CHAPTER 1
INTRODUCTION

1.1 Phenolic Compounds in the Environment

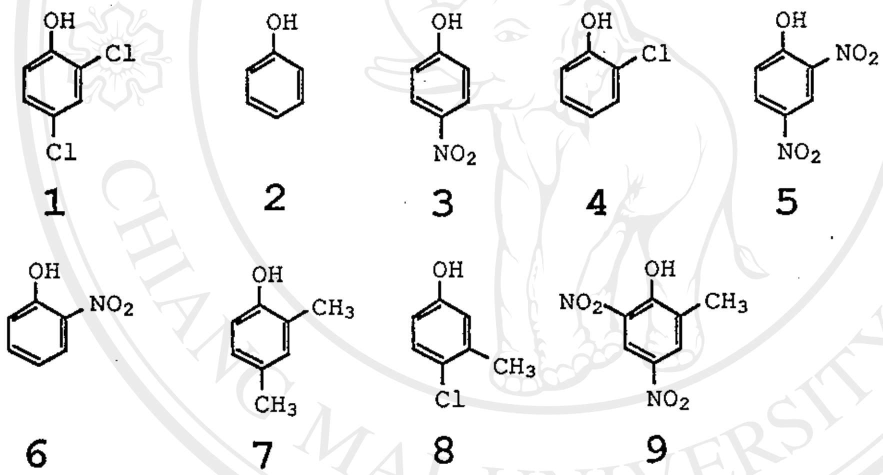
Phenolic compounds are a diverse group of organic chemicals consisting of a basic benzene ring and one or more hydroxyl groups. Simple phenolic compound (hydroxybenzene, C_6H_5OH) was isolated in 1834 from coal tar. Although this was the only source of phenol until the World War I, synthetic production gradually grew in importance and, by 1930, exceeded natural production. Phenolic compounds are ones of the most important and versatile industrial organic chemicals.

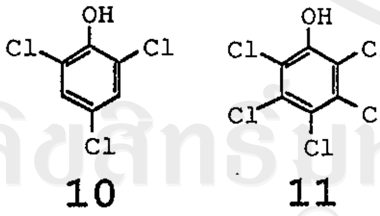
Phenolic compounds are often considered to be an indication of man-made organic pollution.[1] They may be present in raw water owing to the discharge of wastewater from coke distillation plants, the petrochemical industry, numerous other industries where phenols serve as intermediates, and also present in municipal wastewater.[2] They have also been extensively used as pesticides, herbicides and fungicides. Moreover they are important pollutants of river water, being degradation products of many organic compounds, such as pesticides used in agriculture and waste products from a variety of chemical industries.[3]

1.1.1 Phenolic Compounds and Its Derivative

Today almost all phenol is manufactured by sulfonation of benzene and hydrolysis of sulfonate. The more complex phenolic compounds are obtained by replacing one or more of the hydrogen atoms attached to the benzene ring with various atoms (such as chlorine) or more complex substituents (such as methyl or nitro groups).[4]

The environmental aspects of phenolic compounds have been increasingly important in the recent years. The U.S. Environmental Protection Agency (US EPA) and the European Community (EC) authorities have included phenolic compounds in their priority pollutants lists.[5] The structures of priority pollutant phenols (PPP) listed by the (US EPA) are shown as Figure 1.1. General informations of 11 priority pollutant phenols are attached in Appendix 1.1.[62]





Components:

1	2,4-dichlorophenol
2	phenol
3	4-nitrophenol
4	2-chlorophenol
5	2,4-dinitrophenol
6	2-nitrophenol
7	2,4-dimethylphenol
8	4-chloro-3-methylphenol
9	4,6-dinitro-2-methylphenol
10	2,4,6-trichlorophenol
11	pentachlorophenol

