโตของรากหอมแดงเมื่อเจริญเติบโตในน้ำทิ้งจากโรงงานผลิตแบตเตอรี่ที่มีความเข้มข้น 3 ระดับคือ 50, 75 และ 100%v/v เป็นระยะเวลา 96 ชั่วโมง

จากโรงงานที่ 1 พบว่าการเจริญเติบโตของรากหอมแดงถูกยับยั้งอย่างมีนัยสำคัญ ($p\text{-value}<0.05$) เมื่อเปรียบเทียบกับกลุ่มควบคุม ความเข้มข้นของน้ำทิ้งที่มีผลต่อการเจริญเติบโตของรากที่ระดับความเป็นพิษ 30%, 50% และ 70% (IC_{30}, IC_{50} และ IC_{70}) คือ 47%, 74% และ 100%v/v ตามลำดับ ความเป็นพิษของน้ำทิ้งต่อสารพันธุกรรมวัดได้โดยใช้ค่าดัชนีการแบ่งเซลล์และความผิดปกติของโครโมโซมในเซลล์รากหอมแดง เมื่อได้รับน้ำทิ้งที่มีความเข้มข้นในระดับความเป็นพิษ 3 ระดับ คือ 47%, 74% และ 100% v/v หรือมีตะกั่วปนเปื้อนที่ระดับความเข้มข้น 0.19 mg/L, 0.30 mg/L และ 0.41 mg/L ตามลำดับ เป็นระยะเวลา 48 ชั่วโมง โดยที่ดัชนีการแบ่งเซลล์และความผิดปกติของโครโมโซมในกลุ่มน้ำทิ้งมีความแตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญ ($p\text{-value}<0.05$) ความผิดปกติที่พบมากได้แก่ Fragments, laggards และ bridges ซึ่งชี้ชัดได้ว่าน้ำทิ้งจากโรงงานผลิตแบตเตอรี่มีศักยภาพในการยับยั้งการแบ่งเซลล์และชักนำให้เกิดความผิดปกติของโครโมโซม

จากโรงงานที่ 2 น้ำทิ้งถูกเติมด้วยสารละลายตะกั่วให้มีระดับความเข้มข้นเท่ากับ 0.28 mg/L เพื่อเปรียบเทียบกับน้ำทิ้งโรงงานที่ 1 ซึ่งมีระดับตะกั่วอยู่ที่ 0.30 mg/L (IC_{50}) และพบว่าค่าดัชนีการแบ่งเซลล์ไม่มีความแตกต่างกัน ในขณะที่ความผิดปกติของโครโมโซมมีความแตกต่างกันอย่างมีนัยสำคัญ ($p\text{-value}<0.05$)

ทั้งหมดนี้สรุปได้ว่าน้ำทิ้งจากโรงงานผลิตแบตเตอรี่ที่ปนเปื้อนด้วยตะกั่วในระดับสูงเกินมาตรฐาน มีผลต่อการแบ่งเซลล์และเหนี่ยวนำให้เกิดความผิดปกติในโครโมโซมได้

130 หน้า

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

Table **Page**

LIST OF TABLES (continued)

Table **Page**

LIST OF TABLES (continued)

Table **Page**

LIST OF FIGURES

LIST OF FIGURES (continued)

Figure **Page**

LIST OF ABBREVIATIONS

ACGIH	=	American Conference of Governmental Industry Hygienists
DNA	=	Deoxyribonucleic acid
EPA	=	Environmental Protection Agency
INVITTOX	=	In Vitro Techniques in Toxicology
IARC	=	International Agency for Research on Cancer
USEPA	=	United States Environmental Protection Agency
WHO	=	World Health Organization

CHAPTER I
INTRODUCTION

1.1 Background

With the rapid growth in transportation, communication and electronic technologies, the lead-acid battery has become one of the most extensively used consumer products of this century. Lead-acid batteries help to power or start cars trucks, buses, boats, trains, recreational vehicles, electric wheel chairs, golf carts, backup power supplies for lighting, computers and buildings. Today, it is estimated that over 70% of the world's lead production goes into lead-acid battery manufacturing (Lyn, 2004).

Most battery manufacturers are take a number of steps to minimize pollution risks and reduce the volume of hazardous materials and wastes. Manufacturers of lead-acid batteries follow strict guidelines for minimizing lead emissions. Advanced environmental control devices and water treatment systems filter air and remove lead from process water to control lead emissions. Battery manufacturing uses and generates many potentially hazardous chemicals and materials and some like lead, mercury and cadmium represent a serious threat to human health. In older battery plants, manual operations pose a potential for environmental and occupational risk in every step (Aigenvironmental, 2004).

Heavy metals have become essential in this manufacturing process. They have been used in the form of raw materials such as lead, copper, nickel, chromium etc. These heavy metals, after processing, still remained in wastewater. If this wastewater is discharged into the public water resource, such heavy metals and toxic substances are harmful to the ecosystem, especially to aquatic lives.

Allium test provides a rapid screening procedure for chemicals, pollutants contaminants, etc. which may represent environment hazard. Observation of the root tip systems of onion (Allium cepa) and shallot (*Allium ascalonicum* L.) have shown that these plants are particularly sensitive to the harmful effects of such environmental contaminants. Gross effects can be quantified by measurement of inhibition of growth of the newly developing root system, whereas examination of the chromosome of the individual cells of the root tip can indicate likely mutagenic effect. (Bottum invittox.com, 2002).

The modified Allium test was developed to screen genotoxicity of complex mixtures especially industrial effluents. The method is easy for non cytologists, such as Rank and Neison proposed that the modified method of scoring chromosome aberration only in anaphase and telophase is more sensitive and higher levels of significance. This study shallot (*Allium ascalonicum* L.) is used as test organism because it is local spicy and low cost material.

1.2 Research Objectives

1.2.1 General Objective

To study the effects of effluents from battery industries on clastogenicity of shallot (*Allium ascalonicum* L.*)*

1.2.2 Specific Objectives

1.2.2.1 To determine toxicity levels (IC_{30}, IC_{50} and IC_{70}) of effluents from battery industries on root elongation of shallot (*Allium ascalonicum* L.) at 96 hours of exposure.

1.2.2.2 To determine the effects of effluents from battery industries at different toxicity levels (IC_{30}, IC_{50} and IC_{70}) on mitotic index of root tip cells of shallot (*Allium ascalonicum* L.) at 48 hours.

1.2.2.3 To determine the toxicity levels of effluents from battery industries that cause chromosomal aberration in anaphase and early telophase of mitosis in root tip cells of shallot (*Allium ascalonicum* L.) at various 48 hours.

1.3 Research Hypothesis

1.3.1 The length of root bundle of *Allium ascalonicum* in treated groups will be shorter than that of control.

1.3.2 Mitotic index of treated groups will be less than that of control.

1.3.3 The IC at toxicity level of 50 will cause chromosome aberration in root tip cells of shallot (*Allium ascalonicum* L.)

1.4 Research Variables

1.4.1 Independent variables

- Concentrations of effluents from battery industries (50%, 75% and 100%v/v)

1.4.2 Dependent variables

- Root bundle length (centimeters)(root elongation)
- Number of mitotic cell (Mitotic index)
- Number of chromosome aberration cells

1.4.3 Conditions of the experiment

- pH of effluent and control (6.4-7.3)
- Source of shallot

1.5 Scope of Study

1. In this experiment, effluents were taken from Battery industries in Samutprakarn Province.
2. Shallot bulbs used in the experiments were obtained from Lumphun Province and the age of them were about 3 to 6 months after the harvest.
3. Tap water was used as negative control and dilution for effluents.
4. Root bundle lengths were measured by using a ruler at the time interval of 24, 48, 72 and 96 hours. The longest and shortest root lengths were ignored. Then, a mean of root bundle length was calculated for each treatment.
5. Type of chromosome aberration observed in anaphase and early telophase cells were bridges, fragment, vagrant (laggard) chromosome and combinations of abnormalities.

1.6 Conditions and Limitations of the Study

1. The effluents from battery industries located in Samutpakarn Province were obtained by grab sampling technique and kept in refrigerator 4°C laboratory section of Sanitary Engineering Department, Faculty of Public Health, Mahidol University.
2. Lead concentrations were determined in mg/L.
3. Tap water (pH around 7) was obtained from the stainless storage tank of Faculty of Public Health, Mahidol University.

4. All experiments were carried out under the natural conditions at the corridor of the third floor of the building six, Faculty of Public health, Mahidol University.

1.7 Definition of Keywords

1. Clastogenicity: Effects are damages to chromosomes, such as breaks in or change in the amount of protein.
2. Genotoxicity: Genotoxicity is the study of the adverse effect of compounds on the genetic material of cells (DNA) and the subsequent expression of these changes (chromosome).
3. Chromosome aberration: Chromosome aberration is a modification of the chromosome structure or number.
4. Mitotic index: In a population of cells, the ratio of the number of cells undergoing mitosis (cell division) to the number of cells not undergoing mitosis.

1.8 Conceptual Framework

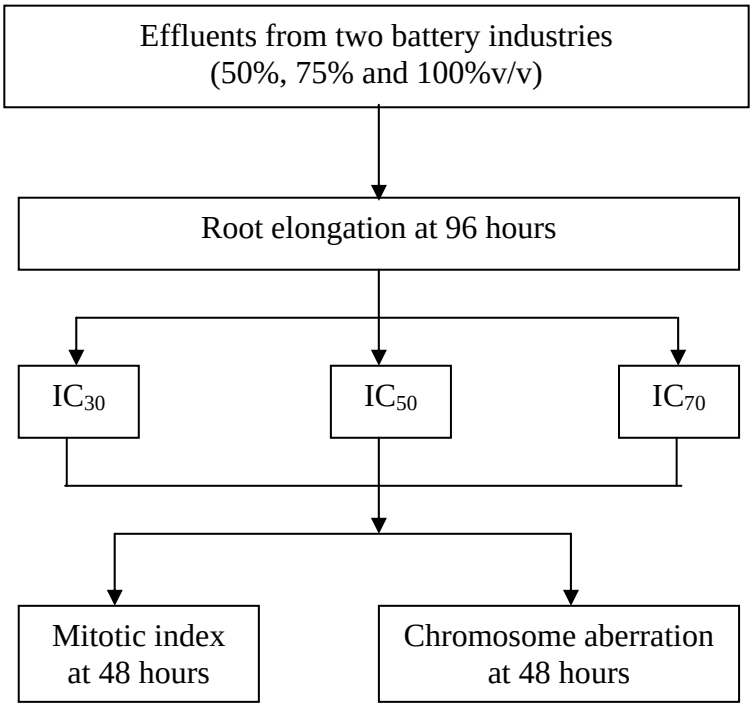


Figure 1.1 Conceptual framework

CHAPTER II
LITERATURE REVIEWS

2.1 Battery Industry

2.1.1 Nature of battery Industries

Batteries are stored and supply energy to operate many types of industrial and consumer products such as automobiles, radios, cameras and hearing aids. In the past, many battery manufacturers are relied heavily on product replacement sales. Batteries are divided into two major groupings; dry cell and wet cell. Dry cell batteries or household batteries are portable and commonly used in appliances and other consumer products such as watches, flashlights, toys, cameras, radios, hearing aids, computer equipment, and cordless appliances. Wet cell or lead acid batteries are mostly used to power vehicle engines. Automotive batteries are considered to be a distinct secondary battery market. All batteries save and transport electrical energy by passing electrons between positive and negative elements in reaction to an external circuit or load (Aigenvironmental, 2004).

2.1.2. Lead acid battery

Lead acid battery is an electrical storage device that uses a reversible chemical reaction to store energy. It uses a combination of lead plates or grids and an electrolyte consisting of a diluted sulphuric acid to convert electrical energy into potential chemical energy and back again (Enviroharvest, 2007).

1. Process description of lead acid battery

Lead-acid battery is a battery that composed of battery plates. The plate composed of paste and grid which is cased from a lead/antimony alloy. The paste is made by lead oxide processing in modern mills, where lead balls or complete lead pigs are rotated in a large perforated drum. The friction generates a high temperature and air in introduced to from an oxide on the surface of the lead. The movement rubs this oxide off and further oxidation takes plate on the surface of the lead. A paste is mixed manually off mechanically from lead oxide, carbon black and sulfuric acid for positive plates and from lead oxide, barium sulfate, lignin etc., for negative plates. Then paste is pressed manually or mechanically into grids which are then dried in an oven. After this the pastes plated are "formed" by placing them in trans of acid and by passing the electric current through them to produce positive and negative plates. After forming, plates are washed to remove acid remained from their surfaces and then they are dried. The plates which have been cast in pairs are parted on various machines to cut to the wanted size. Finally the plated are sent to assembly line and inserted into the battery box, covered by a lid and sealed with an acid-proof joint. The finished batteries are finally filled with acid (Figure 2.1).

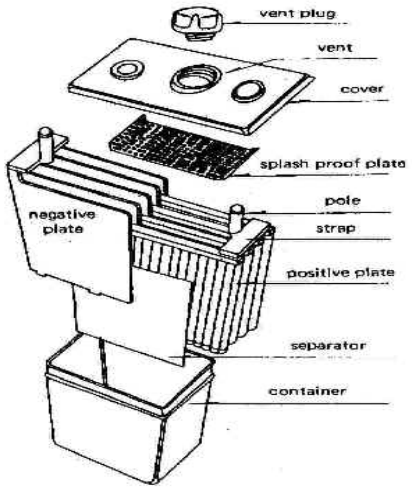


Figure 2.1 Structures of a lead-acid battery (thaigov, 2007)

The battery box may also be made in the same plant either from composition, hard rubber or plastics. The separators which divide the negative from the positive plates may also be produced by the battery manufacturer (Figure 2.2).

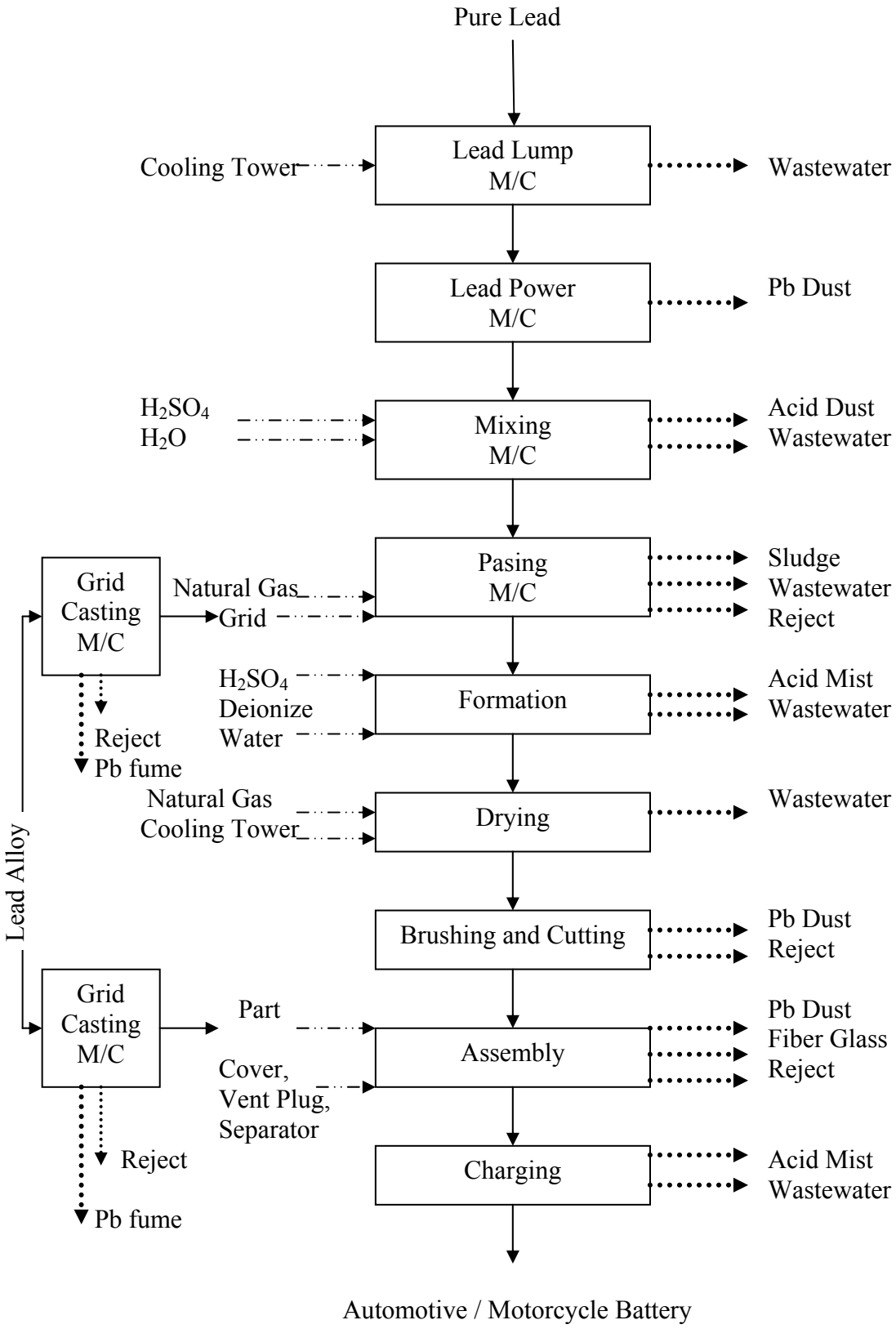


Figure 2.2 Manufacture of lead acid batteries.

2.1.3 Waste characteristics and source

The water supply used in most industries is the ground water received from deep well. Mainly, it is for washing equipments, products from each process and the floors around the working areas. Water used for cooling in some process such as plate mixing and body making of battery box is used by continuously recycling in most factories (Chen, 1974).

The major sources of wastewater are the paste making area, the pasting area and the charged plates washing (ILO, 1976). From paste making and pasting area, ground water was used for washing the floor, the equipment and the area around as well. Distilled water is used for pasting; it is sprayed into the grids so that the paste is more smoothly spread into the grids. Wastewater flows directly from pasting process to the same drainage that received washing wastewater mentioned above.

After charging, the plates are washed with ground water. Wastewater continuously flows from washing basins and/or washing oven flows from direct washing which water is spread manually onto the plates, to the drainage in the charged plates washing area.

In this factory types, production was noted to be nearly the same amount everyday and the same types, of products were manufactured. Therefore, the characteristic of wastewater of each factory are not fluctuated (Chen, 1974).

Wastewater of every process mentioned above consisted of lead. The low pH of charged plates washing wastewater was caused by sulfuric acid used in charging process. Although, the pH of wastewater of the other processes is rather high but the composted wastewater of all lead-acid battery factory is low. This type of wastewater is called lead-acid wastewater (ILO, 1976).

Wastewater from lead acid battery is chemical wastewater treatment plant. Some can be recycled in the factory and some is released to the public area (Pornsuda, 2005).

Table 2.1 List of parameters of effluent from lead acid battery

Parameters	Units	Standard Values	Method	Reference
pH	-	5.5-9.0	pH Meter	Standard Method
Suspended solids (SS)	mg/L	not more than 50	Glass Fiber Filter Disc	Standard Method
Biochemical Oxygen Demand (BOD)	mg/L	not more than 60	Azide Modification at temp.20C, 5 days	Standard Method
Lead (Pb)	mg/L	not more than 0.2	Atomic Absorption Spectrophotometer	Standard Method
Cadmium(Cd)	mg/L	not more than 0.03	Atomic Absorption Spectrophotometer	Standard Method
Manganese(Mn)	mg/L	not more than 5.0	Atomic Absorption Spectrophotometer	Standard Method
Mercury (Hg)	mg/L	not more than 0.005	Atomic Absorption Cold Vapour Techique	Standard Method
Zinc (Zn)	mg/L	not more than 5.0	Atomic Absorption Spectrophotometer	Standard Method
Copper (Cu)	mg/L	not more than 2.0	Atomic Absorption Spectrophotometer	Standard Method
Chromium (Cr)	mg/L	not more than 0.75	Atomic Absorption Spectrophotometer	Standard Method
Arsenic (As)	mg/L	not more than 0.25	Atomic Absorption Spectrophotometer	Standard Method
Nickel (Ni)	mg/L	not more than 1.0	Atomic Absorption Spectrophotometer	Standard Method

Sources: Notification the Ministry of Science, Technology and Environment, No. 3, B.E.2539 (1996) issued under the Enhancement and Conservation of the National Environmental Quality Act B.E.2535 (1992), published in the Royal Government Gazette, Vol. 113 Part 13 D, dated February 13, B.E.2539 (1996)

2.2 Heavy metal

2.2.1 Lead

2.2.1.1 Physical and chemical properties of lead

Elemental lead (Pb) is an odorless silver-bluish white soft metal that is insoluble in water. Its appearance varies depending on the physical structure of the specific compound. Lead exists in the valence states of +2 and +4 and has four naturally occurring isotopes: 204, 206, 207, and 208. Inorganic lead compounds usually consist of lead in the divalent state (+2). The chemistry of divalent lead is similar to that of other group 2 metals, which include beryllium, magnesium, calcium, strontium, and barium. Its melting point 327.43 °C, boiling point 1,740 °C and density 11.4 g/cc are determined at 20°C (ATSDR, 2005a).

2.2.1.2 Source of lead in the environment

Lead occurs naturally in the environment. However, most of the high levels found throughout the environment come from human activities. Lead can enter the environment through releases from mining lead and other metals, and from factories that make or use lead, lead alloys, or lead compounds. Lead is released into the air during burning of coal, oil, or waste. Sources of lead in dust and soil include lead that falls to the ground from the air, and weathering and chipping of lead-based paint from buildings, bridges, and other structures. Landfills may contain waste from lead ore mining, ammunition manufacturing, or other industrial activities such as battery production. Disposal of lead-containing products contribute to lead in municipal landfills. Past uses of lead such as its use in gasoline are a major contributor to lead in soil, and higher levels of lead in soil found along roadways. Most of the lead in inner city soils comes from old houses with paint containing lead and previous automotive exhaust emitted when gasoline contained lead. The overall consumption of lead is growing, mainly due to increased production of lead-acid batteries (ATSDR, 2005a).

Transport action of lead environmental compartments also takes place with resource pathways of human exposure (Figure 2.3).

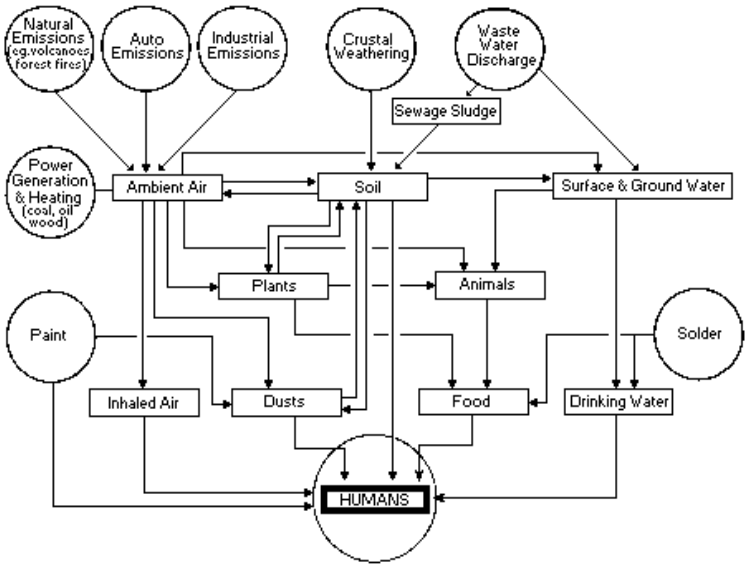


Figure 2.3 Pathways of human exposure (OECD, 1993)

2.2.1.3 Health effects and genotoxicity

The effects of lead are the same whether it enters the body through breathing inhibition or swallowing. The main target for lead toxicity is the nervous system. Long-term exposure of adults to lead at work has resulted in decreased performance in some tests that measure functions of the nervous system. Lead exposure may also cause weakness in fingers, wrists, or ankles. Lead exposure also causes small increasing blood pressure as well as anemia. At high levels of exposure, lead can severely damage the brain and kidneys in adults or children and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. In addition, high-level exposure in men can damage the organs responsible for sperm production.

Lead induced chromosomal aberrations in most studies in plants and in mammals *(in vitro* and *in vivo*) and DNA damage and fragmentation in mammals (*in vivo*; conflicting results were observed in *vitro* studies) and cell-free systems (in the presence of hydrogen peroxide) and inhibited DNA and RNA polymerase in cell-free systems and mammalian cells (*in vitro*). Lead was not mutagenic in bacteria, and conflicting results were observed in mammalian *in vitro* systems (ROC, 2003).

Table 2.2 Regulations and guidelines applicable to Lead and Lead compounds (ATSDR, 2005a)

Agency	Description	Information	Reference
International			
Guidelines:			
IARC	Carcinogenicity classification		IARC 2004
	Lead compounds, inorganic	Group 2A[a]	
	Lead compounds, organic	Group 3[b]	
WHO	Air quality guidelines	0.5 μg/m^3	WHO 2000
	Drinking water quality guidelines	0.01 mg/L	WHO 2004
National			
Regulations and Guidelines:			
Air;			ACGIH 2004
ACGIH	TLV (TWA)		
	Lead, inorganic	0.05 mg/m^3	
	Lead chromate (as Pb)	0.05 mg/m^3	
Water;			
EPA	National primary drinking water standards		EPA 2002
	MCLG	Zero	
	MCL	Treatment technique[c]	
	Action level	0.015 mg/L	

[a]Group 2A: probably carcinogenic to humans

[b]Group 3: not classifiable as to carcinogenicity to humans

[c]Treatment Technique: Lead is regulated by a Treatment Technique that requires systems to control the corrosiveness of their water. If more than 10% of tap water samples exceed the action level, water systems must take additional steps. For lead, the action level is 0.015 mg/L.

2.2.2 Cadmium

2.2.2.1 Physical and chemical properties of cadmium

Cadmium is a lustrous, silver-white, ductile, very malleable metal. Its melting point 321°C, boiling point 765°C and density 8.7 $g.cm^{-3}$ are determined at 20°C. Its surface has a bluish tinge and the metal is soft enough to be cut with a knife, but it tarnishes in air. The chemical symbol is Cd. It is soluble in acids but not in alkalis. It is similar in many respects to zine but it forms more complex compounds (ATSDR, 1999a).

2.2.2.2 Source of cadmium in environment

Cadmium is a natural element in the earth's crust. It is usually found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or sulfur (cadmium sulfate, cadmium sulfide). All soils and rocks, including coal and mineral fertilizers, contain some cadmium. It does not corrode easily and has many uses, including batteries, pigments, metal coatings, and plastics. Cadmium may be released to water by natural weathering processes, by discharge from industrial facilities or sewage treatment plants, or by leaching from landfills or soil (EPA 1985a). Cadmium is more mobile in aquatic environments than most other heavy metals (e.g., lead). In most natural surface waters, the affinities of complexing ligands for cadmium generally follow the order of humic acids > CO_3^{2-} > $OH^- \geq Cl^- \geq SO_4^{2-}$ (Callahan et al., 1979). In unpolluted natural waters, most cadmium transported in the water column exist in the dissolved state as the hydrated ion $Cd(H_2O)_6^{2+}$. Minor amounts of cadmium are transported with the coarse particulates, and only a small fraction is transported with the colloids. In unpolluted waters, cadmium can be removed from solution by exchange of cadmium for calcium in the lattice structure of carbonate minerals (Callahan et al., 1979). In polluted or organic-rich waters, adsorption of cadmium by humic substances and other organic complexing agents plays a dominant role in transport, partitioning, and remobilization of cadmium. Cadmium concentration in water is inversely related to the pH and the concentration of organic material in the water (Callahan et al., 1979). Because cadmium exists only in the +2 oxidation state in water, aqueous cadmium is not

strongly influenced by the oxidizing or reducing potential of the water. However, under reducing conditions, cadmium may form cadmium sulfide, which is poorly soluble and tends to precipitate (EPA, 1983).

