### **HALALSPHERE**

International Islamic Univerity Malaysia - INHART

# Moss and Polyaromatic Hydrocarbon in Malaysia: A Recent Ten-Year Evaluation

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Abstract

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#### 1. Introduction

Combating air pollution is becoming increasingly important as the world becomes more crowded and hotter, with all of us pumping harmful gaseous and particulate matter into the air. Microscopic pollutants in the air enter our bodies and bypass our defence systems, deteriorating human health. Despite the availability of conventional technologies for monitoring air quality, biomonitoring with plants has emerged as a method of interest and has gained global attention in recent years. Mosses serve as air quality indicators and are sensitive to pollutant deposition. Heavy metal industries include sulfur dioxide (SO2), nitrogen oxides (NOX), hydrocarbons, and other air pollutants associated with bryophytes. Mosses (bryophyta) are non-vascular plants that play significant roles in ecosystems, such as stabilizing soil, reducing the risks of flooding and erosion and strongly influencing carbon, water, and nutrient cycling. Polycyclic aromatic hydrocarbons (PAHs) have been regarded as highly toxic, carcinogenic, and mutagenic to all various life forms. They are mainly formed from incomplete combustion materials at high temperatures. Once in the atmosphere, they can bind to and react with other pollutants.

Malaysia is discussed in this paper review. Mosses are excellent biomonitors and bio-indicators of PAH deposition in the environment, often by considering the diversity and development of naturally growing bryophytes and moss bags and have been extensively used in studies of atmospheric contamination over the past decades. The review also highlighted the sources of PAHs atmospheric pollution in the environment. The sample preparation, storage, and extraction methods are discussed and reviewed. Recent applications of gas chromatography for assessing PAH compound emission by mosses are also discussed. Aside from that, current efforts to reduce PAH pollution are mentioned.

The ten-year evaluation of moss species related to Polyaromatic Hydrocarbons (PAHs) in

The Toyyiban aspect must ensure the quality and safety of human life, concerning the air quality issue is not just a minute issue; it may affect an entire city, street, or suburb. People feel the effects of poor air quality in their neighbourhoods, so the message must reflect this. Breathing clean and fresh air is a slew of other health benefits and is necessary for humans to thrive and survive. Cleaner energy sources and the various sustainability requirements to combat air pollution must become more affordable and widely available. Since mosses are plants with substantial cation exchange capacity (CEC), high surface-to-volume ratio, and lack of true root system and welldeveloped cuticles, they can efficiently accumulate PAHs via dry and wet deposition over their surface. Since it is possible to sample many mosses that scatter relatively short distances from their origins, measuring pollution levels in mosses is a cost-effective and easy-to-manage method. A downside of the moss method is the comparatively small increments of pollutants relative to the pre-exposure concentrations for a few elements, at least in today's relatively clean Western environments. The use of mosses in biomonitoring describes pollution levels in background areas; nevertheless, locations classed as background sites that are barely polluted or have the lowest degree of pollution are becoming increasingly similar to



urban areas. Consequently, increases in pre-exposure concentrations are only detectable after prolonged exposure or at high pollution levels. PAH-related moss research PAHrelated moss research is certainly threatened due to a lack of information and research. Most studies were focused on other pollutants like heavy metals. Efforts were made to integrate mosses with engineering to create a variety of industrial applications, such as a green wall integrated system to filter air pollution and the employment of mosses in moss bags for biomonitoring of environmental pollution.

## 2. Moss distribution and its ability to absorb pollutants

Mosses are relatively small phylum Bryophyta and nonvascular plants with at least 12000 species that carpets most of the forest floor except in salt water and predominantly in a moist, damp area. The bryophytes represent a large group of three unique seedless plants which comprise three phyla: Bryophyta (mosses), Anthocerotophyta (hornworts) and Marchantiophyta (liverworts) (Ishizaki, 2017; Jiang, 2018). Research studies have shown these plants are numbered between 11,000-13,000 mosses, 7,000-9,000 liverworts and 200- 250 hornworts, which make a total of 18,000 to 23,000 worldwide (Sabovljević and Sabovljević, 2020). Mosses represent about 90% (ca. 260,000 species) of all extant land plant species; mosses are observed in rapid diversification, outnumbered (Roberts et al., 2012), rich in various ranges of forms, sizes, and heights, and play a significant role in various ecological niches and land plant diversity (Geffert et al., 2013).