Figure 1.1: **Structural Formulas of Priority Pollutant Phenols (PPP)**

1.1.2 Sources and Discharges of Phenolic Compounds

Phenolic compounds arise from the distillation of coal and wood (including burning of the forest); from oil refineries; chemical plants; livestock dips; human and other organic wastes; hydrolysis, chemical oxidation and microbial degradation of pesticides; and from naturally occurring sources (e.g. humification) and substances. Some compounds are refractory to biological degradation and can be transported long distances in water.[6]

Since phenol is a high-volume industrial chemical with multiple uses, it is commonly found in a variety of municipal and industrial discharges (Table 1.1). Some of the highest reported concentrations are from petroleum refineries and other hydrocarbon processing industries. Although phenol residues contents are much lower in municipal effluents, the total amount of material discharged from these sources constitute a considerable input of phenolic compounds to the environment.

Relatively less information is available on the concentrations of other phenols in industrial and municipal discharges. Jungclaus et al.[7] reported the presence of at least nine alkylphenols in the wastewater of a specified chemical plant. Of these 2,6-di-*t*-butylphenol was most the common, reaching a concentration of 800 μg/l, followed by 2,4-di-*t*-butylphenol (max. 600 μg/l) and 2,4-di-*t*-amylphenol (400 μg/l). Although quantitative data are not available, it is known that the combustion and pyrolysis of building materials containing phenolic resin produce methylphenols, dimethylphenols and trimethylphenols. Such materials include foam insulation and adhesives in laminates.

Table 1.1: Concentration (μg/l) of Various Phenols in Municipal and Industrial Wastewater[4]

Compound	Concentration	Source
Phenol	0.03–20	Treated sewage, European cities
	200–3,016,000	3 petroleum refineries (USA)
	3200	2 coal gasification plants (USA)
	38,000–1,240,000	Coke plant (USA)
	10–300	Specialty chemical plant (USA)
2,4-Dichlorophenol	51–330	Chemical plant (Vancouver)
	<0.1	Wood preservation plant (British Columbia)
	<0.1	Landfill leachate (Vancouver)
2,6-Dichlorophenol	220	Chemical plant (Vancouver)
	2.4	Wood preservation plant (British Columbia)
	1.2–5.6	Landfill leachate (Vancouver)
2,4,5-Trichlorophenol	<0.05	Treated sewage, 4 plants (Vancouver)
	0.5–2400	2 chemical plants (Vancouver)
	<0.05	Wood preservation plant (British Columbia)
	0.05–2	Landfill leachate (Vancouver)
2,4,6-Trichlorophenol	<0.05–1	Treated sewage, 4 plants (Vancouver)
	<0.05–3120	3 chemical plants (Vancouver)
	0.5–1	Wood preservation plant (British Columbia)
	0.4–1.0	Landfill leachate (Vancouver)
	25–115	Pulpmill effluent (Vancouver)
2,3,4,6-Tetrachlorophenol	0.6–28	Treated sewage, 4 plants (Vancouver)
	1.2–8270	4 wood preservation plants (British Columbia)
	0.3–166	3 chemical plants (Vancouver)
	0.2–0.8	Landfill leachate (Vancouver)
Pentachlorophenol	0.5–4.7	Treated sewage, 4 plants (Vancouver)
	0.25–1.3	Treated sewage, 6 cities (Ontario)
	0.05–2760	Wood preservation plants (British Columbia)
	5,400,000	Chemical plant (Philadelphia)
	0.6–42	Landfill leachate (Vancouver)

As might be expected, the type of feedstock chemical and industrial process play a key role in determining the presence of phenols in wastewater. Wise and Fahrenthold suggested that most industrial processes were not sources of priority pollutants because the processes do not involve critical precursor/process combinations. In addition, synthetic production methods generally lead to an increase in complexity of priority pollutant molecules. These in turn exhibit variable toxicity and persistence, which may be comparable to related priority pollutants.[8]

In Chiang Mai city area, there are only small industries which all do not concern with phenolics emission. In the domestic wastewater, the uses of phenol as disinfectant, antiseptic and food preservative could be seen. Burning of forest which oftenly found, the trees which contain high lignin contents could lead to phenolic pollutants in the air as the dust. Phenolic compounds could be then transferred to soil and washed out to waterbody by rain or surface runoff. The natural process like humification or metabolism by cattle and human beings could also produce phenol. Burning of gasoline in heavy traffic like the present situation in Chiang Mai could be one source of phenol pollution in air, soil and consequently to water.

