

**ANALYSIS OF POLYCHLORINATED BIPHENYLS IN SEWAGE SLUDGE  
USING SUPERCRITICAL CARBON DIOXIDE AND SUBCRITICAL  
WATER EXTRACTION**

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*Dedication to my beloved family,  
my fiancé and my friends...*

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## ABSTRACT

Extractions of polychlorinated biphenyls (PCBs) for sewage sludge are often time consuming and involved the use of large volume of organic solvents. In this study, a supercritical fluid carbon dioxide (SFCO<sub>2</sub>) extraction and subcritical water extraction (SWE) were developed with the aim of overcoming the disadvantages associated with the conventional Soxhlet extraction. The SFE conditions utilised extraction temperature of 60°C, extraction pressure of 200 bar, 15% methanol as co-solvent, extraction flow rate at 2.0 mL/min, 30 minute extraction and *n*-hexane as the analyte trapping solvent for PCBs extraction from sludge. A lab-made SWE extraction system was successfully developed for the dynamic SWE of PCBs from sludge samples. For the SWE, the optimized condition employed an extraction temperature of 250°C, extraction pressure of 50 bar, 1 min static followed by 10 min dynamic extraction and analyte collection in dichloromethane. Both the SFCO<sub>2</sub> extraction and SWE showed good PCB recoveries (50 to 99%) compared to Soxhlet extraction (52 to 75% PCB recoveries). Comparison of PCBs extracted with Soxhlet extraction revealed that SFCO<sub>2</sub> extraction and SWE to be fast, easy, and environmentally clean techniques. From the two extraction methods investigated, it was clear that the SFCO<sub>2</sub> technique was better in terms of recovery, good reproducibility and speed of analysis compared to the SWE method and was therefore chosen as the method of choice for the analysis of real sludge samples. The PCBs under study (PCB 28, 52, 101, 118, 138, 153 and 180) when analysed using gas chromatography electron capture detector gave low detection limit in the range of 0.016 to 0.048 µg/mL. The concentration of PCBs in sludge samples extracted using SFCO<sub>2</sub> were in the range of 31.2 to 82.0 µg/kg as compared to that using Soxhlet extraction with PCB levels from 20.2 to 60.1 µg/kg. The characterization of sludge samples from other aspects was also carried out. The sludge samples showed slightly acidic pH values ranging from 4.3 to 6.6. All samples were found to be chemically stable and the percentages of nutrient (nitrogen, phosphorus and potassium) were in the range of 0.059 to 0.98%, 0.05 to 0.15% and 0.12 to 0.16%, respectively. Heavy metal studies showed that metal content in municipal sewage sludge samples were below the United States Environmental Protection Agency limit.

## ABSTRAK

Pengekstrakan bifenil politerklorinat (PCBs) dalam enapcemar menggunakan masa pengekstrakan yang lama dan melibatkan isipadu pelarut yang banyak. Dalam kajian ini, pengekstrakan lampau genting karbon dioksida (SFCO<sub>2</sub>) dan pengekstrakan sub lampau genting air (SWE) telah dibangunkan dengan tujuan untuk mengatasi kelemahan pengekstrakan konvensional soxhlet. Parameter pengekstrakan bagi SFCO<sub>2</sub> suhu pengekstrakan pada 60°C, tekanan pengekstrakan pada 200 bar, 15% metanol sebagai pelarut kedua, kadar alir 2.0 mL/min, 30 minit masa pengekstrakan dan *n*-heksana digunakan sebagai larutan pemerangkap analit bagi pengekstrakan PCB di dalam enapcemar. Satu sistem pengekstrakan dinamik SWE telah berjaya dibangunkan di makmal. Bagi SWE, parameter yang dipilih untuk pengekstrakan PCB melibatkan suhu pengekstrakan pada 250°C, tekanan pengekstrakan pada 50 bar, 1 minit pengekstrakan statik diikuti dengan 10 minit pengekstrakan dinamik dan analit dikumpul dalam pelarut diklorometana. Kedua-dua kaedah SFCO<sub>2</sub> pengekstrakan dan SWE menunjukkan peratus pengembalian PCB yang baik (50 hingga 99%) berbanding pengekstrakan soxhlet (52 hingga 75%). Perbandingan pengekstrakan PCB dengan pengekstrakan soxhlet menunjukkan bahawa pengekstrakan SFCO<sub>2</sub> dan SWE adalah cepat, senang dan teknik yang mesra alam. Daripada dua pengekstrakan yang dikaji, kaedah pengekstrakan SFCO<sub>2</sub> adalah yang lebih baik berdasarkan peratusan pengembalian, keboleholungan yang baik serta masa analisis berbanding dengan pengekstrakan SWE dan seterusnya digunakan bagi analisis kandungan PCB di dalam sampel sebenar enapcemar. PCB yang dikaji (PCB 28, 52, 101, 118, 138 153 dan 180) dianalisis menggunakan gas kromatografi pengesan penangkap elektron memberikan had pengesanan yang rendah dalam julat antara 0.016 hingga 0.048 µg/mL. Kepekatan PCB di dalam enapcemar menggunakan pengekstrakan SFCO<sub>2</sub> adalah antara 31.2 hingga 82.0 µg/kg berbanding dengan pengekstrakan soxhlet yang memberikan aras PCB antara 20.2 hingga 60.1 µg/kg. Pencirian sampel enapcemar dari aspek lain juga dijalankan. Sampel enapcemar menunjukkan nilai pH adalah sedikit berasid (julat 4.3 hingga 6.6). Semua sampel enapcemar adalah stabil dari segi kimia dan peratus nutrien (nitrogen, phophorus dan kalium) adalah masing-masing 0.059 hingga 0.98%, 0.05 hingga 0.15% dan 0.12 hingga 0.16%. Kajian logam berat menunjukkan kandungan logam dalam sampel enapcemar adalah di bawah tahap yang dibenarkan oleh Agensi Kawalan Alam Sekitar Amerika Syarikat.

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**LIST OF SYMBOL**

Bp	-	Biphenyls
CE	-	Capillary electrophoresis
CO <sub>2</sub>	-	Carbon dioxide
DCM	-	Dichloromethane
ECD	-	Electron Capture Detector
GC	-	Gas Chromatography
HPLC	-	High Performance Liquid Chromatography
IS	-	Internal Standard
IWK	-	Indah Water Konsortium
LOD	-	Limit of Detection
mg/kg	-	milligram per kilogram
mL	-	millilitre
mL/min	-	millilitre per minute
MeOH	-	Methanol
°C	-	Celsius degree
ODS	-	Octadecylsilane
PCBs	-	Polychlorinated Biphenyls
PE	-	Population Equivalent
ppm	-	Part per million
RSD	-	Relative Standard Deviation
SF CO <sub>2</sub>	-	Supercritical Fluid Carbon Dioxide
SFE	-	Supercritical Fluid Extraction
SPE	-	Solid Phase Extraction
SWE	-	Subcritical Water Extraction
TKN	-	Total Kjeldahl Nitrogen
t <sub>r</sub>	-	Retention Time
USEPA	-	United State Environmental Protection Agency
WTP	-	Wastewater Treatment Plant
ε	-	Dielectric Constant

$\mu\text{L}$	-	microlitre
%	-	Percent
$P_c$	-	Critical Pressure,
$T_c$	-	Critical Temperature,
$T_p$	-	Triple point
$C_p$	-	Critical point.