Mosses are particularly prominent in the tropics; however, they have a significant presence in the boreal forest due to the shadiness and damp conditions, the woodlands of the temperate zones, and tundra regions. Some moss species are drought or desiccation-tolerant though they typically are found in moist environments. Climatic factors (Song et al., 2015); soil factors including type, moisture, and pH (Rousk et al., 2018); topography attributes such as slope, altitude and gradient (Okuda et al., 2011; Staniaszek-Kik et al., 2019), vegetation type and coverage; and the type of substrate that mosses grow on (Staunch et al., 2012; Sale et al., 2016) are all critical environmental factors affecting the distribution of mosses. Humidity can be interpreted as the high-water content in a place. Khujjah and Ekowati (2018) mentioned what makes moss required to grow in humidity place because water outside male gametes body (sperm from antheridium) help them to reach female gametes to facilitate sexual reproduction.

Besides having phylogenetic diversity, mosses have also been reported to render physiological tolerance to environmental stresses (Roberts et al., 2012). Cytological stress signals and vitality tests have been carried out to compare the moss towards potential pollution stressors sensitivity and accumulation capacities with other biomonitors and materials (Spagnuolo et al., 2011), where mosses have higher and better potential capabilities in capturing variances of atmospheric pollutants compared to the lichens (Ndlovu et al., 2019). Their ability to trap pollutants from the air on the surface and tissues is due to (1) the absence of roots, and they receive all nutrients primarily direct from the air, hence, simultaneously allowing the pollutants to concentrate over the moss surface, (2) large surface-region-to-volume ratio that enhances the absorption and retentions of pollutants (Parmar et al., 2016), (3) absence of cuticle layer and lignified cell walls; thus, allow high capacity of metal ions exchange (González & Pokrovsky, 2014), (4) stable and homogenous species population becomes the best bioaccumulator to represent sampling area, (5) low-growing features provide the information over extended periods which is not limited to current pollution status. These factors allow real-time detection, easier sampling, and cost-effective biomonitoring of pollutants at multiple sites (Jiang *et al.*, 2018) and (6) easily grow on bare rock surfaces, building up ground soils, therefore become a primary succession and colonisers in urban areas (Haynes *et al.*, 2019).

Plants biomonitoring is widely distributed, relatively inexpensive, and offers large surface areas that can accumulate numerous amounts of organic pollutants than those in a smaller area (Baldantoni *et al.*, 2014). According to (Nowak *et al.*, 2022), active moss biomonitoring methods have been preferred, and these methods involve transplanting moss samples from clean, pristine areas to the study sites. Since native mosses may adapt to their surroundings and spontaneously respond to new pollutants, this method choice was observed to produce different results from passive biomonitoring.

The use of bryophytes to monitor fluorine pollution from industrial emissions started in the 1950s (De Agostini et al., 2020). In the 1980s, mosses were a reliable air quality tool for evaluating atmospheric organic pollutants. Relatively, mosses are highly sensitive to many air pollutants. It will turn the colour to brown or black, reflecting its surrounding. They will decline and become extinct if exposed to long term pollution (Cen, 2015). The first work that utilises moss species as a bioindicator for atmospheric heavy metal depositions was initiated by Ruhling and Tyler (1968) in Sweden (Donovan et al., 2016). It was chosen merely because this leafy bryophyte receives all nutrients and pollutants directly from the air since they do not have a protective epidermis, as well as they have a cation exchange capacity to accumulate high high concentration of heavy metals to a high degree without damaging the cells (Shakya et al., 2014). They once collected moss samples and vascular plants near the busy roadside, resulting in the moss accumulating Pb better than the vascular plants, which provided emissions data within 7-15 months (Donovan et al., 2016). Likewise, Wilkie & La Farge (2011) mentioned that mosses could accumulate, sequester, and tolerate concentrations that are often toxic to other taxa. In 1990, the European moss Biomonitoring Network was established and coordinated by the UNECE ICP Vegetation Programme to quantify the deposition of heavy metals in naturally growing mosses (Shetekauri et al., 2018). The programme was conducted every five years until 2010, aiming to study heavy metal concentrations on temporal and spatial patterns in Europe at a high spatial degree (Palma, 2016). In 2005, a pilot study of nitrogen, persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) concentrations in mosses was carried out in selected countries using the pleurocarpous species since these species spread a thick cushion mats which has high surface-to-volume ratio that favour high accumulation of pollutants (Harmens et al., 2013). Schröder & Nickel, (2019) found that 74 % of the moss biomonitoring studies employes Pleurozium schreberi, Hypnum cupressiforme, Hylocomium splendens and Psedoscleropodium purum as biomonitors in Europe and North America. Pleurozium schreberi was able to accumulate PAHs and PCBs road traffic (Sucharová & Holá, 2014a) (Danielson et al., 2016). Among these PAHs, Hypnum plumaeformae show good relation with Phenanthrene concentration and traffic volume was observed in Hungary

(Janković-Mandić et al., 2015).