1.1.3 Toxicity

1.1.3.1 Aquatic Plant and Invertebrates

Symptoms of sublethal intoxication by phenols are numerous and may include a reduction in fecundity and survival of progeny. Under natural conditions, this can result in a change in the development and structure of invertebrate communities owing to the differential response of various species of phenols. Since algal metabolism is also influenced by phenol, there may be a reduction in oxygen levels in surface waters, leading to further changes in the density of sensitive species.[4] Phenols have been implicated in neural dysfunction and decrease in the rate of synthesis of ATP in invertebrates.

1.1.3.2 Fish[4]

Toxicity of phenols to fish generally increases with the degree of substitution on the carbon ring. The parasubstituted compounds are more toxic than *ortho* and *meta* compounds. Biochemical changes in fish exposed to phenols include

(i) increased energy utilization and catabolism of fatty acids resulting in increased oxygen consumption,

(ii) denaturation of enzymes such as succinic dehydrogenase, acid and alkaline phosphatases, fumarase and cytochrome oxidase, and

(iii) changes in blood chemistry.

1.1.3.3 Human Health

Instances of phenolic poisoning following the consumption of contaminated water or fish are extremely rare. Simple phenol is probably not carcinogenic or teratogenic but apparently induces mutations in various bacteria and yeast.[9] Although the same may be said about most chlorinated phenols, some of these substances, notably trichlorophenol, may be contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a known teratogen. Methylated derivatives of phenol are other carcinogenic an mutagenic, whereas the majority of nitrophenols are mutagenic but not carcinogenic.

A major aesthetic problem associated with phenolic compounds is their organoleptic properties in water and fish flesh. Threshold odor for 4-methylphenol is 55 μg/l.[10] The chlorinated phenols present problems in drinking water supplies because phenol is not removed efficiently by conventional water treatment process to form persistent odor-producing compounds. Estimated threshold fish flesh tainting concentrations for o-chlorophenol, p-chlorohenol, and 2,4-dichlorophenol range from 0.1 μg/l to 15 μg/l were reported.[12-14]

1.1.4 Behavior of Phenolic Compounds in the Environment

Phenol is fairly soluble in water and nonpolar solvents such as benzene and oils conducting waterbody as the pollutant reservoir. There are many process which can be occurred in natural water such as sorption, volatilization, oxidation and hydrolysis, photolysis and biotransformation[15-19], which some will be attached in Appendix 1.2.1-1.2.5.

Phenolic compounds are somewhat bactericidal but can be broken down aerobically under suitable conditions. Bacteria which are able to utilize phenols have been isolated from sewage, faeces and soil, and belong chiefly to the genera *Achromobacter, Vibrio, Micrococcus, Pseudomonas* and *Norcadia.*[20] The oxidative degradation of phenol by aerobic bacteria is shown in Appendix 1.2.6.

1.2 Water Pollution in Chiang Mai City

During the last decade, Chiang Mai city has been growing rapidly. This expansion of the municipality certainly creates many problems especially concerning water supply and water pollution caused by domestic and industrial wastewaters.