It enters water and soil from waste disposal and spills or leaks at hazardous waste sites. Some cadmium dissolves in water. It doesn't break down in the environment, but can change forms. Fish, plants, and animals take up cadmium from the environment. Cadmium stays in the body a very long time and can build up from many years of exposure to low levels.

2.2.2.3. Health effects and genotoxicity of cadmium

Breathing high levels of cadmium severely damages the lungs and can cause death. Eating food or drinking water with very high levels severely irritates the stomach, leading to vomiting and diarrhea. Long-term exposure to lower levels of cadmium in air, food, or water leads to a buildup of cadmium in the kidneys and possible kidney disease. Other long-term effects are lung damage and fragile bones. Animals given cadmium in food or water had high blood pressure, iron-poor blood, liver disease, and nerve or brain damage. Cadmium is a cumulative toxicant, and the human exposure conditions of most concern are long-term exposure to elevated levels in the diet. Cadmium concentrates in freshwater and marine animals to concentrations hundreds to thousands of times higher than in the water (Callahan et al., 1979). Bioconcentration in fish depends on the pH and the humus content of the water (John et al. 1987). Because of their high ability to accumulate metals, some aquatic plants have been suggested for use in pollution control. For example, it has been suggested that the rapidly-growing water hyacinth *Eichhornia crussipes* could be used to remove cadmium from domestic and industrial effluents (Ding et al., 1998) Regulations and guidelines applicable to cadmium is showed in the Figure 2.3.

Table 2.3 Regulations and guidelines applicable to cadmium (ATSDR, 1999a)

Agency	Description	Information	Reference
International:			
IARC	Carcinogenic classification	Group 2A	WHO 1996
WHO	Chemical of Health Significance	0.003 mg/L	WHO 1996
	Provisional tolerable weekly Intake	7 ug/kg	
National:			
Air;			
OSHA	PEL TWA	5 ug/m^3	29 CRC 1910.1027a
	All cadmium compounds		OSHA 1992
Water;			
EPA	maximum contaminant level	0.005 mg/L	EPA 1995
	Office of drink water		

Group 2A = probably carcinogenic to humans

2.2.3 Manganese

2.2.3.1 Physical and chemical properties of manganese

Manganese formula is Mn. Pure manganese is silver-colored, with melting point 1,244 °C and boiling point 1962 °C (IPCS, 1995). Manganese can exist in both inorganic and organic forms. This profile will discuss is key manganese compounds in both forms, with inorganic compounds discussed first. The inorganic forms include manganese chloride ($MnCl_2$), manganese sulfate ($MnSO_4$), manganese acetate (MnOAc), manganese phosphate ($MnPO_4$), manganese oxide (MnO_2), and manganese tetroxide (Mn_3O_4) (ATSDR, 2005). Manganese and its compound can exist as solids in the soid and as solutes or small particle in water. Most manganese salts are readily soluble in water, with only the phosphate and the carbonate having low solubilities.

2.2.3.2 Source of manganese in the environment

Natural source, Manganese and manganese compounds exist naturally in the environment as solids in the soil and as small particles in water. Crustal rock is a major source of manganese found in the atmosphere. Ocean spray, forest fires, vegetable, and volcanic activity are other major natural source of manganese in atmosphere (Schroeder et al., 1978). Manganese may also be present in small dust-like particles in the air. These manganese-containing particles usually settle out of the air within a few days depending on their sizes, weight, densities, and the weather conditions. Manganese exists naturally in rivers and lakes, and is also naturally present in some underground water. Plankton in the water can consume some manganese and concentrate it within themselves.

Anthropogenic sources of environmental manganese include municipal wastewater discharge, sewage sludge, mining and mineral processing, emission from alloy steer, and iron production. Manganese released from burning a gasoline additive may also be a source of manganese in the air. Manganese from these human-made sources can enter surface water, groundwater, and sewage waters. Small manganese particles can also be picked up by water flowing through landfills and soil. The chemical state of manganese and the type of soil determine how fast it moves through the soil and how much is retained in the soil (ATSDR, 2000a).

2.2.3.3 Health effects and toxicity

Some individuals exposed to very high levels of manganese for long periods of time in their work developed mental and emotional disturbances and slow and clumsy body movements. This combination of symptoms is a disease called “manganism.” Workers usually do not develop symptoms of manganism unless they have been exposed to manganese for many months or years. Manganism occurs because too much manganese injures a part of the brain that helps control body movements. Exposure to high levels of airborne manganese can affect motor skills such as holding one’s hand steady, performing fast hand movements, and maintaining balance. Exposure to high levels of the metal may also cause respiratory problems and sexual dysfunction (ATSDR, 2000a).

Exposure to manganese above essential levels can have toxic consequences. Chronic occupational (inhalation) has been linked to neurological deficits, evidenced as deficits in the ability to perform rapid hand movements and some loss of coordination and balance. Very high chronic inhalation exposure, as in former manganese miners, result in permanent neurological damage, which includes mask-like facial expression, slow and clumsy gait, fine tremor, and sometimes psychiatric disturbances.

Food chain bioaccumulation**.** It has been established that while lower organisms (e.g., plankton, aquatic plants, and some fish) can significantly bioconcentrate manganese, higher organisms (including humans) tend to maintain manganese homeostasis (EPA, 1984). Regulations and guidelines applicable to manganese. In Table 2.4 (ATSDR, 2000a).

Table 2.4 Regulations and guidelines applicable to manganese

Agency	Description	Information	Reference
International:			
WHO	Guideline value for drinking water for aesthetic quility	0.1 mg/L	WHO1996a
National:			
Regulation:			
Water;			
EPA OWRS	General permit under NPDES For total manganese	yes	40 CRF 122.21 April 2, 1992, AppendixATable IV
Guideline:			
Air;			
ACGIH	TLV TWA Manganese dust and compounds, As manganese	5 mg/L	ACGIH 1998

OWRS: Office of water regulations and standards

NPDES: National pollutant discharge elimination system

2.2.4 Mercury

2.2.4.1 Physical and chemical properties of mercury

Mercury formula is Hg. It is a naturally occurring metal which has several forms. Its melting point -38.87°C and boiling point 365.72°C. The metallic mercury is a shiny, silver-white, odorless liquid. If heated, it is a colorless, odorless gas. Mercury combines with other elements, such as chlorine, sulfur, or oxygen, to form inorganic mercury compounds or "salts," which are usually white powders or crystals. Mercury also combines with carbon to make organic mercury compounds. The most common one, methylmercury, is produced mainly by microscopic organisms in the water and soil. More mercury in the environment can increase the amounts of methylmercury that these small organisms make. Metallic mercury is used to produce chlorine gas and caustic soda, and is also used in thermometers, dental fillings, and batteries (ATSDR. 1999b). At room temperature, some of the metallic mercury will evaporate and form mercury vapors. Mercury vapors are colorless and odorless. The higher the temperature, the more vapors will be released from liquid metallic mercury.

2.2.4.2 Source of mercury in the environmental

Inorganic mercury (metallic mercury and inorganic mercury compounds) enters the air from mining or deposits, burning coal and waste, and from manufacturing plants. It enters the water or soil from natural deposits, disposal of wastes, and volcanic activity (ATSDR. 1999b). Mercury can enter and accumulate in the food chain. The form of mercury that accumulates in the food chain is methylmercury. Inorganic mercury does not accumulate up the food chain to any extent. When small fish eat the methylmercury in food, it goes into their tissues. When larger fish eat smaller fish or other organisms that contain methylmercury, most of the methylmercury originally present in the small fish will then be stored in the bodies of the larger fish. In the past, excess amounts of mercury were used in batteries. The batteries are used in hearing devices, digital watches, exposure meters, pocket calculators, and security installations but their use has been declining as non-mercury replacement battery production has increased. Some electrical lamps use mercury vapors in discharge tubes (IARC, 1999b).

The primary pathways of mercury exposure for the general population are from eating fish or marine mammals that contain methylmercury. Some people may be exposed to higher levels of mercury in the form of methylmercury if they have a diet high in fish, shellfish, or marine mammals (whales, seals, dolphins, and walruses) that come from mercury-contaminated waters. Methylmercury accumulates up the food chain, so that fish at the top of the food chain will have the most mercury in their flesh. Of these fish, the largest (i.e., the oldest) fish will have the highest levels. Breathing vapors in air from spills, incinerators, and industries that burn mercury-containing fuels. Releases of mercury from dental work from the dental amalgam used for fillings and medical treatments (ATSDR, 1999b).

2.2.4.3 Health affect of mercury

The nervous system is very sensitive to all forms of mercury. Methylmercury and metallic mercury vapors are more harmful than other forms, because more mercury in these forms reaches the brain. Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, and developing fetus. Effects on brain functioning may result in irritability, shyness, tremors, changes in vision or hearing, and memory problems. Short-term exposure to high levels of metallic mercury vapors may cause effects including lung damage, nausea, vomiting, diarrhea, increases in blood pressure or heart rate, skin rashes, and eye irritation (ATSDR, 1999b). Short-term exposure (hours) to high levels of metallic mercury vapor in the air can damage the lining of the mouth and irritate the lungs and airways, causing tightness of the breath, a burning sensation in the lungs, and coughing. Other effects from exposure to mercury vapor include nausea, vomiting, diarrhea, increases in blood pressure or heart rate, skin rashes, and eye irritation.

There are inadequate human cancer data available for all forms of mercury. Mercuric chloride has caused increases in several types of tumors in rats and mice, and methylmercury has caused kidney tumors in male mice. The EPA has determined that mercuric chloride and methylmercury are possible human carcinogens (ATSDR, 1999b). Regulations and guidelines applicable to mercury in Table 2.5.

Table 2.5 Regulation and guidelines applicable to mercury

Agency	Description	Information	Reference
International:			
WHO	Drinking water guideline value for health related for organics (applied to all form of mercury)	0.001 mg/L	WHO1984
	Permissible tolerable weekly intake	5ug/kg total 3.3ug/kg CH_3Hg	WHO1976
National:			
Water;			
EPA	National Primary Water Regulation		
	Maximum Contaminant Level for inorganic compounds	0.002 mg/L	EPA 1992b
Air;			
OSHA	Air contaminants		
	permissible exposure limit(PEL) 8-hr. time weight average (TWA)	0.1 mg/m3	OSHA1974a
ACGIH	Cancer ranking-metallic mercury	A4[g]	ACGH1996
EPA	Cancer classification		
	Elemental (metallic) mercury	D[h]	
	Methyl mercury	C[i]	
	Mercuric chloride	C[i]	

[g] A4: The substance in not classifiable as a human carcinogen. There are inadequate data on which to classify the substance for human and/or animals.

[h] Cancer classification D mean that the substance is not classifiable to its carcinogicity. There is inadequate or no human and animals evidence of carcinogenicity

[h] Cancer classification C means that the substance is a possible human carcinogen

2.2.5 Zinc

2.2.5.1 Physical and chemical properties of zinc

Zinc is a lustrous, blue-white solid metal, its melting point 419.5 °C, boiling point 908 °C and density 7.14 g/cm3 at 25°C. It is insoluble in the water but soluble in acetic acid and alkali. It is stable in dry air, but upon exposure to moist air, it becomes covered with a film of zinc oxide or basic carbonate isolating the underlying metal and retarding further corrosion (ATSDR, 2005b).

2.2.5.2 Source of zinc in the environment

Zinc is an element commonly found in the Earth's crust. It is released to the environment from both natural and anthropogenic sources; however, releases from anthropogenic sources are greater than those from natural sources. The primary anthropogenic sources of zinc in the environment are related to mining and metallurgic operations involving zinc and use of commercial products containing zinc. Worldwide, releases to soil are probably the greatest source of zinc in the environment. The most important sources of anthropogenic zinc in soil come from discharges of smelter slags and wastes, mine tailings, coal and bottom fly ash, and the use of commercial products such as fertilizers and wood preservatives that contain zinc. Zinc does not volatilize from water but is deposited primarily in sediments through adsorption and precipitation. Sludge and fertilizer also contribute to increased levels of zinc in the soil. In air, zinc is present mostly as fine dust particles. This dust eventually settles over land and water. Rain and snow aid in removing zinc from air. Most of the zinc in lakes or rivers settles on the bottom. However, a small amount may remain either dissolved in water or as fine suspended particles. The level of dissolved zinc in water may increase as the acidity of water increases. Fish can collect zinc in their bodies from the water they swim in and from the food they eat. Most of the zinc in soil is bound to the soil and does not dissolve in water. However, depending on the type of soil, some zinc may reach groundwater, and contamination of groundwater has occurred from hazardous waste sites (ATSDR, 2005b). Zinc may be taken up by animals eating soil or drinking water containing zinc. It is also a trace mineral nutrient and as such, small amounts of zinc are needed in all animals. The zinc background

concentrations in surface waters are usually <50 μg/L (EPA, 1980b), but concentrations in surface waters and groundwater c an range from 0.002 to 50 mg/L (NAS, 1977).

Zinc bioconcentrates moderately in aquatic organisms, and this bioconcentration is higher in crustaceans and bivalve species than in fish. Zinc may concentrate in plants grown on contaminated soils. However, it does not biomagnifies through the terrestrial food chain (ATSDR, 2005b).

2.2.5.3 Health affect and genotoxicity of zinc

Zinc is an essential element in our diet. Too little zinc can cause problems, but too much zinc is also harmful. Harmful effects generally begin at levels 10-15 times higher than the amount needed for good health. Large doses taken by mouth even for a short time can cause stomach cramps, nausea, and vomiting. Taken longer, it can cause anemia and decrease the levels of your good cholesterol. We do not know if high levels of zinc affect reproduction in humans. Rats that were fed large amounts of zinc became infertile. Inhaling large amounts of zinc (as dusts or fumes) can cause a specific short-term disease called metal fume fever. We do not know the long-term effects of breathing high levels of zinc. Putting low levels of zinc acetate and zinc chloride on the skin of rabbits, guinea pigs, and mice caused skin irritation. Skin irritation will probably occur in people (ATSDR, 2005b). Regulations and guidelines applicable to Zinc and Zinc compounds in Table 2.6.

Table 2.6 Regulations and guidelines applicable to Zinc and Zinc compounds

Agency	Description	Information	Reference
International:			
Guidelines:			
IARC	Carcinogenicity classification	No data	
WHO	Drinking water and air quality guidelines.	No data	
National:			
Regulations and Guidelines:			
OSHA	PEL (8-hour TWA) for general Industry		OSHA 2003a 29 CFR 1910.1000
	Zinc chloride (fume)	1 mg/m^3	
	Zinc oxide (Fume and respirable fraction of dust)	5 mg/m^3	
	Zinc oxide (total dust)	15 mg/m^3	
EPA	National secondary drinking water Regulations; secondary maximum contaminant level for zinc	5 mg/L	EPA 2003f 40 CFR 143.3

2.2.6. Copper

2.2.6.1 Physical and chemical properties of copper

Copper is displays four oxidation states: Cu(O), Cu(I), Cu(II), and Cu(III). Along with silver and gold, it is classified as a noble metal and, like them, can be found in nature in the elemental form. Its melting point 1,083 °C and boiling point 2,595°C. Properties of metallic copper such as electrical conductivity and fabric ability vary markedly with purity (ATSDR, 2004). Copper is positioned below hydrogen in the electromotive-force series, so it will not displace hydrogen ions from dilute acid. Accordingly, copper will not dissolve in acid unless an oxidizing agent is present. Therefore, while it readily dissolves in nitric and hot concentrated sulfuric acid, it only

dissolves slowly in hydrochloric and dilute sulfuric acid, and then only when exposed to the atmosphere. It is also attacked by acetic acid and other organic acids. When exposed to moist air, a characteristic green layer of the basic copper carbonate slowly forms Cu(I) or the cuprous ion disproportionate rapidly (<1 second) in aqueous solution to form Cu(II) and Cu(0). The only Cu (I) compounds that are stable in water are extremely insoluble ones such as CuCl (ATSDR, 2004).

2.2.6.2 Source of copper in the environmental

Copper is a metal that occurs naturally throughout the environment, in rocks, soil, water, and air, like volcanoes, windblown dusts, decaying vegetation, and forest fires (Georgopoulos et. al., 2001). Copper is released into the environment by industrial releases are only a fraction of the total environmental releases of copper and copper compounds. Industrial applications of copper sulfate include use as an activator in froth flotation of sulfide ores, production of chromated copper arsenate wood preservatives, mordant for textile dyes, petroleum refining and in the manufacture of other copper compounds such as copper hydroxide and copper carbonate. Other sources of copper release into the environment originate from domestic waste water, combustion processes, wood production, phosphate fertilizer production. In agriculture, copper compounds are used as fungicides and to prepare copper fungicidal products, algicides for reservoirs and streams and nutritional supplements in animal feed and fertilizers. Products containing copper compounds frequently contain other chemicals and may be sold under various trade names. Formulation may be in wettable powders or aqueous solutions. Copper is an essential element in plants and animals (including humans), which means it is necessary for us to live. Therefore, plants and animals must absorb some copper from eating, drinking, and breathing. Drinking water may have high levels of copper if your house has copper pipes and acidic water that are above the acceptable drinking water standard of 1,300 parts copper per billion parts of water (ppb). The average concentration of copper in tap water ranges from 20 to 75 ppb. The concentration of copper in lakes an average concentration of 10 ppb. The average copper concentration in groundwater (5 ppb) is similar to that in lakes and rivers; This copper generally stays strongly attached to the surface layer of soil that exposed to this copper by skin contact (ATSDR, 2004).

2.2.6.3 Health affect and genotoxicity of copper

Everyone must absorb small amounts of copper every day because copper is essential for good health. High levels of copper can be harmful. Breathing high levels of copper can cause irritation of your nose and throat. One of the most commonly reported adverse health effect of copper is gastrointestinal distress. Nausea, vomiting, and/or abdominal pain have been reported, usually occurring shortly after drinking a copper sulfate solution, beverages that were stored in a copper or untinned brass container, or first draw water (water that sat in the pipe overnight). The observed effects are not usually persistent and gastrointestinal effects have not been linked with other health effects. Animal studies have also reported gastrointestinal effects (hyperplasia of forestomach mucosa) following ingestion of copper sulfate in the diet. Copper is also irritating to the respiratory tract. Coughing, sneezing, runny nose, pulmonary fibrosis, and increased vascularity of the nasal mucosa have been reported in workers exposed to copper dust (ATSDR, 2004).

The carcinogenicity of copper has not been adequately studied. An increase in cancer risk has been found among copper smelters; however, the increased risk has been attributed to concomitant exposure to arsenic. Increased lung and stomach cancer risks have also been found in copper miners. However, a high occurrence of smoking and exposure to radioactivity, silica, iron, and arsenic obscure the association of copper exposure with carcinogenesis. Animal studies have not found increased cancer risks in orally exposed rats or mice (ATSDR, 2004).

No studies were located regarding genotoxicity in humans after inhalation, oral, or dermal exposure to copper or its compounds. Several studies have assessed the genotoxicity of copper sulfate following oral or parenteral exposure; the results of these *in vivo* genotoxicity. The increase in sister chromatid exchange in Chinese hamster cells is consistent with the clastogenic effects observed in *in vivo* assays. Regulations and guidelines applicable to Copper in the Table 2.7 (ATSDR, 2004).

Table 2.7 Regulations and guidelines applicable to Copper

Agency	Description	Information	Reference
International:			
Guidelines:			
IARC	Carcinogenicity classification		IARC 2002
	Copper 8-hydroxyquinoline	Group 3[a]	
National:			
Regulations and Guidelines:			
Air;			
OSHA	PEL (8-hour TWA) for general industry		OSHA 2002c 29CFR1910.1000
	Fume (as Cu)	0.1 mg/m^3	
	Dusts and mists (as Cu)	1.0 mg/m^3	
Water;			
EPA	Drinking water standard		
	Action level (Cu)	1.3 mg/L	EPA 2002c
	maximum contaminant level goal (Cu)	1.3 mg/L	EPA2002d 40CFR141.51 (b)
EPA	Carcinogenicity classification (Cu)	Group D[b]	IRIS 2004

[a] Group 3: unclassifiable as to carcinogenicity to humans

[b]Group D: not classifiable as to human carcinogenicity

2.2.7 Chromium

2.2.7.1 Physical and chemical properties of chromium

Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases. Chromium is present in the environment in several different forms. The most common forms are chromium (0), trivalent (or chromium (III)), and hexavalent (or chromium (VI)). Chromium (III) occurs naturally in the environment and is an essential nutrient required by the human body to promote

the action of insulin in body tissues so that sugar, protein, and fat can be used by the body. Chromium (VI) and chromium (0) are generally produced by industrial processes. No known taste or odor is associated with chromium compounds. The metal chromium, which is the chromium (0) form, is a steel-gray solid with a high melting point.

2.2.7.2 Source of chromium in the environment

Chromium enters the air, water, and soil mostly in the chromium (III) and chromium (VI) forms. In air, chromium compounds are present mostly as fine dust particles which eventually settle over land and water. Chromium can strongly attach to soil and only a small amount can dissolve in water and move deeper in the soil to underground water. Fish do not accumulate much chromium in their bodies from water. It is used mainly for making steel and other alloys. The naturally occurring mineral chromite in the chromium (III) form is used as brick lining for high temperature industrial furnaces, for making metals and alloys (mixtures of metals), and chemical compounds. Chromium compounds, mostly in chromium (III) or chromium (VI) forms, produced by the chemical industry are used for chrome plating, the manufacture of dyes and pigments, leather tanning, and wood preserving. Smaller amounts are used in drilling muds, rust and corrosion inhibitors, textiles, and toner for copying machines (ATSDR, 2000b).

The level of chromium in air and water is generally low. The concentration of total chromium in air (both chromium (III) and chromium (VI)) generally ranges between 0.01 and 0.03 microgram per cubic meter of air ($\mu g/m^3$). Chromium concentrations in drinking water (mostly as chromium (III)) are generally very low, less than 2 parts of chromium in a billion parts of water (2 ppb). For the general population, eating foods that contain chromium is the most likely route of chromium (III) exposure. Chromium (III) occurs naturally in many fresh vegetables, fruits, meat, yeast, and grain. Various methods of processing, storage, and preparation can alter the chromium content of food. Acidic foods in contact with stainless steel cans or cooking utensils might contain higher levels of chromium because of leaching from stainless steel. Chromium (III) is an essential nutrient for humans. People may also be exposed to chromium from using consumer products such as household

utensils, wood preservatives, cement, cleaning products, textiles, and tanned leather (ATSDR, 2000b).

2.2.7.3 Health effect and toxicity

Chromium (III) is an essential nutrient that helps the body use sugar, protein, and fat. Breathing high levels of chromium (VI) can cause irritation to the nose, such as runny nose, nosebleeds, and ulcers and holes in the nasal septum. Ingesting large amounts of chromium (VI) can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. Skin contact with certain chromium (VI) compounds can cause skin ulcers. Some people are extremely sensitive to chromium (VI) or chromium (III). Allergic reactions consisting of severe redness and swelling of the skin have been noted (ATSDR, 2000b).

Several studies have shown that chromium (VI) compounds can increase the risk of lung cancer. Animal studies have also shown an increased risk of cancer. The World Health Organization (WHO) has determined that chromium (VI) is a human carcinogen. The Department of Health and Human Services (DHHS) has determined that certain chromium (VI) compounds are known to cause cancer in humans. The EPA has determined that chromium (VI) in air is a human carcinogen.

2.2.8 Arsenic

2.2.8.1 Physical and chemical properties of arsenic

Arsenic is color silver-gray or tin-white Solid, Its melting point 817 °C, boiling point 613 °C , density 5.778 g/cm^3 at 25 °C. It is insoluble in the water, caustic and non oxidizing acids. In the environment, arsenic is combined with oxygen, chlorine, and sulfur to form inorganic arsenic compounds. Arsenic in animals and plants combines with carbon and hydrogen to form organic arsenic compounds. However, arsenic is usually found in the environment combined with other elements such as oxygen, chlorine, and sulfur. Arsenic combined with these elements is called inorganic arsenic. Arsenic combined with carbon and hydrogen is referred to as organic arsenic. Some of the organic forms are less harmful than the inorganic forms. Most inorganic and organic arsenic compounds are white or colorless powders that do

not evaporate. They have no smell, and most have no special taste. Thus, you usually cannot tell if arsenic is present in your food, water, or air. Inorganic arsenic occurs naturally in soil and in many kinds of rock, especially in minerals and ores that contain copper or lead (ATSDR, 2005c).

2.2.8.2 Source of arsenic in the environment

Arsenic occurs naturally in soil and minerals and it therefore may enter the air, water, and land from wind-blown dust and may get into water from runoff and leaching. Volcanic eruptions are another source of arsenic. Arsenic is associated with ores mined for metals, such as copper and lead, and may enter the environment during the mining and smelting of these ores. Arsenic cannot be destroyed in the environment. It can only change its form, or become attached to or separated from particles. It may change its form by reacting with oxygen or other molecules present in air, water, or soil, or by the action of bacteria that live in soil or sediment. Small amounts of arsenic also may be released into the atmosphere from coal-fired power plants and incinerators because coal and waste products often contain some arsenic. Arsenic released from power plants and other combustion processes is usually attached to very small particles. Arsenic contained in wind-borne soil is generally found in larger particles. These particles settle to the ground or are washed out of the air by rain. Arsenic that is attached to very small particles may stay in the air for many days and travel long distances. Many common arsenic compounds can dissolve in water. Thus, arsenic can get into lakes, rivers, or underground water by dissolving in rain or snow or through the discharge of industrial wastes. Although some fish and shellfish take in arsenic, which may build up in tissues, most of this arsenic is in an organic form called arsenobetaine (commonly called "fish arsenic") that is much less harmful (ATSDR, 2005c).

2.2.8.3 Health effect and toxicity

The single-most characteristic effect of long-term oral exposure to inorganic arsenic is a pattern of skin changes. These include a darkening of the skin and the appearance of small "corns" or "warts" on the palms, soles, and torso, and are often associated with changes in the blood vessels of the skin. A small number of the

corns may ultimately develop into skin cancer. Swallowing arsenic has also been reported to increase the risk of cancer in the liver, bladder, kidneys, prostate, and lungs. If breath high levels of inorganic arsenic, then you are likely to experience a sore throat and irritated lungs. Longer exposure at lower concentrations can lead to skin effects, and also to circulatory and peripheral nervous disorders. Almost no information is available on the effects of organic arsenic compounds in humans. Studies in animals show that most simple organic arsenic compounds (such as methyl and dimethyl compounds) are less toxic than the inorganic forms and that some complex organic arsenic compounds are virtually non-toxic. However, high doses can produce some of the same effects. Thus, if you are exposed to high doses of an organic arsenic compound, you might develop nerve injury, stomach irritation, or other effects, but this is not known for certain (ATSDR 2005c).

Food chain bioaccumulation**,** bioconcentration factors have been measured for several freshwater and marine species. While some species (mainly marine algae and shellfish) tend to bioconcentrate arsenic (EPA, 1980a), it is not biomagnified through the food chain (Eisler, 1994). However, further research on the uptake of arsenic by a variety of plants in a wide range of arsenic polluted sites (e.g., mining area, orchards previously treated with lead arsenate) would be valuable in assessing human exposure near such sites through the consumption of vegetables from home gardens. Regulations and guidelines applicable to arsenic and arsenic compounds in Table 2.8 (ATSDR, 2005c).

Table 2.8 Regulations and guidelines applicable to arsenic and arsenic compounds

Agency	Description	Information	Reference
International:			
Guidelines;			
IARC	Carcinogenicity classification for arsenic and arsenic compounds	Group 1[a]	IARC 2004
WHO	Drinking water quality guidelines for arsenic	0.1 mg/L[b]	WHO 2004
National:			
Regulations and Guidelines:			
Air;			
ACGIH	TLV (TWA) for arsenic and inorganic compounds	0.01 mg/m^3	ACGIH 2004
OSHA	PEL (8-hour TWA) for general industry for arsenic organic compounds	0.5 mg/m^3	OSHA2005d 29 CFR 1910.1000
Water;			
EPA	Drinking water standards and health advisories for arsenic drinking water equivalent level	0.01 mg/L	EPA 2004a

a Group 1: carcinogenic to humans

b Provisional guideline value: as there is evidence of a hazard, but the available information on health effects is limited.

