

**COMPARATIVE STUDIES OF SUBCRITICAL WATER EXTRACTION
OF POLYCYCLIC AROMATIC HYDROCARBONS
IN MUNICIPAL SEWAGE SLUDGE**

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ABSTRACT

The quantitative determination of organic contaminants in sewage sludge has received little attention so far, as compared to the voluminous amount of information that is available for the analysis of inorganic compounds in sludge. Many organic chemicals, termed as 'priority pollutants' due to their potentially toxic effects, are known to occur in sludge. In the specific context of wastewater and sewage sludge, polycyclic aromatic hydrocarbons (PAHs) are generally regarded among the most critical in terms of toxicity. Land application of sludge as fertilizers is a way of disposal and recycling of sludge. However, public concern has arisen due to the fact that organic contaminants in sludge may ultimately enter the food chain. Hence the need to analyse the organic contaminants such as PAHs in sludge. Therefore, in this study, subcritical water extraction (SWE) was utilised as a viable extraction method for the analysis of PAHs from municipal sludge samples. Various extraction procedures, such as pure SWE, surfactant-modified SWE and SWE coupled with membrane discs were attempted. Several factors were investigated for the pure SWE procedure coupled with SPE extract clean-up, which yielded the best recovery in comparison with the other methods. Recovery studies of PAHs from spiked sludge samples were performed using gas chromatography with flame ionisation detection. Although a recovery range of 38.84 – 56.06 % was obtained using the above mentioned method, the results indicated the usefulness of the technique as an alternative to Soxhlet extraction for the analysis of PAHs in sludge samples.

ABSTRAK

Penentuan kuantitatif pencemar organik dalam sampel enapcemar kurang mendapat perhatian, berbanding dengan maklumat yang sedia ada untuk analisis pencemar tak organik dalam enapcemar. Terdapat banyak pencemar organik, diistilahkan sebagai pencemar utama akibat daripada kesan toksik bahan tersebut yang wujud dalam enapcemar. Dalam konteks spesifik air sisa dan enapcemar, hidrokarbon aromatik polisiklik (PAH) merupakan salah satu pencemar yang paling kritikal dari segi ketoksikannya. Aplikasi enapcemar ke atas tanah sebagai baja adalah salah satu cara pelupusan dan penggunaan semula enapcemar. Namun, kebimbangan orang ramai telah timbul kerana pencemar organik dalam enapcemar ini akhirnya akan masuk ke dalam rantai makanan. Oleh itu, adalah perlu untuk menganalisis pencemar organik seperti PAH. Dalam kajian ini, pengekstrakan air sub-genting (*subcritical water extraction*, SWE) telah digunakan sebagai kaedah pengekstrakan untuk PAH dari sampel enapcemar. Beberapa prosedur pengekstrakan, seperti SWE tulen, SWE yang diubahsuai dengan surfaktan dan SWE yang diganding dengan cakera membran telah dicuba. Beberapa faktor telah dikaji untuk prosedur SWE tulen dengan pembersihan ekstrak SPE, iaitu kaedah yang telah memberikan hasil pengembalian analit yang terbaik berbanding dengan kaedah-kaedah yang lain. Kajian pengembalian semula analit telah dijalankan dengan sampel pakuan menggunakan teknik kromatografi gas dengan pengesanan pengionan nyala. Walaupun pengembalian analit dalam julat 38.84 – 56.06 % telah diperolehi dengan menggunakan kaedah di atas, keputusan ini mencadangkan bahawa teknik ini boleh digunakan sebagai alternatif kepada Soxhlet untuk analisis PAH dalam sampel enapcemar.

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LIST OF SYMBOLS/ABBREVIATIONS/NOTATIONS/TERMS

SWE	-	<i>Subcritical Water Extraction</i>
PAH	-	Polycyclic Aromatic Hydrocarbons
PCB	-	Polychlorinated Biphenyls
OCP	-	Chlorinated Pesticides
US EPA	-	United States Environmental Protection Agency
PFE	-	Pressurized Fluid Extraction
ASE	-	Accelerated Solvent Extraction
SPE	-	Solid Phase Extraction
SPME	-	Solid Phase Microextraction
FID	-	Flame Ionization Detector
CD	-	Cyclodextrin
SFE	-	Supercritical Fluid Extraction
CHClF ₂	-	chlorodifluoromethane
N ₂ O	-	nitrous oxide
CO ₂	-	carbon dioxide
ϵ	-	dielectric constant
g/L	-	gram per liter
CE	-	Capillary Electrophoresis
CZE	-	Capillary Zone Electrophoresis
EKC	-	Electrokinetic Chromatography
HPLC	-	High Performance Liquid Chromatography
LLE	-	Liquid-Liquid Extraction
mg/kg	-	milligram per kilogram
mL	-	milliliter
SDS	-	Sodium Dodecyl Sulfate

d	-	diameter
ppm	-	parts per million
eV	-	electron volt
μ A	-	micro Ampere
μ L	-	micro liter
NR	-	Not Reported
DBS	-	dodecylbenzene sulfonic sodium salt
CMC	-	Critical Micelle Concentration
SDB	-	Styrene divinylbenzene

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CHAPTER I

INTRODUCTION

1.1 Background

Agriculture plays an important role in waste recycling thereby reintroducing substances as plant nutrition elements into natural cycles [1]. For example, sewage sludges contain appreciable amounts of sodium, phosphorus, potassium and magnesium and have significant inorganic fertilizer replacement value for these major plant nutrients. Crop productivity can also be increased by improving soil physical properties through application to soil of organic matter contained in sludge and biowaste. Thus the application of sewage sludge as fertilizer to agricultural lands represent an economical way to use the high amounts of sludge produced by wastewater treatment plants [2].