1.2.1 Domestic Wastewater

Gray water is commonly discharged into drainage systems and water bodies without any treatment. It is the major pollutant source in Chiang Mai area. In addition, part of garbages are also discarded directly to small canals and rivers in many places because of people's convenience and inadequate solid waste management systems of the municipality. As the result the river and canal water is extremely polluted and hygienic levels of the environment remain undesirable.[21]

The Office of National Environment Board (ONEB) of Thailand established the guidelines for domestic effluent standards in 1985 but they are not widely implemented. Chiang Mai city has recently implemented the standards for hotels, shopping complexes and condominiums. New buildings are therefore required to install gray water treatment facilities. In government offices, most of gray water are discharged into seepage pits. There have been three wastewater treatment plants existing namely Nakornphing hospital, McCormic hospital and Chiang Mai University. [22] Although the Chiang Mai Municipality should enforce big buildings to built gray water treatment facilities, there have been insufficient inspections of the system performances by the authorities concerned. It has been found that some facilities have not yet properly operated.

1.2.2 Industrial Wastewater

In Chiang Mai city, there have been 751 industries as classified by Ministry of Industry, e.g. garages, rice mills, noodle factories, printing shops, etc. Most of them are small scales. Approximate 70% of the industries generate no wastewater while the remainings discharge small amounts of effluent ranging from 10 to 40 m^3/d.

[21] Although there are regulations for the industrial wastewater treatment, small factories are always neglected by the authorities and they normally discharge their waste directly to the public sewers. In Chiang Mai city, the pollution loads from small-scale industries are reported to be insignificant comparing to domestic loads.

As present, three major water bodies passing through Chiang Mai city are the Ping river, the Mae Kha canal and the city moat. The Ping river is the biggest river passing through the city and is used as a water resource for Chiang Mai Waterworks. The Mae Kha canal is a small stream passing through a high population density area of the city center and flows into the Ping river at about 6 km south of the city center. The city moat is an artificial canal surrounding the old city area of Chiang Mai and was originally used for defense purpose. At present, it is used more or less as a drainage of wastewater and storm water.

1.2.3 General Water Characteristics of Chiang Mai

From the flora, fauna and chemical characteristics examined during 1984-85, watercourses in Chiang Mai city was characterized as in Figure 1.2. This classification shows the general pollution levels of the water bodies in Chiang Mai city.[21]

Around Chiang Mai area, there have been some problems on wastewater discharge to natural water resources. Many studies concerning water quality have been taken[23-24] but there have been no baseline data of phenolic compounds so far. Therefore, this study monitors the phenolic compounds in Chiang Mai area in order to assess the anthropogenic effect on the quality of water in terms of phenolic compounds as an indication of organic pollution.