2.2.9 Nickel

2.2.9.1 Physical and chemical properties of nickel

Nickel formula is Ni. Pure nickel is a hard, silvery-white metal. Its melting point 1,455 °C and boiling point 2,730 °C. Density 8.91 g/cm^3 which in its bulk form is resistant to attack by air and water at ordinary temperatures. However, powdered nickel is reactive in air and may spontaneously ignite. Nickel has typical metallic properties; it can be readily rolled, drawn into wire, forged, and polished. It is also ferromagnetic and a good conductor of both heat and electricity. It reacts more rapidly with nitric acid. Nickel is highly resistant to attack by strong alkalis. Nickel can exist in oxidation states -1, 0, +2, +3, and +4, its only important oxidation state is nickel (+2) under normal environmental conditions (ATSDR, 2005d).

2.2.9.2 Source of manganese in the environment

Nickel is a very abundant natural element. Nickel can be combined with other metals, such as iron, copper, chromium, and zinc, to form alloys. These alloys are used to make coins, jewelry. Most nickel is used to make stainless steel. Nickel compounds such as nickel salts are used in electroplating, ceramics, pigments, and as catalysts. Nickel is also used in nickel cadmium (NiCd) and nickel-metal hydride (NiMH) batteries. Nickel is released into the atmosphere by industries that make or use nickel, nickel alloys, or nickel compounds. It is also released into the atmosphere by oil-burning power plants, coal-burning power plants, and trash incinerators. Nickel released in industrial waste water ends up in soil or sediment where it strongly attaches to particles containing iron or manganese. Nickel does not appear to accumulate in fish or in other animals used as food (ATSDR 2005d).

2.2.9.3 Health effect and toxicity of nickel

The most common harmful health effect of nickel in humans is an allergic reaction. Approximately 10-20% of the population is sensitive to nickel. People can become sensitive to nickel when jewelry or other things containing it are in direct contact with the skin for a long time. The most common reaction is a skin rash at the site of contact. Some sensitized people react when they consume food or water

containing nickel or breathe dust containing it. Damage to the lung and nasal cavity has been observed in rats and mice breathing nickel compounds. Eating or drinking large amounts of nickel has caused lung disease in rats and has affected the stomach, blood, liver, kidneys, and immune system, as well as their reproduction and development (ATSD, 2005d). Regulations and guidelines applicable to nickel and nickel compounds in Table 2.9.

Table 2.9 Regulations and guidelines applicable to Nickel and Nickel compounds

Agency	Description	Information	Reference
International;			
Guidelines:			
IARC	Carcinogenicity classification		IARC 1990
	Nickel compounds	Group 1[a]	
	Nickel, metallic	Group 2B[b]	
WHO	Air quality guideline		WHO 2000
	Nickel unit risk	3.8x10 ($\mu g/m^3$)-1	
	Drinking water guideline		WHO 1998
	Nickel	0.02 mg/L	
National;			
Regulation and guideline;			
Air;			
OSHA	PEL (8-hour TWA) for general industry Nickel, metal and insulable compounds	1.0 mg/m^3	OSHA 2003a
	(as Ni) Nickel, soluble compounds (as Ni)	1.0 mg/m^3	
Water;			
EPA	Drinking water health advisories	0.1 mg/L	EPA 2002a

a Group 1: carcinogenic to humans
b Group 2B: possibly to humans

2.3 Background of Toxicity

Toxicity is the properties of a toxicant that produces a harmful effect on a biological system and cause a deleterious biological effect when organisms are exposed to it (Connell, 1997).

2.3.1 Toxic effects

Toxicity results from a number of dynamic processes, including absorption, distribution, and metabolism of the parent compound storage and excretion. To produce a toxic effect on an organism, a toxicant or biotransformation product must be transferred to the site of action in a target organ at a sufficiently high concentration and for a sufficient length of time (Newman & Rank, 1994). There are generally three types of toxic entities; chemical, biological, and physical.

1. Chemicals include inorganic substances such as lead, hydrofluoric acid, and chlorine gas, organic compounds such as methyl alcohol, most medications, and poisons from living things.

2. Biological toxic entities include those bacteria and viruses that are able to induce disease in living organisms. Biological toxicity can be complicated to measure because the "threshold dose" may be a single organism. Theoretically one virus, bacterium or worm can reproduce to cause a serious infection. However, in a host with an intact immune system the inherent toxicity of the organism is balanced by the host's ability to fight back; the effective toxicity is then a combination of both parts of the relationship. A similar situation is also present with other types of toxic agents.

3. Physically toxic entities include things not usually thought of under the heading of "toxic" by many people: direct blows, concussion, sound and vibration, heat and cold, non-ionizing electromagnetic radiation such as infrared and visible light, and ionizing radiation such as X-rays and alpha, beta, and gamma radiation.

2.3.2 Relationship dose-response

Any chemical substances can exhibit harmful effects when the amount introduced to an organism is high enough and sufficient length of time. The critical importance is the dose, or actual amount of chemical that enters the organism. The distribution is a typical sigmoid curve, and they are refers to the cumulative response (dead) organisms (Landis, 1995).

2.3.3 Measures of toxicity

Toxicity can be measured by the effects on the target (organism, organ, tissue or cell). Because individuals typically have different levels of response to the same dose of a toxin, a population-level measure of toxicity is often used which relates the probability of an outcome for a given individual in a population. All measures of toxicity refer to either a concentration or dose that causes a toxic effect (Wikipedia, 2008). Concentration-based measures of toxicity state the concentration in the surrounding environment such as water, air and soil. The particular toxic effect is expressed in unit such as ml/l, mol/l. On the other hand, dose-based measures of toxicity are expressed on a mass per mass of organism-tested basis such as milligrams of toxicant per kilogram of tissue (Connell, 1997).

The principle measure of the toxic effect used in toxicity studies is the 50% effect level, where 50% of the individuals are more tolerant and 50% are less tolerant. This represents the average organism in the population and exhibits the greatest consistency in experiment measurement (Connell, 1997). The toxic potency of the compound is commonly expressed using one of the following terms, depending on the nature of the experiment.

LD_{50} (Lethal Dose) = The dose that cause mortality in 50% of the organisms tested estimated. That one way to measure the short-term poisoning potential (acute toxicity) of material (OSH, 2005).

LC_{50} (Lethal Concentration) = The concentration that causes mortality in 50% of the organisms tested estimated usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water (OSH, 2005).

EC_{50} (Effective concentration) = The concentration that has an effect on 50% of the organisms tested estimated. This parameter is used effects that are not death.

IC_{50} (inhibitory concentration) = That reduces that normal response of an organisms by 50% estimated by graphical or computational means. Growth rates of algae, bacteria and other organisms are often measured as an IC_{50}.

2.4 Background of Bioassay

A bioassay involves use of a biological organism to test for chemical toxicity. For environmental testing, bioassays provide an integrated picture of overall toxicity of an effluent or a sample of water, sediment, or soil from a contaminated site (Conell, 2006). Fathead minnows, various aquatic invertebrates, earthworms, protozoans, and seeds all are used for bioassays of aquatic samples.

Bioassay is a technique by which organisms or biological processes used to measure effect of chemical exposure under control conditions (Connell ed al., 1999). Results from bioassay testing are often used to establish environmental quality criteria, to calculate permit limitations for effluent discharge, and to project the potential impacts of chemical accident. Also, they can determine actual or potential effects of hazardous waste disposal and clean up (Lagrega et al., 1997).

2.4.1 Type of bioassays

The exposure of organisms in variable time period produces four main types of bioassays; acute, sub acute, multigenerational and chronic. Acute toxicity experiments are conducted for short periods of time 24 to maximum of 96 hours. In contrast, chronic tests are conducted for normal expected lifetime of the test organisms. Sub acute tests are designed to determine effect of a toxicant or toxicants to organisms in several generations. The length of chronic, sub acute and muligenerational bioassay varies extensively, depending on the test organism (Connell, 1997)

2.4.2 Types of control

In the experiment, there are three types of basic control (Connell, 1999).

1) A negative control is the same test medium as the treatments groups without the test substance or solvent carrier.

2) A solvent/carrier control may be required in situations where a poorly soluble test substance is firstly dissolved in a small amount of organic solvent.

3) A positive control is provided by exposing the test organisms to a toxicant known to produce a well-defined response on them. The aim is to ascertain the health and sensitivity of the organisms to be used in the test. On this basis, reference toxicants should be toxic levels, stable under testing conditions and relatively nonspecific.

2.4.3 Selections of test organism

The test organisms are very important toxicity test. A number of criteria should be considered (Connell, 1999). The organisms are very important in toxicity test. A number of criteria should be considered. The test organisms should be successfully maintained in the laboratory environment and available in sufficient quantities.The genetics, genetic composition and history of the culture should be known. The relative sensitivities to various classes of toxicants of the test species

should be known relative to the endpoints to be measured. The sensitivity of the test species should be representation of the particular class or phyla that the species represents. In multi-species toxicity tests the interactions among the component species should be understood (Connell, 1999).

2.4.4 The exposure system

Apart from selection of suitable test organisms, the exposure system used can also have an important influence on toxicity results. Basically four techniques are commonly used (Connell, 1999).

1) Static tests involve exposing the test organisms in still test medium without any change of medium for the duration of the test.

2) In a recirculation test, test medium is usually pumped into the test chamber from a reservoir, returned and circulated within the closed system.

3) A renewal test is essentially similar to a static test except that the test medium is completely or partially renewed at fixed intervals such as every 24 hours.

4) For flow-through tests, the test solution is passed though the test chambers, usually from a large reservoir, and the medium is not returned after it has passed through the test chamber.

2.4.5 Calculating toxicological data

The simplest way to estimate toxicity is to plot the logarithm of concentration or dose of the toxicant (x-axis) against the biological response of the test organisms (y-axis). To estimate the effective concentration (EC_{50}) from this graph, a horizontal line is drowning from the point of 50% effect to where it interests to the toxicity curve. From this intersection a vertical line is drawn down until it intersects the x-axis. This point is the concentration of toxicant that effects 50% of the test organisms exposed to the toxicant under the stated experimental conditions (Connell, 1999).

2.5 Background of Genotoxicity

Genotoxicity is the study of the adverse effects of compounds on the genetic material of cell (DNA) and the subsequent expression of these changes (Connell, 1997). The induction of genetic damage may cause an increased incidence of genetic disease in future generations and contribute to somatic cell (other cells except sex cell) diseases including cancer in the present generation. Therefore, it is significantly important to detect compounds that affect the genetic material and to avoid human exposure to them (Watson, 1992).

2.5.1 Basic term using in genotoxicity

Genotoxicity used in many terms such as clastogenic, genotoxic and mutagenic effect. These terms are related to different endpoints used to detect such effects. Clastogenic effect is the effects of chromosome structure including the chromosomes or the chromatid. Genotoxic effect is any defined damage of the DNA helix and may also include the organization of DNA such as Chromosomes and chromosome movement. Mutagenic effect is single or mostly an alternative term for genotoxicity, but it is sometimes meant for mutations in single or multiple genes only. (Walum et. al., 1990). Moreover, other terms are used in this field. For example, genotoxics are agents, which alter or rearrange genes. Such alternatives are usually initiated by DNA damage and the rearrangement commonly called mutation. Any agent that causes mutation is a mutagen (Williums et al., 2002).

2.5.2 DNA and molecular genetics

Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms. The main role of DNA molecules is the long-term storage of information. DNA is often compared to a set of blueprints, since it contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. The DNA segments that carry this genetic information are called genes, but other DNA

sequences have structural purposes, or are involved in regulating the use of this genetic information**.**

The molecular basis for genes is deoxyribonucleic acid (DNA). DNA is composed of a chain of nucleotides, of which there are four types: adenine (A), cytosine (C), guanine (G), and thymine (T). Genetic information exists in the sequence of these nucleotides, and genes exist as stretches of sequence along the DNA chain. (Pearson, 2006**)**

DNA normally exists as a double-stranded molecule, coiled into the shape of a double-helix. Each nucleotide in DNA preferentially pairs with its partner nucleotide on the opposite strand: A pairs with T, and C pairs with G. Thus, in its two-stranded form, each strand effectively contains all necessary information, redundant with its partner strand. This structure of DNA is the physical basis for inheritance: DNA replication duplicates the genetic information by splitting the strands and using each strand as a template for synthesis of a new partner strand. The structures of DNA in Figure 2.4 - 2.5.

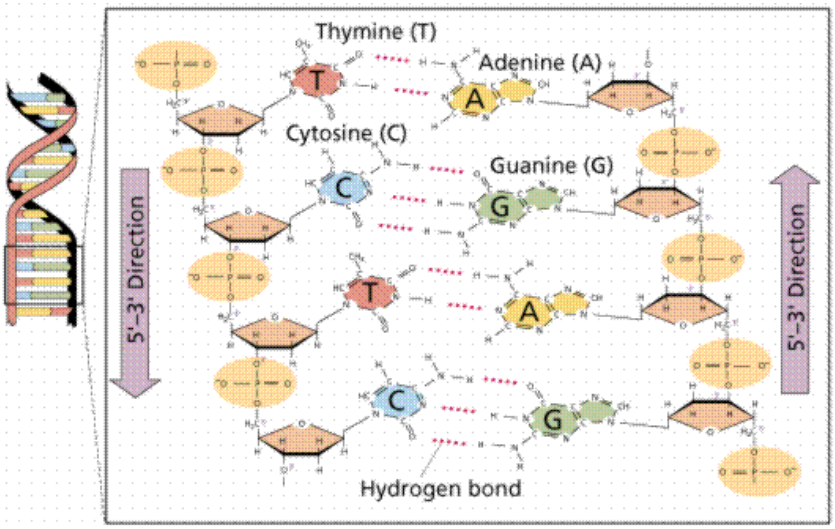


Figure 2.4 The chemical structure of DNA (Wikipedia, 2008)

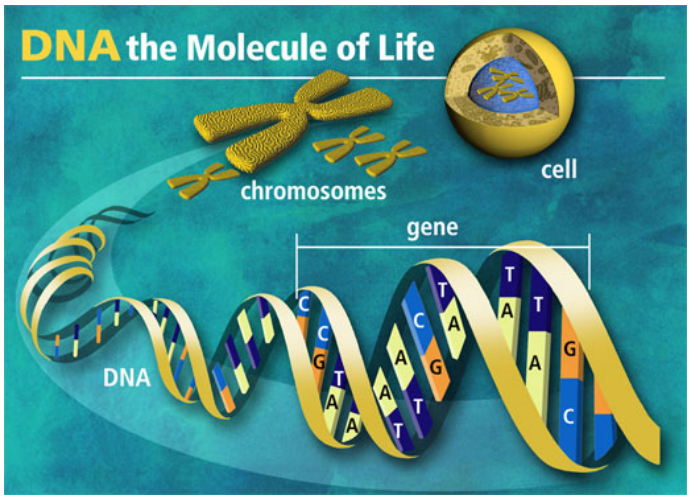


Figure 2.5 DNA stands divide and bases attach themselves to the new stand (Vichakarn, 2005).

Genes are arranged linearly along the long chains of DNA sequence, called chromosomes.

2.5.3 Chromosome structure

Chromosomes are organized structures of DNA and proteins that are found in cells. Chromosomes contain a single continuous piece of DNA, which contains many genes, regulatory elements and other nucleotide sequences. One chromosome is composed of two chromatids. Chromosomes also have another structure feature, the centromere, which is usually located near the center of the chromosome. Spindle fibers are made of microtubule called the spindle apparatus. The mature spindle fibers apparatus is elongated, with its ends pointing to opposite poled of the cell. Fibers from opposite poled of the spindle attach to each chromosome on either side of its centromere, and the structure of chromosome is shown in Figure 2.6 (1) chromatid (2) centromere (3) short aim and (4) long aim **(**wikipedia, 2008**).**

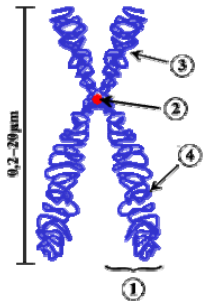


Figure 2.6 Structure of chromosome

2.5.4 Cell cycle

When cells divide, they much progress through a series of step called the cell cycle. One cell cycle is the interval of time between the formation of a cell and its division to form two new cells (Moore et. al., 1995)

In plant, the process of division occurs in special regions called meristems. Merristems are usually found at the tips of roots and shoots. For example, themeristem cell and the root tip are show in Figure 2.7 (Rost et.al., 1998).

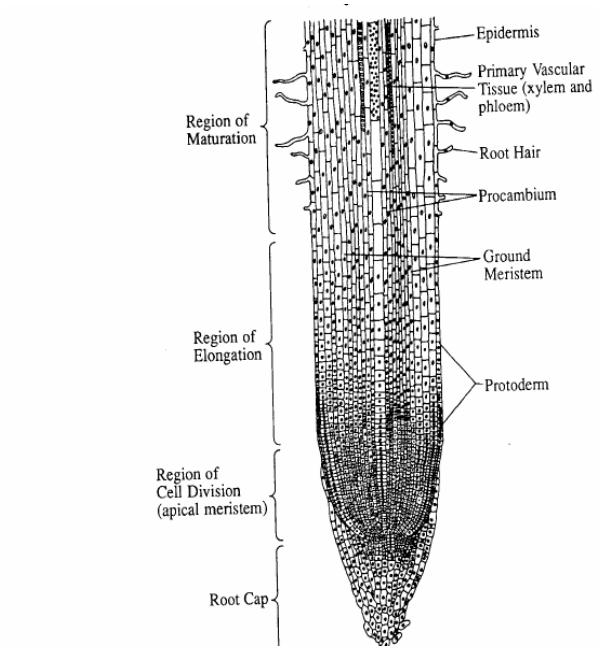


Figure 2.7 Onion root tip (Wikipedia, 2008).

The G1, S, and G2 phases of the cycle are grouped together and called interphase. The remaining phase, cell division (M) is actually composed of two parts: mitosis (nuclear division) and cytokinesis (cytoplasmic division). Specific metabolic events occur in each cell cycle phase. The cell cycle is shown in Figure 2.8. The time required for each stage various with cell type and species.

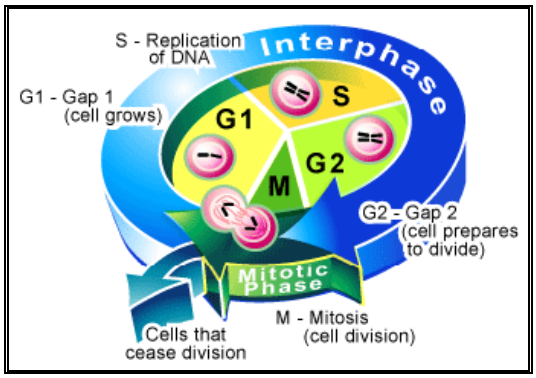


Figure 2.8 Cell life cycles (David, 2003)

1. Interphase

G1 phase of interphase, a period of growth in which protiens and other cellular molecules are synthesized, but DNA is not replicated. During this time, the cell grows in preparation for DNA replication, and certain intracellular components, such as the centrosomes undergo replication. Before a cell begins DNA replication, it must ensure that it is biologically ready to take on such a process. G1 is the phase when this cellular monitoring takes place.

S phase or synthesis is the phase of the cell cycle when DNA packaged into chromosomes is replicated. This event is an essential aspect of the cell cycle because replication allows for each cell created by cell division to have the same genetic make-up. During S phase a number of events additional to chromosome replication take place. Cell growth continues through S phase, as does the rate of synthesis of a number of proteins and enzymes that are involved in DNA synthesis.

Once DNA replication is complete the cell contains twice its normal number of chromosomes and becomes ready to enter the phase called G2.

G2 phase; Similar to G1, G2 is an intermediate phase, a time for the cell to ensure that it is ready to proceed in the cell cycle. Occurring between the end of DNA replication in S phase and the beginning of cell division in mitosis, G2 can be thought of as a safety gap during which a cell can check to make sure that the entirety of its DNA and other intracellular components have been properly duplicated. In addition to acting as a checkpoint along the cell cycle, G2 also represents the cell's final chance to grow before it is split into two independent cells during mitosis.

2. Cell division

Mitosis (Cell division)

Mitosis is nuclear division plus cytokinesis, and produces two identical daughter cells during prophase, prometaphase, metaphase, anaphase, and telophase.

Prophase: Chromatin in the nucleus begins to condense and becomes visible in the light microscope as chromosomes. The nucleolus disappears. Centrioles begin moving to opposite ends of the cell and fibers extend from the centromeres. Some fibers cross the cell to form the mitotic spindle.

Metaphase: Spindle fibers align the chromosomes along the middle of the cell nucleus. This line is referred to as the metaphase plate. This organization helps to ensure that in the next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome.

Anaphase: The paired chromosomes separate at the kinetochores and move to opposite sides of the cell. Motion results from a combination of kinetochore movement along the spindle microtubules and through the physical interaction of polar microtubules.

Telophase: Chromatids arrive at opposite poles of cell, and new membranes form around the daughter nuclei. The chromosomes disperse and are no

longer visible under the light microscope. The spindle fibers disperse, and cytokinesis or the partitioning of the cell may also begin during this stage.

Cytokinesis

Cytokinesis begins before telophase is finished. The partition of the cytoplasm occurs by the formation of the cell plate in the center of the two gaugther cells. Than, the cells return once again to G1 of the cell cycle (Moor et al., 1995; Rost et al., 1998; Singh, 1993).

The cell cycles in root tip of Alliums are shown in Figure 2.9

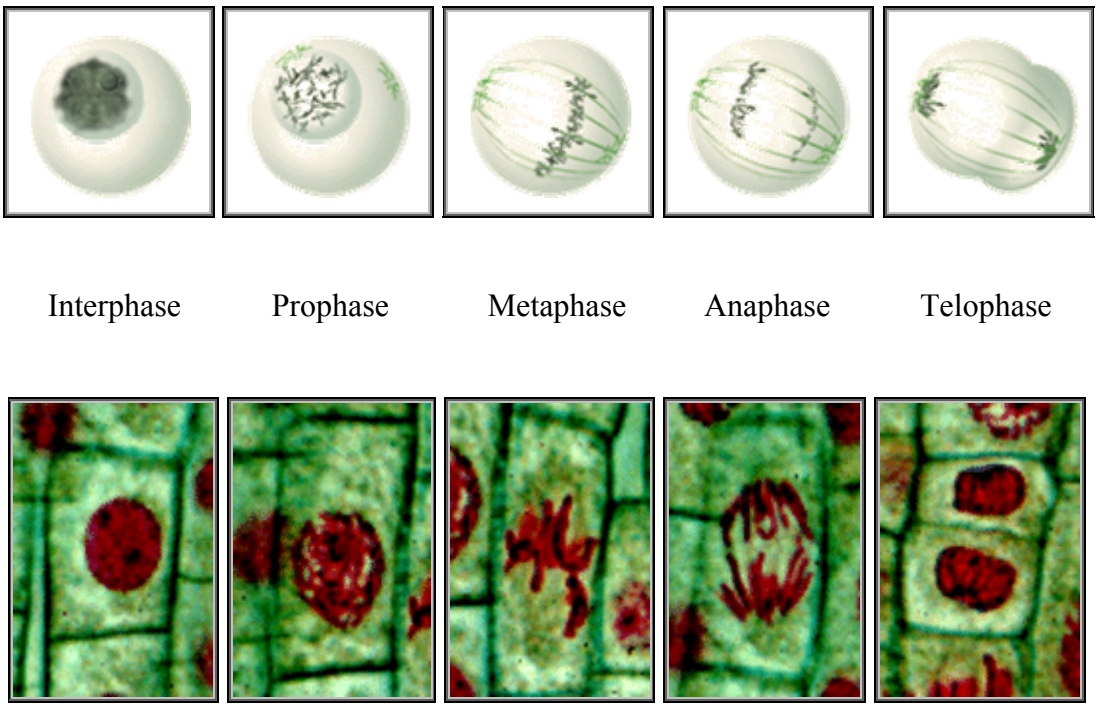


Figure 2.9 Onion root tip interphase and mitosis (Cornell.edu, 1997; Arizona.edu, 1998)

2.6 Genetic Alterations of Classify

DNA damage consists of two broad categories: visible detectable through cytogenic analysis of chromosomes (macrolesions), and non-visible changes which occur at the nucleotide level (Micronesians) (Brusick, 1980) shown in Figure 2.9.

Macrolesion is referred to the genetic lesion visualized by microscopy. Abnormal chromosome numbers result in daughter cell and may be recognized as a chang in the number (gain or loss chromosomes or set chromosomes). Changing in chromosome structure (clastogenic effects) is categorized by the abnormal chromosome morphology (Williams et al., 2002).

For microlesion, gene or point mutation results from the addition or deletion of nucleotides or from the substitution of one nucleotide for another during DNA replication or repair. Such molecular changes may exert their effects by changing the function of single gene and lead to subsequent genetic alteration (Williams et al, 2002; Brusick, 1994).

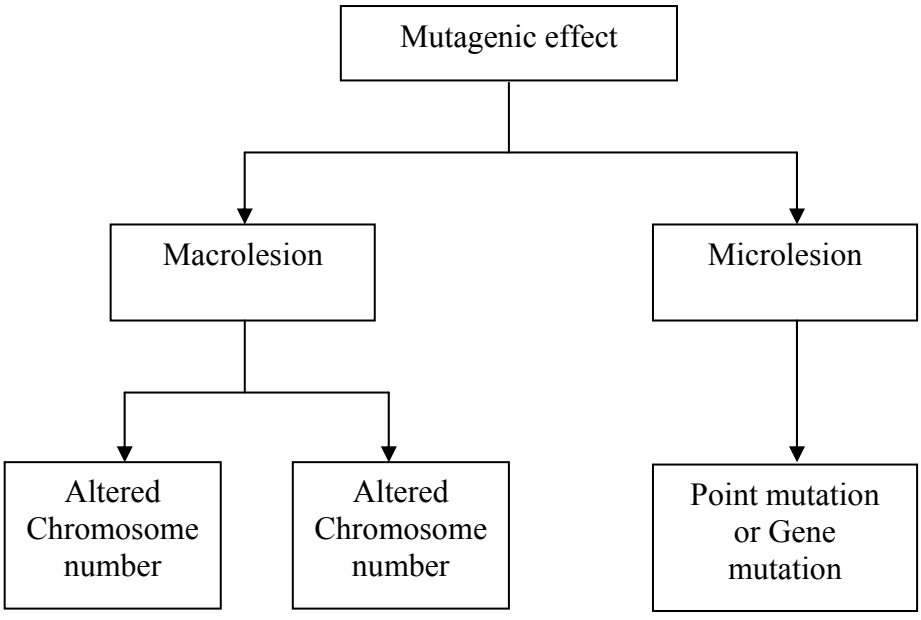


Figure 2.10 Classification of DNA chang (Brusick, 1980).

2.7 Chromosome Aberration

Chromosome aberrations result from the genetic change. This change is much more DNA damage than the point mutations (Connell et.al.,1999). Chromosome aberration is defined as a modification of the genetic material and is detected by light microscope in appropriately prepared cells. Two theories are currently available to explain the mechanism of chromosome aberration. One theory is “breakage-first” hypothesis. The theory assumes that the initial lesion is a break in the chromosome backbone that is indicative of a broken DNA strand. Several possibilities exist following such an event: (Lien, 1993) the ends may repair normally and rejoin to form a normal chromosome; or (Tanoohsin, 1979) they may be mis-repaired or join with another chromosome to cause a translocation for genetic material. A second theory is “chromatid exchange” hypothesis. If the exchange occurs with a chromatid from another chromosome, an “exchange figure” results. This theory assumes that the initial lesion is not a break and that the lesion can either be repaired directly or may interact with another lesion by process called exchange initiation (Williums et al., 2002).

2.7.1 Chromosome aberration classify

Some abnormality chromosome structure or number can occur naturally but mutagenic agents can cause the frequency of these events increasingly. Chromosome aberrations are classified into two major groups: structural aberration and numerical aberration.