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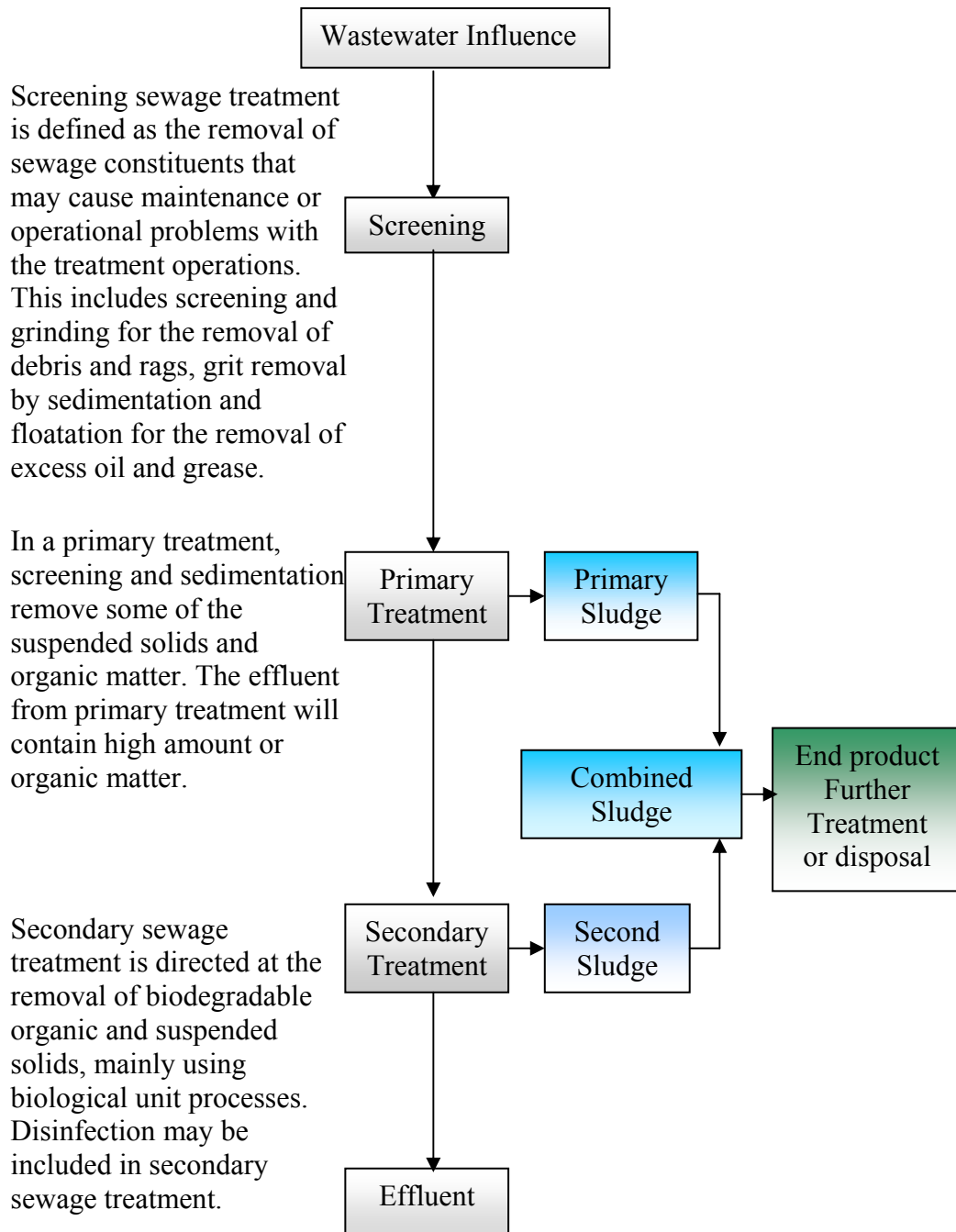
## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Municipal Sewage Sludge**

Municipal sewage sludge is the product of the municipal wastewater treatment plant (Figure 1.1) whereby it consists of the final accumulation of sewage sludges from urban wastewater treatment plants from agriculture, industries and also domestic waste. Its contents is mainly water (approximately 95 %), plus the presence of other substances, such as insoluble and soluble organic matter, nutrients, microorganisms, pathogens, metals, soluble salts, and other minerals (Torrey, 1979). The composition varies due to its function of the infrastructures of the respective residential areas, weather conditions and so on.

The rapid increment in municipal sewage sludge nowadays has brought a serious problem to the environment and has become a major problem in many countries in the world including Malaysia. This is all due to rapid population growth urbanization. Approximately 3 million cubic meters of sewage sludge is produced annually in Malaysia and the total cost of managing it was estimated at RM one billion (Kadir and Haniffa, 1998). By the year 2020, the volume is estimated to increase to 7 million cubic meters, which will require about double the KLCC twin tower to be filled or almost 1.4 million tanker trips to manage (Velayutham and Kadir, 1999).



**Figure 1.1** Flow chart of basic wastewater treatment process.

This solution of the problem is it has to be either disposed or recycled. The methods used to dispose off sludge include incineration and utilization on land in

agriculture, forestry or land reclamation (Hing *et al.*, 1998). However, due to the different composition of sludge caused by the environment, it can cause a problem of environmental relevance if there are substances that are toxic mixed into the sludge. Sludge application on soil is possible only when the concentrations of contaminants which pose a threat to the environment and human health are below permissible limits (EU, 2000). The disposal of sewage sludge on soils as a fertilizer for agriculture or as a regenerative for soil is the most attractive application since the sludges will act as a source of nutrients for crop production owing to their high content of organic matter (Hing *et al.*, 1998). Though an agricultural use of quality sludge is widely practiced in the United Kingdom and the European Union, but is rarely practiced in Malaysia.

However, this practice has the potential to create an environmental pollution problem since the heavy metal contents (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn), toxic organic compounds and pathogenic organisms are still present in the sewage sludge (EU, 2000). Pollution problems may arise if toxics are mobilized into the soil solution and are either taken up by plants or transported in to drainage waters. The pollutant may then enter the human food chain through the consumption of such plants or through the intake of contaminated waters. Nevertheless, the characterization and long term observation of organic contaminants in sludge have received little attention so far.

## **1.2 Criteria in Re-use and Disposal of Sludge**

Certain regulations have been made regarding the re-use and disposal of sewage sludge. Some of the more desirable materials in sludge are also needed to be monitored for the most beneficial reuse of the sludge. These parameters include:

- i. Water and plant nutrients (nitrogen, phosphorus and potassium)
- ii. Organic matter and non-toxic trace elements
- iii. Microorganisms

Furthermore, parameters such as organic content, toxic organics, nutrients, pathogens and hazardous metals have to be taken into account if they will be distributed for re-use (USEPA, 1984). Some of these hazardous elements are:

- i. Potentially toxic elements (eg: zinc, copper, nickel, cadmium, lead, mercury, chromium, arsenic and selenium).
- ii. Organic micro-pollutants (eg: linear alkylbenzene sulphonated (LAS), di (2-ethylhexy) phthalate (DEHP), nonylphenole (NP), polychlorinated biphenyls (PCBs) and others).
- iii. Pathogenic organisms

The above mentioned parameters, together with other important considerations need a careful attention when deciding on the best alternative for the reuse or disposal of sewage sludge.

### **1.3 Beneficial Uses of Sludge**

The benefit of using is that they can be recycled in soil for the application in farming practices, horticulture, land restoration and reclamation, landfill cover, forestry energy value and product.

#### **1.3.1 Farming Practices**

The agriculture use of sludge is linked to the fertilizing value of nitrogen and phosphorus. About 1 to 5 % of dry matter consist of phosphorous and nitrogen (Lasa *et*

*al.*, 1997). Other compounds present in agricultured sludge are potassium, sulphur, magnesium, sodium and elements such as boron, cobalt, and selenium.

Sewage sludge can improve soil quality (nutrients, pH balance, trace elements), give better physical characteristics (improves organic matter, water holding capacity, irrigation, stability and workability) and enhances biological activity (greater water retention and aeration stimulating root growth, increases worm and micro-organisms populations) Hing *et al.*, 1998. The net effect is improved soil quality and agricultural yields.

### **1.3.2 Horticulture**

Sludge can be thermally processed or composted using crop residues or municipal solid wastes, green or wood processing wastes. The products are aesthetically acceptable and suitable for soil conditioning and fertilizer applications in situations where a direct sludge application might not be acceptable or practical, such as in garden, public parks and highway verges (Stabnikova *et al.*, 2005). Even in Malaysia, the waste sludge was mixed with compost to cut the costs for gardening.

### **1.3.3 Land Restoration and Reclamation**

The surface of direct and disturbed land is often deficient in organic matter, nitrogen and phosphorus (Speir *et al.*, 2003). On some sites, where nutrient soil may not be available, cover materials are needed which can then be converted into suitable topsoil for sustaining plant growth. Sewage sludge contains the organic matter and fertilizer value needed to provide a stable medium for the site and also to help plant growth (Mantovi *et al.*, 2004).

### **1.3.4 Landfill Cover**

Sewage sludge can be used as daily and final cover for landfill sites, providing a consistent blanket that serves to reduce nuisance during ongoing operation and ultimately restore the filled site for a subsequent beneficial use (Skousen and Clinger, 1986; Hing *et al.*, 1998).

### **1.3.5 Forestry**

Some soils are more suitable for developing woodlands, especially in producing energy crops and wood products. Sludge improves tree growth by providing the appropriate nutrients to fertilize or improve forestry soils (Marx *et al.*, 1995; Hing *et al.*, 1998).

### **1.3.6 Energy Value**

Sludge has an organic content that can be transformed into or used as fuel. A combination of technologies such as digestion, drying and incineration makes this possible (Hing *et al.*, 1998). By sludge digestion, biogas is produced that consists mainly of methane, which can be used on site for other process purposes such as heating, filtration and etc. Alternatively, it is converted into electricity distribution through national grids. Biogas can also be converted into a renewable fuel and be used by Natural Gas Vehicles (NGV) buses, trucks and cars, which is adapted from fossil fuel (Wang *et al.*, 1997).

