THESIS

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BSc Environmental engineering

Godollo

2024

Hungarian University of Agriculture and Life Science

Szent István Campus

BSc Environmental Engineering

STUDY OF THE DISTRIBUTION OF ORGANIC MICROPOLLUTANTS (PAHs) IN SURFACE WATER & SEDIMENT SYSTEM

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ABBREVIATIONS

- Nap = Naphthalene
- Acy = Acenaphthylene
- Ace = Acenaphthene
- Flu = Fluorene
- Ant = Anthracene
- Phe = Phenanthrene
- Fla = Fluoranthene
- Pyr = Pyrene
- BaA = Benz [a] anthracene
- Chr = Chrysene
- BbF = Benzo [b] fluoranthene
- BkF = Benzo [k] fluoranthene
- BaP = Benzo [a] pyrene
- DBA = Dibenz [a,h] anthracene
- Ind = Indeno[1,2,3-cd]pyrene
- BghiP = Benzo [g,h,i] perylene
- HMW = High Molecular Weight
- LMW = Low Molecular Weight
- PAHs = Polycyclic Aromatic Hydrocarbons
- GC = Gas Chromatography
- HPLC = High-Performance Liquid chromatography
- FID = Flame Ionization Detector
- ECD = Electron Capture Detector
- TCD = Thermal Conductivity Detector
- MS = Mass Spectrometer
- NPD = Nitrogen-Phosphorus Detector
- AED = Atomic Emission Detector
- EQS = Environmental Quality Standards
- PBTs = Persistent Bio-accumulative Toxic substances

- CAS = Chemical Abstracts Service
- AA = Annual Average
- MAC = Maximum Allowable Concentration
- PTFE = Polytetrafluoroethylene
- SIM = Selected Ion Monitoring
- TIC = Total Ion Chromatogram
- SPE = Solid Phase Extraction
- ACN = Acetonitrile

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAH) are a group of organic compounds that contain two or more fused benzene rings. Their presence in the environment causes great concern because of the possibility of causing carcinogenic issues (*Manoli et al., 2000*). This group of organic compounds has been included in the priority list of pollutants in the US Environmental Protection Agency (EPA) and in the European Union (EU). For instance, the US EPA focused on the 16 parent PAHs (*Keith, 2015*) whereas 8 PAHs are listed as a priority compound in the EU. Polycyclic aromatic hydrocarbons (PAHs) can be divided into two types mainly those with Low Molecular Weight (LMW) and those with High Molecular Weight (HMW). Low Molecular Weight PAHs are those organic compounds consisting of two to three fused benzene rings some examples include Fluorene, Naphthalene, and Anthracene. High Molecular Weight (HMW) PAHs are organic compounds consisting of four or more fused or bonded benzene rings such as pyrene, benzo[a]pyrene, etc. (*Ofori et al., 2020*).

However, the lower the molecular weight the less time they remain in the environment because of their higher volatility. (LMW) PAHs are less likely to cause carcinogenic issues since they are slightly soluble in water which can later have a toxic effect on humans if they end up consuming food products obtained from aquatic animals that have been exposed to them. On the other hand, HMW PAHs are classified as persistent chemicals in the environment since they possess low volatility and high oxidative resistance (*Ofori et al., 2020*).

There are various ways PAH gets into the environment such ways include; Petrogenic source and Pyrogenic sources. Petrogenic sources are those that come from petroleum while pyrogenic sources are those obtained from the process of incomplete combustion of organic materials such as cigarette smoke, and vehicle emissions.

The aim of this research is to study the partition of three PAH components (Naphthalene, Fluorene, and Phenanthrene) with relatively low octanol-water partition coefficients (log K_{ow} between three and five). These are slightly soluble in water and bound to the sediment/suspended particulate matter in the surface water. According to the water framework directive, these priority pollutants should be monitored in both phases. The adsorption properties of the above-mentioned PAHs were also investigated with four different soil samples and recoveries and adsorption equilibria were determined.

2. Literature overview

PAHs are typically lipophilic substances with a strong attraction for organic materials, however, the physiochemical characteristics of each PAH vary greatly from one another. Properties like vapor pressure and water solubility, which range from two to six benzene rings in the PAH molecule are displayed in Table 1 and span five and twelve orders of magnitude respectively. As a result, compared to their high molecular weight (HMW) counterparts, low molecular weight (LMW) is significantly more volatile and water-soluble whereas HMW compounds exhibit greater hydrophobicity. As seen in Table 1, the symmetricity of each structure also influences the physicochemical properties like vapor pressure and solubility. The more symmetric a structure the lower the vapor pressure and the less soluble the molecules. Parallel with the increasing number of rings the molecular weight solubility and vapor pressure decrease. Water solubility of PAHs is generally low as they are nonpolar compounds. K_{ow} is the partition coefficient of the PAHs (Table 1), which is the ratio of solubility in octanol to water. This parameter (K_{ow}) is given as the log K_{ow} value (or logP) and it is related to the polarity of compound. The higher the log K_{ow} value, the lower the polarity. Log K_{ow} is increasing by the number of rings.

Table 1. *US EPA's 16 priority-pollutant PAHs and selected physical-chemical properties (Lee, 2010)*

PAHs	Ring Number	Molecular weight	Solubility (mg/L)	Vapor pressure (Pa)	Boiling point (°C)	Log K_{ow}	Melting point (°C)
Naphthalene *(Nap)*	2	128.17	31	$1.0x10^{2}$	218	3.29	80.26
Acenaphthene *(Ace)*	3	154.21	3.8	$3.0x10^{-1}$	279	3.98	93.6
Acenaphthylene *(Acy)*	3	152.20	16.1	$9.0x10^{-1}$	280	4.07	91.8
Anthracene *(Ant)*	3	178.23	0.045	$1.0x10^{-3}$	340	4.45	218
Phenanthrene *(Phe)*	3	178.23	1.1	$2.0x10^{-2}$	340	4.45	100
Fluorene *(Flu)*	3	166.22	1.9	$9.0x10^{-2}$	295	4.18	116-117
Fluoranthene *(Fla)*	3	202.26	0.26	$1.2x10^{-3}$	375	4.90	110.8
Benz [a] anthracene *(BaA)*	4	228.29	0.011	$2.8x10^{-5}$	438	5.61	158
Chrysene *(Chr)*	4	228.29	0.0015	$5.7x10^{-7}$	448	5.9	254
Pyrene *(Pyr)*	4	202.26	0.132	$6.0x10^{-4}$	404	4.88	156
Benzo [a] pyrene *(BaP)*	5	252.32	0.0038	$7.0x10^{-7}$	495	6.06	179-179.3
Benzo [b] fluoranthene *(BbF)*	5	252.32	0.0015	-		6.04	168.3
Benzo [k] fluoranthene *(BkF)*	5	252.32	0.0008	$5.2x10^{-8}$	480	6.06	215.7
Dibenz [a,h] anthracene *(DBA)*	6	278.35	0.0005	$3.7x10^{-10}$		6.84	262
Benzo [g,h i] perylene *(BghiP)*	6	276.34	0.00026	$1.4x10^{-8}$	550	6.50	276.34
Indeno[1,2,3-cd]pyrene *(Ind)*	6	276.34	0.062	-	530	6.58	163.6

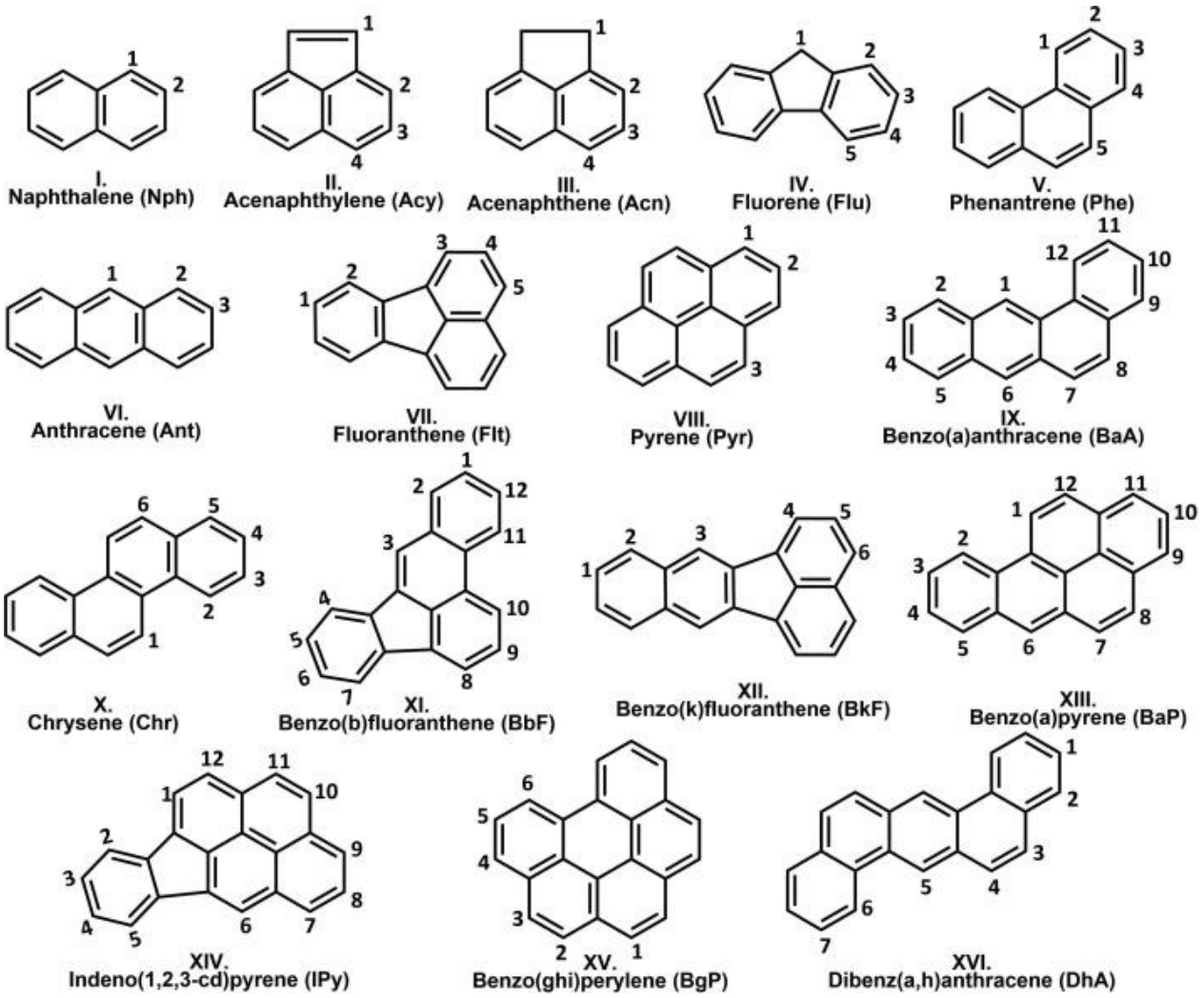

Figure 1. *Diagram showing the structure of the 16 PAHs (Reizer & Fiser, 2022).*

2.1. Occurrence and environmental fate of PAHs

Surface water contains polycyclic aromatic hydrocarbons (PAHs) that can be volatilized, photolyzed, oxidized, biodegraded, adsorbed to suspended particles or sediments, or accumulate inside aquatic organisms with bio-concentration factors often reaching the 10-10,000 range). In addition to volatilizing, PAHs in soil can also biodegrade, accumulate in plants, and go through abiotic breakdown (photolysis and oxidation). Additionally, PAHs in the soil can permeate groundwater and move through an aquifer*(Lee, 2010).*

2.1.1. PAHs in Air

Polycyclic aromatic hydrocarbons (PAHs) are released into the air through incomplete combustion of organic materials. Natural sources may include Forest fires, Volcanoes, etc. Anthropogenic sources may be related to vehicle emissions, cigarette smoking, and emissions from manufacturing companies such as steel, aluminum, and coal. PAHs are emitted into the atmosphere in two different forms, a vapor phase (gaseous until their vapor pressure) and a solid phase alone or together with other substances in aerosol in which the PAHs are sorbed onto particulate matter *(Z. Wang et al., 2013).*

2.1.2. PAHs in Soil

PAHs found in soils are usually due to anthropogenic activities. Industries also play a key factor in the reason. Soil contamination by PAHs depends on the distance from the pollution source, exposure time, and the soil depth. PAHs are deposited in the surface of the soil due to dry and wet deposition contaminating the plants and vegetables cultivated in the area.

2.1.3. PAHs in Water

PAHs generally enter the water bodies through dry and wet deposition surface run-off spillage from industrial activities and leaching. The solubility of PAHs in water commonly diminishes as the molecular weight increases, while their boiling and melting point increases(*Adeniji et al., 2017*). Because of this characteristic, they can attach to the surface of particulate matter, and this mechanism is remarked as the main transport pathway of PAHs from land and air to aquatic systems, as well as from the sea surface to lower depths (*Vagge et al., 2018*).