2.7.1.1 Structure chromosome aberrations

Structural aberrations are the abnormal chromosome morphology. The structure aberrations are further divided into two; chromatid -typed and chromosome typed aberrations. The production of that by an agent depends on the nature of the clastogen (chromosome-breaking agent) and cell cycle stage of target cells. For example, the chromosome type occurs when DNA strand is broken in G1. In contrast, the chromatid type occurs when cells are exposed is S or G2 phase (Brusick, 1980).

2.7.1.2 Numerical chromosome aberrations

Numerical chromosome aberrations caused by an incomplete separation of replicated chromosomes during call division are categorized into two types; aneuploidy and polyploidy. Aneuploid and polyploid cells have chromosome numbers that differ from the normal number for the species. In aneuploid, the deviation in chromosome number involves one or a few chromosomes while in polyploidy, the alterative involves complete sets of chromosomes especially haploid (n). For instance, in humans, where the normally diploid (2n) chromosome number is 46, cell with 45 or 47 chromosome would be described as aneuploid. In contrast, cells with 69 chromosomes would be described as polyploidy, in this case triploid (3n) (Amdur et al., 1992).

2.8 Genotoxicity Tests by Plant Bioassays

Higher plants valuable genetic assay systems for monitoring and screening pollutants in the environmental. Plan cells can be investigated under a wide range of environmental conditions such as water content, pH, and temperature. Chromosome organization is similar to the human system (Li & Hefich, 1991). Higher plant genetic assays results contribute to protect the public from agents that cause mutation and cancer, moreover, higher plant genetic assays are inexpensive and easy to handle and ideal for use in developing countries (Grant, 2002). Gene-Tox Programe of U.S.EPA are listed that criteria to ease of use, well-developed methodology, used by number of investigation, large data base on chemical mutagens, adaptability of protocols to different climatic conditions and ease of distribution of source material (Ecobichon, 1997).

2.9 The allium Test

The allium test system using onion (Allium cepa) has been widely exploited for such purposes since it was first introduced as a test system by Levan in 1938. Since that time, the basic test system has been developed to use as environmental monitoring. The allium test is one of the most useful and convenient methods to monitor toxicity and genotoxicity. The Allium test provides a rapid screening procedure for chemicals, pollutants contaminants, etc. which may represent environmental hazards. Root growth inhibition and adverse effects upon chromosomes provide an indication of likely toxicity (Fiskesjo, 1998). The root tip is often the first part of any plant that is likely to come into contact with chemical and pollutants found is soil and water supplies. Observation of the root tip system is particularly sensitive to the harmful effect of such environmental contaminants. Gross effect can be quantified by measurement of root growth inhibition, whereas examination of the chromosomes in the root tip cells can indicate mutagenic effect (Hare & Atterwill, 1995). Also, the root cell posses certain enzymes, the mixed function oxidases, which activate many promutagens to mutagens. This activating system will improve the detection of those chemicals, which exert their toxic effect in a reactive metabolite (Fiskesjo, 1998). The simple growth test method may be performed by anybody without an specific training. The result demonstrated as shot or long root bundles are easy to interpret as environmental damage. The *Allium* chromosome being quit large and allows a detailed analysis. The bulbs can be easily stores and handled. In addition, root with meristems can grow at profusely at regular intervals (Sharma et al., 1999).

National Swedish Environment Protection Board, the Ergatt/FRAME Data bank of in Vitro Techniques in Toxicology (INVITTOX) and U.S.EPA in Gene-Tox Programe is gradually in the parts of genotoxicity testing in various organizations because of many advantages of the Allium test (Ecobichon, 1997; Fiskesjo, 1993).

2.9.1 Technical materials and methods of the Allium test

1) The test organism

The Allium test should be performed in normal room temperature and protected from direct sunlight (Fiskesjo, a). Bulb of *Allium cepa* should be stored under dry conditions at 10-20 °C. It may be kept more than a year until material from the next season is available. However, some 20% bulbs may dry up or be destroyed by mold. Thus number of onions should be stored about 3 or 4 timed the number needed for experiments. The variation within the population is compensated for the use of series of equal-size onion (Fiskesjo, 1985). Fiskesjo recommended that the Allium test of river water or industrial wastewater should be placed the series of onion bulb directly in the test solutions without previous germination of the root tip (Fiskesjo, 1985). Twelve small amounts of samples are available; five to six onions are set up in each series (Fiskesjo, 1994).

2) Growth medium for the Allium test

- Growth medium

a. nutrients solution for plant growth

nutrient	stock	final concentration
$Ca(NO_3)_2.4H_2O$	1.0 mM	0.1 mM
KNO_3	2.0 mM	0.2 mM
$MgSO_4.7H_2O$	1.0 mM	0.1 mM
KH_2PO_4	1.0 mM	0.1 mM
Fe-EDTA.$3H_2O$	1.0 mM	0.1 mM

b. Trace elements

$MnSO_4$	3.6 μM	0.364 μM
$CuCl_2$	0.48 μM	0.048 μM
Na_2MoO_4	0.0078 μM	0.00078 μM

$ZnSO_4$	0.0042 μM	0.00042 μM
H_3OB_3	3.7 μM	0.37 μM

The stock solution should be ten-fold diluted with distilled water, and the pH adjusted to 7 before test starts (Fiskesjo, 1994).

- Tap water as growth medium

The most convenient growth medium is tap water for control and for the dilution of tested chemicals. The tap water must be good quality; with a pH around 7, without any toxic ions such as chlorine, copper from copper pipes or aluminium in water from private well with low pH. If copper popes are used for the transfer of drinking water, health authorities in Sweden recommend running the water for three minutes before sampling. Because copper concentration is highter than 0.05 mg/L, it will inhibit the allium roots (Fiskesjo, 1994).

2.9.2 Test Procedure

2.9.2.1 Inhibition of root growth

To study the effect of tested chemicals on the root growth of the allium by measuring the root growth as percent of control to estimate the toxicity levels (Fiskesjo, 1994).

1) Prior to test start, the outer scales of the bulbs and the brownish bottom plate should be removed. If many onions are to be the same time, the peeled bulbs should be put into fresh water during the continued cleansing procedure to protect the root primordial from drying.

2) Bulbs from this pool are placed on a soft layer of paper and directly put on test tubes, filled with the test liquid. The experiments should be performed in relatively constant room temperature at about 20^0C and protect against direct sun light. The test procedure may follow either the original form or the modified form of the method. In original form, the root growth is growth is started in tap water

of good quality. When the roots have reached the length of 1-2 cm, the onions are placed directly in the test liquids without the previous germination of the tips.

3) Twelve onions are placed in test in each concentration for 4 days (96 hour), and the liquid is changed every 24 hours. After 4 days, the ten onions that appear to be developing the best in each series are selected for measuring root length (Fiskesjo, 1985).

2.9.2.2 Mitotic index and chromosome aberration

To assesses chromosome damage and cell division disturbances providing additional information about genotoxicity (ed. Hare & Atterwill, 1995).

1) Three or five onions are sufficient for this part (Fiskesjo, 1994). The prepared onions for the experiment are the same as the toxicity test. The different is to place the onions in the control water for two days or begin root germination about two centimeters. Then, the onions are change to test samples and negative and positive controls. MMS is recommended as positive control in 10 mg/L (Nielsen & Rank, 1994). The test solutions should be changed every 24 hours for 48 hours. After the growing period of 48 hours, root tips are cut and fixed for the later preparation of slides.

2) There are two kinds of fixatives used in the experiment. Fixative for direct preparation of slide is composing of nine parts of 45% acetic acid and one part of HCL. Fixative for storage of root tips in a freezer consists of three part of absolute alcohol and one part of concentrated acetic acid (Fiskesjo, 1994).

3) The staining method of direct and indirect fixative is quit different. After fixation with direct fixative for 5 minutes, three root tips on each of three slides are cut two drop of orcein are added to the root tips, and root tip cells is spread with needle or stainless stick for 3 minutes. The cover glass in placed on the slide from one side to the other with squash technique. This technique is to press slight down with the thumb on layers of tissue paper on cover glass (Fiskesjo, 1994). From this technique, the cells are flattened out in a thin layer strain and without the air

bubbles. Then, the nail vanish is sealed along the cover glass edges. This slide will keep a few mounts in refrigerator without the loss of color and contrast within the cells.

However, the root cells are fixed for 24 hours at room temperature and hydrolyzed with 1 M HCl for 5 minutes for the direct fixative before they are stained and prepared in the same procedures as the direct fixative (Fiskesjo, 1994).

2.9.3 Expression of result

2.9.3.1 Macroscopic parameters

Macroscopic parameters may be used in standard observations (ed. Hare & Atterwill, 1995; Fiskesjo, 1985).

- Root form

Crochet hooks is the bending of the roots that occur especially after treatment with certain metal salts.

C- Tumor formation is observed as a swelling of the root tips. This may be observed after 3-5 days of cultivation after various types of treatment, but it is more obvious after a longer time period.

- Root length

A special comment on the procedure for measuring of the root lengths may be needed. Normally, a ruler measures the length of the whole root bundle outside the test tube. A method gives one value for each bulb. A more accurate way to measuring would be to measure each root each bulb, requiring the removal of the roots and the termination. In comparison of the two different ways of root length measuring, the mean values of bundle length are somewhat lower than the mean value of all roots. However, the relationship of both results is nearly the same (about 40%). Therefore, this whole bundle measuring is to be preferred because it is reliability, time saving and continuity in observation (Fiskesjo, 1985).

Plot root lengths are in a diagram between log concentration of treatment and mean root length in % of control to find the toxicity levels such as the

EC_{10}, EC_{50}, and EC_{90} respectively. They mean effective concentration inhibiting 10, 50, and 90% root growth in relation to control. Of these values, EC_{50} is the most commonly used parameter showing the degree of toxicity. The example of the graph for the inhibiting of Allium roots exposed to maleic hydrazide to find EC_{50} in show Figure 2.11.

The root growth inhibited over 45% strongly indicates the presence of toxic substances (Kincl et. al., 1996). The green leaves sprouting from the growing onions may also be observed and photograph to indicate toxicity between the tested chemicals and the control (Fiskesjo, 1985).

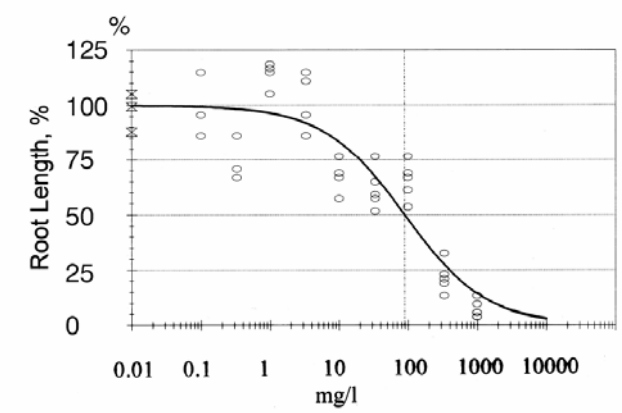


Figure 2.11 Growth inhibition of Allium test exposed to maleic hydrazide (Rank, 2003)

2.9.3.2 Microscopic parameters

Genotoxicity test is suggested for following parameters (Fiskesjo, 1985; Fiskesjo, 1994).

- Mitotic index (MI): The number of dividing cell per 300 observed cells in each slide or 2,000 cells in each concentration is sufficient.
- Chromosome aberration: Chromosome aberration cells are observed and classified in 100 cells of normal metaphase and anaphase (Figure 2.12).

1) Stickiness Sticky chromosomes usually are not reversible leading to cell death. It indicates highly toxic.

2) Chromosome bridges and/or fragments: They refer to clastogenic effect and are used as the indicator of mutagenicity. These effects result from chromosome and/or chromatid breaks.

3) Laggard (Vagrant) chromosome (weak c-mitosis): It indicates the risk of aneuploidy.

4) c-mitosis: It is described as inactivation of the spindle followed by a random scattering of the condensed chromosome in the cell. It indicates weak toxic effect that may be reversible.

5) Micronuclei : Small nuclei are found in the cytoplasm of cell.

6) Multipolar: There are many polar in one nucleus (Liu & li, 1992; ed. Wang, Gorusuch & Hughes, 1997).

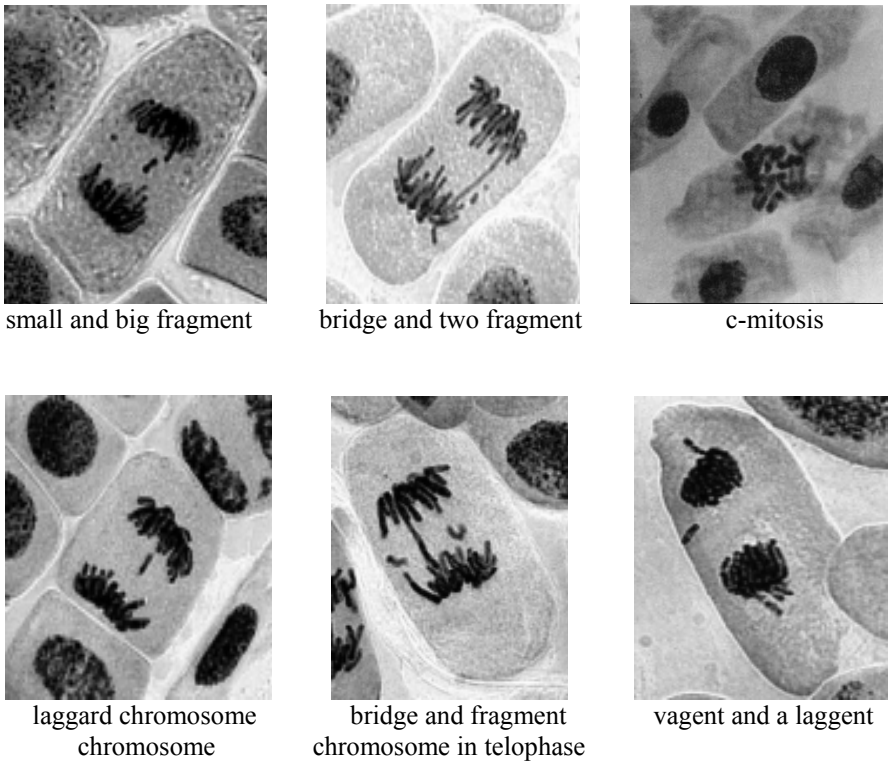


Figure 2.12 Type of chromosome aberration (Rank, 2003)

2.10 Shallot (*Allium ascalonicum* L.)

In Thailand, high domestic consumption and potential for export, it is economical importance. Because of shallots are grown extensively in the humid regions of the tropic. The main planting areas are here in the north and the Northeast such as Chaing Mai, Lampoon, Srisaket and Uttraradit (Robonowitch & Brewster,1990).

2.10.1 Taxonomic classification and botany

Class Monocotyledones
Superorder Liliiflorae
Order Asparagus
Family Amaryllidaceae
Genus *Allium*
Species *Allium ascalonicum* L

Shallot belongs to the gnus Allium, which includes several cultivated crops such as garlic, leek, and onion. Shallot bulbs are pear-shaped, narrowed in the upper part into a rather long point and covered with a russet colored skin of a coppery red color in a lower part. Shallot is distintguished from normal bulb onions by their habit of multiplying. A single shallot bulb usually contains several initial shoots, and after the bulb is planted, several leafy shoots grow out from it. Each shoot rapidly produces a small bulb; thus a cluster form is attached to the original base plate. The bulbs can be separated and the process repeated during the next growing season. Each shallot bulb produces a cluster of 6 or more bulbs. The basic chromosome numbers (n) of shallots is a diploid, 8 or 2n=16 (Ecobichon, 1997). The shallot bulbs are shown in Figure 2.13.

Figure 2.13 Shallot bulb

2.10.2 Production

1. Soil and climate

Shallots are grown in different kinds of soils but they favor a well structured of sandy loam soil with proper drainage and absence of persistent weeds. The size and the quality of bulb depend on the soil type, fertility, and cultivate. The pH interval from 5.5 to 6.5 is the major requirement for the successful production of shallots. Soils that are too alkaline or acidic are not suitable for bulb growth. The optimum temperature is around 13-25°C, and photoperiod is 8-10 hours per day (Botany.com, 2002).

2. Propagation and cultural management

Shallot can be grown from seed. After three months of dormancy the shallot bulbs can be planted. The “mother” bulbs divide forming several bulbs. For uniformity in production, planting similar size bulbs is essential. The weak clumps and the smallest bulbs are discarded. To save bulbs for the following year, only the highest quality bulbs from the quality clumps are stored. These clumps should be as free from diseases as possible. Shallots are shallowing roots; thus, they should be irrigated frequently. Green shallots can be harvested in 30 to 60 days, mature bulb in 90 to 120 days (Botany.com, 2002).

3. Storage

Shallots should be stored in good aeration. This is important to remove excessive moisture and to minimize diseases. Low relative humidity and low temperature are important to keep high quality of shallot and free from sprouting and root growth (Botany, 2002).

2.11 Related Researches

Plant bioassay

Grant, W.F. (1978) studied chromosome aberrations in plants as a monitoring system**.** The use of plant tissue (primarily root tips and pollen mother cells) for studying the induction of chromosomal aberrations is one of the oldest, simplest, most reliable and inexpensive methods available. Pesticides which cause chromosome aberrations in plant cells also produce chromosome aberrations in cultured animal cells. The aberrations are identical. Studies have shown that compounds which have a c-mitotic effect on plant cells have the same effect on animal cells. It is recommended that plant systems be accepted as a first-tier assay system for the detection of possible genetic damage by environmental chemicals.

Allium test

In 1994, Rank, J. and Nielsen, M.H. studied the Allium anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. Five mutagenic or carcinogenic chemicals usually found in wastewater were tested in the Allium anaphase-telophase test. Sodium dichromate (25 μM), benzene (100 μM), dichloromethane (175 μM) and 1,1,1-trichloromethane (175 μM) increased the frequency of chromosome aberrations in the root cells, whereas formaldehyde (1 mM) was found to be non-mutagenic in this test system. Other studies where chemicals were tested in the Allium test were also reviewed. For 15 chemicals the results were compared with results from the Ames test, the Microscreen assay, and carcinogenicity tests in rodents. The sensitivity of the Allium test was calculated to be 82%. In

conclusion the Allium test is recommended for the screening of wastewater because it has a high sensitivity, is cheap, rapid, easy to handle, and because it can be used on wastewater without pretreatment of the sample.

Possible use of Allium test in environmental monitoring was studied by Kumar, S. et al. in the year 2000. This test system indicated that morphological changes i.e. root growth served indicator of toxicity of various chemicals including complex mixtures. The microscopic observations such as mitotic activity and chromosomal abnormalities indicated genotoxic potential of the test compounds. The available data suggests that Allium test can be very well used in monitoring the toxicity of pure chemicals, complex mixture of chemicals, effluents of various industries, river water and also contamination in drinking water etc. along with other test systems for initial screening purposes.

HCl and NaOH

In 1985, Fiskesjo, G. studied the effects of pH on the root growth under pH values between 2 and 12 for 5 days. The pH values were sets by addition of HCl and NaOH to tap water (pH 7.3). The result indicated that the pH interval ranged from 3.5 to 11 did not cause growth restrictions and pH of about 6.7 can permit growth of root cells.

Tap water and distilled water

A comparison of tap water and distilled water using as control water was studied for 10 days. The results indicated that the root growth in tap water at pH 7.8 (copper less than 0.05 mg/L) increase nearly to around 12 centimeters in ten days, whereas the growth in distilled water at pH 5.6 stopped completely after the fourth day (2.5 centimeters). Therefore, tap water was recommended as control water, because the distilled water usually lacks of nutrition for root growth (Fiskesjo, G., 1985).

MMS and EMS

Methyl Methane Sulfonate and Ethyl Methane Sulfonate are use as positive control in mutagenicity testing (Rank, J. & Niesen, M.H., 1997). Recommend that MMS should be used as positive control in the Allium test because MMS (mg/L) was found to be about ten times more potent inducing chromosome aberrations than EMS (100 mg/L). Record of miclonuclei in interphase cells showed that this endpoint does not give more information for clastogenicity than recording of chromosome aberrations in anaphase-telophase.

Lead nitrate

The effects of lead on root growth, cell division, and nucleolus of Allium cepa were investigated. The concentrations of lead nitrate($Pb(NO_3)_2$) were 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M. Lead reduces root growth and causes mitotic irregularities, including c-mitosis, anaphase bridges, and chromosome stickiness. The c-mitotic effect reached its maximum in the meristem at above 10^{-4} M Pb, when practically almost all the anomalous dividing cells are of this type. After treatment with Pb, there were many similar silver-stained particulate materials scattered in the nucleus in short, rounded meristem cells and in long, oblong root cap cells (Liu, D. et. al., 1994).

In 1992, Lerda, D. determined the effects of lead nitrate (at the concentrations of 0.1, 1.0, 10, 50, 100, or 200 ppm) on root growth, cell proliferation, and chromosomal aberrations in *Allium cepa*. It was found that root growth was completely inhibited at concentrations of 100 or 2oo ppm. At lower concentrations, the rate of growth was reduced in a dose-dependent manner. Likewise, cell proliferation was progressively reduced at high concentrations. The frequency of chromosomal aberrations in onion cells from exposed to lead nitrate at the concentration of 0.1 or 1.0 ppm. did not differ from that of the non-exposed controls. Chromosome aberration in plant cells exposed to Pb at the concentration of 10 ppm were foun to be significantly p-value<0.05) much more than those of non-exposed controls.

The effects of lead on genetic systems in the context of lead's various other toxic effects as wells as its abundance and distribution in the environment were

studied. Lead has been tested and found to be capable of eliciting a positive response in an extraordinarily wide range of biological and biochemical tests; among them tests for enzyme inhibition, fidelity of DNA synthesis, mutation, chromosome aberrations, cancer and birth defects. It reacts or complexes with many biomolecules and adversely affects the reproductive, nervous, gastrointestinal, immune, renal, cardiovascular, skeletal, muscular and hematopoietic systems as well as developmental processes. It is likely that lead is a selective agent that continues to act on and influence the genetic structure and future evolution of exposed plant and animal populations (Johnson, F.M., 1998)

Wierzbicka, M. (1993) studied lead can easily enter the food chain. Using the root test, the relationship between the amount of lead in plant tissues and the level of the root growth inhibition was examined for twelve food plant species. At least 96.6% of lead was bound in cells of root tips. Most of the lead was accumulated in cell walls, vacuoles and sometimes in dictyosomal vesicles. Lead accumulating in these cell compartments is separated from cell cytoplasm and therefore is no longer toxic for root cells. The results show that amounts of lead much bigger than those observed in the environment can now easily enter the food chain via plants. High tolerance to lead in plant roots is quite unfavourable for other members in the food chain, including man.

In the year 1998, he further studied on onion (*Allium cepa* L.) adventitious roots were treated with lead, which was found to be accumulated in increasing amounts in the apoplasts of root tips if the metal continued to be present in the roots' external environment. Ultrastructural observations made it possible to formulate the hypothesis that there are two protective mechanisms against lead in onion root tip cells: (i) the amount of polysaccharides in the cell wall and the thickness of the wall increases, this allows more lead to be accumulated in a manner that is not harmful to the cell; and (ii) lead is expelled from inside the cell through plasmatubules to the root tip apoplast, this makes it possible to inactivate the lead that has a toxic effect on the root symplast Wierzbicka, M. (1998)

Later, he studied the effect of lead on the cell cycle in the root meristem of *Allium cepa* L. and adventitious roots were treated with lead at the concentration of 2.5 mg/ dm^3 of Pb^{2+} [$Pb(NO_3)_2$]. It was found that lead prolonged the cell cycle and that cells in two phases of the cycle, G_2 and S, differed in their sensitivity to lead. Data are presented supporting the hypothesis that during the initial period of exposure of A. cepa to lead, this metal enters both the root apoplast and symplast, exerting a destructive effect on cells, while later, lead penetrates only into the root apoplast, and in this way remains harmless to cells (Wierzbicka, M., 1999).

Zinc

Gyana & Premananda, (2003) studied the effects of metal (Zinc) toxicity on plant growth and metabolism. The major change was seen in the nucleus of the root tip cells due to zinc toxicity. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane in presence of 7.5 mM zinc. The cytoplasm became structureless, disintegration of cell organelles and the development of vacuoles were also observed. The number of nucleoli also increased in response to zinc resulting in the synthesis of new protein involved in heavy metal tolerance. This review may help in interdisciplinary studies to assess the ecological significance of metal stress.

Chromium

Liu, D., Jiang, W., & Li, M. (1992) studied the effect of trivalent and hexavalent chromosome on root growth and cell division of *Allium cepa* by using chromium nitrate and potassium dichromate at the concentration of $2x10^{-1}$ to $2x10^{-7}$ M. The result showed that both trivalent and hexavalent chromium inhibited root growth and caused mitotic irregularities comprising c-mitosis, anaphase bridges, chromosome stichiness, chromosome fragmentation and lagging chromosome. When compared with chromium nitrate on the equimolar basis, potassium dichromate showed a stronger inhibitory and toxic effect on root growth and cell division, respectively.

Copper chloride

In 2003, Seyin studied cytogenetic effects of copper chloride on root tip cells of *Helianthus annuus*. Seeds were treated with 10, 25, 50 and 100 mg/L (ppm) of copper chloride for 24 h. It was found that copper chloride had a marked mitodepressive action on mitosis. Mitotic abnormalities were increased and the mitotic index was decreased depending on the concentrations of the copper chloride applied.

copper, zinc, lead

In 2005, correlation between heavy metal ions (copper, zinc, lead) concentrations and root length of *Allium cepa* L. in polluted river water was investigated. The test waters were collected from two sampling sites: at the beginning and the end of the Toledo River. The bulbs of *Allium cepa* L. were grown in test water with nine concentration levels of copper, zinc and lead from 0.1 to 50 ppm. For test liquids containing dissolved Cu below 0.03-ppm the root growth was reduced by 40%. However, the same reduction occurred for 1-ppm dissolved Zn. For dissolved Pb, results reveal toxicity at above 0.1 and 0.6 ppm of the beginning and the end of the Toledo river water, respectively (Soraya et. al., 2005).

Milan, et al., (1995) studied acute toxicity of heavy metals (copper, lead, zinc), phenol and sodium on *Allium cepa*, *Lepidium Sativum* L. and *Daphnia magna* St. In term of comparative investigations and the practical application. The investigations of the effects of various heavy metal: Cu (as $CuSO_4$), Pb (as $Pb(NO_3)_2$) and Zn (as $ZnSO_4$), phenol and Na (as Na_2SO_4 and NaCI) concentrations on root length of onion bulbs *(Allium cepa* L.) and garden cress *(Lepidium sativum* L.), as well as on the survival rate of great water flea *(Daphnia magna* St.) showed varied sensitivity of the above test organisms. Based on IC_{50} value (50% inhibitory concentration) for *Allium. cepa* L. and *L. sativum* L. and on LC_{50} value for *D. magna* St., the acute toxicity of the tested substances decreased after a 48-hour exposition in the following order: *A. cepa* L.:Cu > Pb > Zn > phenol > Na_2SO_4 > NaC1; *L. sativum* L.: phenol > Cu > Pb > Zn > Na2SO 4 > NaCI; D. *magna* St.: Cu > Zn > phenol > Pb > Na_2SO_4 > NaC1.

Chromium, nickel iron

Biomonitoring of leachates from different hazardous solid waste using Allium test was compared. The adverse effects of leachates from a metal and dye industry were investigated using the *Allium cepa* chromosome aberrations assay. The results revealed that both metal waste leachate (MWL) and dye waste leachate (DWL) contained high concentrations of chromium, nickel and iron. The investigations inferred that abnormalities caused by MWL were higher than DWL both in soil and aqueous media. These toxic responses may have relied on raised heavy metal concentrations of metal-based than dye industrial wastes (Chandra, S. et. al., 2005).

Chromium

Liewrungruang, T. (2003) studies application of the modified allium test using shallot (*Allium ascalonicum* L.) for screening toxicity and genotoxicity of chrome plating wastewater. Wastewater at the concentrations of 2.5-40%v/v inhibited root growth. The result showed that the modified Allium test detected total chromium concentration in the range of 5-6 mg/L at EC_{50} value. The gonotoxicity evaluated by percentage of mitotic index and chromosome aberration in root of *Allium ascalinium* at EC_{50} (Cr 5.292 mg/L) and EC_{70} (Cr 8.706) caused chromosome abnormalities compare with control group. Fragments, laggard and bridge were the most types of chromosome aberration found.