### **1.3.7 Alternative Product**

The physical and chemical properties have made sewage sludge suitable for a range of alternative products, although most of these are currently not economically viable. A number of products could be derived directly from sludge or as an admixture with other material. These include building materials such as bricks and cement (Hing *et al.*, 1998; Suzuki and Tanaka, 1997). Sludge can also be used as material for producing the watertight top layer of a landfill. Ash from incineration is a potential source of phosphorus for fertilizer manufacture or a potential building material such as blocks and aggregate or as a raw material in cement industry (Favoni *et al.*, 2005).

## **1.4 Organic Sludge Contaminants**

The presence of organic micro pollutants such as Polychlorinated Biphenyls in municipal sewage sludge is a major problem on account of risks associated with the agricultural use of the sludge. Sewage sludge contains many other organic contaminants such as organic halogen compounds (AOX), linear alkylbenzene sulphonated (LAS), di (2-ethylhexy) phthalate (DEHP), nonylphenole (NP) and nonylphenole ethoxylates (NPE) with 1 or 2 ethoxy groups, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-furan (PCDD/PCDFs) (Abad *et al.*, 2005). PCBs are the major contaminants frequently found in sewage sludge (Gibson *et al.*, 2005).

## **1.5 Polychlorinated Biphenyls**

PCBs are organic chemicals with characteristics similar to that of Dichlorodiphenyltrichloroethane (DDT). They are produced commercially by catalytic

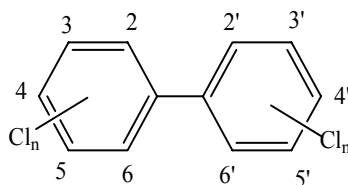


chlorination of biphenyls producing a complex mixture of multiple isomers with different degrees of chlorination yielding up to 209 products called congeners (Hutzinger *et al.*, 1974). PCB congeners with the same number of chlorine atoms are known as homologues, and the homologues with different chlorine positions are called isomers (Erickson, 1992). As a result of the widespread application and their stabilities, PCB has since become a major environmental concern worldwide.

### 1.5.1 Properties of PCBs

PCBs are made up of a biphenyl nucleus with 1–10 chlorine atoms having a chemical formula of  $C_{12}H_{10-n}Cl_n$ . The basic structure of PCB is shown in Figure 1.2. During PCBs manufacturing process, a mixture of compounds with molecular weight ranging from 188 to 439.7 g/mol is produced depending on the number of chlorine atoms attached to the biphenyl ring. Toxic congeners carry between 5 to 10 chlorine atoms, mostly in *para*- and *meta*-positions, however, the congener substituted at the 3,4-*ortho* positions are considered the most toxic (Hutzinger *et al.*, 1974).

PCBs are poorly soluble in water but extremely soluble in oils and fats. Their solubility in water decreases with the increase in the degree of chlorination. The solubility ranges from 6 ppm to 0.007 ppm for monochlorobiphenyl and octachlorobiphenyl respectively. Although decachlorobiphenyl has higher chlorine content, its solubility in water is twice that of octachlorobiphenyl. The solubility also varies among congeners which have the same number of chlorine atoms (Erickson, 1992).

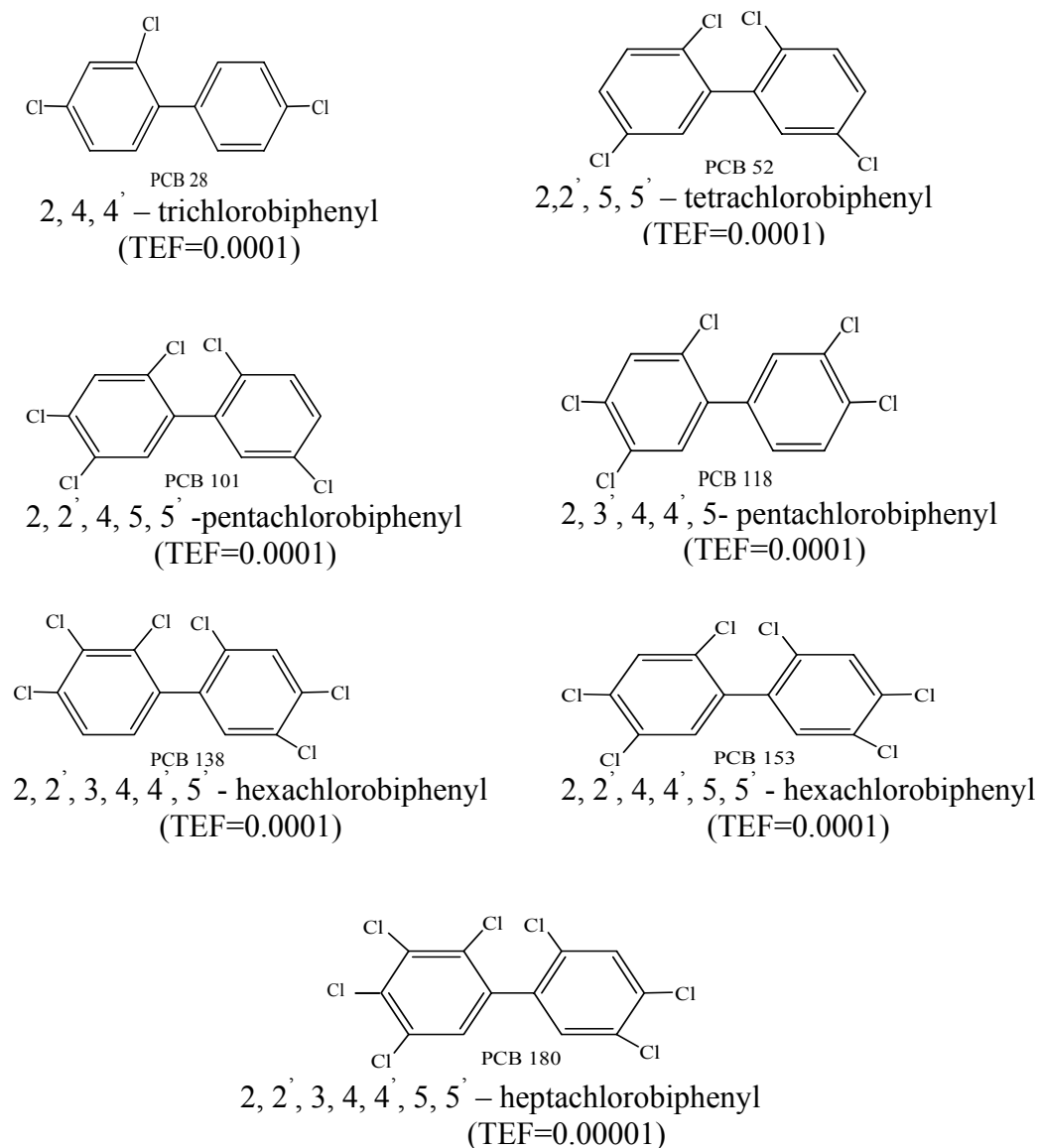


**Figure 1.2** General structural formula of PCBs

PCBs are marketed with respect to the percentage of their chlorine content (by weight) and are available under several trade names, for example Clophen (Bayer, Germany), Aroclor (Monsanto, USA), Kanechlor (Kanegafuchi, Japan), Santotherm (Mitsubishi, Japan), Phenoclor and Pyralene (Prodolec, France) (Hutzinger *et al.*, 1974). The PCB homologues according to IUPAC and their chlorine position atom in the biphenyl ring are show in Appendix A. Only seven out of the 209 PCBs listed as priority pollutants by the USEPA were considered in this study due to their common occurrence in sewage sludge. The target PCBs were PCB No 28, 52, 101, 118, 138, 153 and 180 (Figure 1.3).

### **1.5.2 Uses of PCBs**

For several decades, PCBs were used extensively in a wide range of industrial applications such as oil in transformers, dielectrics in capacitors, hydraulic fluids in hydraulic tools and equipment and heat exchange liquids. PCBs were also used as lubricants for turbines and pumps, in the formulation of cutting oils for metal treatment and to a lesser extent, in applications such as plasticizers, surface coatings, adhesives, pesticides, carbonless copy paper, inks, dyes, and waxes (Hutzinger *et al.*, 1974).