However, besides beneficial plant nutrients, these waste matrices also contain hazardous heavy metals and organic pollutants which enter food chains and are of great concern to the public. For sewage sludge and compost, limit values for heavy metals have been established as a quality criterion. These inorganic compounds are analysed on a routine basis. However, the characterization and long term observation of organic contaminants in sludge has received little attention so far [3], possibly because the methods of analysing these compounds are laborious and often complicated [4].

The range of organic compounds known to exist in sludge is extensive and diverse and is potentially transferred to sludge-amended agricultural soils. Sewage sludge is known to be contaminated with a wide array of xenobiotics (organic pollutants) [4] which has negative impact on soil organisms. Among these organic contaminants, non polar, persistent compounds exhibiting a high accumulation potential are important.

Typical representatives are polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs). However OCPs are present only in extremely minute concentrations and generally does not pose a danger. The chemical-physical properties of the compounds mentioned are similar. They show high octanol-water distribution coefficients, are highly lipophilic and therefore tend to adsorb to solid particles of sewage sludge.

Monitoring the levels of these pollutants in sludge matrices is very important as introduction into the food chain can occur through grazing animals, direct plant intake and through leaching into ground water [5]. There are four main pathways through which a chemical from the soil can enter plants: (i) root uptake and subsequent transport in the transpiration stream; (ii) foliar uptake of vapour from the surrounding air; (iii) uptake by external contamination of shoots by soil and dust, followed by retention in the cuticle or penetration through it; and (iv) uptake and also transport in oil channels which are found in some oil containing plants such as carrots [6].

Concern about the environmental effects of recycling sewage sludge on agricultural land would appear to be legitimate given that many of the organic chemicals designated as 'priority pollutants' due to their potentially toxic effects, are known to occur in sludge [3]. Considering these problems, it is necessary to control the pollutant levels. Germany and the USA have indicated maximum levels for several pollutants in sludges and soils. Other countries, such as Sweden are opposed to the use of the sludges as fertilizer in agricultural soil [6].

1.2 Criteria in Re-Use and Disposal of Sludge

Sludge is defined as the by-product of liquid sewage treatment. It is any solid material containing large amounts of entrained water collected during wastewater treatment. If not handled carefully, it can pose detrimental effect to the human environment.

Certain regulations have therefore been made regarding the re-use and disposal of sewage sludge [7]. Of paramount interest are the chemical and biological characteristics of the sludge, as these factors influence its application. Parameters such as organic content, toxic organics, nutrients, pathogens and hazardous metals have to be taken into account before it can be distributed for re-use. 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.: PAHs, dioxins, OCPs, and PCBs)
- iii. Pathogenic Organisms (eg.: viruses and human/ animal pathogens)

Some of the more desirable materials in sludge also need to be monitored [7], for the most beneficial reuse of the sludge. These parameters include:

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

The above mentioned parameters, together with other important considerations needs careful attention when deciding on the best alternative for the re-use or disposal of sewage sludge.

1.3 Management of Sludge

Desludging in sewerage facilities was done on a need to basis and only less than one percent of the total sludge volume was given due attention. In actual fact, the sludge levels in existing systems keep building up and overflows with the effluent into the receiving water courses.

This clearly speaks for the fact that why most of our rivers systems are polluted with domestic sewage. It has been reported that over 70 percent of the rivers in Malaysia are classified as polluted and the major source of pollutant has been identified as human wastes. The problems were getting serious by the day and this has directly posed a major threat to our water resources.

1.4 Current Sludge Issue in Malaysia

The annual sludge volume produced in Malaysia was estimated to be at 3 million cubic meters [8]. This equates to filling the twin-tower at KLCC to the 78th floor in the first year and requires some 600,000 tanker trips to transport the sludge to designated treatment and disposal sites. 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 fill, or almost 1.4 million tanker trips to manage. These alarming figures indicate the need for better disposal or reuse or recycling of sludge in Malaysia.

1.5 Beneficial Uses of Sludge

The beneficial use of sludge is receiving considerable attention [9] because of the decline in available landfill, and the interest in using the beneficial nutrient and soil conditioning properties of sludge.

1.5.1 Distribution and Marketing

In Europe, sludge that is distributed and marketed is used as a substitute for topsoil and peat on lawns, golf courses, parks and in vegetable gardens. Usually the sludge used for these purposes is composted. The rates of application of sludge may be limited based on whether it is used for food or non-food crops [3].

1.5.2 Chemical fixation

In Europe, the chemical fixation process has been applied to the treatment of industrial sludge and hazardous wastes to immobilize the undesirable constituents [3]. The process has also been used to stabilize municipal sludge for use as landfill cover and for land reclamation projects. The chemical fixation process consists of mixing treated or untreated liquid or dewatered sludge with stabilizing agents such as cement, sodium silicate and lime so as to chemically encapsulate the sludge.

1.6 Disposal of Sludge

Final disposal of sludge usually involves some sort of land disposal. Ocean disposal is prohibited and is being phased out because of water pollution control regulations. There are various methods of ultimate sludge disposal, which can be carried out in the form of liquid or dried sludge. The methods that will be employed here in Malaysia are [8]:

1.6.1 Agriculture/ Forestry Land Improvement

Sludge utilization on agricultural and forestry land has proven to be most resourceful. Sludge contains most of the organic loads from the sewage, which can

help farmers reduce their fertilizer requirements and improve soil fertility. Sewage sludge contains significant proportion of nitrogen and phosphorus and can supply a large part of the requirements of most crops. The organic content of sludge can also improve the water retaining capability and the structure of certain soils. It is also a very useful product for reforestation. In Malaysia, sludge has been used as fertilizer in several plantations in Johor [10].

1.6.2 Land reclamation

Sludge cake can be very effective in improving disturbed soils or providing a growing media where no soil exists. For example, soil is normally stripped and stockpiled prior to mineral extraction for reinstatement on completion of the operation. When reinstatement takes place, the stockpiled soil is generally structurally damaged and the addition of sludge cake provides extra organic matter, improving both the physical and hydraulic properties of soil. In areas where no top soil exists, sludge cake can be used as a soil forming material providing a cheap alternative as top soil. In Malaysia, land reclamation techniques will be most suited to ex-mining lands.