Mercury

In the year 2007, application of the modified allium test using shallot (*Allium ascalonicum* L.) for determination of mercury contamination in sediment at gold mining site was studied. The effect of mercury on shallot root growth was significantly different at the toxicity level of EC_{30} (163 μg/l), EC_{50} (440 μg/l) and EC_{70} (870 μg/l), respectively. The gonotoxicity evaluated by percentage of mitotic index was decreased as Hg increased and chromosome aberration increased as Hg concentration increased. At the Hg concentration of 169 μg/l in mining sediment as well as spiked supernatant of 620 μg/l, the mitotic index was not significantly different whereas the chromosome aberration showed sensitivity to very low concentration of Hg (Teetong, S., 2007).

Wastewater

Nielsen, M.H. & Rank, J. (1994) studied screening of toxicity and genotoxicity in wastewater by the use of the *Allium* test. Two municipal wastewater treatment plants and twelve different industries representing five lines of business (chemical, metallic, petrochemical, pulp- and paper, and textile dye industries) were investigated. The most toxic effect was found in wastewater from one of the pulp- and paper plants. *Allium* root tip cells were analyzed for chromosome aberrations after 24 h of exposure. The textile dye industry was the only line of business which did not show any genotoxic effect. Three of the plants (municipal wastewater, metallic, and pulp-and paper) showed genotoxicity in spite of being nontoxic in the growth inhibition experiment.

Grover, I.S. & Kaur, S. (1999) studied the genotoxicity of wastewater samples from sewage, and industrial effluent from the Amritsar, India, area were investigated using the Allium micronucleus and anaphase aberration assays. Raw sewage samples and acetone extracts of the dehydrated sewage were use for treatment of the Allium roots. Industrial effluents were collected and stored in the form of sludge (semi-dried matter). The acetone extracts of the sludge samples were also used for treatment of the Allium roots. From the Allium root micronuclei tests on the sewage extracts, no significant increased in the number of micronuclei was found in comparison with negative controls. All the other extracts from industrial effluent showed positive responses both in the micronucleus and anaphase aberration assays.

CHAPPER III
MATERIALS AND METHODS

3.1 Introduction

This study was to determine the toxicity root growth inhibition and clastogenicity of effluents from battery industries on shallot root (*Allium ascalonicun*). The toxicity was calculated in terms of toxicity levels of root growth inhibition. The genotoxicity was determined by mitotic index and chromosome aberration.

3.2 Experimental Set-Up

3.2.1 Tap water preparation using as growth medium

The tap water was used as a growth medium for control and dilution of effluents from battery industries. The criterion of tap water used in this experiment were pH around 7.

3.2.2 Effluent from battery industries

Effluents were obtained from battery industries at the wastewater treatment plants located in Samut Prakan Province. Each effluent was mixed together in a storage tank with a 200 L capacity. Primary compositions of the effluents were characterized as lead, cadmium, manganese, nickel, zinc, copper, and mercury. The concentrations of these heavy metals in effluents were analyzed by using the procedure prescribed in the standard method. The effluent was diluted with tap water. The final pH of the test solutions were ranged from 6.4 to 7.3, adjusted with 1M NaOH.

3.2.3 Shallot bulb preparation

Shallot bulbs, a Bangchang variety obtained from Lumphun Province, were stored in a cool, good air flow and dry area after they were harvested around three months. The root which had no growth in this period was called dormancy stage. Shallot bulbs, 2.5 - 5.0 grams by weight as a test organism, had not been treated with any kind of growth regulation and fungicide. Dried and mould-attacked bulbs should be discarded. Also, the shallots should not have started shooting of green leaves because restriction of leaf growth may be used as a parameter for the effects of test chemicals. The loose outer scales were carefully removed, and the dry bottom plate was scraped away without destroying the root primodia to prior the experiment. There are some poorly grown shallots. Thus, the series of shallots should be used in the experiment.

3.3 Equipment and chemical reagents

3.3.1 Experimental Equipment

1. Shallot bulbs
2. Test tubes
3. Circular acrylic plastics
4. Plastic container 700 ml.

3.2.4 Laboratory equipment and analysis

1. pH meters
2. Hot plate
3. Volumetric flasks
4. Beakers
5. Pipette
6. Slides and cover slips
7. Stainless steel stick
8. Ruler
9. Forceps

10. Dropper bottles
11. Nail varnish
12. Electronic balance
13. Filtration paper
14. Atomic Absorption Spectrophotometer
15. Light microscope
16. Razor-blades
17. Petri dishes
18. Graduated Cylinders
19. Thermometer

3.2.5 Chemical reagents

1. 70% ethyl alcohol
2. Hydrochloric acid (1M HCl)
3. 2% acetoorcein
4. Sodium hydroxide (1 M HCl)
5. Concentrated nitric acid (conc. HNO_3)
6. Mcclintock Solution for fixative (absolute ethyl alcohol 3 parts : glacial acetic acid 1 part)
7. Lead nitrate

3.4 Experimental Strategies

3.4.1 Phase I

Determine toxicity levels (IC_{30}, IC_{50} and IC_{70}) of effluents from battery industries on root elongation of shallot (*Allium ascalonicum* L.).

3.4.1.1 Experimental procedure

In each concentration, eighteen samples of shallot bulbs were prepared and placed on the special designed test tubes for this experiment. Effluents

from battery industries were prepared with three definition concentrations (50, 75, and 100 %v/v). The test solutions and control (tap water as a growth medium) were changed every day for 4 days (96 hours). The lengths of root bundles were measured at time a interval of 24 hours. The mean root bundle length of 18 samples in each concentration was expressed as a percentage of control (relative root growth). The toxicity levels on root growth inhibition; IC_{30}, IC_{50}, and IC_{70} were calculated from a plot of root bundle length as a percent of control against the concentrations (Figure 3.1).

3.4.1.2 Statistical analysis

The toxicity levels (IC_{30}, IC_{50}, and IC_{70}) were estimated from the root growth curve using regression models; linear graphs. From the equation, the inhibition concentrations at the toxicity levels of 30, 50, and 70% were calculated.

3.4.2 Phase II

Determine the effects of effluents from battery industries on mitotic index and chromosome aberration (genotoxicity) of root tip cells of shallot (*Allium ascalonicum* L.).

3.4.2.1 Experimental procedure

At concentration of IC_{30}, IC_{50}, and IC_{70} values on root elongation inhibition, the genotoxicity tests were carried out with tap water as negative control and MMS as positive control at 10 mg/L. Six-shallot bulbs were exposed to each concentration. During the first 48 hours, the shallot bulbs were grown in tap water. Then, they were exposed to test solutions for 48 hours, and the test solutions were changed every day. After 48 hours the shallot roots were fixed and macerated with fixative reagents for one day at room temperature (Figure 3.1).

3.4.2.2 Data analysis

One slide was prepared for every shallot bulb. Shallot root tips were cut and placed on a slide and add 2 ml Farmer's fluid. The root tips were rinsed with cold water, and the excess of liquid was removed by tissue paper. Then, they

were transfered to pre-heat with 1M hydrochloric acid at 60^0C for 5 minutes and washed with cold water again. The root tip was taken out of water and placed on a micro slide. Each root tip was cut about 1-2 mm from the growing tip. One drop of 2% acetoorcein was added and mixed with the root tips by stirring and knocking with a stainless steel stick for 3 to 5 minutes. A cover slip was placed on the root tip cells. Then, two layers of tissue paper were placed on the cover glass and the glass was pressed slightly with the thumb in order to squash the cells. The cover slip was fixed carefully with nail varnish. The slides can be kept fresh for a month in a freezer.

From each slide, the mitotic index was determined by counting all of stages of mitotic cell out of 400 cells per slide, six slides per concentration following this equation.

$$\text{Mitotic index} = \frac{\text{Number of mitotic cells x 100}}{\text{Total number of cells}}$$

The chromosome aberration was determined by examination of the first 100 normal anaphase and early telophase cells per slide, six slides per concentration. Bridges, fragments, bridges and fragments, stickiness, vagrant (laggard) chromosome and c-mitosis were scored. Then, the total chromosome aberration in each concentration was calculated as percentage following the equation.

$$\% \text{ Chromosome aberration} = \frac{\text{chromosome aberration cells x 100}}{\text{100 cells in anaphase and early telophase}}$$

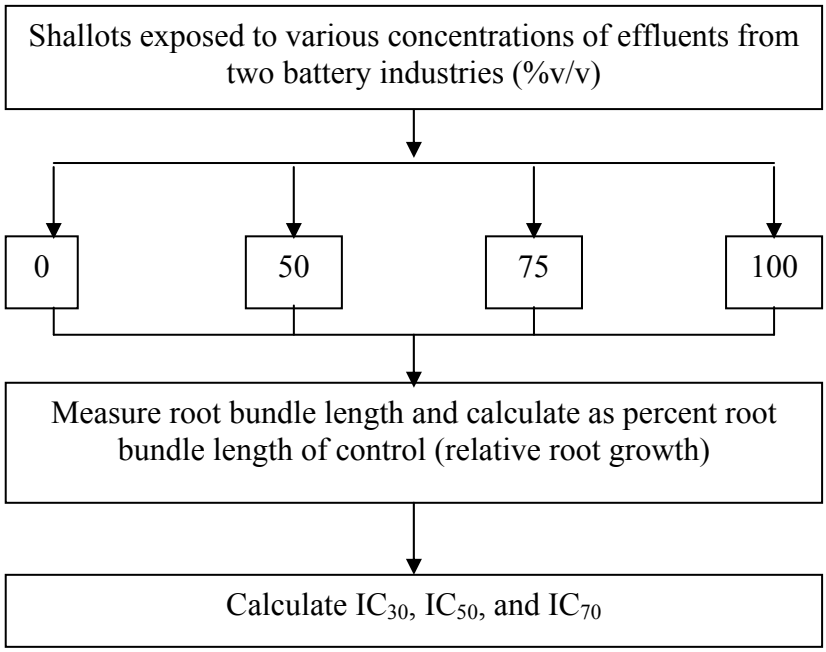
3.4.2.3 Statistical analysis

One way ANOVA was used to compare mitotic index in each test. To detect the significance of difference between($P\text{-value}<0.05$) of variables, a multiple comparison (Duncan) test was performed. Chi-square was used for comparing the chromosome aberration of shallot root cells in various concentrations of effluents from

battery industries. It was also used to determine whether two variables with multiple categories are homogenous. In case, the study had three or more treatments and the test of homogenous yielded a P-value less than or equal to 0.05, that the treatment was not identical. It could not be conclude that there was a treatment difference without further testing. Then, Chi-square was calculated three or more treatments.

The significant level in this study was determined at $\alpha = 0.05$

Phase I To determine toxicity levels (IC_{30}, IC_{50} and IC_{70}) of effluents from battery industries on root elongation of shallot (*Allium ascalonicum* L.)



Phase II To determine the effects of effluents from battery industries on mitotic index and chromosome aberration (genotoxicity) of root tip cells of shallot (*Allium ascalonicum* L.).

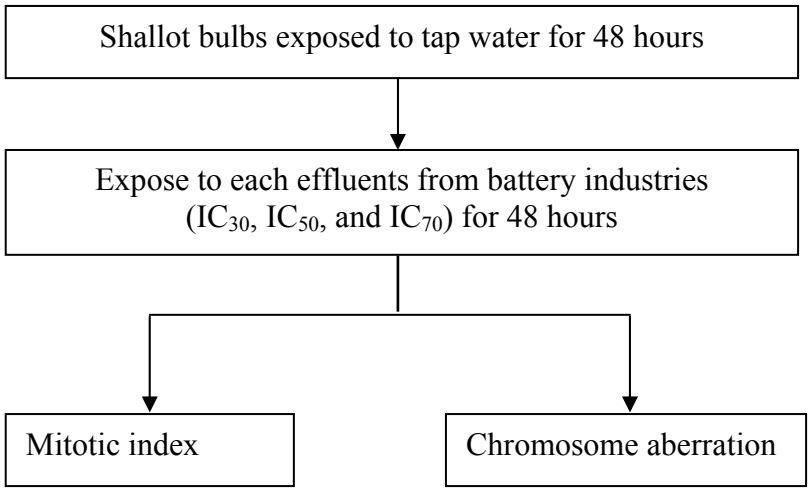


Figure 3.1 Experimental strategies diagram

CHAPTER IV
RESULTS AND DISSCUSSION

4.1 The Characteristics of Effluents from Two Battery Industries

The heavy metals of effluents from two battery industries were characterized. All heavy metals in effluents from battery industries were found not exceed the standard issued by Ministry of Industry, except lead in factory 1 and mercury in factory 2 which exceeded the maximum permissible concentration for industries. Two factories in this study were lead-acid battery industries. The production capacity was more than 300,000 pieces of car and motorcycle battery per year (Table 4.1).

Table 4.1 Heavy metals in effluents from two battery industries used in this experiment

Heavy metals	Factory 1 (mg/L)	Factory 2 (mg/L)	Effluent standards (mg/L)
Lead	0.4060	0.0500	0.2
Cadmium	0.0010	0.0010	0.03
Manganese	0.0110	0.0140	5
Mercury	0.0037	0.0065	0.005
Zinc	0.1460	0.2040	5
Copper	0.0080	0.0070	2
Chromium	0.0250	0.0180	Cr^{+6}0.25Cr^{+3}0.75
Arsenic	0.0018	0.0004	0.25

4.2 Allium test for Effluent from Factory 1

4.2.1 Toxicity level (root growth inhibition test)

The toxicity of effluents from battery industries in the Allium test was studied by using root growth inhibition of *Allium ascalonicum* as the end point.

The effluent from battery industry (factory 1) at the concentration of 0, 50, 75, and 100 %v/v was tested to find the concentration range at the toxicity levels of 30, 50, and 70 with en exposure period of 96 hours (Figure 4.1 and 4.2).

Figure 4.1 Root elongation of shallot at various concentrations of effluent from battery industry (Factory 1) 96 hours

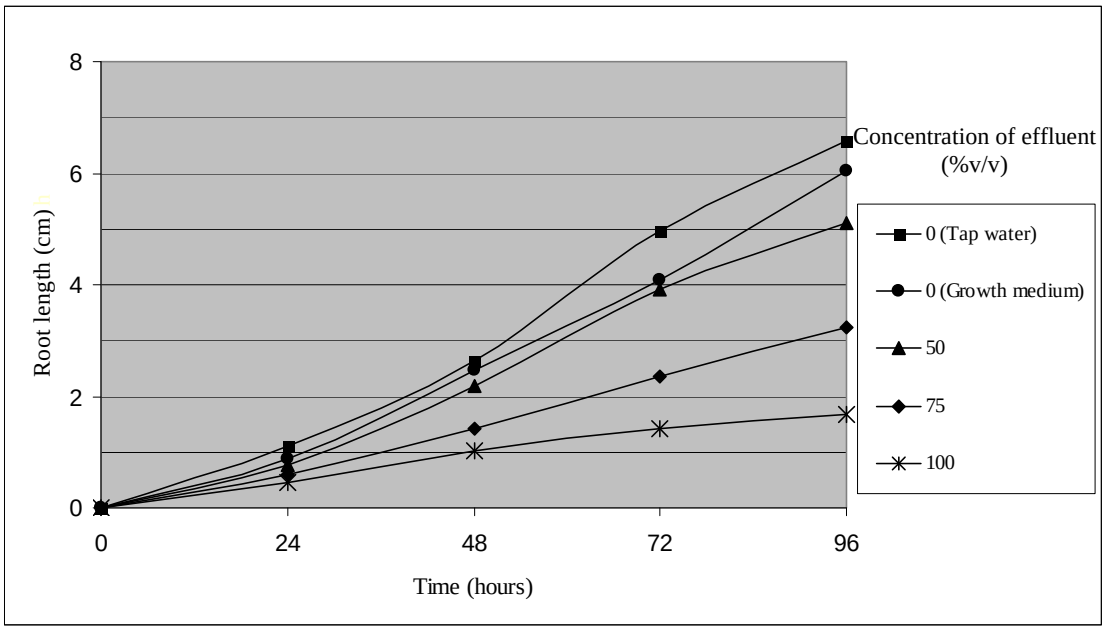


Figure 4.2 The effect of effluent from battery industry (factory 1) at various concentrations within 96 hours

At 24 hours, the root elongation slightly increased at all treatments and rapidly increased even at lower concentration of effluent at 48 hours. The root growth at 24-hour interval decreased with increasing duration of exposure and increasing effluent after 48 hours. The root growth of shallot slightly increased at the highest concentration of effluent (100%v/v) compared with the other concentrations (50 and 75%v/v) that yielded rapid growth (Liu, D. et al., 1994).

The root growth of shallot at various effluent concentrations of 50, 75, and 100 %v/v was different from the control group (a growth medium and tap water) for exposure period of 24, 48, 72, and 96 hours as shown in Table 4.2, 4.3, 4.4, and 4.5, respectively.

Table 4.2 The root bundle lengths at various concentrations of effluent (Factory 1) at 24 hours

Effluent concentration (%v/v)	Experiment	Root length x ± SEM*	Relative growth (%)
0 (Growth medium)	1	0.97 ± 0.04	100.00
	2	0.92 ± 0.05	100.00
	3	0.74 ± 0.03	100.00
0 (Tap water)	1	1.06 ± 0.06	100.00
	2	1.10 ± 0.04	100.00
	3	1.15 ± 0.05	100.00
50	1	0.81 ± 0.04	76.42
	2	0.74 ± 0.04	67.27
	3	0.71 ± 0.03	61.74
75	1	0.57 ± 0.03	53.77
	2	0.59 ± 0.02	53.64
	3	0.66 ± 0.02	57.39
100	1	0.47 ± 0.02	44.34
	2	0.43 ± 0.02	39.09
	3	0.51 ± 0.02	44.35

SEM* = Standard error of the mean

Table 4.3 The root bundle length at various concentrations of effluent (Factory 1) at 48 hours

Effluent concentration (%v/v)	Experiment	Root length x ±SEM*	Relative growth (%)
0 (Growth medium)	1	2.41 ± 0.07	100.00
	2	2.49 ± 0.13	100.00
	3	2.48 ± 0.08	100.00
0 (Tap water)	1	2.56 ± 0.12	100.00
	2	2.87 ± 0.13	100.00
	3	2.52 ± 0.09	100.00
50	1	2.33 ± 0.07	91.02
	2	2.06 ± 0.08	71.78
	3	2.12 ± 0.08	84.13
75	1	1.44 ± 0.06	56.25
	2	1.37 ± 0.08	47.74
	3	1.43 ± 0.08	56.75
100	1	0.96 ± 0.03	37.50
	2	1.01 ± 0.05	35.19
	3	1.08 ± 0.05	42.86

SEM* = Standard error of the mean

Table 4.4 The root bundle length at various concentrations of effluent (Factory 1) at 72 hours

Effluent concentration (%v/v)	Experiment	Root length x ±SEM*	Relative growth (%)
0 (Growth medium)	1	3.74 ± 0.10	100.00
	2	4.12 ± 0.13	100.00
	3	4.43 ± 0.16	100.00
0 (Tap water)	1	4.75 ± 0.17	100.00
	2	5.01 ± 0.12	100.00
	3	5.09 ± 0.11	100.00
50	1	4.07 ± 0.16	85.68
	2	3.85 ± 0.12	76.85
	3	3.78 ± 0.11	74.26
75	1	2.33 ± 0.08	49.05
	2	2.29 ± 0.08	45.71
	3	2.44 ± 0.11	47.94
100	1	1.38 ± 0.04	29.05
	2	1.37 ± 0.04	27.35
	3	1.46 ± 0.06	28.68

SEM* = Standard error of the mean

Table 4.5 The root bundle length at various concentrations of effluent (Factory 1) at 96 hours

Effluent concentration (%)	Experiment	Root length x ±SEM*	Relative growth (%)
0 (Growth medium)	1	5.38 ± 0.16	100.00
	2	6.08 ± 0.15	100.00
	3	6.69 ± 0.12	100.00
0 (Tap water)	1	6.43 ± 0.23	100.00
	2	6.88 ± 0.12	100.00
	3	6.45 ± 0.12	100.00
50	1	5.21 ± 0.12	81.03
	2	4.96 ± 0.14	72.09
	3	5.13 ± 0.15	79.53
75	1	3.30 ± 0.11	51.32
	2	3.21 ± 0.10	46.66
	3	3.18 ± 0.07	49.30
100	1	1.71 ± 0.04	26.59
	2	1.62 ± 0.04	23.55
	3	1.72 ± 0.05	26.67

SEM* = Standard error of the mean

At 96 hours period of the time, relative root lengths at various effluent concentrations percentages of inhibition calculated were using shown in Table 4.6. In addition, relative growths and toxicity levels were showed in Figure 4.3 and 4.4, respectively. The root lengths of shallot at the concentrations of 50, 75, and 100 %v/v were significantly different from those of the control group (tap water)(p-value < 0.05) with relative growth values at about 64.7 %, 45.89 %, and 27.08 %, respectively (Figure 4.3). Although polynomial equation yielded maximum R^2, relative growth value was estimated from linear equation. This was because the values from the experiment and R^2 were not much different and more than 0.9. The results supported the hypothesis that root bundle lengths in the treated group were significantly shorter than the control group (tap water).

Table 4.6 Root bundle length inhibition at various concentrations of effluent (Factory 1) at 96 hours

Effluent concentration (%v/v)	Root length (cm)	Relative root length (%)	Inhibition (%)
0	6.43	100.00	0
0	6.88	100.00	0
0	6.45	100.00	0
50	5.21	81.00	19
50	4.96	71.99	28
50	5.13	79.50	20
75	3.30	51.30	49
75	3.21	46.57	53
75	3.18	49.35	51
100	1.71	26.51	73
100	1.62	23.57	76
100	1.72	26.70	73

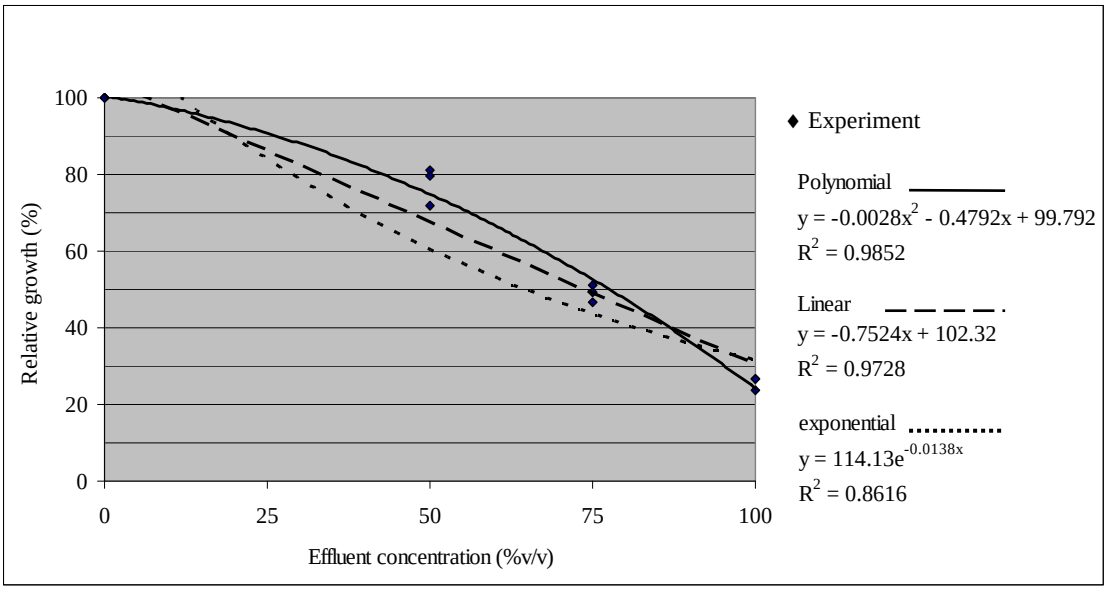


Figure 4.3 Effects of effluent concentrations (Factory 1) on relative root growths of shallot

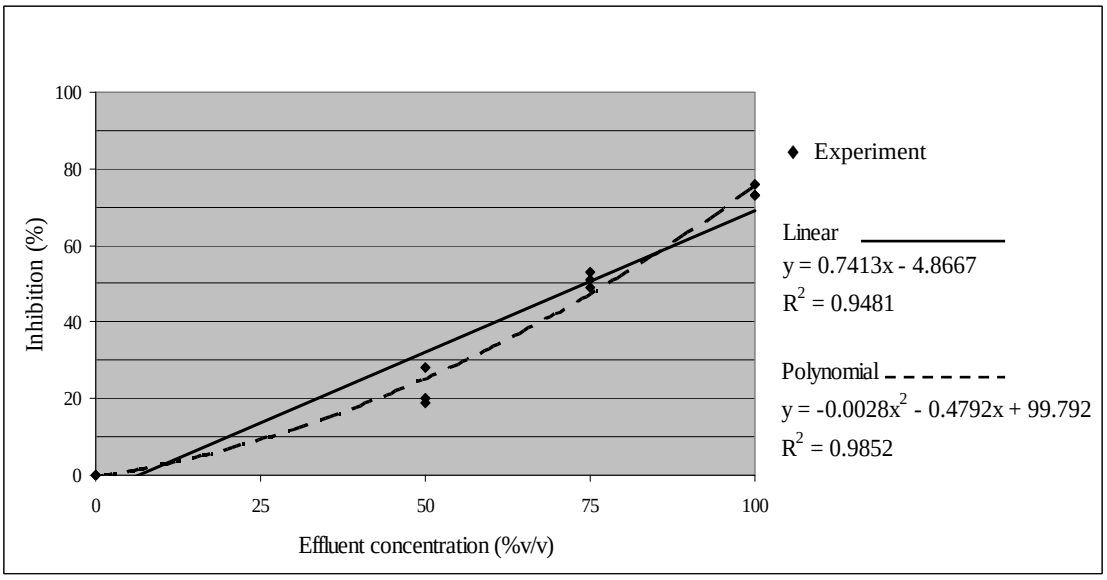


Figure 4.4 Linear equation for calculation of toxicity levels of effluents from battery industry (Factory 1).

The linear and polynomial model in Figure 4.4 showed the determination of the inhibit concentrations: IC_{30}, IC_{50}, and IC_{70}, which retarded 30%, 50% and 70% of root growth. R^2 value of both model were higher than 0.9. The IC_{30}, IC_{50} and IC_{70} values were estimated from linear equation; $y = 0.7413x - 4.8667$ ($R^2 = 0.9481$). The effluent concentrations (%v/v) at toxicity levels of 30%, 50%, and 70% were showed with lead concentration in Table 4.7.

Table 4.7 Effluent and lead concentrations at three toxicity levels (IC_{30}, IC_{50} and IC_{70})

	IC_{30}	IC_{50}	IC_{70}
Effluent concentration (%)	47	74	100
Lead (mg/l)	0.1908	0.3004	0.4060

Industrial effluent standards of lead not more than 0.2 mg/l

4.2.2 Genotoxicity test

The genotoxicity test used in this experiment focused on the damages of chromosome, clastogenicity and the mitotic index. In addition the mitotic index and chromosome aberration were observed in anaphase and early telophase after shallot root were exposed to the effluents for 48 hours. The toxicity levels at the concentrations of IC_{30} (47%v/v), IC_{50} (74%v/v), and IC_{70} (100%v/v) were investigated, respectively.

Mitotic index

The effects of the effluent from factory 1 on mitotic index were shown in Table 4.8. Mitotic index decreased with increasing concentrations of effluent. Mitotic index of shallot root cells in the treated group were ranged from 10.00% to 13.75% and significantly different (p-value<0.05) from the control group (tap water). The results supported the hypothesis that mitotic index of the treated group were less than those of the control group (tap water). The highest value of mitotic activity was

observed in the control group whereas the lowest percentage was noticed after treatment with 100% (IC_{70}) effluent concentration.