2.1.4. PAHs in sediments

Since PAHs are non-polar which prevents them from dissolving in water once PAHs infiltrate sediments their movement is hindered. However, PAHs are completely insoluble especially those with high molecular weight. Small quantities will be dissolved and enter the pore

water where they will become bioavailable. Because PAHs adsorb onto organic colloids in pore water, their concentrations can be raised above their aqueous solubility. As such, PAHs bound to colloids can move quickly across the pore spaces in sediments, which may enhance their mobility and bioavailability in sediments. *(Dong et al., 2012).*

One of the processes controlling the deposition of surface soils is the contribution of PAHs within the sedimentary system. They are deposited into lakes, streams, and oceans through both dry and wet atmospheric pathways. They mix into the sediment through flow and dispersion. Urban areas experience atmospheric accumulation of PAHs through inputs like flood, and highway runoff some of the PAHs eventually absorb onto particles, deposit, and become part of the sedimentary records. Researchers frequently analyze these records from anoxic sediment cores, where oxygen is absent in the water, to discern patterns in PAH deposition over time In contrast, the mobility of suspended sediment is high, and transport of contamination readily occurs.

2.2. Sources of PAHs

2.2.1. Molecular Diagnostic ratios

The molecular diagnostic ratios are used to determine the source of the PAHs in the environment whether they are of pyrogenic origin or Petrogenic origin. (*Sany et al., 2014*) states that ratio values such as an Ant/Ant + Phe and a Fla/Fla + Pyr were used to distinguish between Petrogenic and pyrogenic sources. Ratios < 0.100 were found in cases of Petrogenic inputs while ratio > 0.100 shows that the source is rather Pyrogenic (*Sany et al., 2014*). Many of the LMW PAHs are usually less stable since they are influenced by environmental factors, However, the HMW PAHs ratio are more stable and are less affected by these factors. *(Tobiszewski & Namieśnik, 2012*) . Below is the range of some of the PAH ratios and their emission source.

Table 2. *Diagnostic Ratios reported for PAHs in soils (Jiao et al., 2017)*

PAHs	Diagnostic Ratio	Sources
• Ant/(Ant + Phe)	<0.1 >0.1	Petroleum Combustion
• Fla/(Fla + Pyr)	<0.4 0.4 -0.5 >0.5	Petroleum Liquid fossil fuel combustion Coal, grass, or wood combustion
• Baa/(Baa + Chr)	<0.2 0.2-0.35 >0.35	Petroleum Petroleum or combustion Combustion
• Phe/Ant	<10 >15	Pyrogenic Petrogenic
• Bap/Bghip	>0.6	Traffic
• Ind/(Ind +Bghip)	<0.2 0.2-0.5 >0.5	Petrogenic Fuel combustion Grass/wood/coal combustion
• Bap/(Bap + Chr)	<0.2 0.2-0.35 >0.35	Petroleum Grass/wood/coal combustion Vehicular combustion
• HMW/LMW PAHs	>1 <1	Pyrogenic Petrogenic
• Fla/Pyr	<1 >1	Petrogenic Pyrogenic

2.3. Levels of PAHs

Although many samples were measured in different parts of the world, only some examples from China, Portugal, Nigeria, Egypt, and Hungary were used as study cases for this. These countries were used because their data were more recent than the others.

2.3.1. Levels *of PAHs in Air*

PAH concentration regarding the gaseous atmosphere in Alexandria, Egypt was measured (*Munyeza et al., 2019)* with values ranging from 300 ng/m^3 to 1400ng/m^3 in summer while in winter, values range from 155ng/m^3 to 870ng/m^3 Measurements were taken to determine the sum of the concentration of PAHs in Oporto, the second largest city in Portugal *(Slezakova et al., 2013*), levels were determined in both gas phase and particles and were found to range from 16.8ng/$m^3$to 149ng/m^3 with a mean of 70ng/m^3. Compounds with 3 rings were found to be the most abundant in the air with 53% of the total PAH content. Phenanthrene was observed to have the highest concentration of 27% of the total concentration, while Pyrene had (9.5% of the total concentration), Acenaphthylene had (11%), and Fluorene (9.4%) and Fluoranthene (8.6%). Naphthalene, a compound with two aromatic rings, accounted for (5%).. While at the SW coast of

continental Portugal PAH concentrations were measured also to determine the sum of the 16 EPA-PAHs in the air (*Augusto et al., 2010*). The result ranged between 23.8 and 40.1 ng/m^3 at the industrial site and between 11.0 and 18.9 ng/m^3 at the urban site.

2.3.2. Levels *of PAHs in Soil*

The concentration of the sum of 16 priority PAHs in the southern bank of the Yangtze River near the Three Gorges Dam in the Yichang area, Central China ranged from 8.26ng/g to 397ng/g with an average of 55.8ng/g (*Pu et al., 2022*). The PAH concentration in this area was found to be similar to those in the Dajiuhu sub-alpine wetland in central China with an average of 42.2ng/g(*Xing et al., 2020*) and also found to be higher than those from the karst spring system with an average of 25.8ng/n (*W. Chen et al., 2022*). Also, soils across contaminated sites in China were measured to show the concentrations of the 16 PAHs. The mean values ranged from 3.99mg/kg to 94.94.mg/kg (*You et al., 2024*).

2.3.3. Levels of PAHs in water

The concentration of the total PAH in surface water from three stations along the stretch of Ovia River Edo state in southern Nigeria was analysed (*Tongo et al., 2017*). Total PAHs in water varied from 2.22 to 25.83 µg/L with a mean of 17.56 ± 7.62 µg/L. The downstream (Ekenwan) station showed higher concentration values of 25.83µg/L, the total concentration in Iguiye station was 2.22 µg/L while the last station, Iguoriakhi was observed to be 24.5 µg/L. Naphthalene and Acenaphthylene were the most frequently detected PAHs in the water sample with values of 0.83 to 5.33 and 0.00 to 3.33 respectively. Studies showed that water samples are mostly dominated by 2 or 3-ring PAH (*Kafilzadeh et al., 2011)*. Also, the concentration value in the Danube was measured with a range of 25ng/L to 1208ng/L with a mean value of 122.6 ± 135.6ng/L *(Nagy et al., 2013*)

Samples from the Yongding River Basin in China were monitored to check the concentration levels of PAHs (*Y. Wang et al., 2018*). Total concentration ranged from 41.60 to 1482.60ng/L with a mean of 137.85ng/L in the spring while during the summer values ranged from 53.53 to 506.53ng/L with a mean of 124.43ng/L.

2.3.4. Levels of PAHs in Sediments

The concentration of the total PAH in sediments from three stations along the stretch of Ovia River Edo state in southern Nigeria was analysed (*Tongo et al., 2017)*. 3-ring PAHs showed a considerable predominance from the result obtained from the mean concentration. The concentration ranged from 12.83 µg/kg dry weight to 28.19 µg/kg dry weight for the 2-ringed PAH with a mean of 20.51 µg/kg dry weight while for the 3-ring PAHs had a mean value of 97.55 µg/kg dry weight with concentration ranging from 0 to 76.33 µg/kg dry weight. The mean value for the 4-ring PAHs was 11.01 µg/kg dry weight with a concentration range of 0.39 to 43.83 µg/kg dry weight, a concentration range of 0.64–12.31 µg/kg dry weight with a mean of 0.04 for the 5-ring and 0–1.33 µg/kg dry weight with a mean of 0.80 for the 6-ring PAH. Total PAHs in sediment varied from 5.25 to 573.33 µg/kg dw with a mean of 347.54 ± 301.43 µg/kg dry weight. Also, samples measured from the Bering Sea, Chukchi Sea, and the Canadian Basin of China showed concentration values of 16 US EPA priority PAHs ranging from 27.66 ng/g to 167.48 ng/g dry weight with a mean value of 77.27 ng/g (*F. Chen et al., 2018)*, which was lower compared to the values measured in the summer for the PAHs in water.

2.4. Directives

The EU implemented a directive for the water framework regarding the safety of water bodies which focuses on ensuring the water bodies are not polluted with chemicals and ensuring the environmental quality standards (EQS) for priority substances and other certain pollutants to achieve a good surface water status.

Article 1 spoke of the commission reviewing the adopted list of priority substances at least 4 years from the date of entry into the force of the directive and at least every 6 years afterward. Article 2 was amended and introduced to various directives which stated that the technical specifications for chemical analysis and monitoring of water status shall apply a matrix and a biota taxon. Article 3 was focused on the EQS (Environmental quality standard) stating member states shall apply the EQS laid down in part they are requirements laid down in the regulations and under this article to ensure a good surface water chemical status. The current EU priority list contains 5 PAH components (Table 3) and their corresponding EQS values.

Article 7a focused on the coordination during the discharge, emissions, and losses of priority hazardous substances to ensure the union and member state levels are sufficient to achieve the EQS. It further states that the commission shall report to the European Parliament and the Council on the result of the coordination with any appropriate proposals needed for the control measures. Article 8 aimed at the legislative proposals to amend Annex X to directive 2000/60/EC, proposals to identify new priority substances or hazardous substances, and to set corresponding EQS for surface water, sediment, or biota.

Under this article, provisions for certain specific substances were made. Substances behaving like ubiquitous PBTs (Persistent, Bioaccumulative, and toxic substances), newly identified substances, and substances for which revised, stricter EQS are established. The commission established a watchlist of substances to be monitored by the union worldwide to support future prioritization exercises. Also, specific provisions for pharmaceutical substances were made to reduce the discharges into water bodies. Article 9 focused on the committee and the exercise of the delegation *(Directive 2013/39/EU).*

Table 3*. Environmental quality standards (EQS) for certain priority substances (Directive 2013/39/EU).*

No.	Name of Substance	CAS number	AA-EQS Inland surface waters (µg/L)	AA-EQS other surface waters (µg/L)	MAC-EQS Inland surface waters (µg/L)	MAC-EQS other surface waters (µg/L)	EQS Biota (µg/kg)
1	Naphthalene	91-20-3	2	2	130	130	
2	Benzo(a)pyrene	50-32-8	1.7×10^{-4}	1.7×10^{-4}	0.27	0.027	5
3	Benzo(b)fluoranthene	205-08-9	NB	NB	0.017	0.017	NB
4	Benzo(g,h,i)perylene	191-24-2	NB	NB	8.2×10^{-3}	8.2×10^{-4}	NB
5	Indeno(1,2,3cd)pyrene	193-39-5	NB	NB	Not applicable	Not applicable	NB

- AA-EQS refers to the total concentration of all isomers
- Inland surface water includes rivers, lakes, and any artificial or heavily modified water bodies

- MAC-EQS refers to the maximum allowable concentration where the MAC-EQS are marked as “not applicable”, the AA-EQS values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived based on acute toxicity

- NB: For the group of priority substances the biota EQS and corresponding AA-EQS in water refer to the concentration of benzo(a)pyrene, on the toxicity on which they are based. Benzo(a)pyrene can be considered a marker for the other PAHs, hence only benzo(a)pyrene needs to be monitored for comparison with the biota EQS or the corresponding AA-EQS in water.
- The Biota EQS, in this case, refers to crustaceans and mollusks.

2.5. Analytical Procedure

The analytical protocol for PAH compounds mainly involves preparing the sample and analyzing it. Typical extraction methods for the EPA 16 involve microwave extraction with Hexane and Acetone and solid-phase extraction (Table 4).

2.5.1. Sample Preparation

During the sample preparation of PAHs, various things need to be done before the sample can be analyzed. The first thing that needs to be done is to get a representative sample then we need to store the samples to preserve it, followed by homogenization of the sample. Thereafter comes the extraction process for which various methods can be used according to the matrix. Some examples are mentioned in Table 4.

2.5.2. Instrumental analytical methods (GC/HPLC)

2.5.2.1. Gas chromatography (GC)

Gas chromatographic methods are applied for the separation of non-polar organic compounds that are thermally stable and volatile. PAHs are often determined by GC as they fulfill these requirements and they are sufficiently volatile (semivolatile). Detectors used for the analysis depend solely on the organic compound that is to be determined and the properties of some of the detectors are universal while some are selective. Some examples of the selective detectors used include Electron Capture Detector (ECD works well with halogenated, chlorinated solvents and pesticides), Flame Photometric Detector (FPD used for detecting compounds containing

Table 4*: Different analytical procedures with their sources*

Matrix	Extracting method	Analytical Instrument	Pollution Source	Reference
➢ Soil	Microwave extraction with 25 mL n-hexane and Acetone	GC-MS	Pipeline transport of petroleum products	(*Ogoko, 2014*)
➢ Soil	Soxhlet extraction with 10 mL of methanol and 25 mL of Dichloromethane (DCM)	GC-MS	Solid waste combustion	(*Ekpete et al., 2019*)
➢ Soil	Shake extraction with 10 mL of Dichloromethane (DCM)	GC-FID	Production of asphalt in hot mix asphalt plants	(*Ilechukwu & Osuji, 2013*)
➢ Sediments	Filtration method with 10 mL of Pentane	GC-FID	Gas flaring	(*Inengite et al., 2010*)
➢ Water	Solid-phase extraction with 10 mL of Dichloromethane (DCM)	GC-MS	Oil spillage	*(Obinaju et al., 2015*)
➢ Soil	Agitation extraction with 100 mL DCM/acetone	GC-MS	Oil spillage	(*Ugochukwu et al., 2018*)
➢ Water	Accelerated solvent extraction	GC-MS	Oil spillage	*(Nganje et al., 2012*)
➢ Air	Passive sampling using Polyurethane foams coupled with Accelerated solvent extraction with DCM	HPLC with fluorescence/UV	Ambient air	(*Albinet et al., 2007)*

phosphorus and sulfur only), Nitrogen-Phosphorus Detector (NPD), Atomic Emission Detector (AED). These selective detectors are not suitable for PAH components. Photoionization Detector and Flame Ionization Detector (FID) are not selective detectors but can be used for hydrocarbons including PAHs. Universal detectors also include Thermal Conductivity Detector (TCD), and Mass Spectrometer (MS) but for the study of PAHs. TCD is not applied due to its low sensitivity and high limit of detection (LODs). Mainly FID or more commonly MS detectors are used for PAHs. MS is advantageous because mass spectral identification is possible and the

possibility of eliminating interferences with matrix components by using selective ion monitoring (SIM).