1.6.3 Landfilling

Landfilling of sewage sludge with domestic refuse is the most common method of sludge disposal. The basic procedure is to construct a series of clay sided cells or lagoons which are capable of being filled to an average depth of 3 meters with sewage sludge. Thickened sludge is pumped into the lagoons and allowed to stand for a period of time after which any surface water can be decanted off and additional sludge pumped in. Once the maximum volume of sludge has been passed into the lagoon it is again allowed to stand for a period of time to remove water.

At this point, dry solid wastes are tipped into the lagoon and this absorbs most of the remaining moisture of the sludge. Additional solid waste are then deposited on top of this, up to the final and agreed contour levels. Ground compaction is done and final restoration of the site takes place.

1.6.4 Composting

Composting of sewage sludge is new to Malaysia and maybe considered in the future if the economies are favourable. Liquid or dewatered sewage sludge can be stabilized by mixing it with a bulking agent such as wood chip, straw or municipal waste, provided that non-degradable materials such as metal, plastic and glass is removed.

1.7 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic Hydrocarbons (PAHs) are a group of organic compounds that are principally formed during the incomplete combustion of organic matter such as coal, petrol and oil [11]. They are ubiquitous environmental pollutants which present a potential health concern because of the toxicity, mutagenicity and carcinogenicity of these substances in animals [12]. PAHs have a high persistence in the environment, low biodegradability and high lipophilicity, some of them being highly toxic. Several PAHs have a natural source such as bacterium or terrestrial superior plants. Other have their origin in coal, petroleum and their derived products.

Wastewater catchments receive PAHs from two main sources: industrial and domestic fossil fuel spillages and urban runoff inputs that flush the hydrocarbons deposited on the ground surface from vehicles or heating systems. As a result of their very low aqueous solubility, PAHs are efficiently removed from the water during sedimentation in the wastewater treatment process. During treatment of sewage, most of the PAHs (almost 90 %) are removed from the waste and is concentrated on

the sludge due to the insolubility and adsorption capacity of the PAHs [13]. This results in the formation of sewage sludges that typically contain between 1 and 10 mg/kg of each individual PAH [4, 11, 14].

The determination of PAHs in environmental samples represents an area of analysis where strict US government controls now exists in order to regulate the production, usage and disposal of this groups of materials [15]. A significant proportion of the generated sewage sludge is applied to land as an organic fertilizer or amendment. Because some PAHs are known or suspected carcinogens [16, 17], the fate of these compounds in the soil environment is critical in assessing their potential hazard risk.

1.7.1 PAHs of Common Occurrence in Sludge

The occurrence of alkyl- and nitro- substituted as well as other heteroatom-containing PAHs are most often reported. Parent PAHs are the basic polycyclic aromatic compounds and their carcinogens has mainly been observed for tri-, tetra-, penta-, and hexacyclic compounds [18, 19]. However, the most abundant and routinely monitored PAHs are the 16 listed in Method 610 by the United States Environmental Protection Agency (US EPA) and are also included in the Priority Substances List under the Canada Environmental Protection Act (CEPA) [20].

The 16 PAHs are naphthalene (Naph), acenaphthylene (Ac), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Anth), fluoroanthene (Fluo), pyrene (Pyr), benzo[a]anthracene (BaAn), chrysene (Chr), benzo[b]fluoroanthene (BkFl), benzo[a]pyrene (BaPy), dibenzo[a,h]anthracene (DiAn), benzo[g,h,i]perylene (BePe) and indeno[1,2,3-cd]pyrene (InPy), and have been selected by the US Environmental Protection Agency (US EPA) as “Consent Decree” priority pollutants (Figure 1.1)[18]. These compounds typically contain two or more benzene rings. The carcinogenicity of the PAHs are suspected to arise when four or more benzene rings are present in the compound [16].