**Figure 1.3** Molecular structures and TEF values of the seven PCBs used in present study. (TEF: Toxic Equivalent Factor)

### 1.5.3 General Characteristics of Common PCBs

For determination and understanding of PCBs extractability, the most important parameters are PCBs solubility and boiling point (Hutzinger *et al.*, 1974). Some general characteristics of the PCBs according to the isomer group are listed below in Table 1.1

**Table 1.1** Physical properties of PCB homologues

PCB Isomer Group	Melting Point (°C)	Boiling Point (°C)	Vapour Pressure (Pa) at 25°C	Water Solubility at 25°C (g/m <sup>3</sup> )
Biphenyl	71	256	4.9	9.3
Monochlorobiphenyl	25-77.9	285	1.1	4.0
Dichlorobiphenyl	24.4-149	312	0.24	1.6
Trichlorobiphenyl	28.87	337	0.054	0.65
Tetrachlorobiphenyl	47-180	360	0.012	0.26
Pentachlorobiphenyl	76.5-124	381	$2.6 \times 10^{-3}$	0.099
Hexachlorobiphenyl	77-150	400	$5.8 \times 10^{-4}$	0.038
Heptachlorobiphenyl	122.4-149	417	$1.3 \times 10^{-4}$	0.014
Octachlorobiphenyl	159-162	432	$2.8 \times 10^{-5}$	$5.5 \times 10^{-3}$
Nonachlorobiphenyl	182.8-206	445	$6.3 \times 10^{-6}$	$2.0 \times 10^{-3}$
Decachlorobiphenyl	305.9	456	$1.4 \times 10^{-6}$	$7.6 \times 10^{-4}$

#### 1.5.4 Health and Environmental Effects of PCBs

As for the cause of the presence of PCBs in the environment, it can affect the productivity of phytoplankton and the composition of phytoplankton communities (Ross, 2004). Phytoplankton is the primary food source of all sea organisms and a major source of oxygen in the atmosphere. The transfer of PCBs to the food chain from phytoplankton to invertebrates, fish, and mammals can result a human exposure through the consumption of PCB-containing food source (Ross, 2004).

### 1.5.5 Legislative Measures

While it encourages the usage of sewage sludge, the EU Directive 86/278/EEC regulates its use to prevent harm to the environment, particularly on soil. Table 1.2 shows limit values for concentrations of organic compounds in sludge of different countries. In Germany, the fertilizer effects of sludges have to be taken into account according to the rules of the German Fertilizer Act and its respective ordinances when sewage sludge is to be used in agriculture activities (Hing *et al.*, 1998). It is prohibited to use sludge in fruit and vegetable cultivation, on grassland, in nature conservation areas, in forests and near water catchments wells especially in water protection areas. There are still no regulation on the usage of sewage sludge that stated the extract organic pollutant limits. This was due to the strict industrial regulations practices by the US that banned and restricted the use of hazardous organic compounds. The pollutant is detected infrequently in sludge and it present in only 5 % of sludge sample (Hing *et al.*, 1998).

**Table 1.2** Standard for concentration of organic contaminants in sewage sludge in different countries of the EU (EU, 2000).

	AOX mg/kg dw	DEHP mg/kg dw	LAS mg/kg dw	NP/NPE mg/kg dw	PAH mg/kg dw	PCB mg/kg dw	PCDD/F ng TEq/kg dw
EU 2000	500	100	2600	50	6 <sup>1</sup>	0.8 <sup>2</sup>	100
Denmark	-	50	1300	10	3 <sup>1</sup>	-	-
Sweden	-	-	-	50	3 <sup>3</sup>	0.4 <sup>4</sup>	-
Lower Austria	500	-	-	-	-	0.2 <sup>5</sup>	100
Germany	500	-	-	-	-	0.2 <sup>5</sup>	100

<sup>1</sup> Sum of acenaphthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo (b+j+k) fluoranthene, benzo (a) pyrene, benzo (ghi) perylene, indeno (1,2,3-c,d) pyrene.

<sup>2</sup> sums of 6 congeners PCB 28, 52, 101, 138, 153, 180.

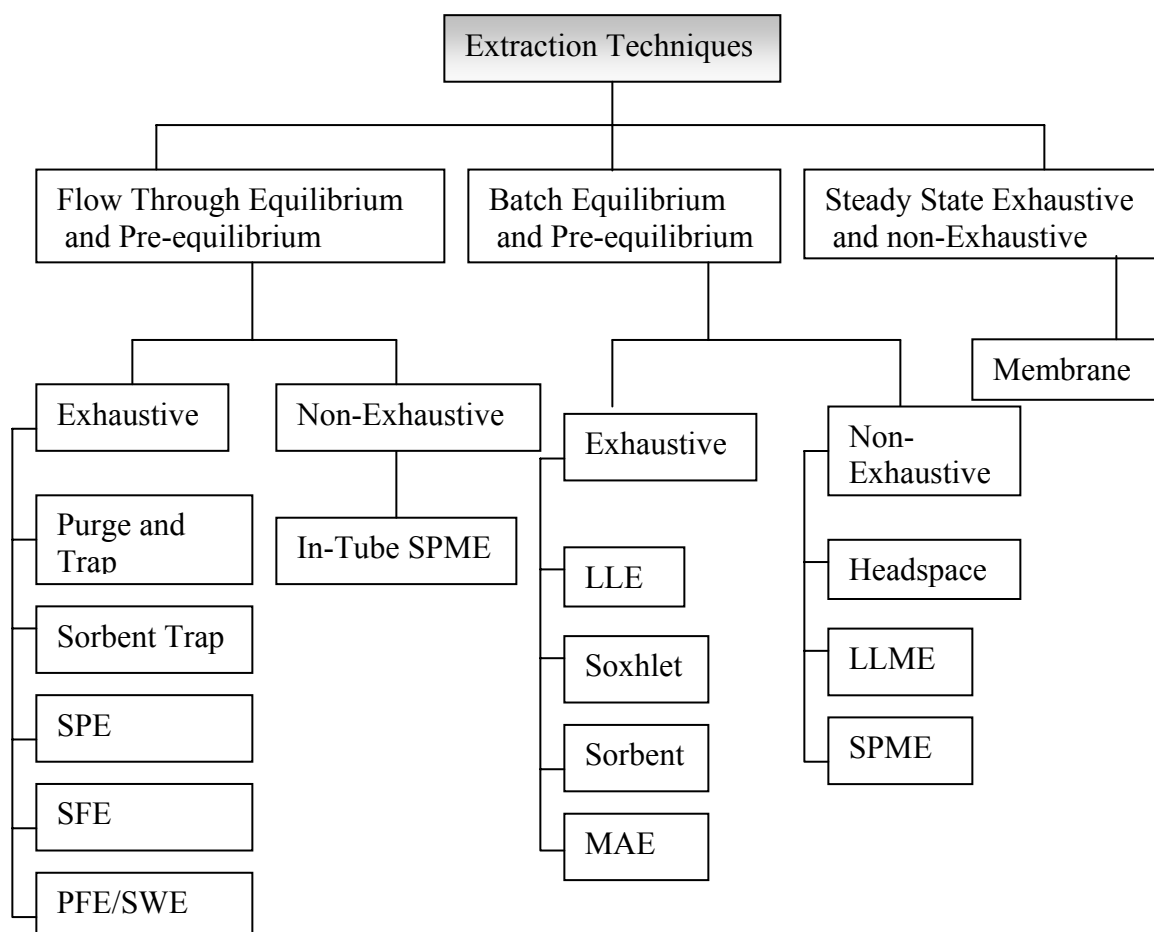
<sup>3</sup> sums of 6 compounds

<sup>4</sup> sums of 7 congeners

<sup>5</sup> each of the six congeners PCB 28, 52, 101, 138, 153, 180.

## 1.6 Extraction Technique

The sample preparation step in an analytical process typically consists of an extraction procedure that results in the isolation and enrichment of components of interest from a sample matrix (Marsin *et al.*, 2004). Increasing interest in sample preparation research has been generated by the introduction of non-traditional extraction technologies (Wang and Weller, 2006). In this context, the development of more rapid and efficient methodologies for the sample preparation of solid matrices is a significant trend in modern analytical chemistry (Fifield and Haines, 1997). A classification of extraction techniques that unifies the fundamental principles behind the different extraction approaches is shown in Figure 1.4.