2.5.2.2. High-Performance Liquid Chromatography (HPLC)

One of the alternative techniques used for the analyses of PAH derivatives is HPLC combined with a detector. It is suitable for thermally liable compounds but target compounds have to be soluble in the eluent. HPLC gives some benefits which include faster speed, high sensitivity, and excellent resolution, especially by using UHPLC. Columns C18 is the type of column commonly used. Due to the volatile nature of some LMW PAH derivatives, HPLC is typically used for aqueous samples and congeners with high molecular weight. In contrast to GC, sample preparation is only the filtration of the aqueous sample, which allows faster determination of the compounds. Detectors used for HPLC include Fluorescence detector (selective), MS(universal),(which are more common due to their lower LODs and higher sensitivity), and UV FLD Agilent application note.(5990-8414EN.Pdf, n.d.)

2.6. Effect of PAHs

PAHs are known to persist in the environment containing air, water, and soil. PAHs harm humans and wildlife, causing reproductive and developmental issues in aquatic and terrestrial organisms. Effects on human health can be grouped based on the length of the effect (short-term/acute or Long-term/chronic effect).

2.6.1) Acute or short-term effect

The concentration of PAHs during exposure, the duration of exposure, the route, and the toxicity of the PAHs determine the acute effect on human health(*Kim et al., 2013*). symptoms such as vomiting, diarrhea, nausea, eye irritation, and confusion have risen from exposure*(Mallah et al., 2022*). Mixtures of PAHs can cause skin irritation and inflammation Direct skin irritants include anthracene, benzo (a)pyrene, and naphthalene. However, skin sensitizers are confirmed to be anthracene and benzo (a)pyrene, i.e., induce an allergic reaction in animals and humans to the skin(*Bil et al., 2018*).

2.6.2. Chronic or long-term effects

Problems like Jaundice, cataracts, diminished immune function, breathing problems such as asthma, and lung function abnormality can be gotten from prolonged or long-term PAH exposure (*Diggs et al., 2011*). The harmful effects of PAHs depend on the way of exposure. Humans have been exposed to PAHs at work mostly through inhalation, while a small number of studies have also included skin contact exposure. The only data available on human exposure to specific Polycyclic Aromatic Hydrocarbons (PAHs) is from a few cases of accidental contact with naphthalene. Moreover, as PAHs have the potential to interfere with hormone systems, they can exert harmful effects on reproduction and immune function. Other effects of PAHs are carcinogenic, genotoxicity, and teratogenicity.

2.6.3. Carcinogenicity

BaP is one of the most potent carcinogens in the PAHs group. A lot of PAHs have been classified for carcinogenicity based on the fact that they have been tested for carcinogenicity or related toxic effects compared to other carcinogenic compounds and it was found to have similar effects *(Nisbet & LaGoy, 1992)*. A method to determine the carcinogenic level was set which was to calculate the total concentration of benzo[*a*]pyrene - equivalent concentration (B[*a*]P_{eq}) by using the relative PAH toxicities. This is calculated with the formula,

$$\text{Total } B[a]P_{eq} = \Sigma_i C_i \times TEF_i. \qquad (1)$$

Where $\Sigma_i C_i$ = the concentration of PAH and,

The TEF_i is the toxicity equivalency factor for each PAH relative to BaP.

Table 5 below shows the TEF of each PAH with their log K_{ow} value.

Table 5. *Details showing the TEF of each PAH* (*Nisbet & LaGoy, 1992)*

PAHs	Aromatic ring	TEF	Partition coefficient log(*K*ow)
Naphthalene	2	0,001	3.28
Anthracene	3	0,01	4.45
Phenanthrene	3	0,001	4.45
Fluoranthene	3	0,001	4.90
Fluorene	3	0,001	4.18
Acenaphthene	3	0,001	3.98
Acenaphthylene	3	0,001	4.07
Pyrene	4	0,001	4.88
Benzo[a]anthracene	4	0,1	5.61
Chrysene	4	0,1/0,01	5.16
Benzo[k]fluoranthene	5	0,01/0,1	6.06
Benzo[a]pyrene	5	1	6.06
Benzo[b]fluoranthene	5	0,1	6.04
Benzo[g,h,i]perylene	6	0,01	6.50
Indeno[1,2,3-cd]pyrene	6	0,1	6.58
Dibenzo[a,h]anthracene	6	1/5	6.84

3. Materials & Methods

Three model compounds were chosen by the octanol-water partition coefficient (K_{ow}) to study their adsorption properties for the four different soil samples.

Table 6. *Details showing the different soil parameters*

	Soil sample 1	Soil sample 2	Soil sample 3	Soil sample 4
Location	Magyarszombatfa	Keszthely	Karcag	Kapolnasnyek
Humus %	0.49	1.45	2.00	3.70
$CaCO_3$%	0.00	0.05	0.13	9.52
K_A	59	30	90	46

Humus%: Soil Organic Matter (SOM) [%] content measured by the Tyurin method.
$CaCO_3$: Calcium carbonate content [%], measured by Scheibler calcimeter method.
K_A: Arany number, which is a special Hungarian method to measure the plasticity of the soils, and has a close correlation with the general plasticity index.

3.1. **Spiking of soil samples**

Three stock I solutions (10mg/mL) were prepared using Fluorene, Phenanthrene, and Naphthalene. 100mg of each of the compounds were measured and then mixed with acetonitrile. Then stock 2 was prepared from the stock 1 solution with 10ml from stock 1 solution containing the individual compounds and addition of 10ml acetonitrile was added, thus 40ml stock 2 solution was obtained and the level was of compounds was 2.5mg/mL.

5g of soil samples was measured into 9 centrifuge containers (three different models: high level 100µg/mL, middle level 10µg/mL, and low level 1µg/mL each level repeated three times). For each level, different spiking solution concentrations were used. To determine the recoveries for 4 soil samples, these were spiked as follows. For the high level (100µg/g), the stock 2 solution was added using a 200µL pipette, for the middle level (10µg/g), the stock solution was diluted (the dilution process includes measuring 100µL of the stock solution and then added with 900µL of acetonitrile after the solution was mixed to get a homogenous solution) after dilution 200µL was added to the soil sample. For the low level (1µL/g), the diluted stock used in the middle level was further diluted (100µL middle-level stock was mixed with 900µL of acetonitrile and then homogenized) 200µL was also added to the soil sample.

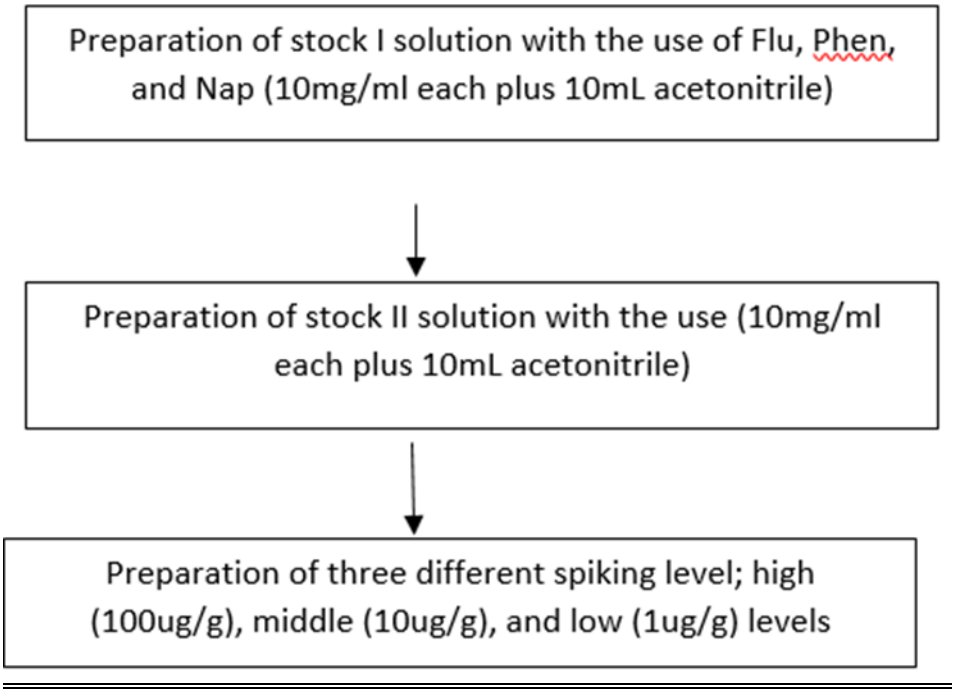

Figure 2. *Illustration of the spiking method (own source)*

3.2. **Sample Preparation**

The target components have been extracted from soil samples according to the procedure presented in Figure 3. Hexane and Acetone were measured in a ratio of 4:1 in a measuring cylinder and then mixed. Then using a 5000µL pipette 15mL was added to each soil sample and then shaken and put into the ultra sonification machine for 3 minutes. The weight was measured before being taken to the centrifuge which operated at 4000rpm at first but then later changed because the centrifuge tube could not handle that speed, the speed was later changed to 2000rpm the samples were centrifuged for 5 minutes. The solution was extracted from the centrifuge tubes to a tube and evaporated to dryness. The same steps from adding the solvent were applied for a second time and then evaporated to dryness, while the solution was evaporated to 5mL the third time. Altogether the extraction was done three times for the same soil. This process was done for all the soil samples (chapter 3.1) at each level to determine recoveries and partition rates.

The blank samples were made for the soil samples using the same steps used for earlier ones but no spiking of the samples before adding the solvent.

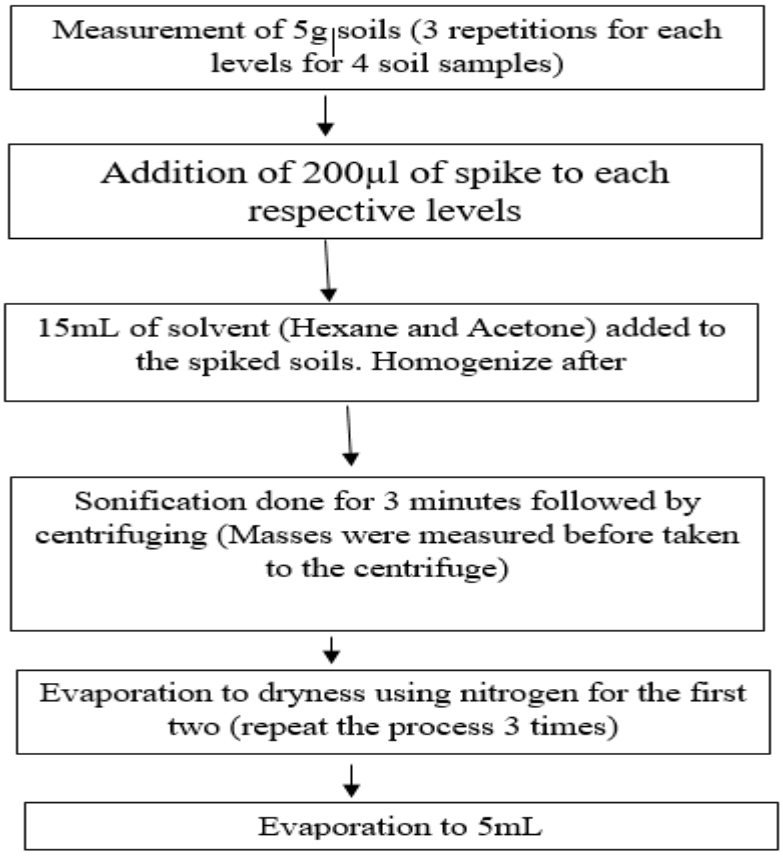

Figure 3*: Illustration of the sample preparation method (own source).*

3.3. GC-MS Method

Two different methods were applied: Selected Ion Monitoring (SIM) and Total Ion Chromatogram (TIC). The SIM method contains the selected ions that contain the most intensity for the PAHs chosen in this study. From the TIC method, the selected ions were the highest. Although there were other ions with similar intensities we chose the molecular ions with the highest intensities. The samples for determination of the calibration points were put into the GC-MS machine using the Selected Ion Monitoring (SIM) method with the carrier gas of Helium, carrier gas flow rate of 1ml/min, and a split ratio of 1:100 with the run time of 10 minutes and initial temperature of 80°C with a final temperature of 260°C. The oven temperature was increased

by 30°/min to the final temperature and kept there for 3 minutes, the pressure depends on the actual temperature, column dimension, and the flow rate. The column length used for this was 15m with an internal diameter of 0.25mm and a film thickness of 0.25um. A gas saver mode was set to 2 minutes and a flow m/min was 15 and the split ratio was lowered to 1:5. MS parameters were also set as follows. The Ion source temperature was set to 200°C with a transfer line temperature of 250°C. Selected ion monitoring was applied (SIM method), the ions were the molecular ions $[M]^+$ of the corresponding components. Three ions were monitored in the process, 128 for naphthalene, 166 for Fluorene, and 178 amu for Phenanthrene with 1.5, 3.5, and 4.7 as the start time in minutes. The Solvent delay when the ionization started was 1.5 min.