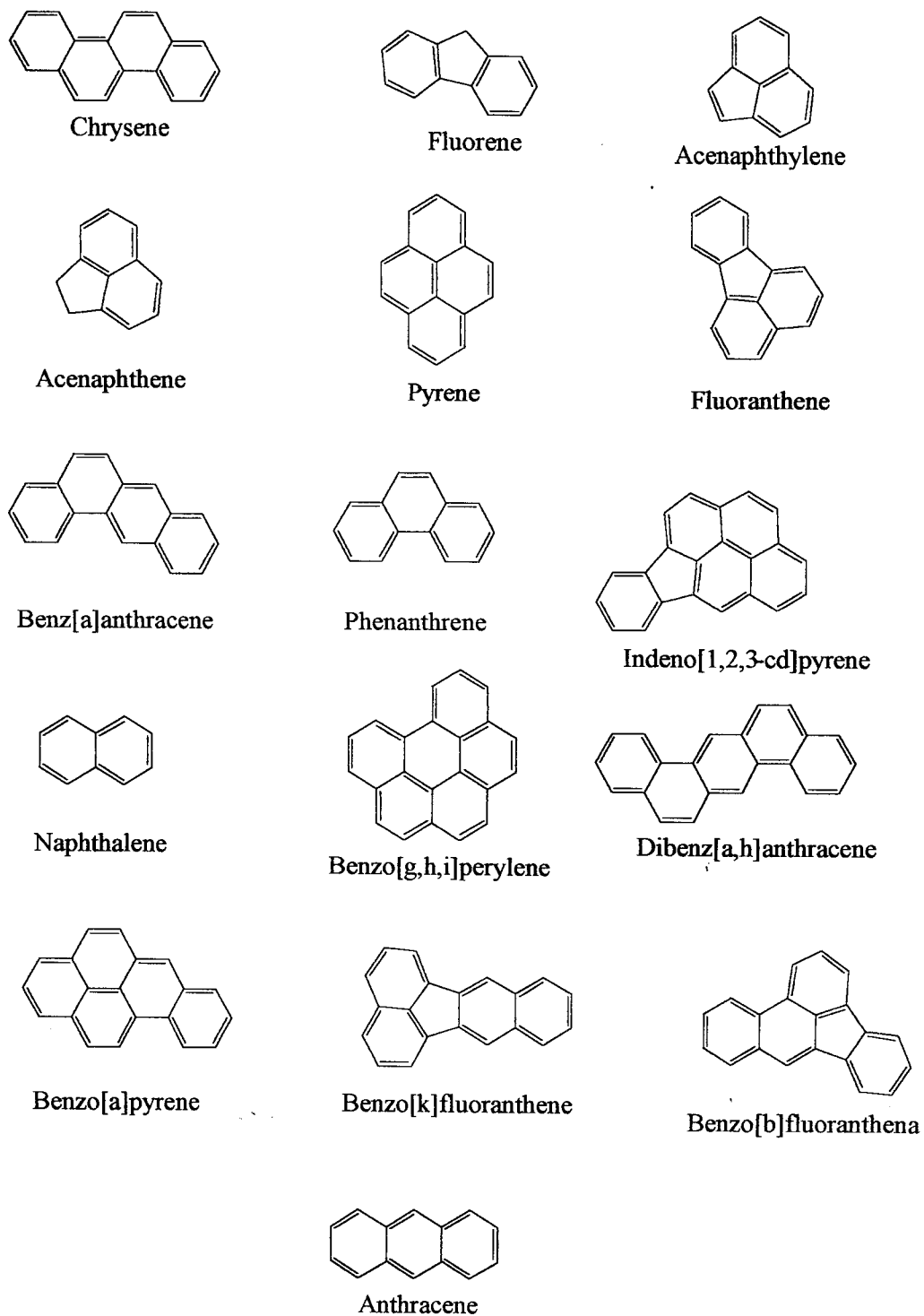


Figure 1.1: Structures of the 16 PAH Termed as Priority Pollutants by the United States Environmental Protection Agency

1.7.2 General Characteristics of Common PAHs

In determining and understanding the extractability of a PAH, the most important parameters are its solubility and also its boiling point. Some of the general characteristics of the common PAHs are listed below in Table 1.1.

Table 1.1 General Characteristics of Common PAHs [21, 22]

Compound	Molecular Mass	Boiling point (°C)	Water Solubility (mg/L)
Naphthalene	128	218	32
1-Methylnaphthalene	142	245	29
2-Methylnaphthalene	142	241	25
Acenaphthylene	152	270	4
Fluorene	166	297	2
Phenanthrene	178	340	1.3
Anthracene	178	340	0.073
Fluoranthene	202	393	0.26
Pyrene	202	394	0.14
Benz[a]anthracene	228	438	0.014
Chrysene	228	436	0.002
Benzo[a]pyrene	252	496	0.0038
Benzo[ghi]perylene	276	500	0.00026

The other common characteristics of PAHs are that they are highly hydrophobic and lipophilic, show high octanol-water distribution coefficients and due to their hydrophobicity, they also tend to adsorb on to organic particles. These compounds are known to be persistent and also have high accumulation potential [1]. As pure chemicals, PAHs generally exist as colorless, white, or pale yellow-green solids.

1.7.3 Sources and Exposure to PAH

The primary sources of exposure to PAHs are inhalation of the compounds in tobacco smoke, wood smoke, and ambient air, and consumption of PAHs in foods. For some people, the primary exposure to PAHs occurs in the workplace. PAHs have been found in coal tar production plants, coking plants, bitumen and asphalt production plants, coal-gasification sites, smoke houses, aluminum production plants, coal tarring facilities, and municipal trash incinerators [23].

Workers may be exposed to PAHs by inhaling engine exhaust and by using products that contain PAHs in a variety of industries such as mining, oil refining, metalworking, chemical production, transportation, and the electrical industry. PAHs have also been found in other facilities where petroleum, petroleum products, or coal are used or where wood, cellulose, corn, or oil are burned. People living near waste sites containing PAHs may be exposed through contact with contaminated air, water, and soil [23].

1.8 Extraction of PAHs from Sludge

In comparison to inorganic pollutants, the determination of organic contaminants in sewage sludge is a field that has received little attention, possibly because no legal limit values have been implemented.

The other more probable reason is the fact that the determination of organic compounds are often complicated and laborious. Analytical methods for analysing these compounds include extraction, one or several clean-up steps, separation and finally the quantification of the analytes. These traditional methods require large amounts of organic solvents and several reconcentration and clean-up steps before final quantification [1]. Some of the common extraction procedures for the analysis of PAHs are described below.

1.8.1 Soxhlet Extraction

Soxhlet extraction is a conventional approach that is effective in its extraction but requires large quantities of toxic solvents that are hazardous to work with and dispose of. It is a labour-intensive process and can take up to 18 hours to complete an extraction.

Extraction efficiencies vary according to the solvent that is used. In a study that employed toluene, cyclohexane and dichloromethane for the extraction of PAH from sewage sludge, toluene exhibited the highest percentage of recovery [24]. There have been several recommendations for the best Soxhlet extraction of solid environmental samples. Among these, acetone, benzene and cyclohexane are all said to be highly efficient in the extraction of PAHs [25]. Soxhlet extraction has also been utilised in this laboratory for the analysis of PAHs and OCPs [26], where approximately 10 g of freeze dried sludge sample was subjected to Soxhlet extraction for 16 hours using a mixture of hexane and dichloromethane.

Some Soxhlet extractions are further coupled with saponification techniques to enhance determination of PAHs [11]. Saponification with bases is applied to increase the availability of contaminants in soil by hydrolysis and solvation of organic matter [27]. Codina *et al.* [11] demonstrated that Soxhlet plus saponification techniques has proven to be yield higher recoveries of PAHs as compared to pure traditional Soxhlet techniques. This method was further improved by using silica gel clean-up and fluorescence detection, in preference over UV detection.