Table 4.8 Mitotic index of shallot exposed to effluent from battery industry (factory 1) at various toxicity levels within at 48 hours

Effluent concentrations (%v/v)	Mitotic cells per 100 cells						Total mitotic cells Per 600 cells	Mitotic index (%) x ±SEM*
	1	2	3	4	5	6		
0 (Growth medium)	64	64	56	67	64	47	362	16.75 ± 0.51
0 (Tap water)	62	57	61	43	51	56	330	15.08 ± 0.77
47 (IC_{30})	51	42	45	47	44	46	275	13.75 ± 0.72
74 (IC_{50})	48	39	38	39	36	40	240	11.46± 0.31
100 (IC_{70})	48	42	32	33	24	30	209	10.00 ± 0.41

SEM* = Standard error of the mean

The effects of mitotic index were shown in Figure 4.5. It was found that when increasing effluent concentrations, the mitotic index decreased. Mitotic index of shallot root tip cells in a growth medium and tap water (as negative control groups) were higher, and decreased significantly when exposed to increasing concentration effluent.

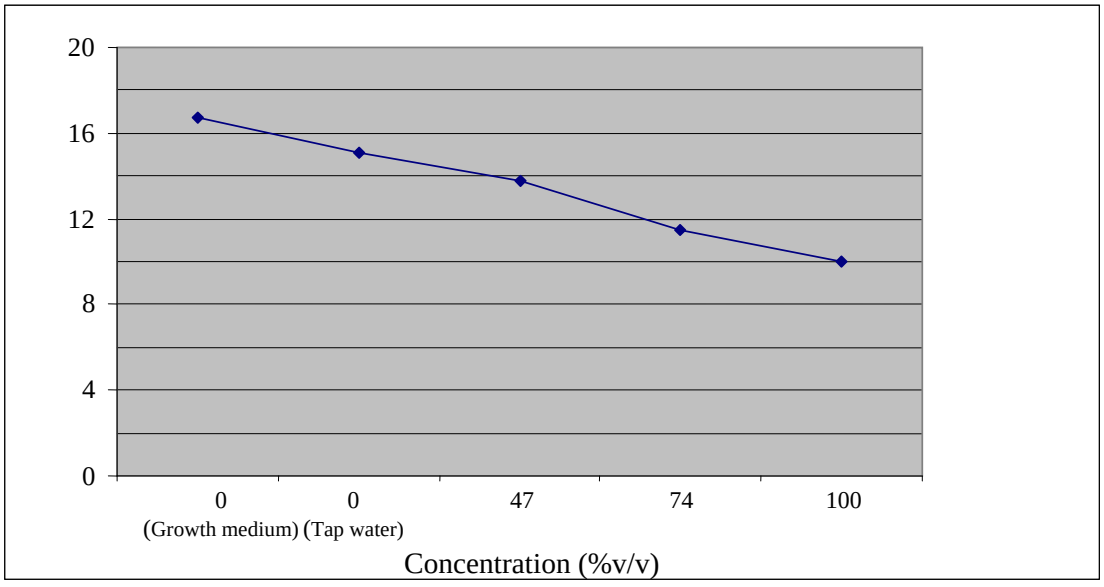


Figure 4.5 Effects of effluent from battery industry (factory 1) at three toxicity levels on mitotic index

Chromosome aberration

Chromosome aberration in anaphase and early telophase of mitosis (somatic cell division) was induced by the effluents from battery industries. The various types of aberration in this study were fragment, laggard and bridge. Laggard was found and observed more frequently than the others (Table 4.9). Moreover, combinations of fragment and laggard, fragment and bridge, laggard and bridge were also found. Chromosome aberration percentages and various types of chromosome aberration were shown in Figure 4.6 and Figure 4.7, respectively.

The chromosome aberration significantly increased as effluent from battery industry increased (p-value<0.05). The result supported the hypothesis that the toxicity level at IC_{50} caused chromosome aberration in root tip cells of shallot. The percentages of chromosome aberration for negative control as tap water and a growth medium were 19.17% and 17.50%, respectively. The percentage of chromosome aberration for negative control (tap water) was lower than those obtained from the study in the year 2007 (Teetong, 2007) with the level of 24.67%. It might be caused by the variation of shallots in different crops and the quality of tap water used that had influences on chromosome aberration of shallot. For negative control, the percentage of chromosome aberration control of a growth medium was lower than that of tap water because there was very little heavy metal contaminated in a growth medium. Furthermore, the amount of chromosome aberration in growth medium was less than that of tap water which was contaminated by heavy metal.

Table 4.9 Chromosome aberrations in root tip cells of shallot exposed to various different effluent concentrations

Effluent	Chromosome aberration (cells)								total	%aberration cell
conc. (%v/v)	Laggard	Bridge	Fragment	c-mitosis	LB[1]	LBF[2]	BF[3]	LF[4]	600	X ± ± SEM*
0 (Growth medium)	42	20	15	19	6	0	0	3	105	17.50 ± 0.26
0 (Tap water)	29	20	19	23	3	5	10	6	115	19.17 ± 0.34
47 (IC_{30})	56	19	16	51	10	6	1	3	162	27.00 ± 0.31
74 (IC_{50})	58	35	19	73	23	11	7	3	229	38.17 ± 0.35
100 (IC_{70})	56	18	31	95	13	9	5	6	233	38.83 ± 0.36

SEM* = Standard error of the mean

1 = Laggard + Bridge, 2 = Laggard + Bridge + Fragment, 3 = Bridge + Fragment, 4 = Fragment + Laggard Bridge

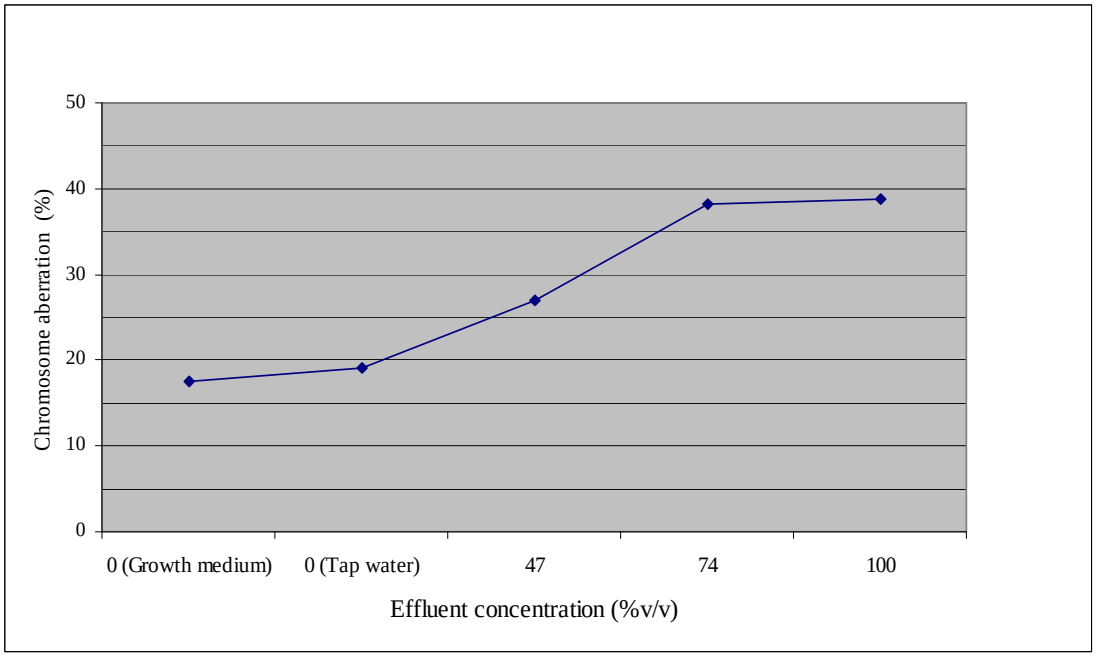
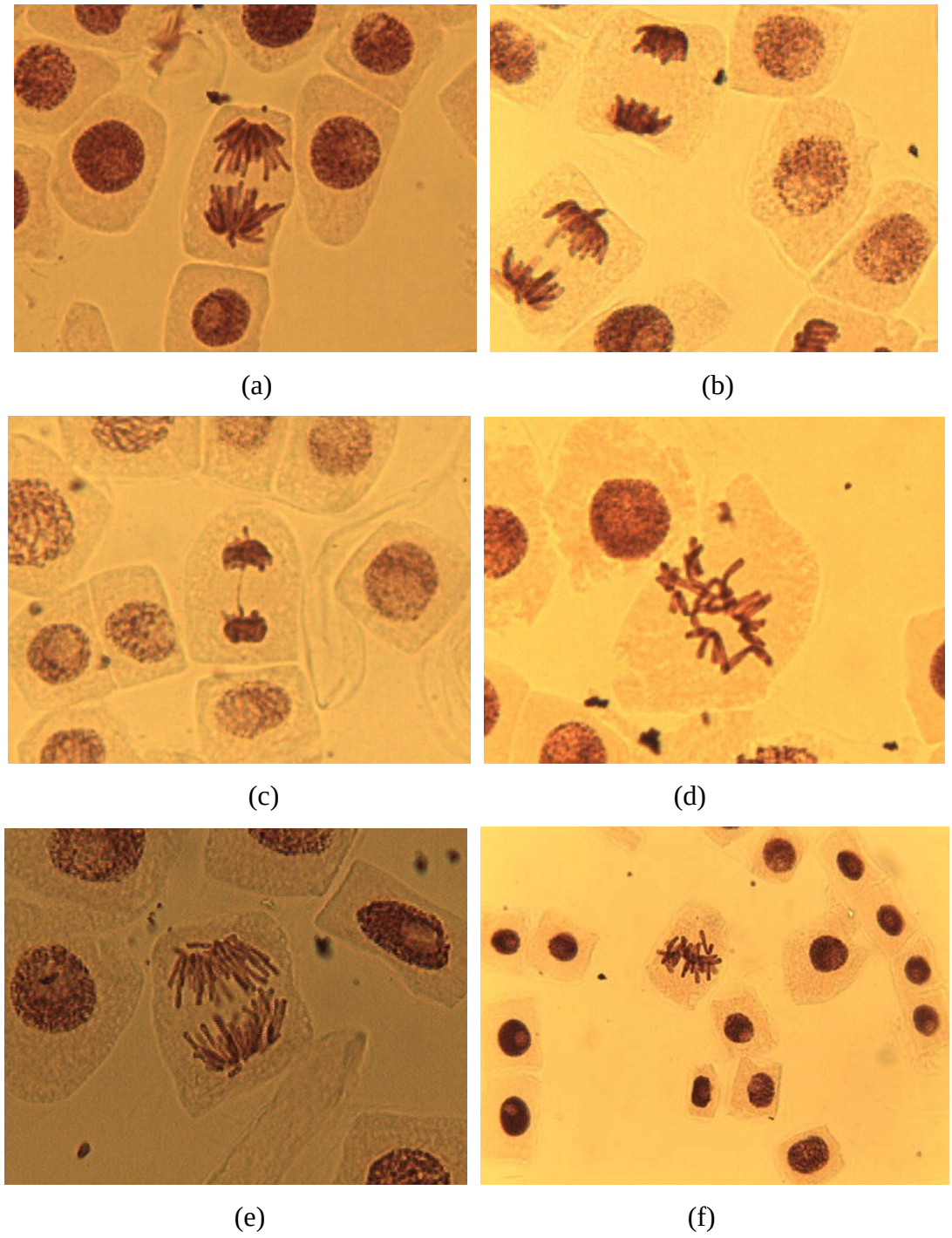


Figure 4.6 Effect of effluent from battery industries (factory 1) on percentage of chromosome aberration

The results corresponded to the hypotheses that mitotic index of shallot root cells of the treated group was significantly lower than that of the control group (both in a growth medium and tap water) and percentage of chromosome aberration was significantly increased as increasing effluent concentrations at the 95% confidence interval (Kumar, et.al., 2000). The effects of lead on genetic systems were different from other toxic effects; tests for enzyme inhibition and chromosome aberrations amoung various toxics, it is likely that lead is a selective agent that continues to act on and influence the genetic structure of exposed plant (Johnson, F.M., 1998). However, this study was opposite to the study of Lerda, that the frequency of chromosomal aberrations in cells from onions (*Allium cepa*) exposed to lead nitrate at the concentration of 0.1 and 1.0 ppm (mg/l) did not differ from that of the non-exposed controls (Lerda, 1992).



(a) (b)

(c) (d)

(e) (f)

Figure 4.7 Chromosome aberrations observed in root tip cells of shallot (a) normal anaphase (b) normal anaphase and normal early telophase (c) bridge (d) c-mitosis (e) fragment and (f) laggard

4.3 Allium Test for Effluent from Factory 2

4.3.1 Toxicity levels (root growth inhibition test)

Effluent from battery industry in factory 2 had no heavy metal excepted mercury that exceeded the standard of industry. The Allium test was studied with the root growth inhibition of *Allium ascalonicum* L. at four concentrations of 0, 50, 75, and 100 %v/v to find the effective concentration range (Figure 4.8 to 4.10).

Root growth of shallot exposed to various concentrations of effluent from battery industry in factory 2 (as treated groups) was significantly different from the control group (tap water) (p-value<0.05). Root growth inhibition of the treated group could be obtained from relative growth (Figure 4.10). Both linear and polynomial equation had R^2 valued more than 0.9. Linear equation with R^2 = 0.9481 was calculated by y = 0.1171x-0.7143. Percentage of inhibition concentration 11% was estimated for the effluent at the concentration of 100 % v/v.

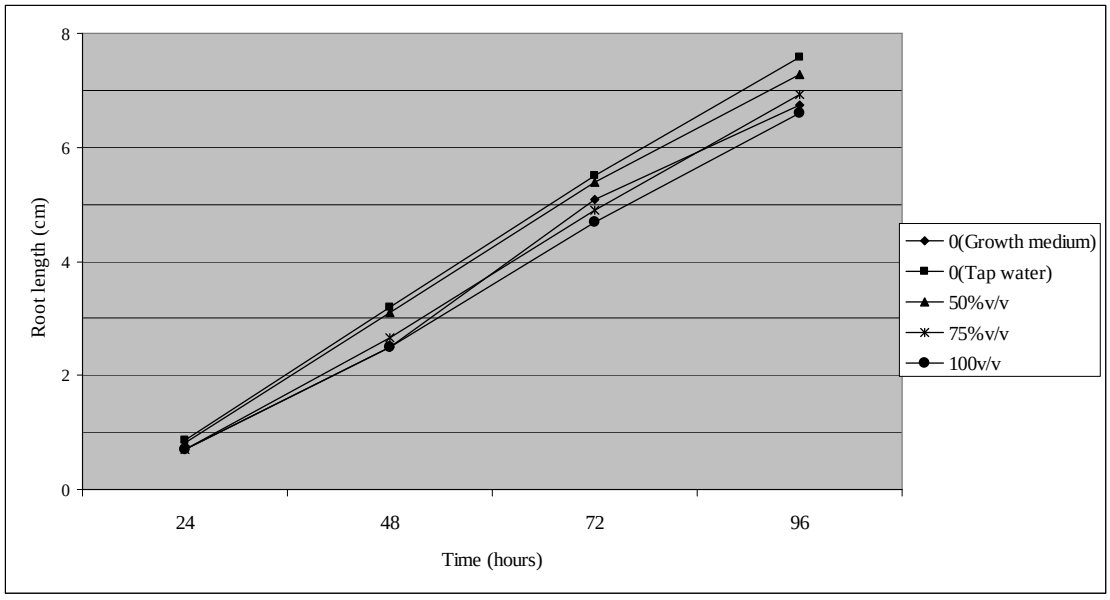


Figure 4.8 Root elongation of shallots exposed to the effluent from battery industries within 96 hours

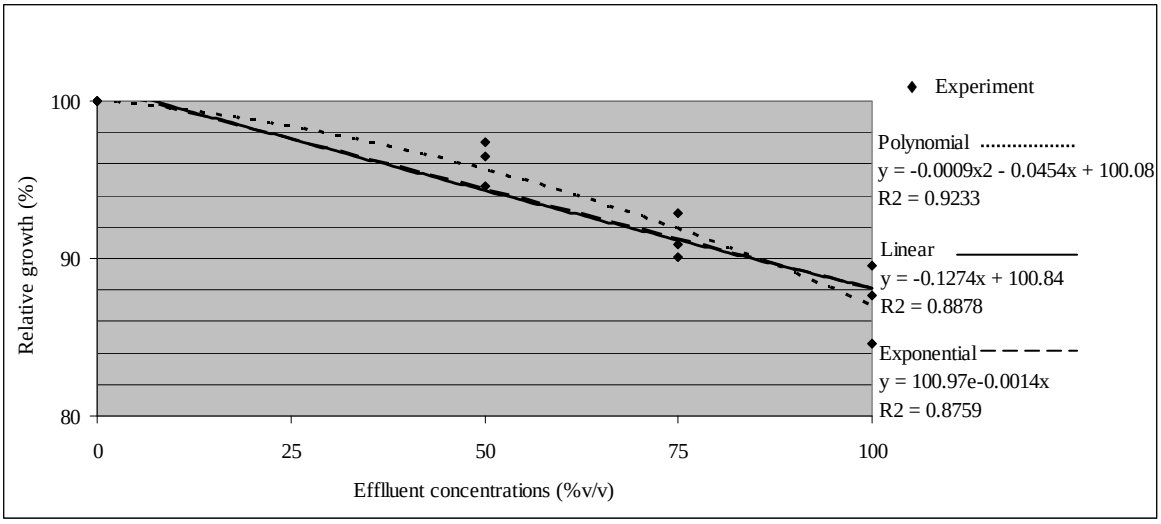


Figure 4.9 Relative root growth of shallot exposed to various effluent concentrations

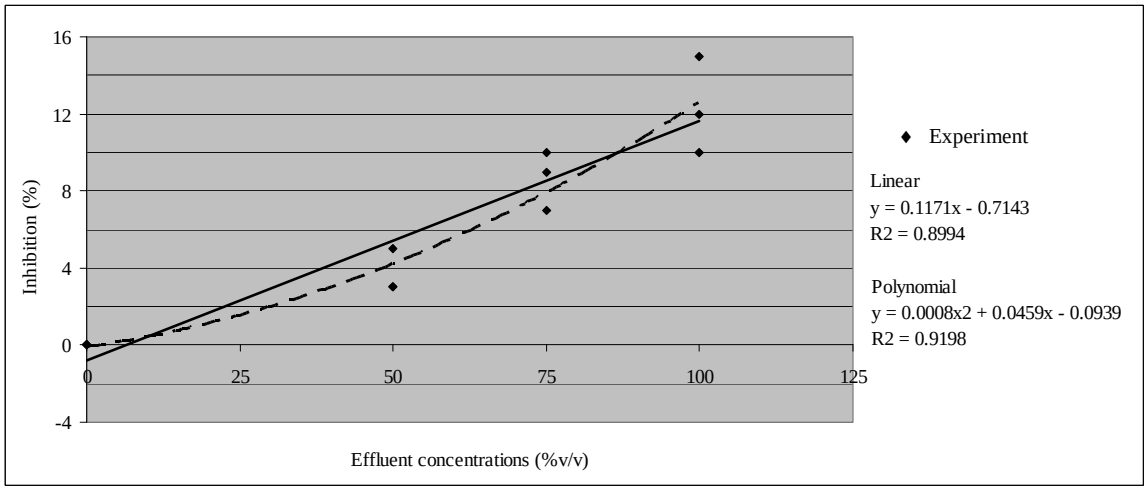


Figure 4.10 Inhibition percentage of root growth at various effluents from factory 2 on root growth of shallot

The heavy metal found in the effluent from battery factory 2 was only excess mercury. There was no lead contaminated in this effluent. In this study, lead was added into the effluents from factory 2 at the concentration of 0.2780 mg/l for Allium test.

Relative growth of shallot in the treated group (spiked 0.2780 mg/l of lead) was significantly different (p-value<0.05) from the control group (tap water)(Figure 4.11 and Figure 4.12)

Figure 4.11 Root elongation of shallot in treated group (0.2780 mg/l as Pb) and control groups (tap water) for 96 hours of exposure

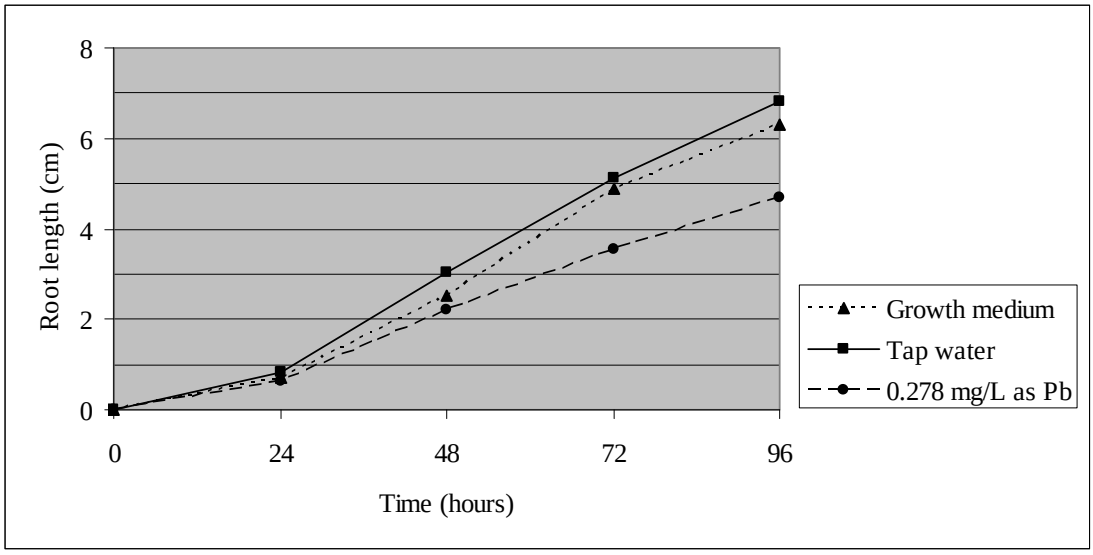


Figure 4.12 The effects of lead (spiked 0.2780 mg/l) in effluent of battery industry factory on root length of shallot (96 hours)

Root growth of the shallot that spiked lead at concentrations of 0.2780 mg/l was different from that of the control group for all exposed period of time as shown in the Table 4.10, 4.11, 4.12 and 4.13, respectively.

Table 4.10 The root bundle length of effluent with lead concentration 0.2780 mg/l (factory 2) at 24 hours

Lead concentration (mg/l)	Experiment	Root length x ±SEM*	Relative growth (%)
0	1	0.76 ± 0 .04	100.00
(Growth medium)	2	0.70 ± 0.05	100.00
	3	0.70 ± 0.02	100.00
0	1	0.77 ± 0.04	100.00
(Tap water)	2	0.88 ± 0.06	100.00
	3	0.84 ± 0.05	100.00
0.2780	1	0.63± 0.03	81.82
	2	0.63 ± 0.03	71.59
	3	0.62 ± 0.04	73.81

SEM* = Standard error of the mean

Table 4.11 The root bundle length of effluent with lead concentration 0.2780 mg/l (factory 2) at 48 hours

Lead concentration (mg/l)	Experiment	Root length x ±SEM*	Relative growth (%)
0	1	2.37 ± 0.16	100.00
(Growth medium)	2	2.69 ± 0.12	100.00
	3	2.46 ± 0.08	100.00
0	1	2.78 ± 0.13	100.00
(Tap water)	2	3.19 ± 0.15	100.00
	3	3.30 ± 0.09	100.00
0.2780	1	2.04 ± 0.13	73.38
	2	2.27 ± 0.09	71.16
	3	2.30 ± 0.15	69.70

SEM* = Standard error of the mean

Table 4.12 The root bundle length of effluent with lead concentration 0.2780 mg/l (factory 2) at 72 hours

Lead concentration (mg/l)	Experiment	Root length x ±SEM*	Relative growth (%)
0	1	5.09 ± 0.094	100.00
(Growth medium)	2	4.93 ± 0.24	100.00
	3	4.62 ± 0.17	100.00
0	1	4.96 ± 0.19	100.00
(Tap water)	2	5.07 ± 0.15	100.00
	3	5.36 ± 0.20	100.00
0.2780	1	3.80 ± 0.09	76.61
	2	3.34 ± 0.18	65.88
	3	3.49 ± 0.22	65.11

SEM* = Standard error of the mean

Table 4.13 The root bundle length of effluent with lead concentration 0.2780 mg/l (factory 2) at 96 hours

Lead concentration (mg/l)	Experiment	Root length x ±SEM*	Relative growth (%)
0	1	6.47 ± 0.12	100.00
(Growth medium)	2	5.82 ± 0.18	100.00
	3	6.67 ± 0.12	100.00
0	1	6.86 ± 0.13	100.00
(Tap water)	2	6.87 ± 0.12	100.00
	3	6.76 ± 0.20	100.00
0.2780	1	3.49 ± 0.22	50.87
	2	4.70± 0.20	68.41
	3	4.53 ± 0.18	67.01

SEM* = Standard error of the mean

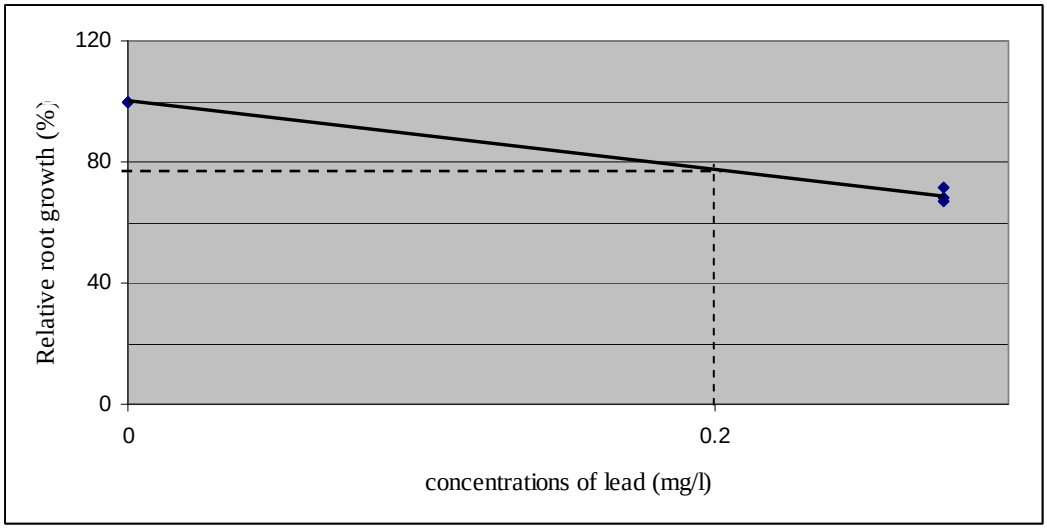


Figure 4.13 Effects of effluent with concentration of lead 0.2780 mg/l (Factory 2) on relative root growth of shallot

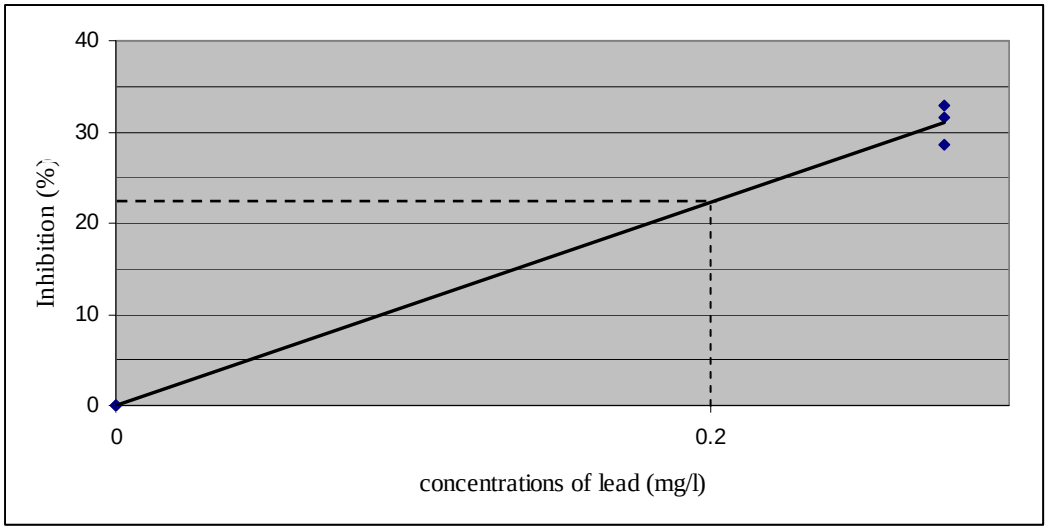


Figure 4.14 Inhibition percentage of root growth of effluent with concentration of lead 0.2780 mg/l (factory 2) on root growth of shallot

4.3.2 Genotoxicity test

The shallot roots exposed to the effluent from battery industry factory 2 spiked with lead concentration 0.2780 mg/l were investigated for genotoxicity of mitotic index and chromosome aberration with 48 hours.