**Figure 1.4** Classification of extraction techniques

SPE = Solid Phase Extraction, SFE = Supercritical Fluid Extraction, PFE = Pressurized Fluid Extraction, SWE = Subcritical Water Extraction, LLE = Liquid-Liquid Extraction, MAE = Microwave Assisted Extraction, LLME = Liquid-Liquid Microextraction

These technologies address the need for reduction of solvent use, fast and environment friendly techniques. These extraction techniques are frequently easier to operate but further optimization steps. Some of the common extraction procedures for PCBs are as follows:

### 1.6.1 Basic Theory of Extraction

The fundamental thermodynamic principle of chemical extraction techniques involved the distribution of analyte between the sample matrix and the extraction phase (Pawliszyn, 2001). When liquid is used as the extraction medium, the distribution constant ( $K_{es}$ ), can be explained by following equation;

$$K_{es} = a_e/a_s = C_e/C_s \quad (1.1)$$

$K_{es}$  defines the equilibrium conditions and ultimate enrichment factors achievable in the technique, where  $a_e$  and  $a_s$  are the activities of analytes in the extraction phase and matrix, which can be calculated using the appropriate concentrations. For solid extraction phase adsorption, the equilibria can be explained by following equation;

$$K_{es}^s = S_e/C_s \quad (1.2)$$

where  $S_e$  is the solid extraction phase surface concentration of adsorbed analytes and  $C_s$  is the concentration of the sample. The above relationship is similar to equation 1.1, with the exception that extraction phase concentration is replaced with the surface concentration. The  $S_e$  term in the numerator indicated that the sorbent surface area available for an adsorption must also be considered.

### 1.6.2 Soxhlet Extraction

Traditional methods such as Soxhlet extraction, which have been used for many decades are very time-consuming and require relatively large quantities of solvents (Castro and Ayuso, 1998). Soxhlet extraction which has been used for a long time is a standard technique and the main reference for evaluating the performance of other solid–liquid extraction methods. Soxhlet extraction is a general and well-established technique which surpasses in performance of other conventional extraction techniques except for the extraction of thermolabile compounds (Fifield and Haines, 1997).

In a conventional Soxhlet system, the sample is placed in a thimble-holder, and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask the solutes is separated from the solvent through distillation process. The solutes are left in the flask and fresh solvent passes back into the solid bed. The operation is repeated until complete extraction is achieved.

The use of soxhlet extraction to determine quantitatively the level of organic contaminants in the environments is well recognized. The solvents typically used in soxhlet extraction included dichloromethane and a combination of acetone and hexane (Castro 1998). The solvent chosen depends on both the solute and solid. Soxhlet extraction is useful in determining the total pollutant contents in soils, though it is not very efficient. Large volumes of both sample and solvent (300 mL) are necessary for traditional soxhlet extraction. The need to dispose of these solvents presents the potential for further environmental contamination and expense. Soxhlet extraction is also very time consuming. Up to 24 hours may be needed for full extraction and concentration of analytes that are tightly integrated into the soil (Sporring *et al.*, 2005).

Abrha *et al.*, (2000) have conducted a study of PCB recovery from spiked organic matrix between soxhlet extraction (SE) and accelerated solvent extraction (ASE).



They demonstrated that PCBs recovery were slightly higher using ASE compared to SE depending on the type and molecular weight of congener, and nature of matrix. Another work was reported by Crespo and Yusty (2005) who also studied the comparison of SFE and soxhlet extraction for the determination of PCBs in seaweed samples. They found that the PCB level in seaweed were in the range of 6.2 to 8.6 ng g<sup>-1</sup> and that concluded SFE method gave the advantage of detecting all PCBs compared to soxhlet extraction due to short time extraction, less solvent consumption and environmental friendly.

Hawthorne and co worker (2000) have studied the comparison of soxhlet, PLE, SFE and SWE for environmental solids. They demonstrated that extracts from soxhlet extraction were much darker in color and yielded more artifact peaks in the gas chromatography (GC)–mass spectrometry compared to other techniques. Sporing *et al.* (2005) performed a comprehensive comparison of classic soxhlet, ultrasonic, SFE, MAE and ASE for the determination of PCB in soil. They found that PCB recoveries for all techniques were similar compared to soxhlet extraction.

In Malaysia, limited research works on PCB analysis have been carried out. A study on the analyses of PCB in fish tissues have recently been reported (Mohd Sani and Syahidah, 2004). They found that the PCB levels in fish tissues were below the permissible limit.

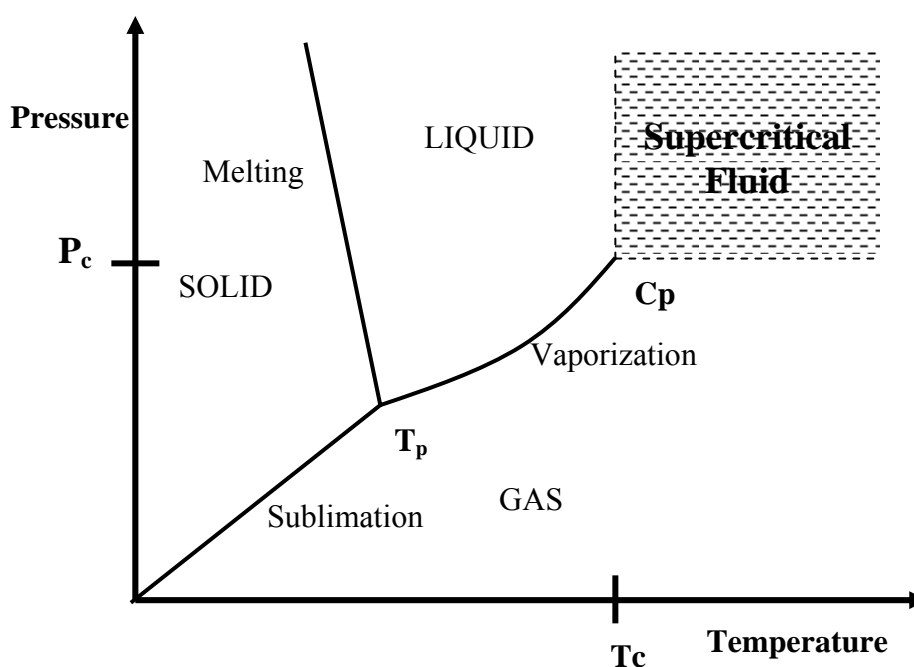
### **1.6.3 Supercritical Fluid Extraction**

Supercritical Fluid Extraction (SFE) has attracted researchers interest for the past 20 years, mainly for the extraction of solid samples because it offers short extraction times and minimum use of organic solvents (Chester *et al.*, 1992). Supercritical fluid is defined as a substance above its critical pressure,  $P_c$  and temperature,  $T_c$  resulting in a fluid that is more penetrative and higher solvating power (Castro *et al.*, 1994). CO<sub>2</sub> has

been popular because of its low cost, availability, safety and its suitable critical temperature (31.2°C) and pressure (72.8 atm; 1 atm = 101,325 Pa) (Smith, 1988).

Figure 1.5 shows a typical phase diagram in which for a pure substance the temperature and pressure regions of the substance occurs as a single phase (solid, liquid, gas, supercritical fluid). Such phases are bounded by curves indicating the coexistence of two phases, which determine the point of sublimation, melting and vaporization. The curves intersect at the triple point ( $T_p$ ) where solid, liquid and gaseous phase coexist in equilibrium (Smith, 1988).

The critical point is defined as a point in the phase diagram designated by a critical temperature ( $T_c$ ) and critical pressure ( $C_p$ ) above which no liquefaction will take place on raising the pressure and gas phase will be formed upon raising the temperature (Wenclawiak, 1992). The critical pressures of several compounds are given in Table 1.3. Supercritical fluids have physico-physical properties somewhat in between those of liquids and gases (Westwood, 1993). Compared to liquid solvent, supercritical fluids have lower viscosities and higher diffusivities, thus allowing more efficient mass transfer of solutes from the sample matrices.



**Figure 1.5** A typical phase diagram.  $P_c$  is critical pressure,  $T_c$  is critical temperature,  $T_p$  is the triple point and  $C_p$  is the critical point.

**Table 1.3** Critical points of typical solvents.**Table 1.3** Critical points of typical solvents

Solvent	Critical Temperature $T_c$ (°C)	Critical Pressure $P_c$ (bar)	Critical Density $\rho_c$ (g/mL)
Carbon Dioxide	31.1	72	0.448
Ammonia	132.4	112.5	0.235
Water	374.15	218.3	0.315
Nitrous Oxide	36.5	71.7	0.45
Xenon	16.6	57.6	0.118
Methane	-82.1	45.8	0.2
Ethane	32.28	48.1	0.203
Ethylene	9.21	49.7	0.218
Propane	96.67	41.9	0.217
Pentane	196.6	33.3	0.232
Methanol	240.5	78.9	0.272
Ethanol	243.0	63.0	0.276
Isopropanol	235.3	47.0	0.273

### 1.6.3.1 Literature Review on SFE

According to Camel (2001), there are several uses of supercritical fluids. The supercritical fluids can be applied for separation, reactions, analytical domain and more specifically chromatography and extraction purposes such as remediation. The development of SFE is due in part to the patent developed by Zosel in 1970 for

decaffeinating green beans with supercritical CO<sub>2</sub> (Taylor, 1996). This process involved soaking the beans in water and submerging them in supercritical CO<sub>2</sub>. In the mid 1980s, SFE was believed to be the new solid samples extraction method (Smith, 1999). Several researchers believed this technique to be more selective and cleaner than conventional organic solvent extraction method (Neude *et al.*, 1998 and Mannila *et al.*, 2002). Rosa and co workers (2005) have reviewed the supercritical technology in Brazil.