The Soxhlet apparatus can be modified for automation purposes. This results in faster, safer and more economic operating procedures. The modified apparatus is known as Soxtec [28]. Comparison of Soxhlet extraction to other extraction techniques for environmental solids have been described [20, 21].

Soxhlet extraction, when not suitable for extraction of PAH compounds with substantially high molecular weights, is often replaced with pressurized fluid extraction (PFE) [29], also known as Accelerated Solvent Extraction (ASE).

1.8.2 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) is a new method for the extraction of organic compounds from soils, sludges and sewage wastes. It uses organic solvents at high pressures and temperatures above the boiling point. With ASE, a solid sample is placed in a cartridge and different solvents are used to extract the sample statically under elevated temperatures (50- 200°C) and pressures (7-20 MPa) to increase the speed of the extraction process with low solvent consumption [30].

The applications of ASE for the determination of contaminants in various environmental samples e.g. the determination of chlorinated pesticides from contaminated soils, the determination of PAHs in heap material and the determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans from fly ash samples have been investigated [30].

In a study conducted by Schantz *et al.* [29], PFE has been evaluated and found suitable as an alternative to Soxhlet extraction for use in the extraction of PAHs, PCB congeners and chlorinated pesticides from natural matrix environmental conditions. It was also demonstrated that Soxhlet extraction with methylene chloride is not quantitative for higher molecular weight PAHs from diesel materials (diesel particulate matter); therefore PFE is the preferred technique for the certification of these materials.

In another study by Popp *et al.* [30], comparison of Soxhlet extraction (18 h) and ASE (2 x 5 min), both studies employing toluene, was performed. The 16 PAHs listed as priority pollutants by the US EPA were analysed. With exception of phenanthrene and benzo[a]anthracene, ASE provided higher extraction yields and was shown to be more effective than the Soxhlet extraction.

ASE was found to meet the demand for increased productivity, faster analyses, more automation and reduced solvent usage [29]. ASE is considered equivalent to standard US EPA extraction methodology in terms of recovery and precision [30]. Comparison of PFE to other extraction techniques such as Soxhlet,

supercritical fluid extraction as well as subcritical water extraction have been described [20, 21].

1.8.3 Solid Phase Extraction

In recent years, solid-phase extraction (SPE) has become an increasingly popular procedure in environmental analysis. SPE is a popular procedure that is used to preconcentrate components to be analyzed and clean-up matrices from sample for analysis [18]. Recently, C₁₈-bonded silica has been introduced as extraction discs in which they are enmeshed in a network of PTFE fibers to form strong porous membrane. These discs have been studied for their suitability to field extractions and storage mechanisms [31].

Another approach for simple extraction method is to couple subcritical water extraction with standard SPE discs (Empore discs). The SPE disc quantitatively collects analytes from the water during the subcritical water extraction step. Empore SPE discs (C₁₈, styrene/divinyl benzene and polymer-based anion exchange resins) are stable under 250°C. Therefore, the SPE discs can be placed in the cell during the heating step to collect extracted organics from the extractant water [32].

Prepacked SPE cartridges provide users of SPE with a variety of stationary phases to selectively separate and concentrate analytes for detection. However since PAHs have low polarity, SPE of pollutants from samples is usually carried out on a bonded octadecyl-silica stationary phase [18]. The combination of the small particle size and high surface area of C₁₈ bonded porous silica ensures contact of the dissolved organics with the adsorbents even when very rapid flow rates are employed. Methanol and acetonitrile are the recommended solvents for the elution of compounds sorbed on to alkyl-bonded porous silica, though many hydrophobic compounds such as PAHs have low solubilities in these two solvents [33].

In contaminated soil, besides the PAHs that are analyzed, other compounds may interfere with the determination. In addition, some PAHs in the extraction

solution from the soil cannot be determined directly because of their lower concentrations [18]. Therefore, SPE as a clean-up procedure is often required for the determination of PAHs in soil samples, while aqueous samples need preconcentration [34].

1.8.4 Solid Phase Microextraction

Recently, solid phase microextraction (SPME) which is fast, solvent free and exhibits excellent performance has been studied as the substitution or alternative to SPE. SPME has been successfully employed to analyze a wide range of pollutants including PAHs [5]. Whereas the SPE technique has been primarily designed for use with liquid matrices and exhaustive extraction, SPME can be used in liquid (aqueous) or gaseous matrices and primarily aims for partial or equilibrium extraction of the analyte [35]. There are two basic types of SPME: direct immersion (DI) and headspace extraction. In the DI extraction mode, the coated fiber is inserted into the sample medium, where some level of agitation is sometimes necessary to enhance transport of the analytes from the bulk of the solution into the vicinity of the fiber.

Although SPME coupled with gas chromatography is a powerful approach for the rapid extraction and analysis of non-polar and moderately-polar organics from water, its use for the analysis of organic pollutants from solid samples has been limited to compounds that can be readily vaporized from solids and show strong partitioning to the SPME phase [36]. In a study employing FID as the detector, FID responses of PAHs increased slightly with increasing water volume for DI-SPME, and increasing the working temperature also increased the extraction efficiencies [37]. A method for the determination of PAHs by SPME coupled with cyclodextrin (CD)-modified capillary electrophoresis using UV detection has also been developed [12], where with 30 kV applied potential, separation of the 16 PAHs was successfully achieved in less than 15 minutes.

Headspace SPME (HSSPME) is a good supplement to conventional SPME because the fiber is not in contact with the sampling medium. This approach serves primarily to protect the fiber coating from high molecular-weight species and other non-volatile contaminants present in the liquid sample matrix. This headspace mode also allows modification of the matrix, such as a change of the pH, without damaging the fiber [35]. Therefore, the background is much cleaner and the useful life of the fiber is prolonged.