#### 2.1 Type of moss monitoring

The biomonitoring method using mosses can be performed in two ways, through passive and active monitoring (Motyka et al., 2011). Passive moss biomonitoring has been used for decades, where native moss species are collected directly at the sampling site, and the moss is analysed to estimate the trace elements of atmospheric deposition. On the contrary, active biomonitoring involves transplanting mosses at the site of interest, especially to monitor pollutants in urban areas. The mosses are packed in nylon-net or fabric bags and exposed for certain periods and height exposure from the ground. Since the height placement of moss influences the accumulation uptake capacity (Di Palma et al., 2017), besides tunnels (Q. Wu et al., 2014), garages (G. Vuković et al., 2013), airports (Turgut et al., 2019), petroleum refinery (Cortis et al., 2016) and bodies of water (Gecheva et al., 2020), moss bags method assists the monitoring of air around volcanoes (Calabrese et al., 2015) and in cold climate regions (Salo et al., 2016). Airborne pollutants accumulated in mosses through wet or dry deposition remain for at least 2 to 3 years and reflect the level of pollution (Fabure et al., 2010; Schröder & Nickel, 2019). Details on studies that used both types of monitoring are shown in Table 1 and Table 1.1. Mosses are passive biomonitors in their natural environments, but in recent years, active biomonitoring with moss transplants has become a more popular method for environmental monitoring (Di Palma et al., 2017; González & Pokrovsky, 2014; G. P. Vuković, 2015). In most studies, mossbags produced positive and satisfying results. Because of their sensitivity to natural climatic conditions, moss-bags are rarely used indoors. Long-term exposure has a significant advantage over technical tools with demanding infrastructure and maintenance typically only applicable in short-term assessments. Despite this, the moss bag method has not been standardised in terms of the amount of moss exposed in the bags, their shape, and the material used to make them. There is also little evidence to support the relationship between pollutant deposition and moss uptake. Another unstandardized aspect is the use of blanks or zero time controls. Typically, results are reported as spatial and temporal concentrations. PAHs can accumulate in plants through absorption and adsorption depending on their availability in the environment, physical chemical properties such as gaseous-to-particle partitioning, ambient variables (such as temperature, radiation, and humidity), and plant characteristics (Harmens et al., 2013; Loppi et al., 2015). These characteristics influence PAH levels and profiles in a given species, even in nearby background areas.

#### 3. Sources of polyaromatic hydrocarbon

Polycyclic aromatic hydrocarbons (PAHs) are large organic substances, a group of about 10,000 compounds, and each of the individual compounds often shares similar characteristics. They generally appear in pale yellow solids or colourless white at room temperature (Han *et al.*, 2015; Hindersmann & Achten, 2018). Chemically, PAHs consist of two or more fused benzene rings formed in linear, cluster or angular arrangements (Moyo *et al.*, 2013; Okonkwo *et al.*, 2014). Most PAH rings comprise six carbon-hydrogen units and are attached with shared edges. Since the structure varies in the ring system, the molecules probably carry different side chains instead of hydrogen atoms. The PAHs have different molecular weights; therefore, they are divided into low molecular weight PAHs (LMW-PAHs) and high molecular weight PAHs (HMW-PAHs). The LMW-PAHs comprise 2 to 3 rings, like Naphthalene, Acenaphtylene, Acenaphthene, Fluorene, Phenanthrene, and Anthracene (Moscoso et al., 2015; Shou & Li, 2015). The HMW-PAHs comprise more than four rings, such as Pyrene, Chrysene, Benzo(a)anthracene and Indeno(1,2,3-cd)pyrene. The LMW-PAHs are less hydrophobic, volatile and soluble in many organic solvents than HMW-PAHs. The HWH-PAHs have a melting point of> 100°C, making them solid and stable in the environment (Adeniji et al., 2018). The solubilities of PAHs are inversely proportional to the number of fused benzene rings. Therefore, HWH-PAHs are well dissolved in oil substances, sediments and soil, while LMW-PAHs are likely to dissolve in water.