3.4. **Calibration**

Calibration solutions were made using the addition of acetonitrile and Stock II solution. Ten (10) different calibration solutions were prepared by dilution (500µg/mL, 100µg/mL, 50µg/mL, 10µg/mL, 1µg/mL, 0.5µg/mL, 0.1µg/mL, 0.05µg/mL, 0.01µg/mL, and 0.005µg/mL). Three working solutions were used to dilute other samples. Calibration 3 (50µg/mL) was the first working solution used to dilute Calibration 4 (10µg/mL) and 5 (1µg/mL) while calibration 5 was the second working solution to dilute Calibration 6 (0.5µg/mL), 7 (0.1µg/mL), 8 (0.05µg/mL), and 9 (0.01µg/mL) while calibration 8 was the last working solution to dilute Calibration 10 (0.005µg/mL). The working solutions were prepared in 2mLs instead of 1mL as the others to be able to be used in the dilution of the needed calibrations. Each point was filled to 1mL using Acetonitrile followed by homogenization using the vortex machine. After this process, the soil samples were filtered using a Syringe filter (Chrom Filter SHANGHAI) with PTFE membrane with a Hydrophilic filter, (pore size = 0.22µm, Diameter = 13mm) using a Kruuse disposable syringe and needle. After this procedure, the filtered sample was put into the GC-MS auto-sampler and each solution injected 3 times. The results from the calibration curve were plotted to generate a calibration curve.

Table 7. *Data showing the calibration volume.*

Calibrations	Calibration points (µg)	Stock II volume (µL)	Acetonitrile Volume (µL)
1	500	200	800
2	100	40	960
3*	50	40	960
4	10	200	800
5*	1	40	960
6	0.5	500	500
7	0.1	100	900
8*	0.05	50	950
9	0.01	10	990
10	0.005	100	900

* Represents calibration values which were done in 2mL for further dilution instead of the regular 1mL

3.5. Preliminary experiment for extraction of water phase

This experiment aimed to determine the best organic solvent for further experiments. For this purpose, two solvents were chosen (Hexane and DCM). The spiking solution was prepared using 20µL of stock II and then diluted 500 folds with Acetonitrile in a 10mL volumetric flask. 50 mL of water was measured using a graduated cylinder into two 100 mL DURAN SCHOTT Square bottles. 20µL of the above-mentioned spiking solution was added to the water and then homogenized. 1mL of Acetonitrile was added to both bottles to facilitate the phase transfer of pollutants between the aqueous and organic phase followed by the addition of 3mL of each organic solvent.

The solvent from the solution in the tube was collected by using a syringe and needle. The addition and extraction process was done three times after evaporation to dryness. 1mL of the corresponding solvent hexane or DCM was added to the residue, vortex for 30 seconds and approximately 200µL was transferred into an insert in the vial, and then taken to the GC-MS auto-

sampler. Theoretically, the levels are 0.1 µg/mL in the volume of the extract if 100% is recovered and 0.002µg/mL was originally in the water. Table 7 below shows the peak areas of the target compounds obtained from the analyses and the solvent applied. Unfortunately, we were not able to pick one out of the two solvents due to the loss of Naphthalene mainly due to evaporation in the Hexane and the value for Phenanthrene in the DCM seems to be lower than that in Hexane.

Table 8. *Data showing the peak areas obtained from the analysis.*

	Target compounds		
	Naphthalene	Fluorene	Phenanthrene
DCM	20351	12230	17060
Hexane	-	12057	21516

3.6. Experiment for extraction of water phase with suspended material

For the soil samples, 500mL of water was measured into a DURAN SCHOTT bottle then spiked with 200ul of stock II followed by the homogenizing of the solution. 5g soil was added to the solution and stirred for 4 hours to help facilitate the pollutants partitioned between the soil and the water phase. 50mL was taken off the stirred solutions into two different bottles. The remaining solution was filtered using filter paper and a 500 mL cylinder while for filtered solution approximately 250ml of filtered water was collected from each soil and was poured into a 1-litre bottle and stored for further experiments.

The two bottles containing 50mL of the solution were separated and labeled one for DCM while the other was for Hexane. 3mL of each solvent (Hexane and DCM) were added to their respective bottles. Extraction of the solvent from the solution was done next using a syringe and needle into a tube. The addition and extraction process was done three times after evaporation to dryness. 1mL of each solvent was added to their respective tubes for evaporation and then homogenized using the vortex machine. Approximately 200µL was extracted using the needle and syringe, but the extracted solvent was filtered using a syringe filter before being inserted into the vial since the solvent contained some particles then the vial was taken to the GC-MS autosampler to be analyzed. The analyses were done in triplicates for the various soils.

3.7. Experiment using 2g soil

After the filtering of the water as discussed in chapter 3.6, the filter paper used was allowed to dry. This experiment aimed to determine the adsorbed PAHs in the soil. After the drying of the filter paper, the soils on the filtered paper were scraped and put into a centrifuge tube. The soil was then measured and also the filter paper was measured after. After measuring, we decided to take out 2g of soil from each soil sample because that was the only way to get a representative result since some soils had more than 2g while some had just 2g soil left.

The solvent which was a mixture of Hexane and Acetone of 6 mL in a ratio of 4:1 was added to the measured 2g soil and then sonification for 5 minutes. The mass of the sonification component was taken since it had to be taken to the centrifuge machine. the steps taken is similar to the steps mentioned in Chapter 3.2. The process was done three times for each soil sample.

3.8 Solid Phase Extraction (SPE) method

For the determination of PAH content in the water phase, we also tested an SPE method. The stored filtered water was removed from the refrigerator rest from 3.6. Soils (spiking level) as mentioned in Chapter 3.6, sealed with parafilm to prevent evaporation. The SPE method was used following Phenomenex applications TN-0042 and 19651(*Polycyclic Aromatic Hydrocarbons (PAHs) by GC/MS and LC/MS (TN-0042)*, n.d.).

Firstly, 250mL of filtered water was measured into 4 graduated cylinders, labeled, and poured into separate Erlenmeyer flasks. 4 cartridges (Strata PAH cartridge made by Phenomenex which could have 1.5g mass of solid phase/6mL as the volume) conditioned with 10 mL of DCM and 10 mL of methanol followed by 20mL distilled water. After the conditioning, the filtered water was passed through the cartridge with the help of a vacuum with tubes in each flask (figure 4). Unfortunately, not all the water was able to pass through the cartridge because it was clogged so we were unable to use the whole 250mL of filtered water. Then the cartridge was washed with 5ml of methanol water in a 1:1 ratio followed by elution of components by DCM. The solution was collected into a glass bottle and then poured into a test tube the water was removed and the

organic solvent was evaporated. 1mL of ACN was added to the dried residues followed by vortex and then it was transferred to the vial and measured by GC-MS. The results were corrected according to the extracted volumes.

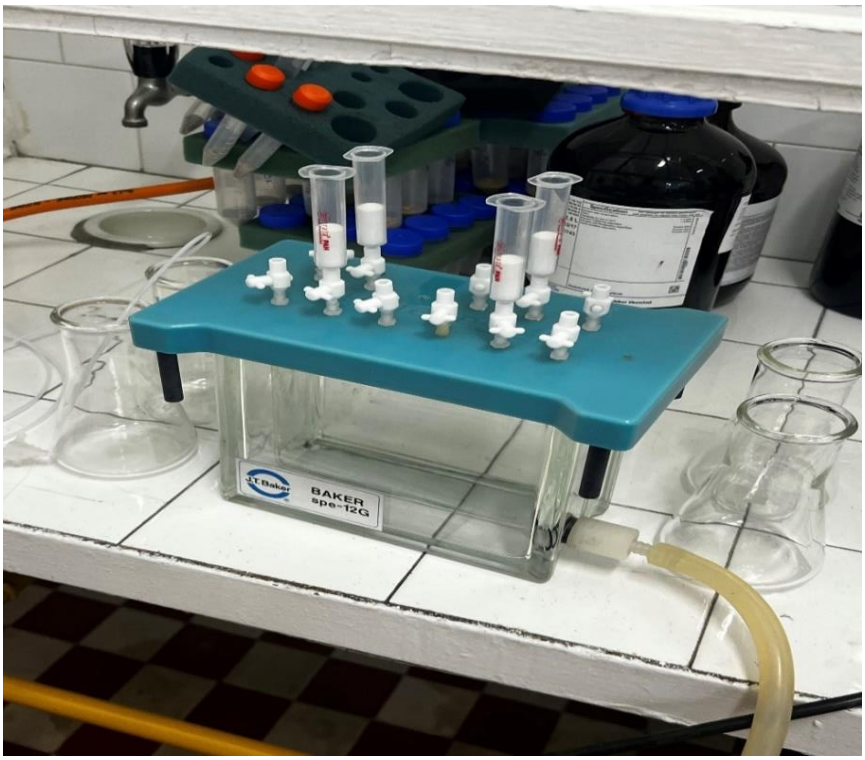

Figure 4. *A picture showing the cartridge and the device used for the SPE.*

4. RESULTS

Three ions were monitored during the analysis method. Nap was monitored in reference to the mass-to-charge ratio of ion 128 amu, and was found to have a retention time of 2.49 minutes while Flu was done in reference to 166amu and a retention time of 4.28 minutes and Phen was also done with reference to 178 amu and its retention time was 5.08 minutes. The typical chromatogram is shown in Figure 5 showing the chromatogram of three monitored components. Figures 6, 7, 8, 9, 10, and 11 shows information about the peak and their respective molecular mass in the spectra.

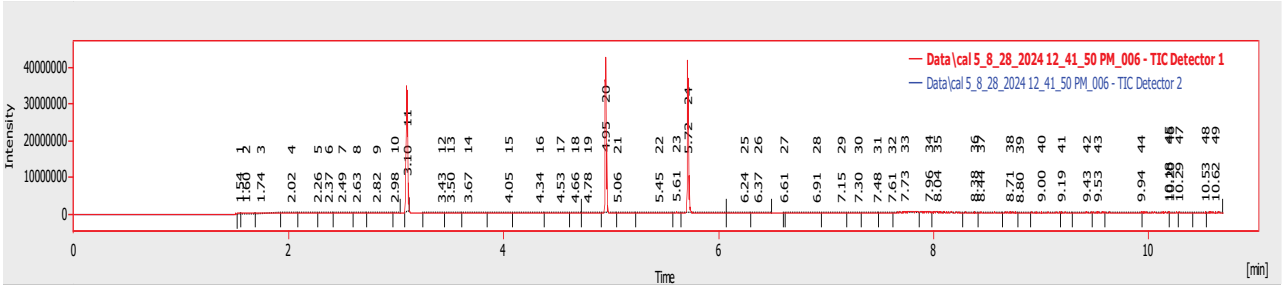

Figure 5. *A diagram showing the chromatogram of a calibration point (1 µg/mL).*

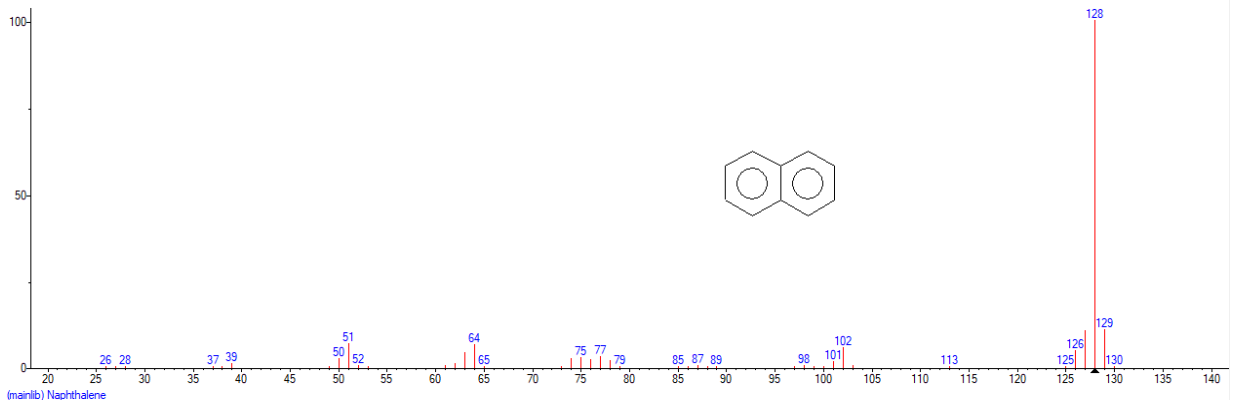

Figure 6. *A diagram showing the structure and library spectrum for Naphthalene*

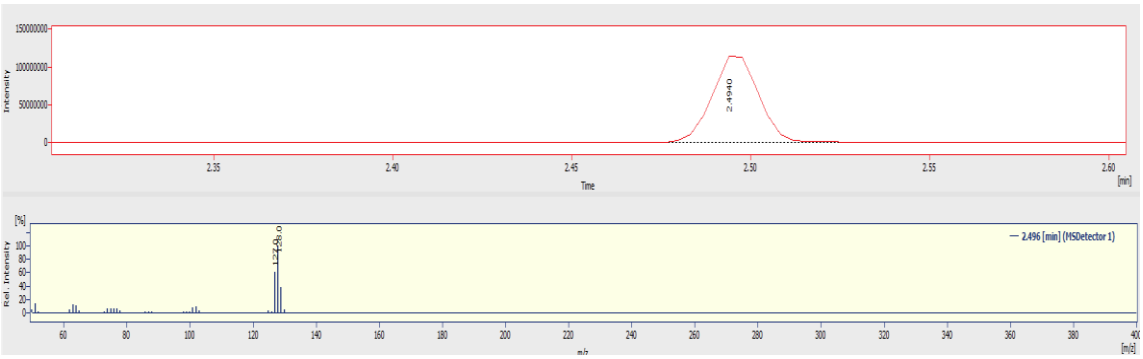

Figure 7. *A diagram showing the peak measured for Naphthalene together with its spectrum.*