In practice, more than 90 % of all analytical SFE is performed with carbon dioxide (CO<sub>2</sub>) for several practical reasons. Apart from having relatively low critical pressure (74 bar) and temperature (32°C), CO<sub>2</sub> is non-poisonous, not flammable or explosive, chemically relatively inactive, available in high purity at relatively low cost, easily removed from the extract, and creates no environmental problems when used for analytical purposes. In the supercritical state, CO<sub>2</sub> has a polarity comparable to liquid pentane and therefore, best suited for lipophilic compounds (Smith, 1988).

The second most common choice of extraction fluid for analytical SFE is N<sub>2</sub>O. This fluid was considered better suited for polar compounds because of its permanent dipole moment (Hawthorne *et al.*, 1992). One of the applications where N<sub>2</sub>O has shown significant improvements when compared to CO<sub>2</sub> is for the extraction of polychlorinated dibenzodioxins from fly ash. Unfortunately, this fluid has been shown to cause violent explosions when it was used for samples contained high organic content. Thus, it is highly recommended to be used appropriately.

SFE has been used for several years for the decaffeination of coffee and tea, in food and seed oil extraction, and in the production of fine powders (Marr and Gamsee, 2000). Recently, the US Environmental Protection Agency (EPA) has adopted SFE as the official analytical method for the removal of total petroleum hydrocarbons and PAHs from environmental matrices (EPA Method 3560 and 3561).

Considerable researches on CO<sub>2</sub> extraction of PCBs from the environmental have been aimed towards the optimization of analytical scale extractions for the

characterization of PCBs in the environmental samples (Zhu and Lee, 2002). Most of the work was done on soils and sediment samples but very little effort in sewage sludges. Supercritical carbon dioxide extraction proved to be a valuable, fast, quantitative and partly selective extraction technique for determination polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs) and chlorinated pesticides (OCPs) in sewage sludge (Berset and Holzer, 1999). The most recent report on the extraction of organic contaminants from the environmental samples was by using dry ice CO<sub>2</sub> based supercritical fluid extraction (Chiu *et al.*, 2005).

An extraction utilizing supercritical fluids can be divided into three sequential steps, which are initial partitioning of the analyte from matrix active sites into the supercritical fluid, elution of analyte from the extraction cell and collection of the analyte in SFE trapping system (Hawthorne *et al.*, 1993). Zhu and Lee (2002) claimed that SFE employing CO<sub>2</sub> was easier to perform and was a feasible alternative extraction procedure for monitoring PCBs in pine needle samples. In a similar study, Nilson *et al.*, (2002) developed a simple selective SFE method for the determination of PCBs desorption behavior in sediment. In another study by Tong and Imagawa, (1995) they determined the optimum pressure, temperatures, time factors and co solvent composition that enabled maximum extraction of all PCBs.

Van Der Velde *et al.*, (1992) reported the comparison of SFE with two other techniques widely use for the extraction of PCBs and OCP in soil. SFE using CO<sub>2</sub> at 50°C and 20 MPa 10 min static followed by 20 min dynamic extraction with collection in *iso*-octane, was found to give the optimum extraction of PCBs from soils. Cheng *et al.*, (1997) studied about the remediation of PCBs contaminated soils and sediments by SFE using condition of 30 min, 40°C and 100 atm. Reutergardh *et al.*, (1998) used SF CO<sub>2</sub> to extract three planar (PCB 77, 169 and 126) and three mono-ortho (PCB 105,156 and 189) PCBs from three different soil types. Bowadt *et al.*, (1997) have developed a single SFE method for field extraction of PCBs in soils for use in combination with capillary GC-ECD without the need of clean up steps after extraction.

PCBs extraction from biological matrices (milk, fat, tissues, and food) is another important area of SFE. Ramos *et al.*, (2000) studied the distribution of PCBs in milk fat globules by sequential extraction of four different lipidic fractions from powdered full fat milk with supercritical carbon dioxide. In another research by Atuma *et al.*, (1998), they have determined non-ortho PCBs 77, 126 and 169 in a number of fish species from Swedish coastal environment, using either supercritical fluid extraction or the traditional liquid-liquid extraction followed by HPLC separation aqueous solution.

Cleanliness and safety are of the main advantages of SFE since it is non-toxic, poses no fire risk and does not introduce more hazardous waste into the matrix. SFE is also typically faster than conventional liquid extraction techniques since supercritical CO<sub>2</sub> is capable of penetrating the soil much faster than liquids (Chester, *et al.*, 1996; Taylor, 1996; Wenclawiak, 1992). Finally, SFE provides selectivity and it can remove a wide range of contaminants such as PCBs, PAHs and heavy metals without extracting integral components of the soil such as humic acid (Castro and Carmona, 2000). SFE also has several disadvantages that impede its application in the field, especially the cost of instrument.

### **1.6.3.2 Basic Mechanism of SFE Process**

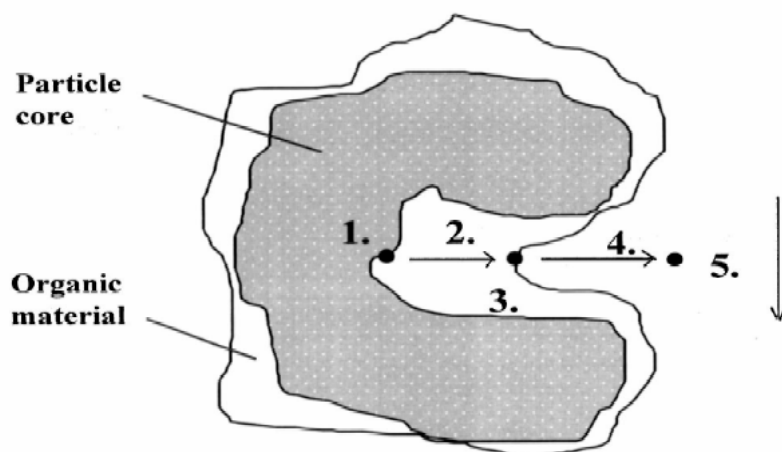
The factors that can influence the mechanism of an SFE process can be grouped into three categories (Chester *et al.*, 1992). Firstly, the thermodynamic factor such as the solubility of the extraction. The second factor is kinetic that include the effective diffusion of the solute and the desorption rate of solute from matrix surface and finally the influence of solid matrix on diffusion and the interaction of the solute within the solid matrix. Several parameters such as oven temperature, pressure, composition of modifier, CO<sub>2</sub> flow-rate, solvent trapping and extraction duration time are some of the factors which affect the process (Burford *et al.*, 1994; Hawthorne *et al.*, 1993).

Reutergardh *et al.*, (1998) reported that the temperature increment from 40°C to 100°C at a constant pressure had negligible effect on the recovery of all congeners. While Hawthorne and Miller (1994) found that by increasing the temperature of supercritical fluid CO<sub>2</sub> the recovery of a range of organic pollutants would be enhanced. These researchers extracted PAHs and PCBs from soil using supercritical fluid CO<sub>2</sub> within a range of temperature up to 350°C. The recovery increased as the temperature rose but leveled off once the threshold of 200°C had been reached.

In a research, Langenfeld *et al.*, (1993) has proven that the extraction temperature was directly proportional to PCBs recoveries from river sediments. A temperature increment from 50°C to 100°C increased the recovery of small molecule weight PCBs but not higher molecular weight. A further increment to 200°C has drastically increased the PCBs recovery. An alternative to the hot-ball model is the kinetic model developed in 1993 by Pawliszyn for SFE, which is based on the mass transfer kinetics and chromatographic elution (Pawliszyn, 1993). The matrix is simplified as the organic layers on an impermeable core where the analyte is adsorbed onto the core surface (Figure 1.6). The extraction process is assumed to consist of several basic steps:

- (1) Desorption from the core surface
- (2) Diffusion through organic matrix
- (3) Solvation in the supercritical fluid at the matrix–fluid interface
- (4) Diffusion through static fluid in porous matrix
- (5) Transport through interstitial pores by flowing bulk of fluid in order to leave the extraction vessel

The kinetic model suggested that desorption kinetics of the analyte can control the extraction rate, especially if the analytes are distributed between the sorption sites of various activation energy (real world samples).



**Figure 1.6** Schematic representations of the individual steps in a supercritical fluid extraction process (Pawliszyn, 1993).