PAHs developed several heat resistances, corrosion resistance, light intensity, conductivity, and physiological action. PAHs have characteristic UV reflectance spectra. Ultraviolet (UV) absorbance spectra of aromatic hydrocarbons contain special absorption bands with considerable fine structure, especially in non-polar solvents (Charriau et al., 2016). Each ring structure presented a unique UV spectrum, crucial in identifying the PAHs molecules. Generally, PAHs molecular weight is divided into two groups, low molecular weight and high molecular weight or heavy weight. PAHs usually dominate low molecular weight PAHs with 2 to 3 aromatic rings; for example, naphthalene, methylnaphthalenes, acenaphthylene, and fluorine, which are present in the gaseous phase (Balmer et al., 2019; Rengarajan et al., 2015) meanwhile high or heavier weight have more aromatic rings (usually four or more rings); for example, Pyrene, Benzo(a)anthracene, Benzofluoranthene which present in the particulate (PM) phase (Khan et al., 2015; Shen et al., 2017).

Chiu et al. (2018) mentioned that sources of PAHs are derived from two major groups: anthropogenic (human-caused) and non-anthropogenic sources. The anthropogenic sources entail pyrogenic (dominant source) and petrogenic sources, while natural sources are the non-anthropogenic sources. Pyrogenic PAHs are formed during the combustion of oil derivatives, coal tar, traffic-related pollution, agricultural fires, waste incineration and anything related to burning organic matter (Balmer et al., 2019; Dat & Chang, 2017). They are readily abundant in the gaseous phase and more abundant when the rings become more than 4 in the exhaust engines' particulate phase (soot). Abdel-Shafy & Mansour (2016) explained that the PAH molecular formation results from the incomplete combustion of carbonaceous material through pyrolysis (thermal decomposition) and pyrosynthesis (subsequent recombination) processes.

As for petrogenic PAHs, most of them are derived from direct contamination, such as refined petroleum products, spillage of oil products, municipal sewage treatment plants and engine oils, and emissions from vehicles, aluminium production facilities, making dyes, pesticides and plasticisers (Liu *et al.*, 2017; Santos *et al.*, 2017). Wildfires and volcanic eruptions produce natural-sourced PAHs (Kozak *et al.*, 2017). Both anthropogenic and non-anthropogenic activities yielded parent PAHs and alkylated PAHs, where the alkylated PAHs are more persistent in the environment than the parent PAHs (Adeniji *et al.*, 2018).

Type of monitoring	Species	Sample preparation	Moss bag description	After exposure	Reference
Active	<ul> <li>Pseudoscleropodium purum</li> <li>1) Wide surface area and temporal and spatial distribution.</li> <li>2) Widely and successfully used.</li> </ul>	<ol> <li>3-4 cm of apical shoots were washed with bidistilled water for 30 min.</li> <li>The excessive moisture was removed using filter paper.</li> <li>Placed 10 g into moss bag.</li> </ol>	<ol> <li>1) Size: 10 cm x 20 cm.</li> <li>2) Material: Polythene net (1 mm mesh).</li> <li>3) Height placement: 3-4 m, on a PVC tube attached vertical to the lamp post.</li> <li>4) Total bags: 50 were placed at the study area, 3 as controls and 4 as blanks in vacuum packed.</li> </ol>	Samples were homogenized, dried at 80°C prior to analysis.	(Ares <i>et al.</i> , 2011)
Active	Hypnum amabile	NM	<ol> <li>1) 600g of mosses placed in 5 bags were exposed at residential, commercial and industrial areas with 30 days duration.</li> <li>2) Control mosses were kept in the chamber with filtered air.</li> </ol>	NM	(Gómez-Arroyo <i>et al.</i> , 2021)
Active	Sphagnum girgensohnii	NM	<ol> <li>1) Size: 10 cm x 10 cm.</li> <li>2) Material: Nylon net (1 mm mesh).</li> <li>3) Height placement: 5-10 m, exposed for 3 months.</li> <li>4) Total bags: 21 were placed in the central zone.</li> </ol>	Samples were homogenized, dried at 40°C to a constant weight for next chemical analysis.	(Hajiyeva <i>et al.</i> , 2017)
Active	Hypnum cupressiforme	NM	<ol> <li>Size: 10 cm x 10 cm.</li> <li>Material: Nylon net (1 mm mesh).</li> <li>Total bags: 30 bags were exposed for 6 weeks, and 10 bags were unexposed as controls.</li> </ol>	Samples were kept at a temperature of 4 °C prior to next analysis.	(F. Capozzi <i>et al.</i> , 2017)
Active	Hypnum cupressiforme	<ol> <li>Dried overnight in a humid chamber.</li> <li>Only green part of moss shoots was selected (10 l per 100 g dry weight) and washed with distilled water.</li> </ol>	<ol> <li>1) Size: 12 cm x 12 cm.</li> <li>2) Material: Nylon net (2 mm mesh).</li> <li>3) Height placement: 3, 6 and 9 m.</li> <li>4) Total bags: 500 mg of fresh moss was placed in 6 bags at each point were exposed for 30 days.</li> </ol>	Refer to (Ares <i>et al.</i> , 2011)	(De Nicola <i>et al.</i> , 2013)
Active	Pleurozium schreberi, Sphagnum fallax	Only the green parts of mosses were selected.	<ol> <li>Size: NM</li> <li>Material: Nylon net.</li> <li>Height placement: 3, 6 and 9 m.</li> </ol>	NM	(Świsłowski et al., 2021)