Mitotic index

In Table 4.14 and Figure 4.15, the results showed that mitotic index of shallot root exposed to negative control, as a growth medium and tap water, and the treated groups were 15.92%, 12.29% and 11.04%, respectively. The mitotic index of root cells slightly decreased in the contaminated effluent from battery industry. Mitotic index was not significantly different between the treated group and the control group of tap water (p-value>0.05). For another negative control, a growth medium was found to have higher mitotic index than that of tap water due to the nutrient in such solution.

Table 4.14 Mitotic index of shallot exposed to spiked lead effluent from battery industry (factory 2) for 48 hours

Lead concentration	Mitotic cells						Total	Mitotic index (%)
(mg/l)	1	2	3	4	5	6	600 cells	x ±SEM*
0 (Growth medium)	62	77	64	74	59	46	382	15.92 ± 1.14
0 (Tap water)	42	62	57	50	44	40	295	12.29 ± 0.90
0.2780 (spiked lead)	55	44	42	36	39	49	265	11.04 ± 0.71

SEM* = Standard error of the mean

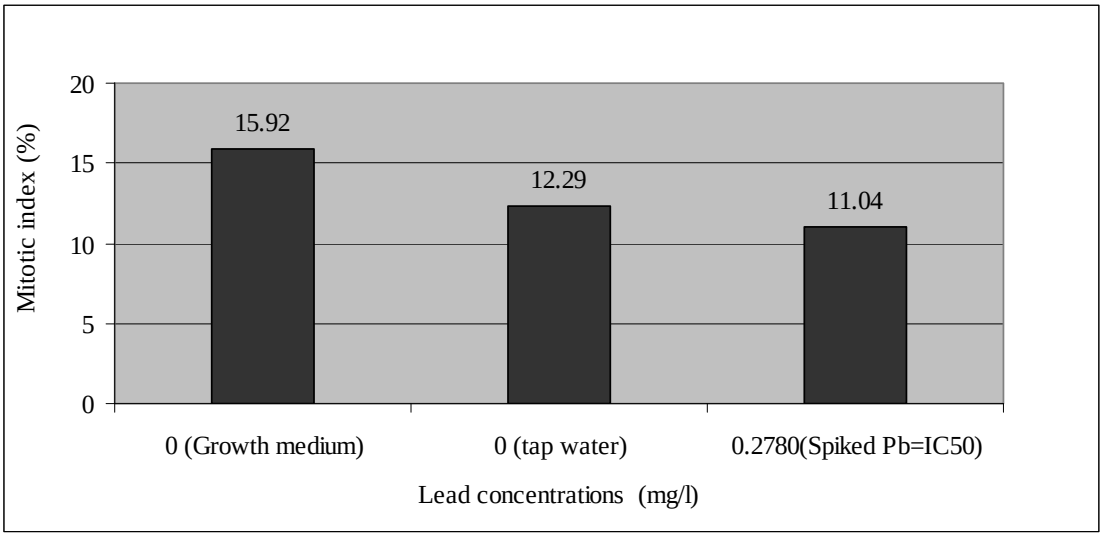


Figure 4.15 Effects of effluent from battery industries on mitotic index (48 hours)

Chromosome aberration

Chromosome aberration at anaphase and early telophase laggard was the most in all the treatment and negative control (tap water) as same as in factory 1. The various types of aberration were meeting such as fragment, laggard and bridge. Moreover, combinations of fragment and laggard, fragment and bridge, laggard were.

The statistical information of chromosome aberration was not significantly different (p-value>0.05) between treated and negative control (tap water). Table 4.15 and Figure 4.16 showed that the chromosome aberration of a growth medium and tap water as negative control, and the treated group were 16.17%, 18.50%, and 23.50%, respectively.

Table 4.15 Chromosome aberrations of shallot exposed to spiked lead effluent from battery industry (factory 2) for 48 hours

Effluent conc.	Chromosome aberration (cells)								Total	% aberration
of lead (mg/l)	Laggard	c-mitosis	Fragment	Bridge	FL[1]	FB[2]	BL[3]	FBL[4]	600	x ±SEM*
0 (Growth medium)	29	14	13	23	3	3	9	3	97	16.17± 0.36
0 (Tap water)	43	30	11	12	9	3	3	0	111	18.50± 0.38
0.2780 (spiked lead)	51	34	24	13	10	5	3	1	141	23.50± 0.11

SEM* = Standard error of the mean

1 = Fragment + Laggard, 2 = Fragment +Bridge, 3 = Bridge + Laggard, 4 = Fragment +Bridge + Laggard

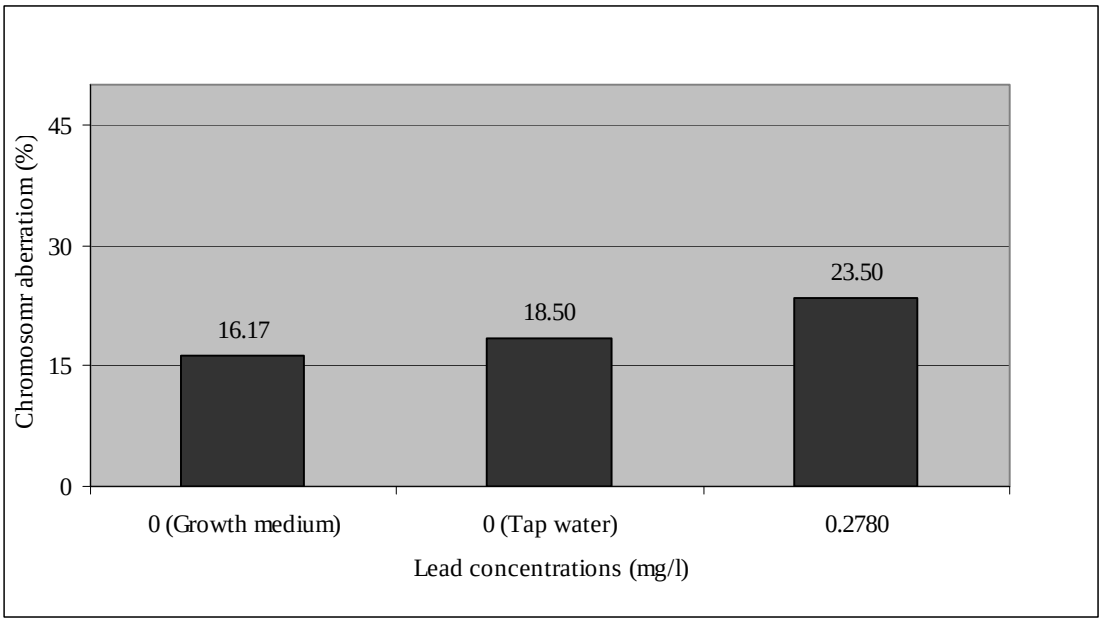


Figure 4.16 Effects of effluent contaminated with lead 0.2780 mg/l on chromosome aberration

4.4 Genotoxicity Comparison of Effluents from Two Battery Industries

Effects of effluent from two battery industries on mitosis (somatic cell division) at molecular level were investigated. The effluent from factory 2 was spiked with lead solution to obtain lead concentration of 0.2780 mg/l in order to compare the effect with that of 0.3004 mg/l from factory 1. The difference in characteristics of both effluents are not only the lead concentration but also their matrix, concentration of minor heavy metals contaminated. Furthermore, the mercury contaminated in effluent from factory 2 with concentration of 0.0065 mg/l exceeded the standard and might have adverse effects on shallot growth.

Mitotic index of shallot exposed to two effluents 0.2780 mg/l in factory 2 and 0.3004 mg/l in factory 1 were not significant different (p-value>0.05). Table 4.16 showed that mitotic index of 0.2780 mg/l and 0.3004 mg/l were 11.04% and 11.46%, respectively. Comparison of factory 1 and factory 2 showed a growth medium, tap water and effluent contaminated with lead (Figure 4.17).

Table 4.16 Mitotic index of shallot exposed to effluents from 2 battery industries

Lead conc. (mg/l)	Mitotic cells						Total Mitotic cells Per 600 cells	Mitotic index (%) x ±SEM*
	1	2	3	4	5	6		
Factory 1								
0 (Growth medium 1)	64	64	56	67	64	47	362	16.75 ± 0.51
0 (Tap water 1)	62	57	61	43	51	56	330	15.08 ± 0.77
0.3004	48	39	38	39	36	40	240	11.46 ± 0.31
Factory 2								
0 (Growth medium 2)	62	77	64	74	59	46	382	15.92 ± 1.14
0 (Tap water 2)	42	62	57	50	44	40	295	12.29 ± 0.90
0.2780 (spike lead)	55	44	42	36	39	49	265	11.04 ± 0.71

SEM* = Standard error of the mean

Tap water 1, Growth medium 1 = produced by September 2007

Tap water 2, Growth medium 2 = produced by December 2007

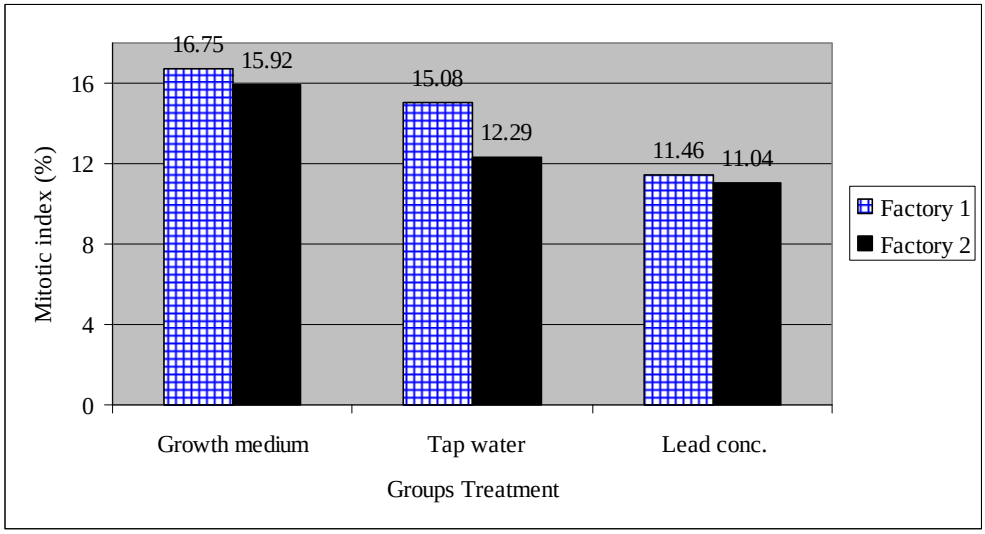


Figure 4.17 Comparison mitotic index of shallot exposed to two effluents

The results showed that the chromosome aberration was significantly different (p-value<0.001). The aberration slowly increased with increasing lead concentration of effluent from battery industry (p-value<0.001). The results of this study corresponded to the study of Grant (Grant, 1978).

In Table 4.17 showed that the chromosome aberration of 0.2780 mg/l and 0.3004 mg/l lead contamination were 23.5% and 38.17%, respectively (Figure 4.18).

Table 4.17 Chromosome aberrations induced in root of shallot by exposure 0.2780 mg/l and 0.3004 mg/l lead concentration of effluents

Lead conc.	Chromosome aberration (cells)								Total	%aberration
(mg/l)	Laggard	Bridge	Fragment	c-mitosis	LB[1]	LBF[2]	BF[3]	LF[4]	600	x ±SEM
Factory 1										
0 (Growth medium)	42	20	15	19	6	0	0	3	105	17.50 ± 0.26
0 (Tap water)	29	20	19	23	3	5	10	6	115	19.17 ± 0.34
0.3004	58	35	19	73	23	11	7	3	229	38.17 ± 0.35
Factory 2										
0 (Growth medium)	29	23	13	14	9	3	3	3	97	16.17± 0.36
0 (Tap water)	43	12	11	30	3	0	3	9	111	18.50± 0.38
0.2784 (spiked lead)	51	13	24	34	3	1	5	10	141	23.50± 0.11

SEM* = Standard error of the mean

1 = Laggard + Bridge, 2 = Laggard + Bridge + Fragment, 3 = Bridge + Fragment, 4 = Fragment + Laggard Bridge

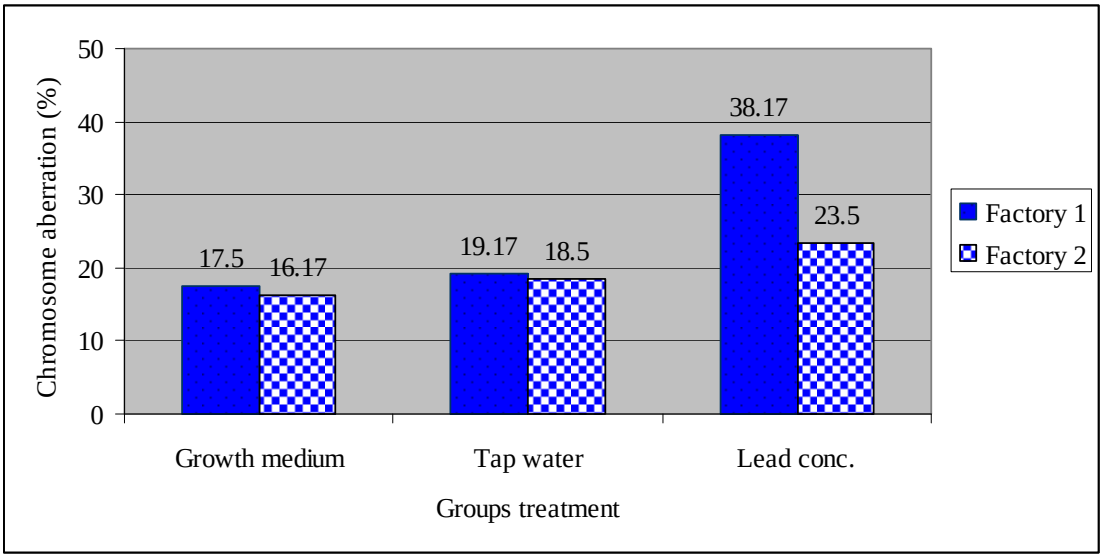


Figure 4.18 Comparison between 0.2780 and 0.3004 mg/l of lead concentration toxicity levels on chromosome aberration

Effluent from battery industry (factory 1) was significantly different (p-value<0.05) in both relative growth and genotoxicity (clastogenicity), whereas effluent from battery industry (factory 2) only spiked with lead concentration of 0.2780 mg/l was significantly different (p-value<0.05) only in relative growth but not in genotoxicity. In comparison of two factories, mitotic index did not have significant difference, whereas chromosome aberration did (p-value<0.05). In addition, the chromosome aberration was increase with increasing lead concentration effluent. These results were supported with the study of Soraya, (2005) that there was correlation between heavy metal (copper, zinc, lead) concentrations and root length of onion (*Allium cepa* L) exposed to polluted river water.

The results also concluded that chromosome aberration was induced by increasing lead contaminated in the effluent from battery industries.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The effects of effluents from two battery industries on clastogenicity of root tip cells of shallot (*Allium ascalonicum* L.) were studied by using Allium test. Moreover, inhibition of root growth was determined by measuring root bundle length. In addition, genotoxicity was examined by evaluating mitotic index. These studies also investigate chromosome aberration at the end point of the root. These results were compared by statistical analysis and concluded as follows:

5.1.1 In factory 1, root bundle lengths of shallot (*Allium ascalonicum* L.) in effluent from battery industries were significantly different from that of the control group. Concentration of lead in effluent from this factory was more than 0.2 mg/L and could inhibit root length of shallot at the toxicity levels of IC_{30}, IC_{50}, and IC_{70} at effluent concentration of 47, 74 and 100%v/v, respectively. Mitotic index of root cell in effluent was significantly different from that of the control group. In addition, effluent concentration higher than IC_{50} (74%v/v) was able to inhibit the root cell division of shallot. The results also revealed that chromosome aberrations significantly increased as the effluent concentration increased at the effluent concentration level of IC_{50} (74%v/v) and IC_{70} (100%v/v), whereas IC_{30} (47%v/v) induced chromosome aberration in root meristem cells of shallot.

5.1.2 In factory 2, the lead concentration in the effluent was not detected and was then added to obtain the lead concentration at the toxicity level of IC_{50} (0.3004 mg/L) to be of 0.2780 mg/L. It was found that at the concentration of 0.2780 mg/L, the mitotic index and chromosome aberration were not significantly different from that of the control.

5.1.3 The effects of effluent from two factories of shallot root growth were compared. The mitotic index as well as chromosome aberration of root cell in each effluent from two battery industries at lead concentration of 0.2780 and 0.3004 mg/L were not significantly different.

5.1.4 It can be concluded that the Allium test using shallot could be used to confirm the standard of effluent (0.2 mg/L) issued by the Ministry of Industries for acid battery industry effluents not only for root growth (mitotic index) but also the abnormalities (chromosome aberration) at the molecular level.

5.1.5 A growth medium could be used instead of tap water even than its mitotic index of growth medium was more than that of tap water and chromosome aberration was less than tap water. They were not significantly difference, therefore tap water could be used instead of a growth medium by test characteristics of tap water.

5.2 Recommendations

1. Lead concentrations in root, bulb and leave should be determined to study the translocation of lead through various parts of shallot.
2. The modified Allium test using the clastogenecity of shallot root tip is appropriate monitoring the effluent from lead-acid battery industries with high Lead concentration exceeding the standard of effluent issued by the Ministry of Industry (Lead < 0.2 mg/L).
3. Sensitivity of Allium test for other industries which have the same type of effluent mixtures concentration should be investigated in comparison.
4. Further study to compare sensitivity of Allium test to the other selected appropriate methods for environmental monitoring in the aspect of economical purpose for decentralized environmental center should be conducted.
5. In factory 2, Lead concentration in effluent would be adding 0.2 mg/L for the treated group. To distinct comparison with standard of effluent issued by the Ministry of Industry (Lead < 0.2 mg/L).
6. Tap water as control would be analyses chemically of heavy metal before test in laboratory.

REFERENCES

Aigenvironmental**.** (2004). *Battery Manufacturers*, [Online], Available form: <http://www.aigenvironmental.com/environmental/public/envindustries/0,1340,63-11-316,00.html> [9 September 2004]

Amdur, MO., Doull, J. & Klassen, C.D. (1992). *Toxicology: the basic science of poison,* New York: MacGraw-Hill.

Arizona. edu. (1998). Determining time spent in different phases of the cell cycle, Available form: <http://www.biology.arizona.edu> [7 August 2004]

ATSDR. (1999a). *Toxicological profile for lead.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (1999b). *Toxicological profile for mercury.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (2000a). *Toxicological profile for manganese.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (2000b). *Public health statement chromium.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (2004). *Toxicological profile for copper.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (2005a). *Toxicological profile for lead.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (2005b). *Toxicological profile for Zinc.* U.S. Department of Health and Human Services, Public Health Service.

ATSDR. (2005c). *Toxicological profile for arsenic.* U.S Department of Health and Human Services, Public Health Service.

ATSDR. (2005d). *Toxicological profile for nickel.* U.S. Department of Health and Human Services, Public Health Service.

Botany. (2002). Allium, [Online], Available from: <http://www.botany.com/allium.html> [2 September 2002]

Brusick, D.J. (1980). *Principles of genetic toxicology.* New York: Plenum.

Brusick, D.J. (1994). *Methods for genetic risk assessment.* Florida: Lewis.

Callahan, M.A., Slimak, M.W., & Gabel, N.W. (1979). *Water-related environmental fate of 129 priority pollutants.* Washington, DC.

Chandra, S., Chauhan, L.K., Murthy, R.C., Saxena, P.N., Pande, P.N., & Gupta, S.K. Comparative biomonitoring of leachates from hazardous solid wasted of two industries using Allium test. *Science of the total Environment*, 347, 46-52.

Chen, Jyi-Hong. (1974). *An Evaluation of industrial Waste Waters in Thailand with Special Reference: to Inorganic and Heavy Metal Waste Waters.* [M. Eng. Environmental Engineering] Bangkok: Faculty of Engineering, Asian Institute of Technology.

Connell, D.W. (1997). *Basic concepts of environmental chemistry.* Florida: Lewis.

Cornell. Edu. (1997). *Cell division/onion root tip.* Availabled form:< http://BioG-101-104.bio.cornell.edu> [15 January 2008]

Connell, D.W, Lam, P., Richaedson, B. & Wu, R. (1999). *Introduction to ecotoxicity.* London: Blackwell.

Cornell. (2006). *Cell division,* [Online], Available from: <http://biog-101-104.bio.cornell.edu/biog101_104/tutorials/cell_division.html> [4 January 2006]

Ding, Y., Vaziri, N.D. & Gonick, H.C. (1998). Lead-induced hypertension: II. Response to sequential infusions of l- arginine, superoxide dismutase, and nitroprusside. *Environ Res,* 76(2): 107-113.

Ecobichon, D.J. (1997). *The basic of toxicology. 2nd ed.* Florida: Lewis.

Eisler, R. (1994). A review of arsenic hazards to plants and animals with emphasis on fishery and wildlife. *Arsenic in the environment: Part II,* New York, 185-259.

Enviroharvest. (2007). *Battery glossary,* [Online], Available from: <http://www.enviroharvest.ca/toc.htm/battery> [22 March 2007]

EPA. (1980a). *Ambient water quality criteria for arsenic.* U.S. Environmental Protection Agency.

EPA. (1980b). *Identification and listing of hazardous waste: Appendix VIII,* U.S. Environmental Protection Agency, Hazardous constituents.

EPA. (1983). *Code of Federal Regulations. 40 CFR 468*. U.S. Environmental Protection Agency.

EPA. (1984). *Code of Federal Regulations. 40 CFR 60*. U.S. Environmental Protection Agency.

EPA. (1985). Controls applicable to gasoline refiners and importers Code of Federal Regulations. 40 CFR 80.20. U.S. Environmental Protection Agency.

Fiskesjo, G. (1985). The Allium test as a standard in environmental monitoring. *Hereditas*, 102, 99-112.

Fiskesjo, G. (1993). The allium test in wastewater monitoring. *Env. Toxicol and water Qual*, 8, 291-298.

Fiskesjo, G. (1994). Technical methods section : Allium test II. *Env. Toxicol and Water Qual*, 9, 235-241.

Fiskesjo, G. (1998). *Protocol number 8: Allium test,* [Online]. Available from: <http://emblyo.ib.amwaw.edu.pl/invittox/prot/8.htm> [15 August 2002]

Fiskesjo, G. (1993), *Technical methods section; Allium Test 1: A 2-3 Day Plant Test for Toxicity Assessment by Measuring the Mean Root Growth of Onions (Allium cepa* L.*)* Department of Genetics, University of Lund, S-223 62 Lund, Sweden.

Gabriela, C. & Lorena, S. (2000) . Evaluation of a bioassay battery for water toxicity testing. *Environment Toxicological, 15*, 331-337.

Georgopoulos, A.R. Yonone-Lioy, MJ. & Opiekun, R.E. (2001). Environmental copper: Its dynamics and human exposure issues. *Toxicol Environ Health Part B Crit Rev* 4(4):341-394.

Grant, W.F. (1978). Chromosome aberrations in plants as a monitoring system. *Environmental Health Perspectives, 27,* 37-43.

Grant, W.F. (2002). *Plant bioassay,* [Online], Available from: <http://www.agrenv.mcgill.ca/plant/grant.htm> [22 August 2002]

Grover, I.S. & Kaur, S. (1999). Genotoxicity of wastewater samples from sewage and industrial effluent detected by the Allium root anaphase aberration and micronucleus assays. *Mutation Research 19; 426(2)*:183-188.

Hare, S.O. & Atterwill, C.K. (1995). *In vitro toxicity testing protocols.* New Jersey: Humana.

ILO. (1976). *Occupational health and safety Vol. 1.* Encyclopedia, Geneva, International Labour Office.

IPCS. (1995). Inorganic lead. *Environmental Health Criteria 165 ed.* Geneva, Switzerland.

Johnson, F.M. (1998). The genetic effects of environmental lead. *Mutation Research* , 410, 123–140.

Keddy, C.J., Greene, M.A. & Bonnell. (1995). Review of whole-organism bioassays: soil, freshwater sediment, and freshwater assessment in Canada. *Ecotoxicology and Environmental Safety*, 30, 221-251.

Kincl, V.S., Stegnar, P., Lovka, M. & Toman, M.J. (1996). The evaluation of waste, surface and ground water quality using the allium test procedures. *Mutat. Res*, 368, 171-179.

Kumar, S., Verma, Y. & Gautam, A.K. (2000). Possible use of Allium test in environmental monitoring. *Pollution Research*, Volume 19, 555-560.

Lagrega, M.D.(1994). *Buckingham PL, Evans JC. Hazardous waste management.* New Yock: McGraw-Hill.

Landis, W.G. & Yu, M.H. (1995). *Introduction to environmental toxcicology*. Florida.

Lerda, D. (1992). The effect of lead on *Allium cepa* L. *Mutat Res*, 281, 89-92.

Li, A.P. & Hefich, R.H. (1991). *Genetic toxicology.* Boca Raton(FL): Lewis.

Liu, D., Jiang, W. & Li, M. (1992). Effect of trivalent and hexavalent chromosome on root growth and cell divisions Allium capa. *Heriditas*, 117, 23-29.

Liu, D. Jiang, W., Wang, W., Zhao, F. & Lu, C. (1994). Effects of lead on root growth, cell division, and nucleolus of Allium cepa. *Environment pollution*, 86(1), 1-4.

Lien, N.T. (1993). *Application of clean technology in small scale electroplating industry*, [MS. Thesis in science] Bangkok: Faculty of Science, Asian Institute of Technology.

Liewrungruang, T. (2003). *Application of the modified allium test using shallot (Allium ascalonicum L.) for screening toxicity and genotoxicity of chrome plating wastewater*, [MS. Thesis in Environmental and Technology] Bangkok: Faculty of Graduate studies, Mahidol University.

Lyn, S. (2004). *BC Lead-acid battery collection program,* Ministry of Water, Land and Air Protection, British Columbia, 1-10.

Milan, B., Arambasic , Sabrija, B. & Gordana, S. (1995). Acute toxicity of heavy metals (copper, lead, zinc), phenol and sodium on Allium cepa.,Lepidium Sativum L. and Daphnia magna St. Comparative investigations and the practical application. *Wat. Res.*29, 497-503.

Moore, R., Clark, W.D., Stern, K.R. & Vodopich, D. (1995). *Botany.* Dubuque (AI), Wm.C. Brown.

NAS (1977). *Drinking water and health--inorganic solutes.* National Academy of Sciences. Washington, D.C.

Newman, M.C. & McInsush. (1991). *Metal ecotoxicology concepts and applications.* U.S.A: Lewis Publisher, Inc.

Nielsen, MH. & Rank, J. (1994). Screening of toxicity and genotoxicity in wastewater using of Allium test. *Hereditas,* 121, 249-254.

OECD. (1993). LEAD – Background and national experience with reducing risk: *Risk reduction monograph No. 1.*Environmental Health and Safety Division, Paris.

OSH. (2005). *LD_{50} and LC_{50},* [Online], Available from: <http://www.ccohs.ca/toxic/OSH Answers.htm> [16 June 2005].