HSSPME has been proven to be effective on the analysis of volatile organic compounds [37]. The feasibility of HSSPME for the determination of high-ring PAHs (4-6 rings) in water and soil samples have been studied [18, 37]. In this study, 100- μm PDMS showed the highest extraction efficiency compared to 85- μm and 30- μm PDMS fibres. Also, the extraction efficiency decreased with increasing molecular weights of the PAHs. HSSPME with a 90 minute extraction time has been proven to be effective for determination of PAHs in water and soil samples. HSSPME at room temperature was successfully applied to the analysis of low ring PAHs. A working temperature of 80 °C provided significant enhancement in sensitivity of high ring PAHs [37].

1.8.5 Supercritical Fluid Extraction

The analytical scale supercritical fluid extraction (SFE) was introduced in the late 1980s and started a new field of research. SFE paved the way not only for reduction in use of organic solvents but also for automation of analytical procedures [38]. High repeatability, accuracy and high selectivity were among the most important advantages of SFE in residue analysis. Extracts with low amounts of co-extractants from the soil matrix were achieved, allowing extracts to be pooled and concentrated with little or without further clean-up steps. Thus, the limited volume of extraction thimbles of the SFE apparatus used could be compensated and insufficiently high limits of determination could be improved [39].

SFE equipment typically consists of an extraction cell that can be heated and pressurized. This cell (containing the sample) is placed in an oven and connected to a high-pressure pump supplying the supercritical fluid. Pumping of the extraction fluid can be done in two different ways, either continuously via a reciprocating pump or by filling of a syringe pump which then delivers the fluid. Analyte collection can also be done in basically two different ways; in solvents or on solid-phase traps [38].

When a supercritical fluid is used as an extractive solvent, it is possible to separate a multicomponent mixture by capitalizing on both the differences in component volatilities and the specific interaction with the solvent. The application of supercritical fluids is based on the observation that many gases exhibit enhanced solvating power when compressed to condition above the critical point [40]. The advantages of using supercritical fluids are that they possess a very low surface tension, low viscosity, and high diffusivity, which translates into fast mass transfer when compared to liquid solvents [5].

The comparison of supercritical chlorodifluoromethane, CHClF_2 (Freon-22), nitrous oxide (N_2O) and carbon dioxide (CO_2) as the supercritical media for the extraction of PAHs and PCBs from environmental solids have been described [21]. The supercritical parameters of carbon dioxide (which is non toxic and non flammable) are easily accessible (72.8 bar, 31.1°C), and CO_2 can be modified with methanol, acetonitrile or acetone to improve its polarity [21]. Freon-22 too has recently been shown to yield higher extraction efficiency than N_2O and CO_2 . However Freon-22 has ozone depleting potential and the advocacy of freon as a SFE solvent is a step backward in environmental protection [20].

Application of SFE for determination of PAHs in sludge samples have been conducted by Berset and Holzer [1] where the procedure was divided into two discrete steps; in the first step, the more volatile PAHs were extracted using pure CO_2 at a rather low density and pressure. In the second step the less volatile PAHs were removed at a higher density and temperature and by adding modifiers. SFE has also been coupled with liquid chromatography employing fluorescence detection for the analysis of PAHs in sludge [24]. However, as have been previously reported [1], most of SFE based studies have been focused on soils and sediments [14, 20, 21, 39].

viscosity of the water at higher (up to 300°C) temperatures. Under these conditions, organic compounds generally considered insoluble in water show dramatic increases in solubility [36]. For example, the PAHs such as anthracene, chrysene and perylene each have solubilities ca. 20000 fold higher in water at 200°C, than at 25°C [21].

Subcritical water has several potential physiochemical as well as practical advantages over supercritical water and supercritical carbon dioxide. As shown in Table 1.2, the polarity (solvent strength) of subcritical water can be controlled over a much broader range than either supercritical water or carbon dioxide. By simply controlling the temperature with enough pressure to maintain the liquid state, a very wide range of solvent polarity can be achieved with pure water, as demonstrated in Figure 1.3 [42].

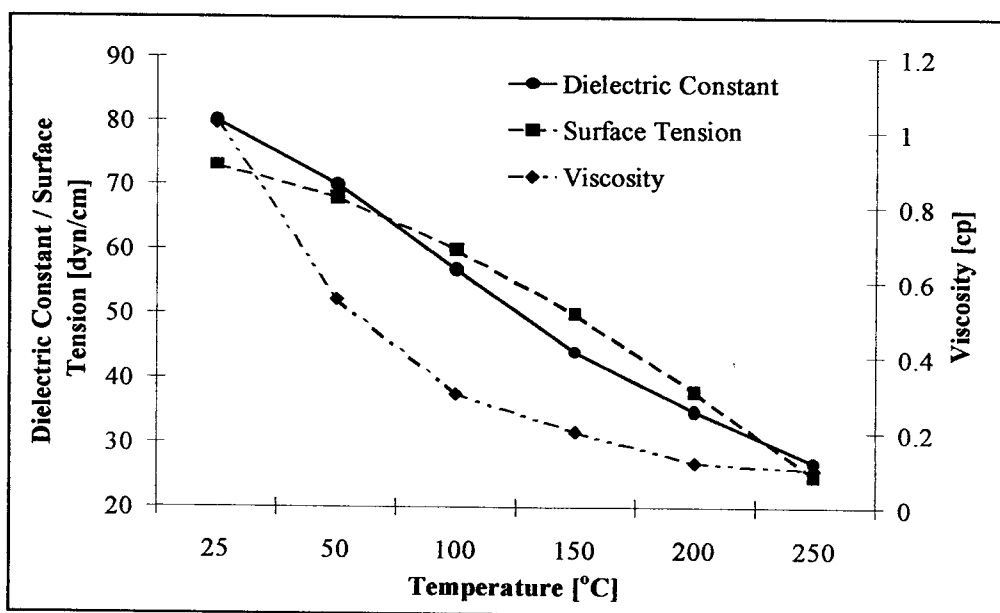


Figure 1.3: Temperature Influence on Physical Properties of Water

In addition to changing the polarity of water, increasing the temperature also lowers the viscosity and surface tension of the water- both factors that enhance water's ability to extract organic compounds from contaminated solids (Figure 1.3). Additional comparisons of subcritical water with supercritical water and carbon dioxide is shown in Table 1.2 below.