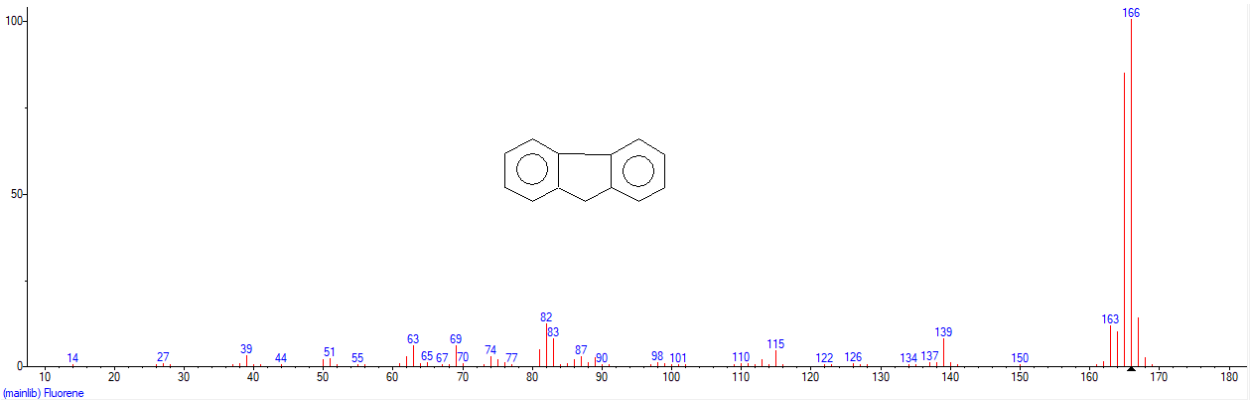

Figure 8. *A diagram showing the structure and library spectrum for Fluorene.*

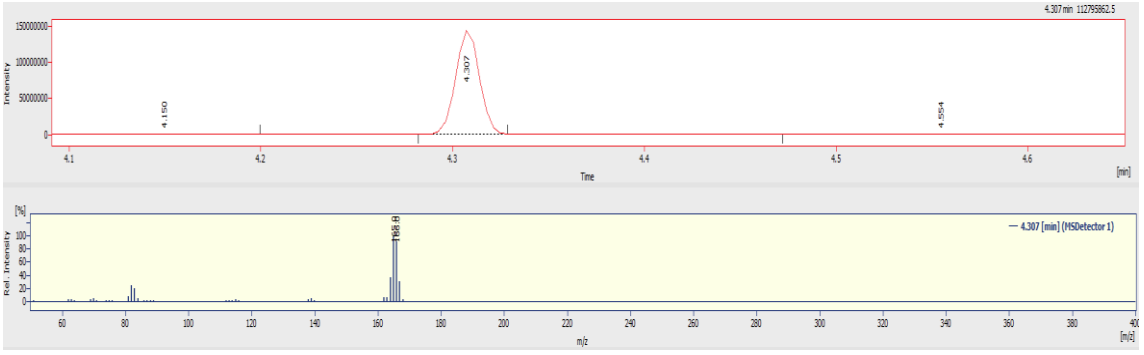

Figure 9. *A diagram showing the peak measured for Fluorene together with its spectrum.*

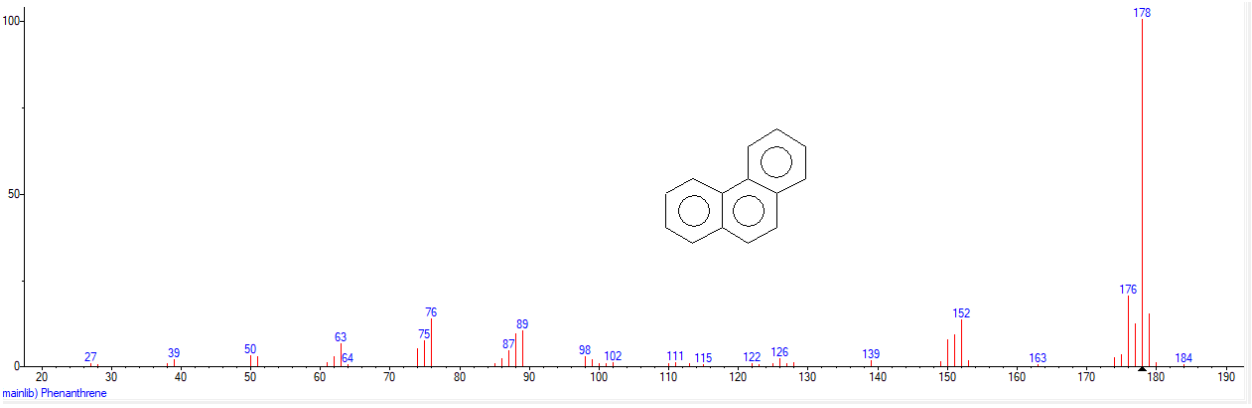

Figure 10. *A diagram showing the structure and library spectrum for Phenanthrene.*

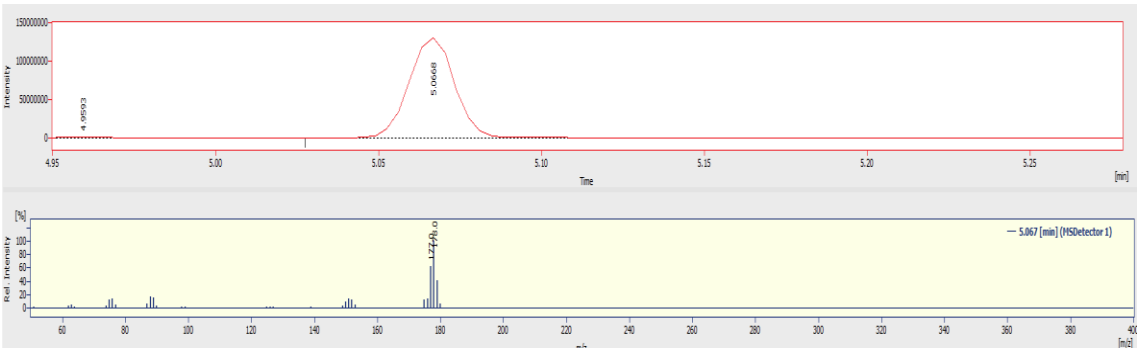

Figure 11. *A diagram showing the measured peak for Phenanthrene together with its spectrum.*

4.1. Calibration curve

The calibration curve was recorded for standard solutions (Chapter 3.4). The calibration curve contains the slope and the R^2 value. The slope value was used to calculate unknown concentrations. As seen in Figures 12, 13, and 14 not all the calibration points were included in the graph as the result obtained for the concentration range was too large, and also the graph was slightly not linear therefore, we used only range relevant to our samples. The Calibration Curve was plotted using the Peak area of each point against the concentration of the points.

When the calibration curve was recorded, the results for Nap did not seem unusual. The calibration curve for Phen and Flu were slightly linear but they had less good fitting. The R^2 value for Nap was better than the other two.

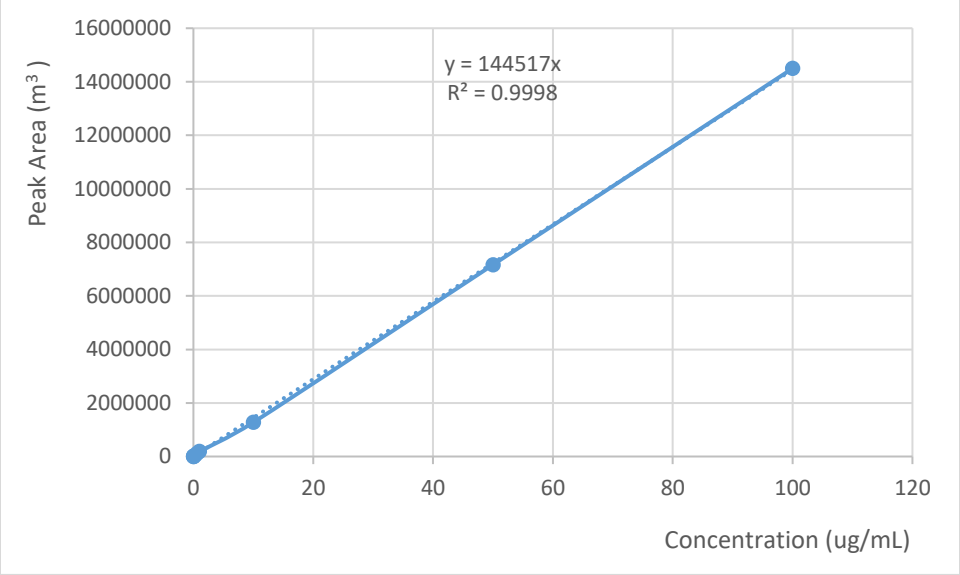

Figure 12. *Calibration curve for Nap.*

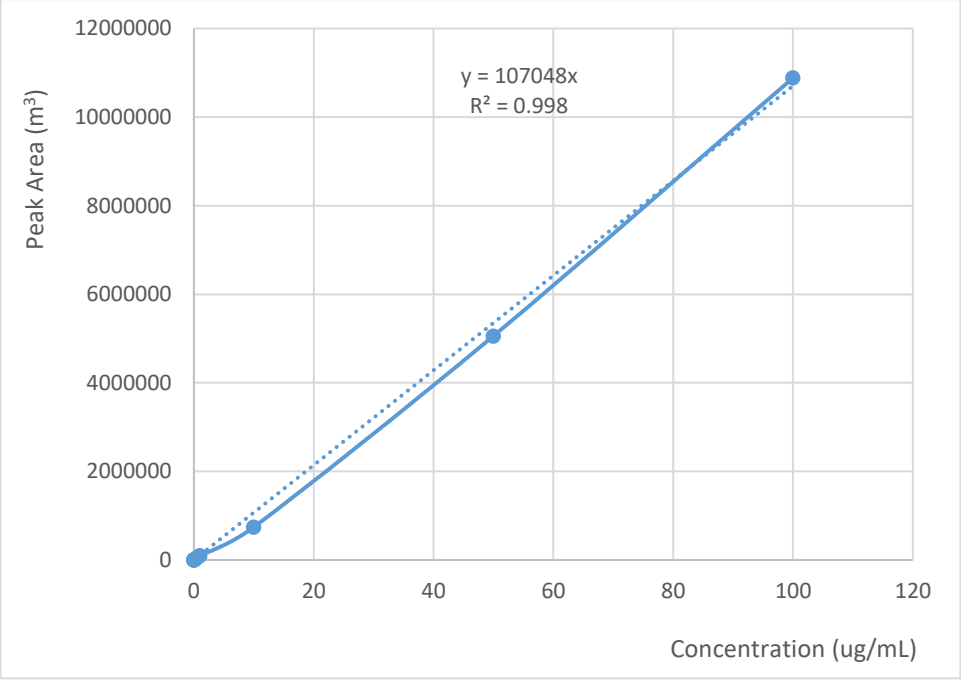

Figure 13. *Calibration curve for Flu.*

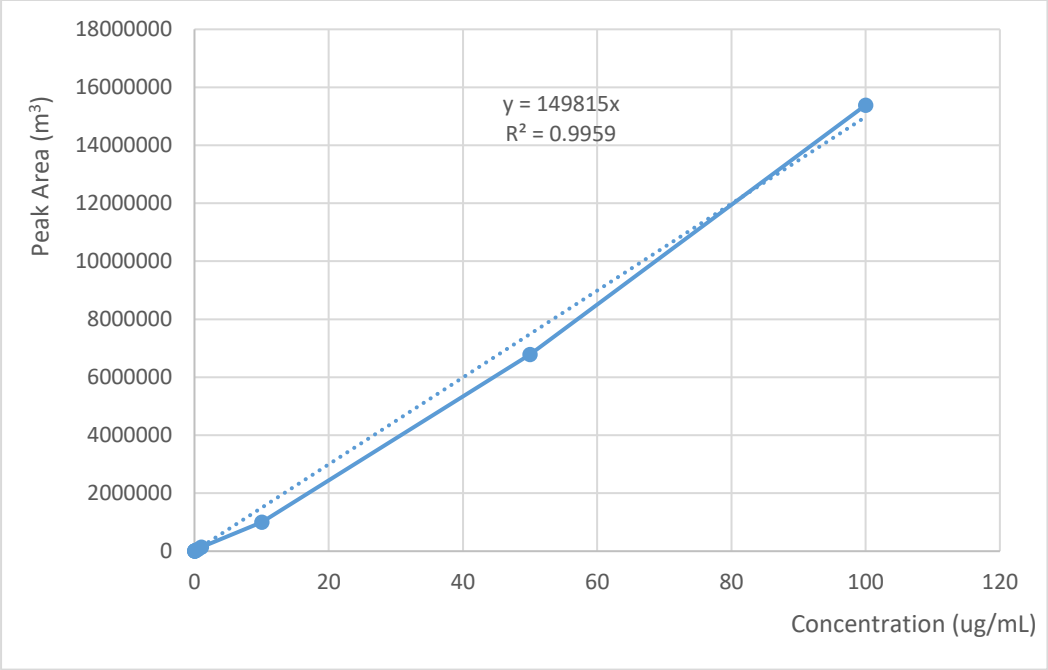

Figure 14. *Calibration Curve for Phen*

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4.2. **Results of recovery determinations from soils**

Spiking for 4 model soil samples (See Chapter 3.2) has been performed. It was found that Nap results were not reliable because there were losses due to evaporation. During the sample preparation process, it is hard to prevent the evaporation of the most volatile PAH component, and insufficient control of the process may result in a higher standard deviation between the parallels.