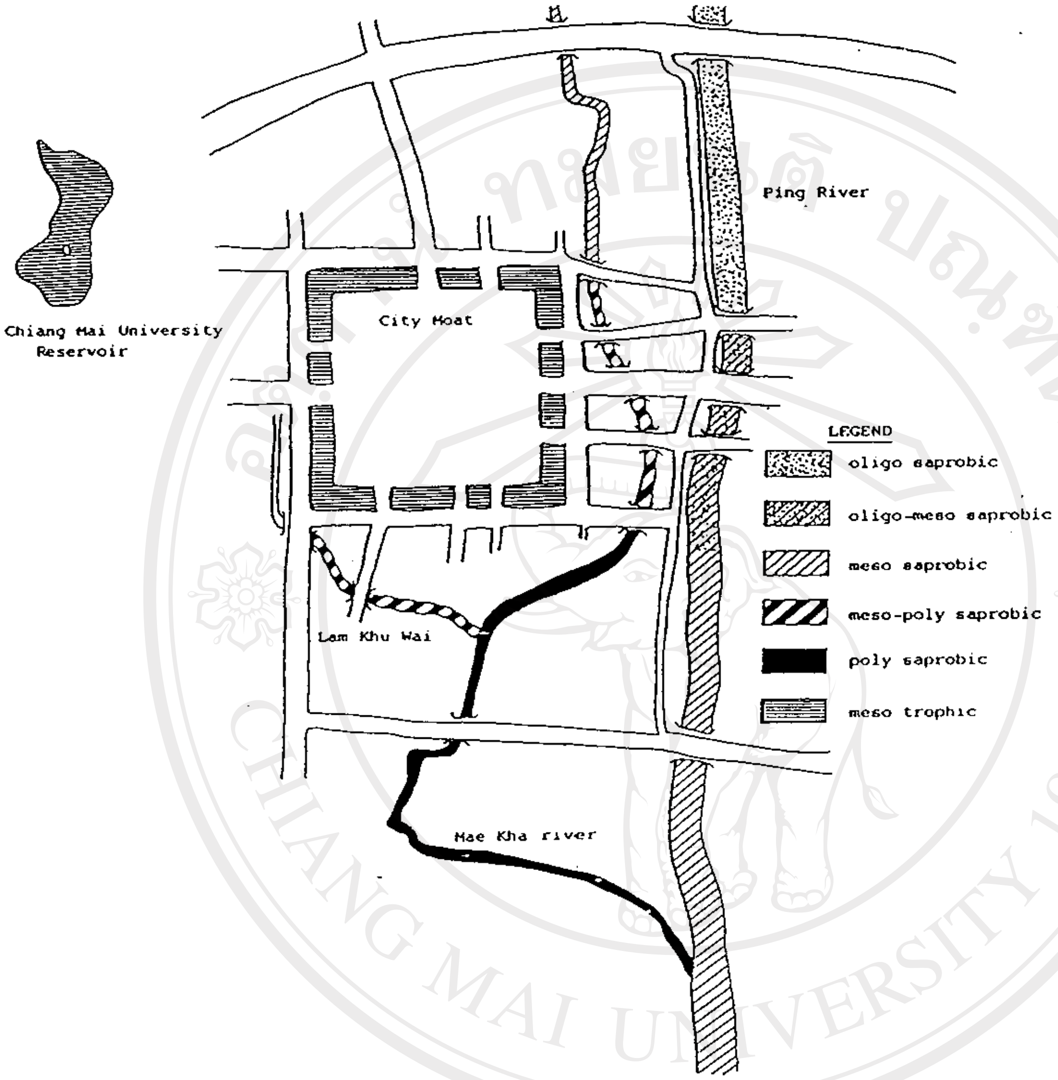


Figure 1.2: Characteristics of Watercourses in Chiang Mai[21]

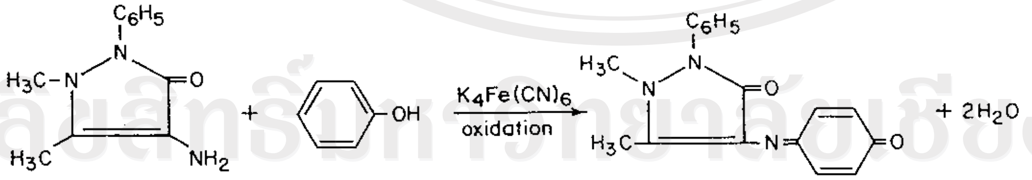
1.3 Determination of Phenolic Compounds

The determination of phenol and its derivatives in wastewater is realized to be important. Many analytical methods for such compounds have been developed. In addition to chromatographic methods, which are suitable for the selective determination of individual phenolic compounds[25], spectrophotometric methods are frequently employed for the determination of the sum of the compounds possessing phenolic moiety.[26, 27]

1.3.1 Spectrophotometric method

The earlier methods used for the determination of traces of phenolic compounds in water samples were usually spectrophotometric or colorimetric. Sensitive colorimetric methods have been developed for determining phenolic compounds in water based on the reactions with various chromogenic reagents including 4-aminoantipyrine in the presence of potassium ferricyanide (at various pHs), p-amino-*N,N*-diethylaniline in the presence of potassium ferricyanide (indophenol blue method), 2,6-dibromoquinone clorimide (Gibbs reagent), pyramidone (i.e. dimethylamino-antipyrine or aminopyrine) in the presence of potassium ferricyanide, nitroaniline diazotization, and 3-methyl-2-benzothiazoline hydrazone.