### 1.6.3.3 Analyte Solvent Trapping

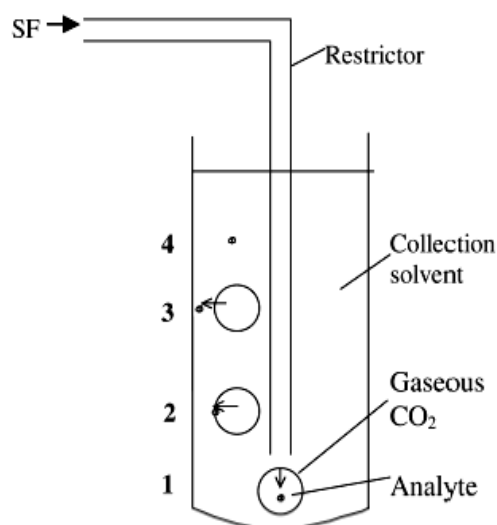
Apart from the extraction process, the single most important process in SFE is the efficient trapping of extracted material. Two different approaches are commonly used for off-line SFE, liquid solvent collection and solid phase trapping (Castro *et al.*, 1994). Both systems have their advantages and disadvantages with respect to ease of handling. Collection in a solvent is most commonly achieved by keeping the restrictor outlet immersed into a vessel containing a small volume of an organic solvent, such as methanol, hexane or acetone. Two common approaches have been used. In the first approach, the end of the flow restrictor is placed directly into the collection solvent, and the CO<sub>2</sub>-analytes mixture is depressurized directly in contact with the solvent. In the second approach, the CO<sub>2</sub>-analytes effluent is first depressurized to the gas phase in a glass transfer tube before contacting the solvent (Castro *et al.*, 1994).

As shown in Figure 1.7, the analytes undergoes four major steps during the collection process. Firstly the analyte existed from the supercritical fluid through the restrictor tube. It was then diffused through the gas bubble to the gas-liquid interphase.



After the analyte has diffused completely, it is then solvated into the liquid solvent phase. At last the analyte maintained its stability in the solvent (Turner *et al.*, 2002).

The first step implies that the analyte should not adsorb to the inside of the restrictor, or by any other means reside inside the restrictor. Ideally, the entire pressure drop is at the outer tip of the restrictor, this means that solutes should have full solubility in the SF all the way out to the tip. The second step is controlled by the diffusion constant of the analyte in the gas phase. Smaller bubble sizes results in shorter average diffusion paths, which means that the analytes will reach the gas–liquid interface faster. The third step, solvation of the analytes into the solvent, is naturally mostly determined by the solvent strength of the collection solvent. A good match of the solubility parameters of solvent and target analytes is an important parameter in analyte collection, which also has been a demonstrated using simple thermodynamic model. In the fourth, part of analyte collection the collected analytes should be maintained in the collection vial during the whole extraction procedure until the samples are taken for further analysis.



**Figure 1.7** Schematic of solvent collection, showing the four main steps of the collection procedure: (1) exit (2) diffusion (3) solvation (4) maintained stability (Turner *et al.*, 2002).

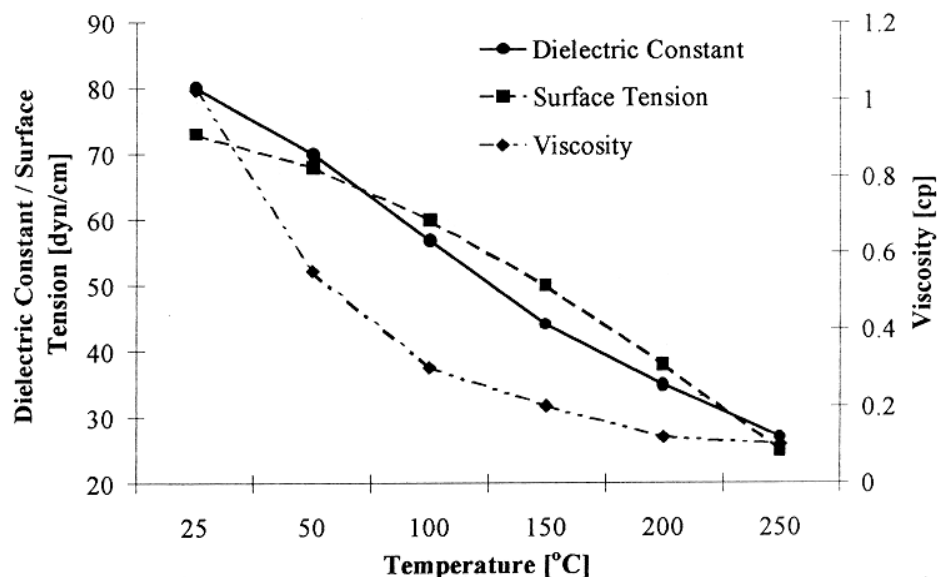
#### 1.6.4 Subcritical Water Extraction

Like CO<sub>2</sub>, water is an “environmental-friendly solvent” and has the additional advantages of being readily available, non-toxic, and cheap. Water is a unique solvent because of its highly hydrogen bonded structure, and at room temperature it has a disproportionately high boiling point for its mass, a high dielectric constant and high polarity. Subcritical water extraction (SWE), also known as hot water extraction, pressurized (Hot) water extraction, high temperature water extraction, superheated water extraction or hot liquid water extraction, is an emerging technique based on the use of water as an extraction solvent at temperatures between 100 °C and 374 °C (critical point of water, 374 °C and 22 MPa) and at a pressure which is high enough to maintain in the liquid state (Yang *et al.*, 1998).

Water is a unique solvent because of its higher hydrogen bonded structure. In addition, ambient temperature it has a disproportionately high boiling point for its mass, a high dielectric constant and polarity. However, when water is heated, its properties change markedly as the hydrogen bonded lattice is disrupted due to an increasing in thermal motion (Meyer, 1993). Pure water at ambient temperature and pressure has electric constant,  $\epsilon = 79$  however, when the temperature is increased to 250 °C at a pressure of 5 MPa (necessary to maintain the liquid state) a significant reduction of electric constant value ( $\epsilon = 27$ ) (Meyer, 1993). This value is similar to that of ethanol at 25 °C and 0.1 MPa and consequently, low enough to dissolve many compounds of intermediate or low polarity (Yang *et al.*, 1997).

The polarity of water can be controlled over a similar range by simply heating pure water with enough pressure to maintain the liquid state (Figure 1.7). While water at ambient conditions has very high surface tension and fairly high viscosity, a high temperature will reduce surface tension and viscosity (Ramos *et al.*, 2002). Heating water at 250 °C reduces its surface tension about the same as solvent programming to 100 % methanol or acetonitrile (as in liquid chromatography) and reduces the viscosity of water even more than solvent programming to 100 % methanol or acetonitrile. Thus,

the mobile phase polarity, surface tension and viscosity can be controlled by simply heating pure water (in the liquid state) over the same ranges achieved by conventional solvent programming to 100 % methanol or acetonitrile (Smith, 1988).



**Figure 1.8** Temperature influence on physical properties of water

#### 1.6.4.1 Literature Review on SWE

When water is heated at low temperature, inert or dipole bonding between analytes and matrices is broken whereas a high temperature is required to break Van Der Waals forces and even higher temperatures are needed to break  $\pi$ - $\pi$  electronic interactions. Yang *et al.*, (1998) examined the solubility of a number of PAHs and found marked changes in solubility as the temperature increased. The high degradation efficiency achieved using metal additives in water at reasonable temperatures and pressure demonstrates the potential for subcritical water dechlorination of PCBs in paint scrapings and potentially in other solid and liquid wastes (Kubatova *et al.*, 2003). Richter *et al.*, (2003) have studied the determination of pesticides in soil using

continuous subcritical water extraction and all pesticides can be screened depending on their polarity.

Ruziyati and Smith, (2005) have studied an on-line coupled superheated water extraction and superheated water chromatography. Hartonens *et al.*, (1997) reported good recovery of PCBs was achieved with the tenax trap, solid phase (florisil) and liquid collection PCBs were recovered with higher efficiency than soxhlet extraction. Another work by Kubatova *et al.*, (2001) showed the comparison of subcritical water and organic solvents for extracting kava lactones from kava root. About 40 minutes of subcritical water extraction yielded essentially the same recoveries of lactones employing 18 hour of sonication.

SWE can efficiently elute both polar and non-polar solutes from normal and reversed phase packing based on the lower polarity and surface. Hawthorne *et al.*, (1998) reported on a simple method for estimating PCBs concentrated on soils and sediments using subcritical water extraction coupled with solid phase membrane extraction. Konda *et al.*, (2002) employed subcritical water extraction to evaluate the desorption behavior of organic pesticides in soil.