Pandec, P.N. & Gupta, S.K. (2005). Comparative biomonitoring of leachates from hazardous solid waste of two industries using Allium test, *Science of the Total Environment*, 347, 46– 52.

Pearson, H. (2006). Genetics: what is a gene. *Nature 441* (7092), 398-401.

Pornsuda Sirisawetvarin (2005). *Lead Leach ability and compressive strength of solidifiednbatter waste and sludge using calium carbage waste and rice hust ash.* [M.Eng. Thesis in Environmental Sanitation]. Bangkok: Faculty of graduate studies, Mahidol University.

Rabinowitch, H.D., & Brewster, J.L. (1990). Onions and Allied crops: *botany, physiology and genetics,* Boca Raton (FL): CRC.

Rank, J. (2003). The method of Allium anaphase-telophase chromosome aberration assay. Department of environment, Technology and social studies, Roskilde University; Denmark 39-42.

Rank, J. & Nielsen, M.H. (1994). Evaluation of the Allium anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research*, 312, 17-24.

Rank, J. & Nielsen, MH. (1997). Allium cepa anaphase-telophase root tip chromosome aberration assay on N-methyl-N-nitrosourea, maleic hydrazide, sodium azide and ethyl methanesulfonate. *Mutations Reserch,* 390, 121-127.

ROC. (2003). *Report on Carcinogens Background Document for Lead and Lead Compounds.* U.S. Department of Health and Human Services, Public Health Service.

Rost, T.L., Barbour, M.G., Stocking, C.R. & Murrphy, T.M.(1998). *Plant biology.* Belmont(CA), Wadworth.

Schroeder, S.R. & Hawk, B. (1987). Psycho-social factors, lead exposure and IQ. *Monogr Am Assoc Ment Defic S*: 97-137.

Seyin, H. (2003). Cytogenetic Effects of Copper Chloride on the Root Tip Cells of *Helianthus annuus L.Biol 27,* 43-46.

Sharma & Sharma A.K. & Shama, A. (1999). *Plant chromosomes*. Analysis, main pollution and engineering. Amsterdam: Harwood, academic.

Singh, R.J. (1993). *Plant cycogenetics.* Boca Raton(FL).

Soraya, Rad (2005). Correlolfo, F., Galate, R., Zenatti, D., Seolatto, A., Lorenze, E., Zacarkim, C., Rossi, N., Rizzutto, M. & Tabacniks, M.Correlation between heavy metal ions (copper, zinc, lead) concentrations and root length of Allium cepa L. in polluted river water. *Brazilian Archives of Biology and Technology*, 48, 191-196.

Staykova1, Evgeniya, N. Ivanova1 & Iliana, G. (2005). Cytogenetic effect of heavy-metal and cyanide in contaminated waters from the region of southwest Bulgaria Teodora A. *Cell and Molecular Biology*, 4, 41-46.

Tanoohsin, U. (1979). *Chromic acid recovery from chroming plating waste by ionexchange.* [M.S. Thesis in Engineering]. Bangkok: Faculty of Engineering, Chulalongkorn University.

Teetong, S. (2007). *Application of the modified allium test using shallot (Allium ascalonicum L.) for determination of mercury contamination in sediment at*

gold mining site. [MS. Thesis I Environmental and Technology] Bangkok: Faculty of Graduate studies, Mahidol University.

Thaigov. (2007). *Powerbattery*, [Online], Available from: <http://powerbattery.thaigov.net/Data/Product/bt_componant.htm>[24 May 2007]

Vichakarn. (2005). *มหัศจรรย์ ดีเอ็นเอ (DNA),* [Online], Available from: <http://www.vcharkarn.com/varticle/296> [4 July 2005]

Walum, E. Stenberg, K. & Jenssen, D. (1990). *Understanding cell toxicology. Principle and practice.* West Sussex (England): Horwood.

Wang, W., Gorsuch, J.W. & Hughes, J.S. (1997). *Plants for environmental studies.* Boca Raton.

Watson, R.R. (1992). *In vitro methods of toxicology.* Boca Raton.

Wierzbicka, M.(1993). How lead can easily enter the food chain - A study of plant roots. *Science of the Total Environment*, Issue SUPPL. Part 1, 423-429.

Wierzbicka, M. (1998). Lead in the apoplast of Allium cepa L. root tips - Ultrastructural studies. *Plant Science*, 133, 105-109**.**

Wierzbicka, M. (1999). The effect of lead on the cell cycle in the root meristem of *Allium cepa* L. *Protoplasma*, 207, 186-194.

Wierzbicka, M. (2003). Effect of metal toxicity on plant growth and metabolism: I. Zinc. *Agronomie* 23, 3–11.

Wikipedia. (2008). *Chromosome,* [Online], Available from: <http://en.wikipedia.org/wiki/Chromosome> [5 January 2008]

Williums, P.L., James, RC. & Robert, SM. (2002). *Principle of toxcicology, environment and industrial application*. 2nd ed. New York.

APPENDIX

APPENDIX A

EXPERIMENTAL DATA

Table A-1 The effects of effluents from battery industry (Factory 1) at 50, 75 and 100%v/v on root length of shallot (*Allium ascalonicum* L.)

Test solution (%v/v)	Time (hrs)	Exp.	Root length(cm)						Mean	S.D
			1	2	3	.	.	.		
0	24	1	1.1	1.0	1.0	.	.	.	0.97	0.19
(Growth medium)		2	1.1	0.6	0.8	.	.	.	0.92	0.21
		3	0.8	0.7	0.9	.	.	.	0.74	0.12
	48	1	2.5	2.5	2.0	.	.	.	2.41	0.31
		2	2.8	2.5	2.9	.	.	.	2.49	0.54
		3	3.0	2.6	2.5	.	.	.	2.48	0.35
	72	1	3.5	3.7	4.5	.	.	.	3.74	0.44
		2	4.2	3.8	5.0	.	.	.	4.12	0.54
		3	4.4	5.0	3.2	.	.	.	4.43	0.70
	96	1	6.4	5.0	5.9	.	.	.	5.38	0.67
		2	6.7	5.0	7.2	.	.	.	6.08	0.63
		3	6.3	6.5	7.1	.	.	.	6.69	0.52
0	24	1	1.4	1.0	0.8	.	.	.	1.06	0.27
(Tap water)		2	1.0	1.0	1.1	.	.	.	1.10	0.17
		3	1.3	1.2	1.5	.	.	.	1.15	0.19
	48	1	2.3	2.5	2.5	.	.	.	2.56	0.52
		2	2.5	1.8	2.7	.	.	.	2.87	0.55
		3	2.5	2.8	3.0	.	.	.	2.52	0.36
	72	1	4.6	3.7	4.7	.	.	.	4.75	0.74
		2	4.6	4.7	5.0	.	.	.	5.01	0.50
		3	5.2	5.0	5.3	.	.	.	5.09	0.46
	96	1	6.4	6.1	4.2	.	.	.	6.43	0.97
		2	6.4	6.8	7.3	.	.	.	6.88	0.53
		3	6.4	6.7	6.3	.	.	.	6.45	0.49
50	24	1	0.9	1.2	0.7	.	.	.	0.81	0.17
		2	0.7	0.7	0.7	.	.	.	0.74	0.15
		3	0.8	0.8	0.5	.	.	.	0.71	0.14
	48	1	2.0	2.5	2.3	.	.	.	2.33	0.30
		2	1.7	2.4	1.6	.	.	.	2.06	0.35
		3	2.0	2.2	2.5	.	.	.	2.12	0.33
	72	1	2.8	3.5	3.6	.	.	.	4.07	0.67
		2	3.8	4.0	2.8	.	.	.	3.85	0.51
		3	4.2	3.9	3.3	.	.	.	3.78	0.46
	96	1	5.2	4.8	5.2	.	.	.	5.21	0.50
		2	4.1	4.1	4.8	.	.	.	4.96	0.59
		3	5.5	4.3	5.5	.	.	.	5.13	0.64

Table A-1 The effects of effluents from battery industry (Factory 1) at 50, 75 and 100%v/v on root length of shallot (*Allium ascalonicum* L.) (continued)

Test solution (%v/v)	Times (hrs)	Exp.	Root length (cm)				Mean	S.D.
			1	2	3	. . .		
75	24	1	0.5	0.8	0.5	. . .	0.57	0.13
		2	0.6	0.6	0.5	. . .	0.59	0.10
		3	0.7	0.5	0.7	. . .	0.66	0.10
	48	1	1.8	1.3	1.3	. . .	1.44	0.27
		2	2.1	1.8	1.9	. . .	1.37	0.34
		3	1.8	1.6	1.0	. . .	1.43	0.35
	72	1	2.2	2.7	2.4	. . .	2.33	0.33
		2	1.8	2.7	2.6	. . .	2.29	0.35
		3	2.9	1.8	1.5	. . .	2.44	0.45
	96	1	3.0	3.9	3.1	. . .	3.30	0.47
		2	3.2	3.8	3.2	. . .	3.21	0.44
		3	3.5	2.6	3.1	. . .	3.18	0.31
100	24	1	0.5	0.5	0.5	. . .	0.47	0.10
		2	0.2	0.5	0.3	. . .	0.43	0.10
		3	0.5	0.5	0.5	. . .	0.51	0.08
	48	1	1.0	0.7	0.9	. . .	0.96	0.12
		2	1.2	0.6	1.1	. . .	1.01	0.21
		3	1.0	1.4	1.2	. . .	1.08	0.22
	72	1	1.4	1.3	1.5	. . .	1.38	0.17
		2	1.5	1.1	1.5	. . .	1.37	0.15
		3	1.3	1.2	1.5	. . .	1.46	0.27
	96	1	1.5	1.5	1.8	. . .	1.71	0.16
		2	1.3	1.7	1.5	. . .	1.62	0.15
		3	1.9	1.6	2.0	. . .	1.72	0.23

Table A-2 The effects of effluents from battery industry (Factory 2) at 50, 75 and 100%v/v on root length of shallot (*Allium ascalonicum* L.)

Test solution (%v/v)	Time (hrs)	Exp.	Root length (cm) 1	2	3	. . .	Mean	S.D.
0	24	1	0.5	0.7	0.6	. . .	0.69	0.17
(Growth medium)		2	0.7	1.0	0.7	. . .	0.71	0.20
		3	0.8	0.7	0.6	. . .	0.72	0.08
	48	1	1.5	1.5	2.1	. . .	2.37	0.62
		2	1.3	2.9	1.7	. . .	2.63	0.58
		3	3.1	2.5	2.2	. . .	2.47	0.37
	72	1	4.7	4.5	5.0	. . .	5.07	0.41
		2	5.2	5.0	5.4	. . .	5.26	0.57
		3	4.5	5.2	4.7	. . .	4.95	0.36
	96	1	6.0	6.2	6.9	. . .	6.51	0.60
		2	7.2	4.8	6.1	. . .	6.91	1.09
0	24	1	0.8	0.8	1.2	. . .	0.87	0.18
(Tap water)		2	1.0	0.8	0.7	. . .	0.84	0.22
		3	0.6	1.0	1.0	. . .	0.84	0.16
	48	1	2.5	2.0	4.0	. . .	3.36	0.66
		2	3.3	2.9	3.0	. . .	3.32	0.52
		3	3.2	3.7	3.0	. . .	2.91	0.52
	72	1	5.1	3.6	5.5	. . .	5.94	1.04
		2	4.5	4.1	4.8	. . .	5.45	0.91
		3	5.0	4.5	5.4	. . .	5.14	0.66
	96	1	7.4	5.0	6.5	. . .	7.71	0.90
		2	7.0	6.5	7.0	. . .	7.44	1.08
		3	7.5	7.9	6.7	. . .	7.58	1.13
50	24	1	0.8	0.8	0.8	. . .	0.82	0.12
		2	1.1	0.8	0.7	. . .	0.92	0.21
		3	0.8	0.8	0.5	. . .	0.73	0.21
	48	1	3.4	3.3	3.2	. . .	3.09	0.28
		2	2.8	3.9	3.6	. . .	3.11	0.44
		3	3.4	3.0	3.6	. . .	3.09	0.40
	72	1	5.7	6.5	6.5	. . .	5.86	0.70
		2	7.0	5.8	5.6	. . .	5.36	0.58
		3	5.5	5.6	3.5	. . .	4.97	0.88
	96	1	6.5	7.6	8.2	. . .	7.29	0.78
		2	6.5	6.2	7.6	. . .	7.18	0.51
		3	7.5	8.3	7.5	. . .	7.38	1.03

Table A-2 The effects of effluents from battery industry (Factory 2) at 50, 75 and 100%v/v on root length of shallot (*Allium ascalonicum* L.)(continued)

Test solution (%v/v)	Time (hrs)	Exp.	Root length (cm)						Mean	S.D.
			1	2	3	.	.	.		
75	24	1	1.0	1.0	1.0	.	.	.	0.76	0.24
		2	0.5	0.9	1.0	.	.	.	0.75	0.23
		3	0.6	0.6	0.7	.	.	.	0.61	0.16
	48	1	3.2	3.0	3.3	.	.	.	2.82	0.36
		2	3.2	3.3	2.0	.	.	.	2.82	0.52
		3	2.5	2.2	2.8	.	.	.	2.38	0.38
	72	1	5.6	5.5	5.3	.	.	.	5.22	0.70
		2	4.4	5.0	4.6	.	.	.	4.66	0.70
		3	5.0	4.3	5.5	.	.	.	4.79	0.56
			5.0	4.9	5.1	.	.	.	4.89	0.44
	96	1	7.2	7.1	7.4	.	.	.	7.17	0.44
		2	7.1	6.8	7.2	.	.	.	6.70	0.54
		3	7.0	7.0	7.1	.	.	.	6.89	0.72
100	24	1	0.5	0.8	0.8	.	.	.	0.69	0.15
		2	0.8	1.0	1.0	.	.	.	0.81	0.18
		3	0.9	0.5	0.2	.	.	.	0.58	0.22
	48	1	2.1	2.4	2.5	.	.	.	2.69	0.46
		2	2.9	2.7	2.5	.	.	.	2.63	0.29
		3	2.2	1.6	2.4	.	.	.	2.17	0.49
	72	1	4.6	5.2	5.6	.	.	.	4.86	0.47
		2	4.0	3.7	3.9	.	.	.	4.72	0.45
		3	5.3	5.3	4.5	.	.	.	4.47	0.71
	96	1	6.0	7.8	7.6	.	.	.	6.52	0.75
		2	5.3	7.0	7.5	.	.	.	6.52	0.71
		3	7.0	6.5	6.7	.	.	.	6.79	0.60

Table A-3 The effects of effluents from battery industry (Factory 2) spiked lead to 0.2780 mg/l on root length of shallot (*Allium ascalonicum* L.)

Test solution (%v/v)	Time (hrs)	Exp.	Root length (cm) 1	2	3	.	.	.	Mean	S.D.
0	24	1	0.4	1.0	0.5	.	.	.	0.76	0.18
(Growth medium)		2	0.6	1.0	0.7	.	.	.	0.70	0.20
		3	0.5	0.7	0.6	.	.	.	0.70	0.09
	48	1	2.5	1.5	2.1	.	.	.	2.37	0.66
		2	2.3	2.9	1.7	.	.	.	2.69	0.49
		3	2.8	2.5	2.2	.	.	.	2.46	0.34
	72	1	5.0	4.5	5.0	.	.	.	5.09	0.40
		2	5.4	3.0	5.4	.	.	.	4.93	1.03
		3	4.8	5.2	3.2	.	.	.	4.62	0.74
	96	1	6.5	6.2	6.9	.	.	.	6.47	0.51
		2	4.0	5.1	6.7	.	.	.	5.82	0.78
		3	6.0	6.8	7.0	.	.	.	6.67	0.52
0	24	1	0.6	1.0	0.5	.	.	.	0.77	0.16
(Tap water)		2	1.0	1.0	0.8	.	.	.	0.88	0.28
		3	0.8	1.0	0.9	.	.	.	0.84	0.20
	48	1	2.8	2.6	3.1	.	.	.	2.78	0.54
		2	3.0	3.6	3.1	.	.	.	3.02	0.61
		3	3.4	2.7	3.5	.	.	.	3.30	0.37
	72	1	4.3	4.8	5.8	.	.	.	4.96	0.79
		2	5.4	4.6	5.9	.	.	.	5.07	0.65
		3	6.0	4.8	5.1	.	.	.	5.36	0.85
	96	1	7.5	7.0	7.5	.	.	.	6.86	0.53
		2	7.5	7.0	5.8	.	.	.	6.87	0.51
		3	6.3	6.5	7.5	.	.	.	6.76	0.86
0.2780	24	1	0.7	0.4	0.6	.	.	.	0.63	0.11
		2	0.7	0.7	0.5	.	.	.	0.63	0.12
		3	0.6	0.5	0.5	.	.	.	0.62	0.15
	48	1	2.4	0.8	2.1	.	.	.	2.04	0.54
		2	2.5	2.5	1.5	.	.	.	2.27	0.40
		3	2.8	4.0	1.7	.	.	.	2.30	0.62
	72	1	3.6	4.5	3.8	.	.	.	3.80	0.39
		2	3.4	2.9	2.1	.	.	.	3.34	0.77
		3	4.2	3.1	1.5	.	.	.	3.49	0.95
	96	1	5.4	1.7	5.3	.	.	.	4.89	1.08
		2	5.0	6.5	4.8	.	.	.	4.70	0.86
		3	5.5	5.7	4.9	.	.	.	4.53	0.78

APPENDIX B
STATISTICAL DATA

Table B-1 Statistical analysis of % root shallot growth at the levels of effluents concentration 0, 50, 75, and 100%v/v on factory 1

Tests of Normality

	Effluent conc. (%v/v)	Shapiro-Wilk		
		Statistic	df	P-value
% Root growth	50	.871	3	.298
	75	.990	3	.807
	100	.795	3	.103

Test of Homogeneity of Variances

% root growth

Levene Statistic	df1	df2	P-value
5.615	3	8	.023

ANOVA

%root growth

Source of variable	Sum of Squares	df	Mean Square	F	P-value
Between Groups	9517.072	3	3172.357	396.110	< 0.001
Within Groups	64.070	8	8.009		
Total	9581.142	11			

Table B-1 Statistical analysis of % root shallot growth at the levels of effluent concentration 0, 50, 75, and 100%v/v on factory 1 (continued)

Homogeneous Subsets

Duncan

Effluent conc.	N	Subset for alpha = .05			
(% v/v)	1	2	3	4	1
100	3	25.5933			
75	3		49.0733		
50	3			77.4967	
0	3				100.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table B-2 Statistical analysis of % root growth of shallot at the levels of effluent concentration 0, 50, 75 and 100%v/v on factory 2

Tests of Normality

	Effluent conc. (%v/v)	Shapiro-Wilk		
		Statistic	df	P-value
% Root growth	50	.951	3	.572
	75	.950	3	.570
	100	.984	3	.757

Test of Homogeneity of Variances

% Root growth

Levene Statistic	df1	df2	P-value
2.798	3	8	.109

ANOVA

% Root growth

Source of variable	Sum of Squares	df	Mean Square	F	P-value
Between Groups	279.006	3	93.002	35.271	< 0.001
Within Groups	21.094	8	2.637		
Total	300.100	11			

Table B-2 Statistical analysis of % root growth of shallot at the levels of effluent concentration 0, 50, 75, and 100%v/v on factory 2 (continued)

Homogeneous Subsets

Duncan (a)

Effluent conc.	N	Subset for alpha = .05			
(% v/v)	1	2	3	4	1
100	3	87.2600			
75	3		91.2733		
50	3			96.1400	
0	3				100.0000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Table B-3 Statistical analysis of % root growth of shallot at the levels of effluent concentration 0.2780 mg/l of lead on factory 2

Tests of Normality (a)

	Pb conc.	Shapiro-Wilk		
		Statistic	df	Sig.
%Root growth	.278	.958	3	.607

a % root growth is constant when Pb concentration = .000. It has been omitted.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
%Root growth	Equal variances assumed	8.102	0.047	24.578	4	<0.001	31.093	1.26507	27.58	34.61
	Equal variances not assumed			24.578	2	.002	31.093	1.26507	25.65	36.54

Table B-4 Statistical analysis of mitotic index of effluent at toxicity levels IC_{30}, IC_{50}, and IC_{70} at 48 hours on factory 1

Tests of Normality

	Test Solution (%v/v)	Shapiro-Wilk		
		Statistic	df	P-value
Mitotic index	0	.941	6	.664
	47	.773	6	.033
	74	.919	6	.497
	100	.965	6	.860

Test of Homogeneity of Variances

Mitotic index

Levene Statistic	df1	df2	P-value
4.377	3	20	.016

ANOVA

Mitotic index

	Sum of Squares	df	Mean Square	F-ratio	P-value
Between Groups	243.299	3	81.100	20.058	< 0.001
Within Groups	80.865	20	4.043		
Total	324.164	23			

Table B-4 Statistical analysis of mitotic index of effluent at toxicity levels IC_{30}, IC_{50}, and IC_{70} at 48 hours on factory 1(continued)

Multiple Comparisons

Duncan

Test solution (%v/v)	N	Subset for alpha = .05		
	1	2	3	1
100	6	6.7917		
74	6	9.1667	9.1667	
47	6		9.7083	
0	6			15.4583
Sig.		.054	.646	1.000

Means for groups in homogeneous subsets are displayed.

Table B-5 Statistical analysis of chromosome aberration of effluent at toxicity level 47%v/v (IC_{30}), 74%v/v (IC_{50}) and 100%v/v (IC_{70}) at 48 hours on factory 1

Test Solution * CA Cross tabulation

Count		Chromosome aberration								Total
		Laggard	c-mitosis	Bridge	Fragment	LB[1]	LF[2]	LBF[3]	BF[4]	
Test solution	100	34	31	24	21	5	7	2	8	132
(%v/v)	74	42	41	21	13	10	3	5	0	135
	47	75	64	23	20	19	3	4	2	210
	0	75	107	26	28	22	6	13	4	281
Total		226	243	94	82	56	19	24	14	758

1 = Laggard + Bridge, 2 = Laggard + Fragment, 3 = Laggard + Bridge + Fragment, 4 = Bridge + Fragment

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	50.793(a)	21	<0.001

a 7 cells (21.9%) have expected count less than 5. The minimum expected count is 2.44.

Table B-6 Statistical analysis of mitotic index of effluent at toxicity levels 0, 0.2780 mg/l as Pb at 48 hours on factory 2

Tests of Normality

	Test Solution (mg/l as Pb)	Shapiro-Wilk		
		Statistic	df	P-value
Mitotic index	0	.917	6	.486
	0.2780	.967	6	.874

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mitotic Index	Equal variances assumed	.728	.414	1.093	10	.300	1.25000	1.14352	-1.29792	3.79792
	Equal variances not assumed			1.093	9.460	.301	1.25000	1.14352	-1.31779	3.81779

Table B-7 Statistical analysis of chromosome aberration of effluent at toxicity levels 0 and 0.2780 mg/l as Pb at 48 hours on factory 2

Test solution * CA Crosstabulation

Effluent conc. (mg/l as Pb)		Chromosome aberration								Total
		Laggard	c-mitosis	Fragment	Bridge	LF[1]	FB[2]	LB[3]	LFB[4]	
Test solution	0	43	30	11	12	9	3	3	0	111
	0.2780	51	34	24	13	10	5	3	1	141
	Total	94	64	35	25	19	8	6	1	252

1 = Laggard + Fragment, 2 = Fragment + Bridge, 3 = Laggard + Bridge, 4 = Laggard + Fragment + Bridge

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.835(a)	7	.799

a 6 cells (37.5%) have expected count less than 5. The minimum expected count is .44.

Table B-8 Statistical analysis of mitotic index compared of factory 1 (IC_{50} = 0.3004 mg/l as Pb) and factory 2 (0.2780 mg/l) at 48 hours

Tests of Normality

	Effluent conc. (mg/l as Pb)	Shapiro-Wilk		
		Statistic	df	P-value
Mitotic index	0.3004	.919	6	.497
	0.2780	.967	6	.874

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mitotic Index	Equal variances assumed	1.085	.322	-2.18	10	.054	-1.875	.859	-3.789	.039
	Equal variances not assumed			-2.18	8.91	.057	-1.875	.859	-3.821	.071

Table B-9 Statistical analysis of chromosome aberration of factory 1 (IC_{50} = 0.3045 mg/l as Pb) and factory 2 (0.2780 mg/l) at 96 hours

Test solution * CA Crosstabulation

		Chromosome aberration								Total
		Laggard	c-mitosis	Fragment	Bridge	LF[1]	FB[2]	LB[3]	LBF[4]	
Test solution	0.3045	58	73	19	35	3	7	23	11	229
	0.2780	51	34	24	13	10	5	3	1	141
Total		109	107	43	48	13	12	26	12	370

1 = Laggard + Fragment, 2 = Fragment + Bridge, 3 = Laggard + Bridge, 4 = Laggard + Bridge + Fragment

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	34.152(a)	7	<0.001

a 3 cells (18.8%) have expected count less than 5. The minimum expected count is 4.57.

Table B-10 Statistical analysis of mitotic index compared of tap water at 96 hours on factory 1 and factory 2

Tests of Normality

	Test solution	Shapiro-Wilk		
		Statistic	df	P-value
Mitotic index	Factory 1	.941	6	.664
	Factory 2	.917	6	.486

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mitotic Index	Equal variances assumed	.804	.391	1.936	10	.082	3.16667	1.63533	-.47708	6.81041
	Equal variances not assumed			1.936	8.655	.086	3.16667	1.63533	-.55530	6.88863

Table B-11 Statistical analysis of chromosome aberration compared of tap water at 96 hours on factory 1and factory 2

Test solution * CA Crosstabulation

		Chromosome aberration								Total
		Laggard	C-mitosis	Bridge	Fragment	LF[1]	BF[2]	LB[3]	LBF[4]	
Tap water	Factory 1	34	31	24	21	7	8	5	2	132
	Factory 2	43	30	12	11	9	3	3	0	111
Total		77	61	36	32	16	11	8	2	243

1 = Laggard + Fragment, 2 = Bridge +Fragment, 3 = Laggard + Bridge, 4 = Laggard + Bridge + Fragment

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.487(a)	7	.119

a 4 cells (25.0%) have expected count less than 5. The minimum expected count is .91.

Table B-12 Statistical analysis of mitotic index compared of growth medium at 96 hours on factory 1 and factory 2

Tests of Normality

Test solution		Shapiro-Wilk		
		Statistic	df	P-value
Mitotic index	growth medium Factory 1	.847	6	.149
	growth medium Factory 2	.908	6	.423

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mitotic index	Equal variances assumed	.334	.576	-.762	10	.464	-1.000	1.312	-3.924	1.924
	Equal variances not assumed			-.762	8.918	.466	-1.000	1.312	-3.973	1.973

Table B-13 Statistical analysis of chromosome aberration compared of growth | medium at 96 hours on factory 1and factory 2

Testsolution * CA Crosstabulation

		Chromosome aberration								Total
		Laggard	c-mitosis	Bridge	Fragment	LF[1]	BF[2]	LB[3]	LBF[4]	
Test solution	Growth medium factory 1	58	73	35	19	3	7	23	11	229
	Growth medium factory 2	29	14	23	13	3	3	9	3	97
	Total	87	87	58	32	6	10	32	14	326

1 = Laggard + Fragment, 2 = Bridge +Fragment, 3 = Laggard + Bridge, 4 = Laggard + Bridge + Fragment

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.514(a)	7	.043

a 4 cells (25.0%) have expected count less than 5. The minimum expected count is 1.79.

BIOGRAPHY

NAME	Miss Patumrat Jaipet
DATE OF BIRTH	20 May 1978
PLACE OF BIRTH	Nakhon Si Thammarat, Thailand
INSTITUTIONS ATTENED	Mahidol University; 2001 Bachelor of Science (Environmental Health Science) Mahidol University; 2008 Master of Science (Environmental technology)
HOME ADDRESS	20/1 moo 3 Koa Pet Hua Sai, District, Nakhon Si Thammarat 80170