Table 1.2: Typical Characteristics Related to Organic Pollutant Extractions for Subcritical Water, Supercritical Water and Supercritical CO₂

Parameters	Subcritical Water	Supercritical Water	Supercritical CO ₂
Temperature range	>25 to 300°C	>374°C	>32 to 150°C
Pressure required	2 to 50 atm	>218 atm	>72 atm
Density range	0.8 to 1g/mL	0.1 to 0.6 g/mL	0.3 to 0.9 g/mL
Polarity range	15 to 85	5 to 15	1 to 2
Corrosivity	Low to moderate	High	Low
Organics solvated	Polar to non-polar	Polar to non-polar	Non-polar
Selectivity	Potentially high	Non-selective	Some selectivity

In contrast to the dependence on pressure commonly reported for SFE with supercritical carbon dioxide, the efficiency of subcritical water extractions depends primarily on the water temperature, as long as sufficient pressure is applied to maintain the liquid state (typically < 40 bar). The least polar organics are the easiest organics to extract with pure CO₂. In direct contrast, subcritical water prefers the more polar analytes, i.e., PAHs can be efficiently extracted from air particulates at 250°C [21].

The dynamic mode is the common approach to performing subcritical water extraction. Quantitative extraction of PAHs using the dynamic mode have been reported by several authors [21, 41]. Lately, a prototype extractor was developed for automated extraction in the dynamic mode (Figure 1.4) [41].

It consists of a stainless steel cylindrical extraction chamber, with closed screws at the end to permit the circulation of fluids through them. Both screw caps contain stainless steel filter plates to ensure that the sample remains in the extraction chamber. A pump with digital flow rates and pressure readouts is used to impel the extractant through the system.

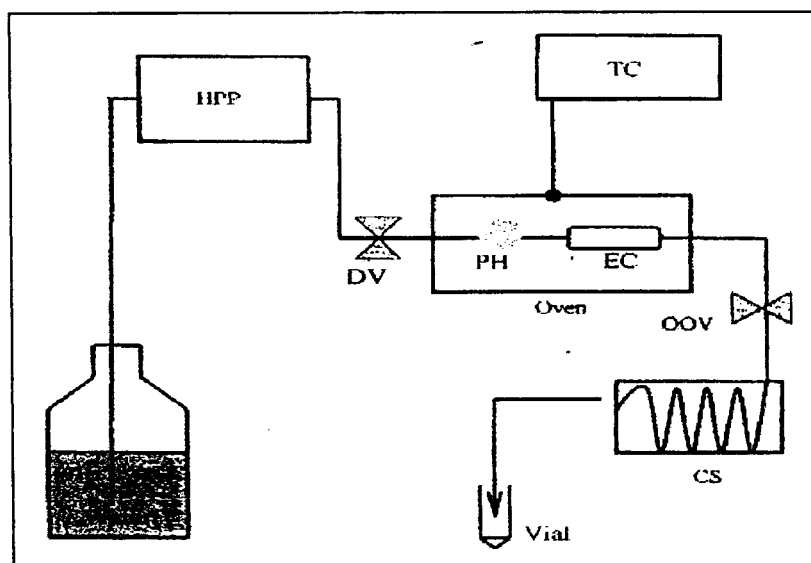


Figure 1.4: Schematic diagram of the prototype extractor [41]

HPP=High Pressure Pump, PH=Preheater,
 CS=Cooler System, EC=Extraction Cell,
 TC=Temperature Controller, DV=Diverting Valve

Static subcritical water extraction is relatively simpler to perform. In this case, the sample is simply placed in the vessel with an appropriate amount of extractant (water) and heated to the desired temperature for the needed duration. After cooling, the extractant is removed and analysed. Good recoveries were also obtained for PAHs and PCBs using this method of extraction [36, 43].

Another approach to subcritical water extraction is the micelle formation extraction methodology [41] that has been applied to the extraction of a wide range of analytes including PAHs, vitamin A, vitamin E, estrogens, progesterones and proteins from aqueous solutions. The analytical interest of the micelle formation is a consequence of the (a) ability to concentrate a variety of analytes; (b) safety and cost benefits; (c) easy disposal of the surfactant; (d) compatibility of the surfactant-rich phase with micellar liquid chromatography; and (e) preclusion of the analytes losses during the evaporation of the solvent used in traditional liquid-liquid extraction [41]. Research has also shown that higher recoveries of PAHs were obtained in the

presence of sodium dodecyl sulfate as the surfactant compared to extraction with pure water.

1.9 Extract Clean-Up

The determination of PAHs in soil, sediment and sludge samples require a good clean-up, while aqueous samples need concentration due to their low concentration levels [34]. As individual PAHs are present in soils in the ng/g level or below, few samples can be analyzed directly without serious interference.

Efficient extraction, preconcentration and clean-up of PAHs from the samples are essential prior to the determination of the PAHs. The two most commonly used techniques for sample clean-up are the conventional column chromatography as well as the SPE clean up.

1.9.1 Conventional Column Chromatography

Silica gel (silicic acid) is often used in column chromatography for the separation of analytes from interfering compounds of a different chemical polarity. It is a regenerative adsorbent of silica with weakly acidic properties, and is produced from sodium silicate and sulfuric acid. It is used deactivated, after heating to approximately 150-160 °C, or deactivated with up to 10 % water [44].