The different recovery rates for each level of the target components were determined using the calculated concentration (µg/mL) against the concentrations of the various levels. Figures 15, 16, 17, and 18 show the data for the recovery of the 4 different types of soils compared to high (100 µg/g), medium (10 µg/g), and low (1 µg/g) spiking levels.

Nap recovery was significantly lower (up to 26%) than the other compounds. A result is said to be acceptable if when the recovery falls between 60-120%. The result for Nap for all samples wasn't acceptable due to the evaporation that had occurred. Soil 1 had an acceptable result for Flu and Phen at the high level. The middle level for soil 1 showed result that weren't acceptable for

all compounds. For the low level, only Flu had acceptable result which was low but was in the acceptable range.

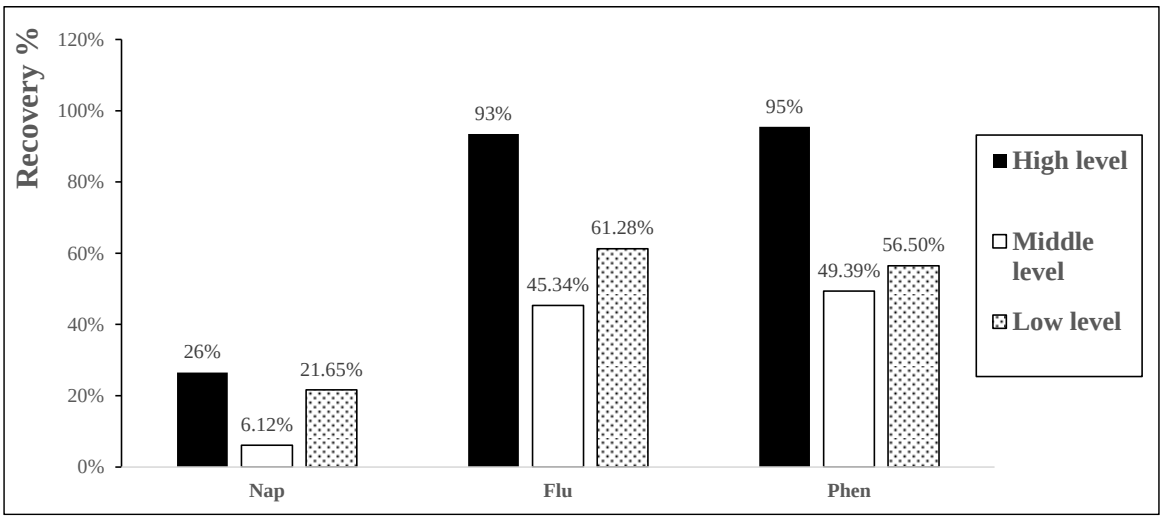

Figure 15. *A chart showing the different recovery percentages of various levels for 3 components in soil 1.*

For Soil 2 (Figure 16), the recovery was lower than other soils. The recovery for Flu and Phen at the high level was acceptable but was low. The middle and low level recovery weren't acceptable since they were not in the reproducibility range.

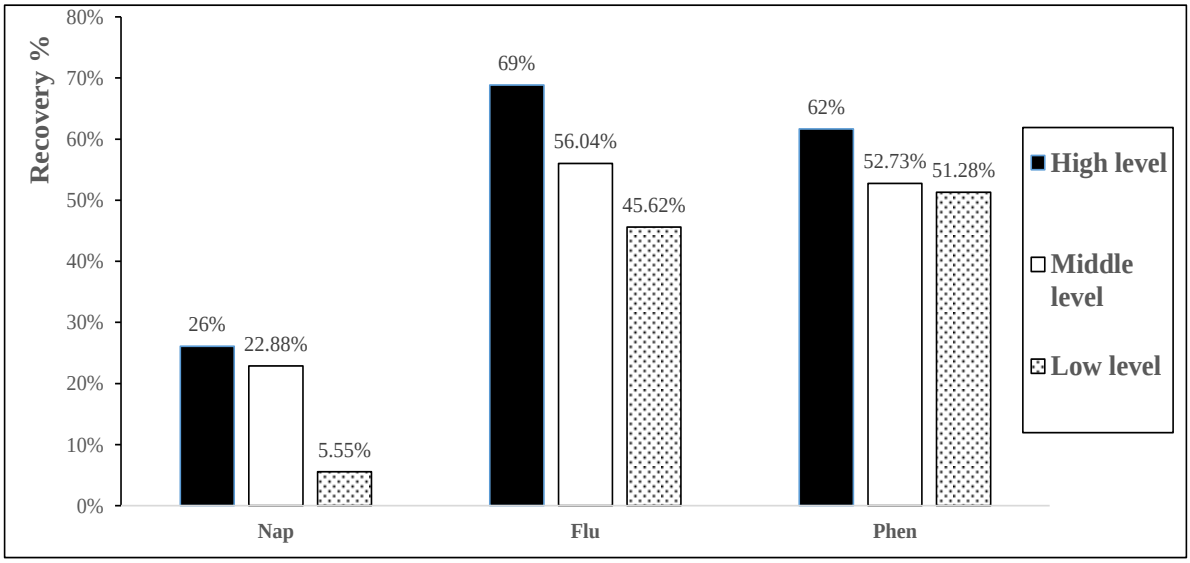

Figure 16. *A chart showing the different recovery percentages of various levels in soil 2.*

Figure 17 shows the recovery for Soil 3 which was acceptable and reproducible for Flu and Phen at the high level and middle level. The low level recovery were very low and were not accepted nor reproducible.

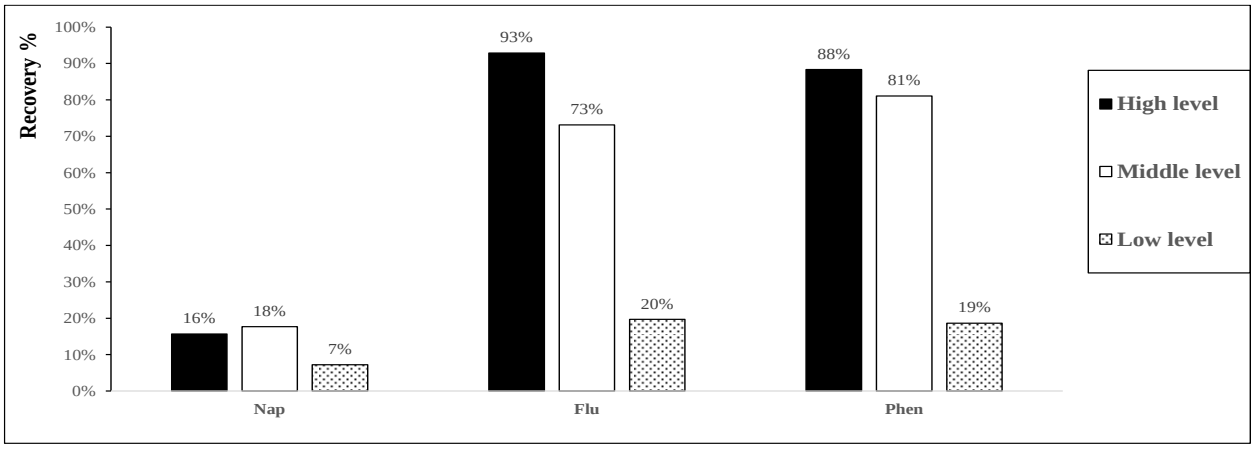

Figure 17. *A chart showing the different recovery percentages of various levels in soil 3.*

In Figure 18, the recovery at high level for Flu and Phen were acceptable and reproducible. The middle level and low level were low and not acceptable.

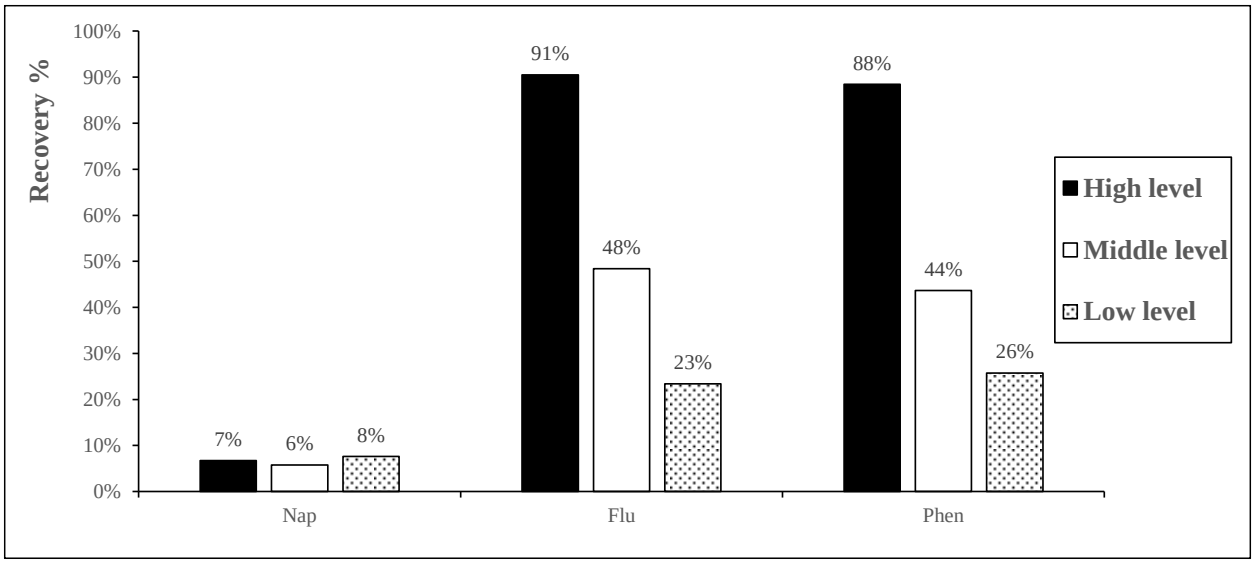

Figure 18. *A chart showing the different recovery percentages of various levels in soil 4.*

Different trends were observed for different model of soils. Good recoveries were observed for soils 1, 3, and 4 in the high level. Lower recovery for soil 2 for Flu and Phen. Comparing soil 3 and soil 1, soil 3 had a better recovery rate compared to soil 1. Despite the fact that soil 4 had higher humus and $CaCO_3$ percentage (as mentioned in Table 6), soil 3 had the best recovery rate. For high level, we had good recoveries (90%) except for soil 2 which was lower but acceptable. The middle level, typical recovery was between (40-50%) except soil 3 which had 75%. The low level, soils with lower humus content (soil 1 and 2) had recoveries between 50-60% while for soils 3 and 4 the recoveries were around 20% (high humus content). For lower spiking levels, we got lower recovery.

4.3. Preliminary Experiment

As discussed in Chapter 3.5, the preliminary experiment was done in order to determine the best organic solvent for further experiments. In Figure 19, the result of the experiment is shown. The recovery of Nap for the Hexane solvent was found to be 0% because of the evaporation while the result of Flu seem practically identical to each other and the Phen recovery with Hexane was slightly better than the recovery which was obtained with DCM. The recovery percentage observed for this experiment was slightly higher than expected (100%) but this proved that the extraction was successful from the water phase only. Both solvents were chosen due to the fact that we couldn't pick one because of the differences observed. Nap showed significantly higher recovery (140%) which was very high, probably to the fact that were some interracting contribution to the intensity. This experiment was done just one time with no parallels.