Lamm and Yang, (2003) reported the off line coupling of subcritical water extraction with subcritical water chromatography via a sorbent trap and thermal desorption. They demonstrated that the off-line coupling technique was efficient and successful. The extract of anilines and phenols in sand has yielded an approximately 80 % recovery. Hinz *et al.*, (2000) studied on the remediation of a nonachloro biphenyl congener with zero-valent iron in subcritical water. Pross *et al.*, (2000) conducted an extraction of PCBs with supercritical fluid carbon dioxide, sulfur hexafluoride and subcritical water. CO<sub>2</sub> and water were found to be suitable for the quantitative extraction of all PCBs. However, water was found to be the best solvent because it is cheap, good availability and environmental safety.

In most of the extraction studies, distilled or deionised water has usually been used alone as the solvent in either static or dynamic extraction modes. Sometimes it is degassed or flushed with nitrogen to remove oxygen to prevent oxidative side reactions. pH control has been employed to enhance the extraction of selected analytes. For example, Crescenzi *et al.*, (2000) found improved extraction efficiency for a range of polar and medium polar pesticides, if a phosphate buffer at pH 7.5 was employed as the superheated eluent and this condition could release the sequestered pesticides from aged soils. In other studies, Pérez and Castro, (2000) added dodecyl sulfate (SDS) as a micellar agent to the extraction water to enhance the extractability of PAHs and thereby reduce the extraction temperature and time required.

The basic components for static SWE are normally used for continuous SWE. After filling the extraction cell with the sample, the cell is placed in a heated oven. An experiment typically starts with pumping the water at selected flow rate until the selected pressure for SWE is reached. The extracted analytes are collected in a solvent or using other collection technique.

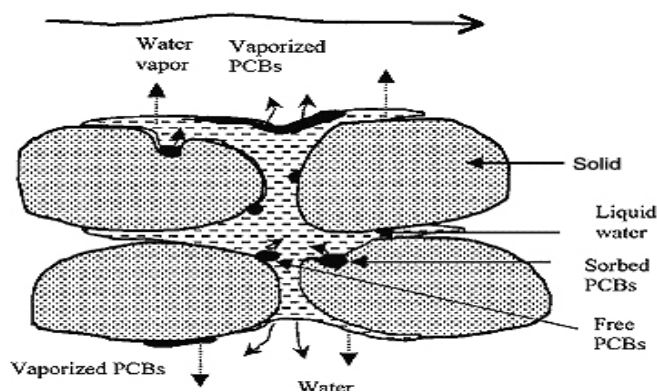
#### **1.6.4.2 Basic Mechanism of SWE Process**

The removal of PCBs from a solid by SWE is complicated, (Pingkuan *et al.*, 2002) and it involves at least three processes (Figure 1.8) which are;

- (i) Heat transfer,
- (ii) Water phase change and transport and
- (iii) PCB phase change and transport among solid and liquid water.

These processes take place simultaneously and are strongly coupled. Pingkuan and co-worker were assuming that the PCBs form a continuous type of liquid film along the water-steam and soil-steam interfaces after start-up of water evaporation by heating.

Second step, they were assumed that the liquid PCB-water interactions resulting after the start-up of water evaporation can be neglected. Then, mass transfer between the liquid PCB and steam phase then takes place through a boundary layer that is a part of the steam phase, the driving force being the difference in the vapor-phase PCB concentration at the liquid PCB interface and in the bulk steam flow. Finally they were assumed that the mass-transfer processes are approximated by a first-order kinetic expression and that the steam behaves as an ideal gas phase.



**Figure 1.9** Conceptual diagrams for interphase mass transfer of PCBs (Pingkuan *et al.*, 2002).

## 1.7 Extract Clean-Up

The determination of PCBs in soil, sediment and sludge samples require a good clean-up, while aqueous samples need concentration due to their low concentration levels. This is due to PCBs which are present in sludge in the range of ng/g or below (Folch *et al.*, 1996; Preira and Kuch, 2005). Two most commonly used techniques for sample clean up are the conventional column chromatography and solid phase extraction (SPE) utilising disposable cartridges.

Silica gel is often used in column chromatography for the separation of analytes from interfering compounds of a different chemical polarity (Jang and Li, 2001). It is used deactivated, after heating to 130°C to 160°C. Generally conventional column chromatography need larger amount of adsorbent and larger volume elution solvent, but with a greater clean-up capability (Martinez *et al.*, 2005). In the standard column clean-up protocol, the column is packed with the required amount of adsorbent, topped with a water adsorbent and then loaded with the sample to be analyzed. Elution of the analytes is accomplished with suitable solvent that leaves the interfering compounds on the column and the eluate concentrated off using nitrogen gas.

In a study employing silica gel for the clean-up of PCBs from sewage sludge, it was found that the use of silica gel to clean up extract was highly suitable for PCB determination (Martinez *et al.*, 2005; Jang and Li, 2001). The silica gel column chromatography method was also used for the clean-up of sludge samples after extraction by Soxhlet. The same study compared the efficiency of the column to that of SPE cartridges. It was found that the silica column yielded better results in terms of lesser interfering peaks but this technique needed larger volume of solvent as compared to SPE cartridge (Folch *et al.*, 1996).

SPE cartridge is a popular technique for pre concentrate and clean up matrices from samples. The cartridge column protocol uses SPE cartridges packed with 1 g or 2 g of adsorbent. The SPE cartridge is placed onto vacuum manifold and the cartridge is solvent washed immediately prior to use. After that, the sample extracts are loaded onto the cartridges, which are then eluted with a suitable solvent. The collected eluate may need further preconcentration prior to analysis.

## 1.8 Instrumental Analysis

Various instrumental analytical procedures have been employed for the determination of PCBs. Gas chromatography (Alvarado *et al.*, 1997), high performance liquid chromatography and capillary electrophoresis are the techniques most commonly used. The most widely used gas chromatographic detector for PCBs is the electron capture detector (ECD) (Bowardt and Johansson, 1994, Booij *et al.*, 1998, Rissato *et al.*, 2005). ECD provides an excellent linear response, sensitivity and reliability. However, the separation of all PCBs remains quite a challenge, even with high resolution capillary GC.

Capillary electrophoresis (CE) methods including capillary zone electrophoresis (CZE) have recently emerged as the most efficient methods available for the separation of PCBs components in a mixture. Electrokinetic chromatography with cyclodextrin derivatives (CD-EKC) was used to achieve the rapid enantiomeric separation of PCBs by electrokinetic chromatography using mixtures of neutral and charged cyclodextrin derivatives (Garcia *et al.*, 2001). In the study reported by Garcia *et al.*, (2001), thirteen out of the nineteen chiral PCBs which were stable at room temperature were individually separated into their two enantiomers by using 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.5) containing carboxymethylated  $\gamma$ -cyclodextrin (CM- $\gamma$ -CD) as pseudostationary phase.

Krup *et al.*, (1977) studied the analysis of PCBs by high-performance liquid chromatography (HPLC) and capillary gas-liquid chromatography. A method to separate PCBs by using HPLC was developed by Lundgren *et al.*, (2002). The HPLC column was packed with Amoco PX-21 activated carbon dispersed on octadecylsilane (ODS). The separation was carried out by gradient elution with *n*-hexane, dichloromethane and toluene in the forward direction followed by reversed elution with toluene. The developed immunosensor assay was able to detect PCB congeners in the range of concentrations usually found in the real matrices (Přibý *et al.*, 2006).



## **1.9 Problem Statement**

As municipal sewage sludge and waste has become a major issue in Malaysia, proactive action has to be taken to either dispose or recycle it safely. To determine the organic toxicity of sludge, soxhlet extraction technique has usually been employed for PCBs determination. This technique is often time-consuming and requires large volumes of organic solvent. The use of large volumes of extracting solvents adds to the additional cost associated with purchasing and disposal of toxic solvents. For these reasons, alternative extraction strategies are much needed especially those involving supercritical fluid CO<sub>2</sub> and subcritical water extraction for determining PCBs in sludge.

This study is therefore undertaken to address the above problems by developing a suitable extraction and instrumental techniques for the analysis of selected PCBs in sludge sample.

## **1.10 Objectives of Research**

The objectives for this research are as follows;

- (i) To develop extraction strategies of PCBs from sludge samples using supercritical fluid CO<sub>2</sub> and subcritical water as extractants.
- (ii) To characterize sewage sludge from other aspects such as pH, total vapor and solid, nutrient content (Nitrogen, Phosphorus, Potassium) and heavy metals.
- (iii) To determine PCB levels in real municipal sewage sludge.

### **1.11 Scope of Research**

The scope for this research encompasses the analysis of seven selected PCBs commonly found in sewage sludge. The PCBs are extracted using SFE and SWE followed by instrumental technique using GC-ECD. Real sludge samples are obtained from Indah Water Konsortium (IWK) treatment plants in Johor.