The commonly used method is based on oxidative coupling of phenols with 4-aminoantipyrine (4-AAP) in alkaline solution[28] which is shown as follow:



Phenols react with 4-aminoantipyrine at a pH of 10 ± 0.2 in the presence of potassium ferricyanide as an oxidizing agent to form a colored antipyrine dye. The compound is extracted from aqueous solution into chloroform and evaluated at 470 nm.[29] The German DIN and U.S. EPA include the method[30,37]

1.3.2 Flow Injection Analysis

With respect to the spectrophotometric method, there is a need for a rapid, reliable and sensitive procedure capability in including a wide variety of phenolic derivatives. Moreover, the susceptibility to interferences should be low. Manual[26-28,30-31], air segmented flow methods[32] and flow injection procedures[1,33], of 4-AAP reactions have been reported.

Flow injection procedures for the spectrophotometric determination of phenol by two different color reactions were developed.[1] The common 4-aminoantipyrine (4-AAP) reaction and the oxidative coupling of phenol with 3-methyl-2-benzothiazoline hydrazone (MBTH) are both readily applicable and permit the determination of phenol at a sampling rate of 60 h^{-1} and the relative standard deviation better than 3%. The result indicated that the oxidative coupling of phenol with 3-methyl-2-benzothiazoline hydrazone (MBTH) in acidic solution, was about four times higher than that of 4-AAP reaction and it was more universal in reactivity and exhibits higher response factor since it reacts with several *para*-substitued phenols. The detection limits were reported to be 12 and 30 μg/l of phenol, respectively.

Recent concerns with the introduction of the flow methods into the framework of German standard methods for water quality control stimulated the examination of the applicability of both spectrophotometric methods for the determination of phenolic compounds under flow-injection conditions using 4-AAP, to take place.[1]

1.3.3 Chromatographic Method

An isocratic HPLC method using 30 mM ammonium acetate pH 5.00-acetronitrile-methanol (56:34:10), NUCLEOSIL-5 C_{18} column, is able to determine ng/l of priority pollutant phenols in water.[34] Optimization of mobile phase composition of three common organic modifiers; methanol, acetronitrile and tetrahydrofuran for HPLC analysis, isocratic system, of eleven priority substituted phenols has been investigated.[25]

Simple gradient device for HPLC with variable volume and automatic regeneration used to separate 11 phenolic compounds was studied. It was reported that a solvent began with a composition of 10% methanol and 90% of water to an end composition of 40% methanol and 60% of water.[35] Better column stability could be obtained, when using different mobile phase containing as solvent A, 0.5% phosphoric acid in water and as solvent B, 0.5% phosphoric acid in methanol.[5]

Effect of cetyltrimethylammonium bromide on the separation of the eleven priority pollutant phenols and transient change in a cetyltrimethylammonium (CTAB) mobile phase produced by a sodium laurylsulphate solution plug permit the elution of hydrophobic pentachlorophenol in high-performance liquid chromatographic was performed.[36] For determination of individual phenolic compounds, flame ionization detector gas chromatographic (FIDGC) method is known to be applicable to the determination of the phenolic compounds. When analyzing unfamiliar samples for any or all of these compounds, confirmed identification by at least one additional qualitative technique, eg. electron capture detector gas chromatography (ECDGC) method, is usually required.[37]

Liquid chromatography or electrochemistry (LCEC) was optimized for the analysis of trace phenols in the 100 parts per trillion (pptr) to 500 parts per million (ppm) concentration range. For wastewater samples with phenol concentrations greater than 20 ppb, direct injection was satisfactory. Municipal drinking water used for beverage production was analyzed for trace phenols and chlorophenols in the 0.1-10 parts per billion (ppb) range using trace enrichment.[38]

Electrochemical concentration modulation (ECM) was used as a sample introduction technique in the correlation chromatographic (CC) trace determination of phenol in water. ECM-CC in combination with fluorescence detection is selective and sensitive enough to be used for the monitoring of the phenol concentration in river water at the draining points for drinking water production.[39]

1.3.4 Sample Preparation

Sample preparation in an analysis is usually required for two reasons: clean up and concentration. The sample matrix frequently give interferences in measurement step. In ultraviolet (UV) analysis, absorbing impurities may render the sample opaque. In gas and liquid chromatography, the life of column may be drastically shorten by impurities. In these cases, sample clean-up is mandatory.[40] In many instances, the analytical concentration falls below the sensitivity range of the analytical method chosen. Only with concentration can the analysis be brought in to a practical range.