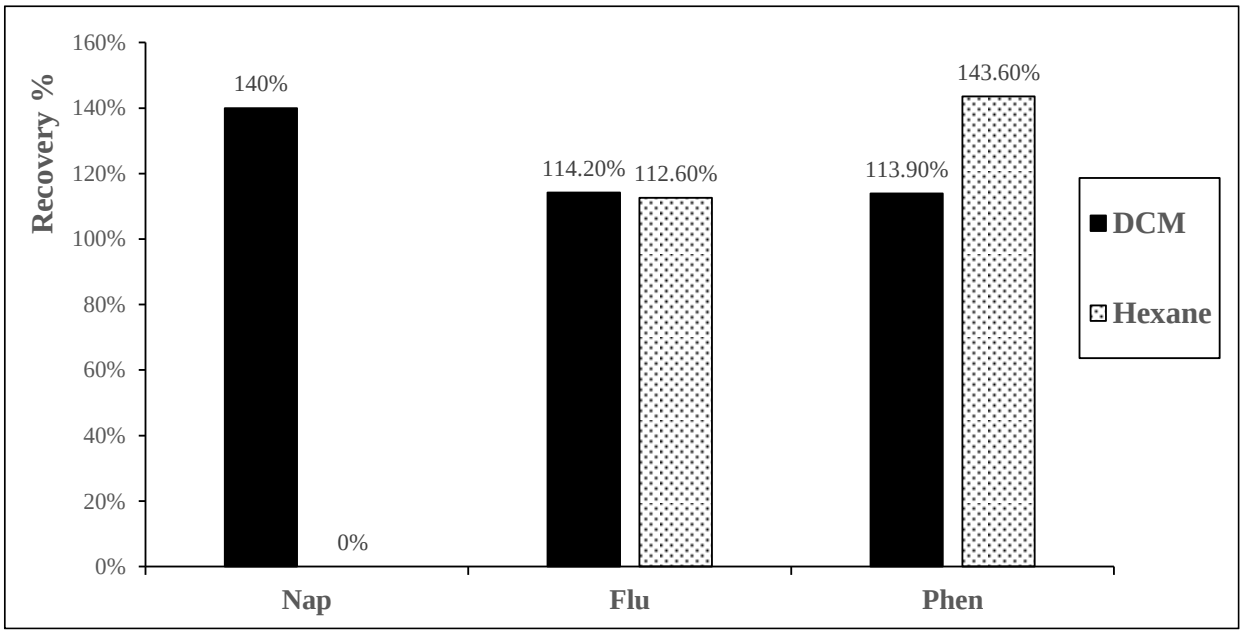

Figure 19. *Prelimimary experiment data.*

4.4. Recovery from water and suspended materials

The recovery from spiked water phase containing suspended particles as well was determined as described in Chapter 3.6. Solutions were stirred in triplicates for four hours and then a portion of the water phase together with suspended particles was immediately removed and prepared. Outliers were found with the results during the analyses. But after comparing the results of the data containing outliers and data without outliers, the difference was almost nothing so the average containing outliers was used for evaluation. The average results were used to determine the recovery rates compared to the spiking amounts (500µg in 500 mL of water) using both Hexane and DCM as solvents. A sample of the chromatogram result obtained using DCM and Hexane (Figure 20) showed the different peaks areas.

As discussed in Chapter 4.3 Nap results were not reliable which also showed in this experiment. In Figure 21, Flu had a better recovery rate than Phen for all the soil samples and it was noticed soil 1 and soil 3 had very similar results, and soil 2 and soil 4 also had practically the same recovery rate. In Figure 22, the recovery rate for both Flu and Phen were the same in each soil sample.

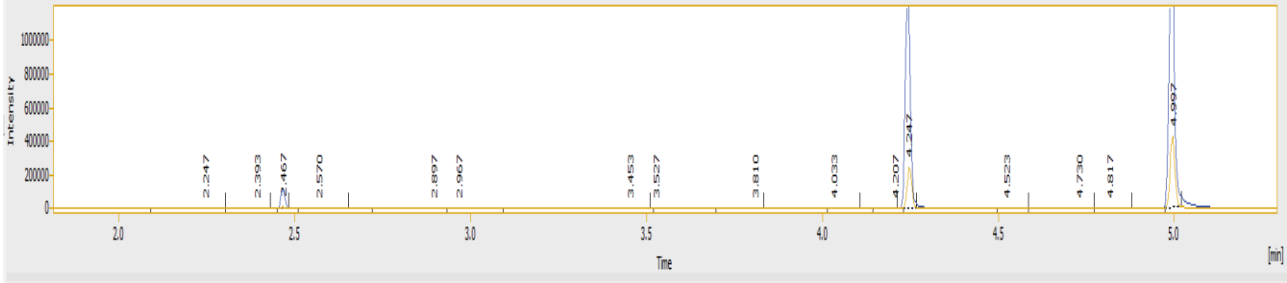

Figure 20. *A diagram showing the chromatogram of the extract of water and suspended phase extracted by both DCM and Hexane (blue = DCM and orange = Hexane).*

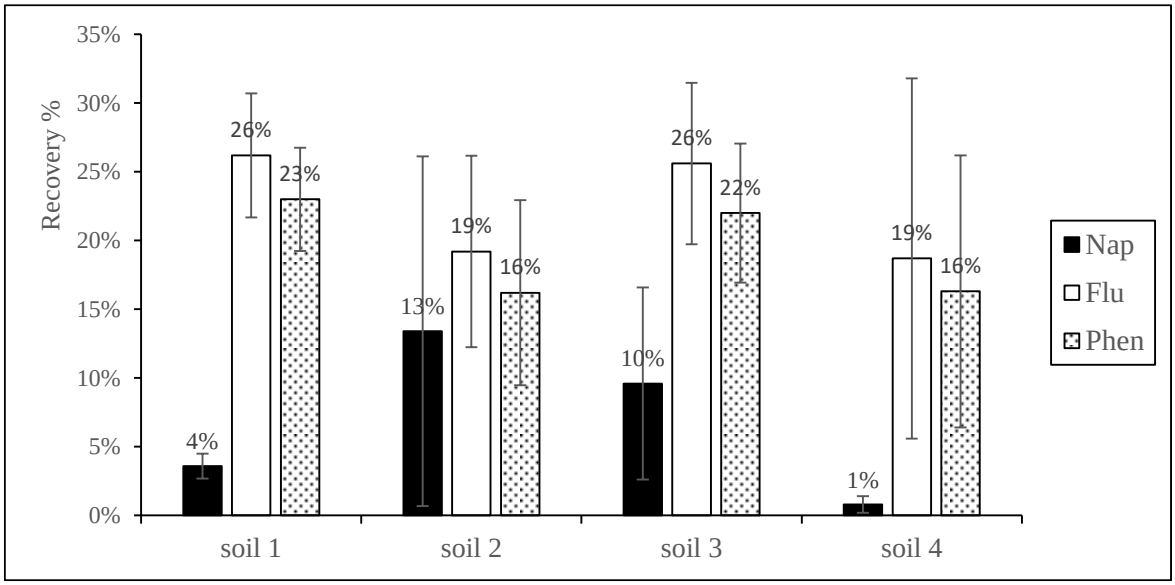

Figure 21. *Average recovery with Hexane.*

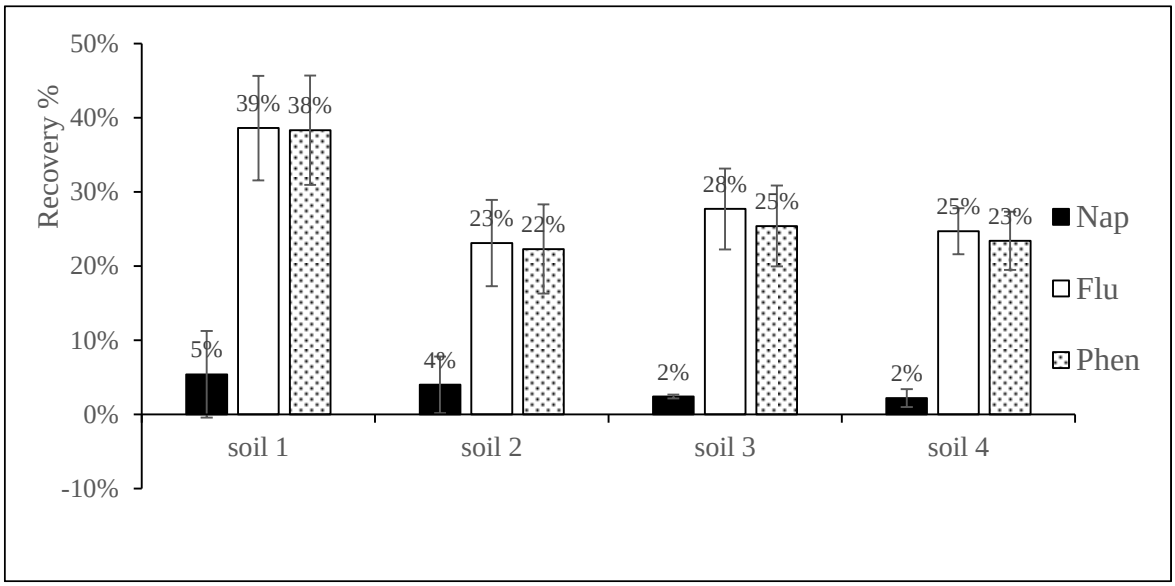

Figure 22. *Average recovery with DCM.*

4.5. Extraction from soils

As mentioned in Chapter 3.7, the soils were filtered from the water-soil system, 2g was extracted and PAH contents were determined. Figures 23, 24, 25, and 26 show the recoveries for individual components and for different extractions (triplicates). A total loss of Nap which was due to the fact the filtered paper was left for three days to dry and during that process, a lot of evaporation occurred. In Figure 26, Phen had the highest recovery level, and the third parallel is followed by the first parallel and the second is the lowest value. The result of Flu was similar to Nap but they were little result very low. As seen in Figure 24 and 26, Flu also had the same as Figure 23 while the second parallel had a higher recovery rate than the third and first parallel. For Figure 25, the first parallel was the result with the best recovery while the third parallel had the second-best rate in the Phen.

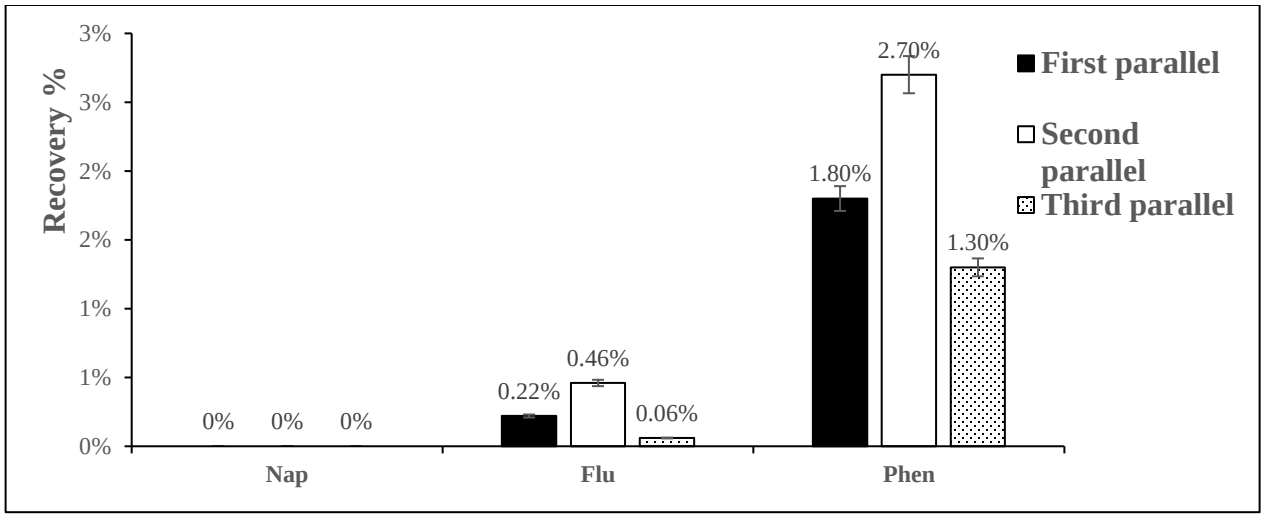

Figure 23. Recovery of 2g soil using soil 1.