Generally, the standard column chromatography, as compared to SPE cartridges, use larger amounts of adsorbent and therefore, have a greater clean-up capacity. 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 a suitable solvent(s) that leaves the interfering compounds on the column. The eluate is then concentrated, if necessary.

adsorbent material for SPE cartridge that is most commonly used are C₁₈ and C₈ bonded porous silica [18, 33, 34] as well as the silica cartridge [26, 41].

1.10 Instrumental Analysis

Various instrumental analytical procedures have been employed for the determination of PAHs. Among the methods, gas chromatography, high performance liquid chromatography and capillary electrophoresis are the techniques most commonly used.

1.10.1 Gas chromatography

The extreme complexity of PAH mixtures demands the greatest resolution possible in their analysis, and in this respect gas chromatography has proven to be efficient. As the number of known carcinogenic polyaromatics have increased considerably, their detection has been carried out by more sophisticated techniques, in particular capillary column gas chromatography (GC) combined with mass spectrometry [45]. This combination (GC-MS) is often favourable for identification of various components in a complex mixture.

Based on structural information, GC-MS is capable of identifying more than 21 PAHs and PAH derivatives present in soil, sludges and sediments [13]. The maximum resolution of mixture components before MS analysis is of utmost importance in providing unambiguous identification of individual compounds. This is especially true in the case of PAHs because the conventional mass spectra of many isomers are identical [25].

The most widely used gas-chromatographic detector for PAHs is the flame ionization detector (FID). This is a result of its universally accepted characteristics of excellent response linearity, sensitivity and reliability [25]. There are various studies

that have employed the use of GC-FID [6, 13, 14, 46]. This method of detection is mostly used for determination of PAHs.

For chlorinated compounds, electron capture detection (ECD) is a better choice. Analysis of PCBs and OCPs usually employs ECD and MS, instead of FID. This is because halogen-containing compounds possess high affinity for thermal electrons and give strong ECD responses [31]. SPME coupled with gas chromatography is a powerful approach for the rapid detection and analysis of non-polar and moderately polar organics from water [36].

1.10.2 Capillary Electrophoresis

Capillary electrophoresis (CE) methods, including capillary zone electrophoresis (CZE) have recently emerged as the most efficient methods available for separation of components in mixtures. However, CZE is limited to the analysis of water-soluble, charged species and is therefore not applicable for the analysis of neutral and water-insoluble PAHs [47].

Analysis of such uncharged species through electrokinetic chromatography (EKC) is feasible through addition of a charged phase to a standard CZE buffer to effect separation based on analyte partitioning into the charged phase [48]. Micellar electrokinetic chromatography employs the same principle as CZE, except that an ionic surfactant is added to the buffer mobile phase. MEKC is able to effect the separation of neutral compounds based on the differential distribution of solutes between an electroosmotically pumped aqueous mobile phase and a slower moving electrophoretically retarded micellar phase [49].

However, of the limitations of MEKC is the need for samples to be reasonably soluble in an aqueous mobile phase. Hydrophobic compounds, such as the PAHs, tend to be completely solubilized by the micelles and co-elute with migration times near to that of the micelles. These problems can be alleviated by silanizing the capillary column walls, coating the walls with polymers, or adding

modifiers to the electrophoretic buffer. Therefore, for the separation of PAHs, which are all neutral, non-ionizable and are of similar hydrophobicity, it is necessary to extend the concept of employing a mobile phase and pseudostationary phase to the utilization of buffer modifiers [49].

Some common buffer modifier that have been used are derivatized cyclodextrins, tetraammonium perchlorate, histidine complex and organic modifiers [49]. One of the most successful schemes for improving the partitioning for PAH separation is the addition of cyclodextrins to the buffer. Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides which consist of α -1,4-linked glucopyranose subunits. The most common forms are α -CD, β -CD and γ -CD. They possess a toroidal structure with a nonpolar interior cavity and can form host-guest inclusion complexes with many hydrophobic complexes [47].

Experience with micellar solutions unfortunately, has shown them to be difficult to prepare reproducibly and MEKC separation methods are highly sensitive to operating parameters such as temperature and applied voltage. Thus method development for MEKC is often cumbersome and time-consuming [47].

1.10.3 High Performance Liquid Chromatography

PAHs are commonly analyzed by HPLC with fluorescence detection. Although capillary gas chromatography is known to have much higher resolution than HPLC methods, but today, HPLC can easily separate the 16 EPA priority PAHs [19]. This method of analysis is known to be selective and requires less clean-up procedures as compared to GC-MS due to the use of the guard precolumn in the HPLC [50].

A number of studies have been conducted to determine PAHs using HPLC [11, 24, 30, 41]. Codina *et al.* [11] have developed a HPLC method with both UV and fluorescence detection to determine PAHs from sewage sludge-amended soil following extraction using Soxhlet plus saponification extraction and silica gel clean-

up. A comparative study of SPE and conventional LLE was conducted using HPLC as the method of analysis [34]. HPLC was also compared to other methods of analysis such as GC and GC-MS for the determination of PAHs and chlorinated pesticides in solid wastes [30].

1.11 Importance of Research

Almost all of sludge based research in Malaysia concentrate on the inorganic aspects of contamination, particularly heavy metals. Little attention is given to the determination of organic pollutants in sewage sludge, although these organic compounds, namely PAHs are toxic and hazardous. Therefore, it is important to develop an analytical method to determine the levels of these compounds in sewage sludge.

1.12 Research Objectives

The objectives for this research are as follows:

- i. To compare three subcritical water extraction methods (pure subcritical water extraction, surfactant-modified subcritical water extraction and subcritical water extraction coupled with membrane discs) for the extraction of PAHs from spiked sludge samples.
- ii. To perform instrumental analysis of the extracted PAHs using GC-FID.
- iii. To perform recovery studies for the PAHs extracted from spiked sludge samples.

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