Liquid-liquid extraction and/or steam distillation are the most commonly used method for isolating phenolic compounds from water samples.[28,31] Batch extractions are simple and straightforward procedures, but sometimes formation of emulsions can hinder phase separation. Moreover, poor recovery of the most hydrophilic phenols, which ultimately reflects on the sensitivity and precision of the analysis, is obtained by a single-step extraction procedure. This can be improved by repeating the extraction steps with several portions of organic solvents. But it is obtained at the expense of simplicity and analysis time, and the sample is more prone to contamination.[41] However this manual method using separatory funnels is inefficient, tedious and costly.[40]

Solid phase extraction (SPE) is attracting in increasing attention and constitutes an alternative to liquid-liquid extraction. This technology is a growing area of research in the development of environmental sample preparation. It is similar to low pressure liquid chromatography. Desorption of retained organic compounds can be carried out by elution with a suitable solvent. SPE is widely used for the trace enrichment of very dilute solutions such as natural waters, where large sample volumes may have to be processed, to yield concentrations of analyte sufficient for detection. Applicants of SPE technique in the form of cartridges have provided equivalent recoveries when compared to liquid-liquid extraction. More recently, advanced SPE technology has resulted in membrane filters or filter disks.

The SPE technique has been reviewed in a variety of areas such as extraction of organic compounds[41-43], on-line precolumn techniques[44] and sample preparation in drug analysis[45], Svoboda[46] reviewed the use of sorbents for the preconcentration of one or a few pesticides. Stability of various pesticides on SPE media have shown the equivalent or greater stability on solid phase extraction disks compared to their storage in water at 4°C, freezing the disk after pesticide loading is the most favorable storage option.[47]

For sample preparation of phenolic compounds, the reverse phase chromatography with non-polar sorbent like octadecyl bonded silica (C_{18}) was widely used. C_{18} extraction catridges have been included in several analytical schemes devoted to monitor phenolic compounds in water sampled from the environment. However the most hydrophilic phenolic compounds, such as phenol, 4-nitrophenol and others have a low tendency to be adsorbed on the C_{18} surface.[48]

Graphitized carbon black (GBC) reversible cartridge showed a far better extraction efficiency for the more highly water-soluble phenolic compounds compared with C_{18}.[49] The feasibility of extracting substituted phenolic compounds from aqueous solutions using supercritical CO_2 was reported with recoveries greater than 90%.[50] SPE using keto-derivatized poly (styrine-divinylbenzene) copolymer was reported the recoveries as 85-104%.[51] A micro-extraction technique, based on fibers coated with poly(dimethylsiloxane) was developed for the extraction of phenols from ground and wastewater.[52]

Direct injection, off-line and on-line trace enrichment and liquid-liquid extractions were examined as sample preparation schemes.[38] It was reported that the direct injection of wastewater samples was satisfactory with concentrations greater than 20 ppb. MacCrehan and Brown Thomas have investigated the purification of phenolic compounds in environmental samples by solid-phase extraction column.[53]

1.4 Purposes of the study

This study have been undertaken with the following objectives:

1) To determine total phenolic contents and individual phenolic compounds in the water samples collected from different sites in and around Chiang Mai area.

2) To find out if there an effect of anthropogenic activity on quality of water in terms of phenolics pollution.

3) To use the results as part of the water pollution assessment

4) To evaluate the appropriate analytical methods employed.