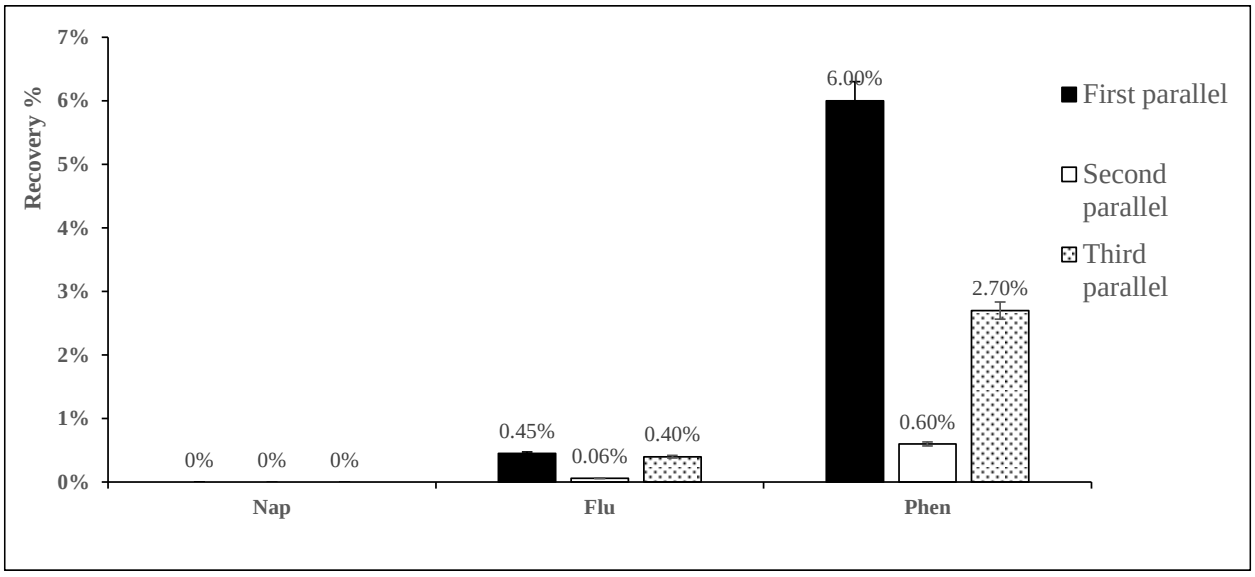

Figure 24. *Recovery of 2g soil using soil 2.*

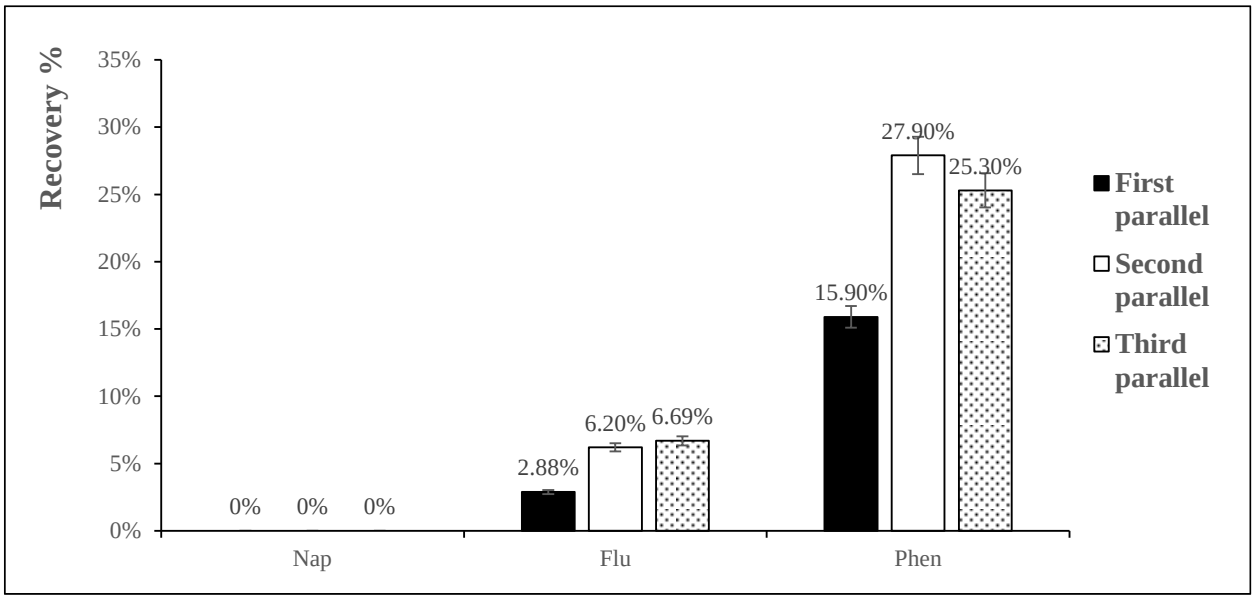

Figure 25. *Recovery of 2g soil using soil 3.*

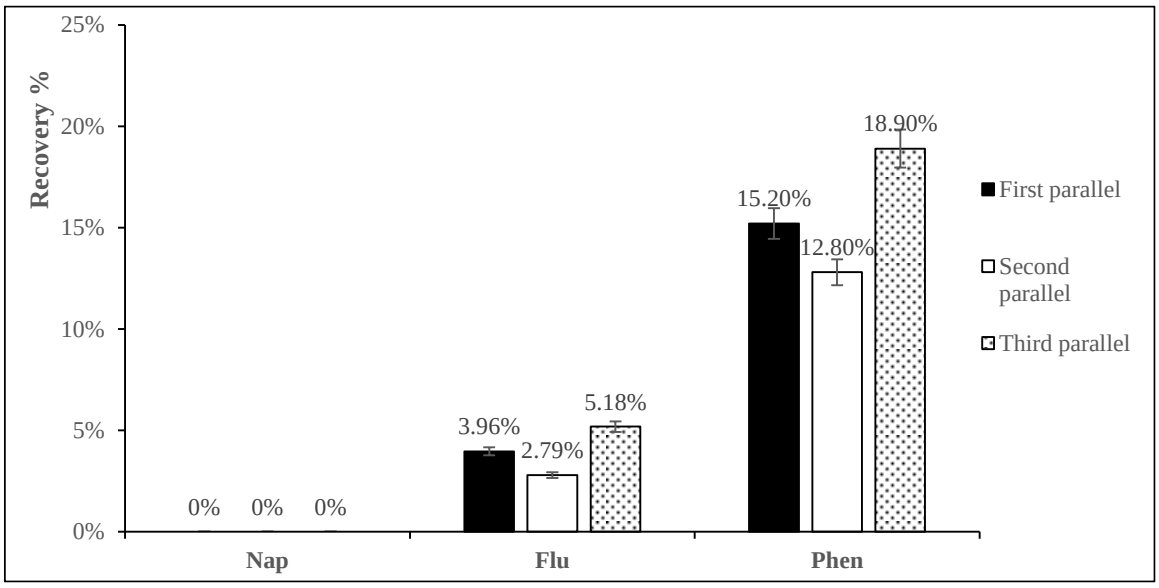

Figure 26. *Recovery of 2g soil using soil 4.*

4.6. Recovery from SPE

In Chapter 3.8, the steps for the SPE determination were explained, and after experimenting the recovery level for each soil sample was calculated and plotted in Figure 27. Here the PAH compounds solved in the water are determined. Soils 1 and 3 had the highest recovery rate for Flu

with 16% and 18% respectively but for soil 4, Nap had a very low recovery of 0.05%. Phen had the best recovery rate in soil 1 (13%) while Nap had the best recovery in soil 3 (16%). The experiment was done once so there was no standard deviation. The concentration of PAHs was checked in reference to 500µg. Soil 1 had 81µg in the Flu, 64µg in the Phen, and 26µg in the Nap. Soil 2 had 8µg in Nap, 65µg in Flu, and 42µg in Phen while Soil 3 had 79µg in Nap, 89µg in Flu, and 47µg in Phen, and Soil 4 had 0.2µg in Nap, 56µg in Flu, and 26µg in Phen.

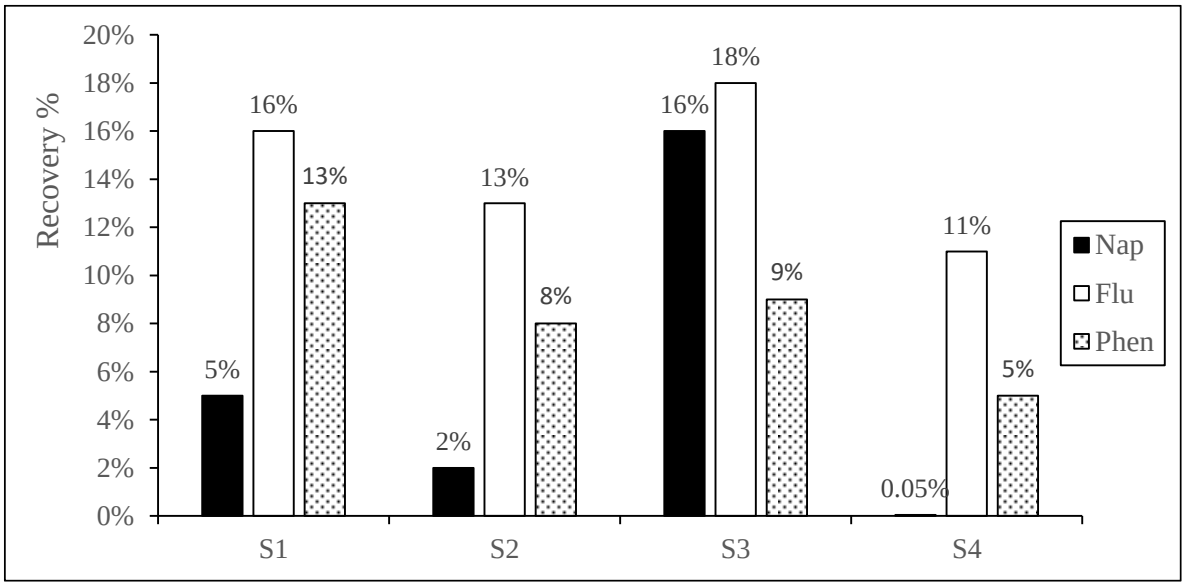

Figure 27*. SPE recovery for the soils.*

4.7. Amounts of PAHs in SPE vs 2g soil

The amounts of PAHs in the soil were compared with the amounts of PAHs in the water phase as seen in Tables 9 and 10. The average results were used for the soil phase since it was done in triplicates. Nap results were not obtained due to losses due to evaporation. Soil 1 and 2 amount were low in both Flu and Phen due to the low humus and $CaCO_3$. Phen had more amounts in the soil phase compared to the amounts obtained in the water phase. Flu had more amounts in the water phase as seen in Table 10. This trend is in accordance with their octanol-water partition coefficient values, as their log*P* are 4.18 and 4.45 for Flu and Phen respectively.

Table 9. *Data showing the amount of PAHs in the soil phase*

	Target compounds		
	Naphthalene	Fluorene	Phenanthrene
Soil 1	-	1.2µg	9.6µg
Soil 2	-	1.5µg	15.4µg
Soil 3	-	26.3µg	115.3µg
Soil 4	-	19.9µg	78.1µg

Table 10. *Data showing the amount of PAHs in the water phase*

	Target compounds		
	Naphthalene	Fluorene	Phenanthrene
Soil 1	26µg	82µg	64µg
Soil 2	8µg	65µg	42µg
Soil 3	79µg	90µg	47µg
Soil 4	0.2µg	56µg	26µg

5. Conclusion

During the recovery of PAHs from the soil, different trends were observed for the different levels of the four spiked soils. Among the soil properties, the Arany number (K_A) showed a possible trend at the high level to influence the recoveries from the four different soils. Higher recoveries were seen for soils with high K_A value, while the lowest recovery was seen for soil 2, which had the lowest K_A value at the high level (Figures 8, 9, 10, and 11). At the middle level, a trend was noticed parallel with the $CaCO_3$ values. Soil 1 had no $CaCO_3$ content and it was noticed to have been the soil with the lowest recovery rate at the middle level. At the low level, soils with low humus content (Soil 1 and 2) had better recoveries than the other two soils. The soils showed greater results for high levels followed by the middle levels and lastly the low levels except for soil 1 in which the low level results were higher than the middle level. Nap results were insignificant because of the losses due to the evaporation during the sample preparation.

For the extraction of the water phase to determine the best organic solvent to be used in further experiments, a preliminary experiment has been performed. The result for the DCM was better in case of Nap, while for Hexane there was no detection of Nap. In the case of Flu, the results were similar for both solvents, whereas for Phen, Hexane proved to be more efficient (Figure 12).

Therefore, two solvents (Hexane and DCM) were used to extract water plus suspended phase, because we couldn't pick a solvent from the two in the preliminary experiment. There was no significant trend observed with the soils with Hexane, and soil 4 had the lowest recovery percentage for Nap. Although Nap results were classified to be unreliable for the whole experiment. Worthy of note, that Soil 4 had the highest humus and $CaCO_3$ content. Flu and Phen had similar recovery results for soils 1 and 3 while soil 2 and 4 had similar recovery percentages for water containing suspended phase as well. With the DCM solvent, the soil with the lowest humus content had the best recovery (soil 1) for the Nap. DCM results were usually better for Flu and Phen than the results with Hexane. Flu was seen to adsorb more to the suspended phase than the other components added to the water phase (Figures 13 & 14).

Regarding the determination of the amounts of PAHs in the soil phase only, the results were low for soils 1 and 2, which could be due to the low $CaCO_3$ content as well as the low humus content in the soil. Soils 3 and 4 had better results, which could also be explained by the humus

and $CaCO_3$ content. It was observed that Phen had higher levels in the soil phase compared to Flu. Nap results were not discussed because of the losses caused due to evaporation.

Considering the SPE results related to the dissolved PAHs in the water phase, Flu had higher amounts (Figure 19), which shows that Flu was more dominant in the water, while Phen had more amount in the soil. The same has been observed with the water and suspended phase, which is in accordance with their hydrophobic properties, characterized by the $\log K_{ow}$ values.

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