Table 1: Active moss monitoring and descriptions

	and Dicranum		4) Total bags: 2 g of fresh moss was placed in 9		
	polysetum		bags were exposed for 3 months (3 bags for each species). Control moss was left for pre-exposure analysis.		
Active	Sphagnum capillifolium and Hypnum cupressiforme	The samples were cleaned with 10 L of distilled water for every 100 g dry weight.	<ol> <li>Size: Diameter ca. 4 cm. (in a spherical shape).</li> <li>Material: Nylon net (2 mm mesh).</li> <li>Height placement: 4 m above the ground and hung up on plastic sticks.</li> <li>Total bags: 500 mg of fresh moss was placed in 3 bags were exposed at 3 different site background (urban, rural and semi-rural) for 10 weeks (3 bags for each species). Control moss was left for pre-exposure analysis.</li> </ol>	Samples were homogenized for the next analysis.	(Vingiani <i>et al</i> ., 2015)
Active	Hypnum cupressiforme	NM	<ol> <li>1) Size: 15 mg cm<sup>-2</sup></li> <li>2) Material: Nylon.</li> <li>3) Height placement: NM.</li> <li>4) Exposed for 6 weeks.</li> </ol>	Samples were kept at a temperature of 4 °C in the freezer until next analysis.	(Fiore Capozzi <i>et al.</i> , 2021)

### Table 1.1: Passive moss monitoring and descriptions

Type of monitoring	Species	Sample preparation	Reference
Passive	Hypnum plumaeforme	<ol> <li>At each 2 m plot, a sample was collected from several points and grouped together. Attached soils and litter were cleaned of mosses, and only green and brown-green moss tips were chosen for further analysis.</li> <li>The average length of moss tips was 2.67 ± 0.47 cm (mean ± SD).</li> <li>The samples were prepared in aluminium foil, air-dried at room temperature, and stored in paper bags secured in polyethylene bags in the dark until PAH analysis.</li> </ol>	(Oishi, 2013)
Passive	Pleurozium schreberii	<ol> <li>The samples were collected from 5 - 10 points in a 2500 m<sup>2</sup> (50 x 50 m) and sealed together in polythene bags.</li> <li>They were brought back to the laboratory with a cooler box and maintained frozen for next analysis.</li> <li>The green living parts were selected for the analysis.</li> </ol>	(Godzik et al., 2014)
Passive	Orthotrichum lyellii	<ol> <li>The samples were taken from 226 randomly selected points within each 1 km grid cell. An additional 52 points were plotted to help in the prediction of PAHs in moss at points along the city's edge with a 1 km extension further than the city boundary. However, 72 resamples within 100 m were taken to estimate the dataset's semivariograms and define the spatial correlation. A total of 350 samples were collected over the course of three weeks.</li> <li>Mosses were sampled at a height of 1 m from the ground to avoid spray from vehicles on the roads.</li> </ol>	(Jovan <i>et al.</i> , 2021)

		3) 5 g dry weight of moss were cleaned for next chemical analysis.	
Passive	NM	The samples were taken at a distance of roughly 5 metres or more from roadways and homes. The same distances were used to gather samples from adjacent parks and other green spaces. Physical impurities were carefully removed from the samples. Moss apical shoots measuring 3 to 4 cm were air-dried at room temperature under 22° C for 72 hours and then homogenised for subsequent analysis.	(Rotaru <i>et al.</i> , 2017)
Passive	Funaria hygrometrica	The samples were collected from diverse substrates and habitats and then packaged in aluminium foil to prevent exposure to sunlight. Before the next analysis, samples were cleansed of any physical contaminants under dry surroundings.	(Adie <i>et al.</i> , 2021)
Passive	Hypnum cupressiforme and Isothedum myosuroides	About 0.5 m2 of each species was gathered, yielding 50 and 25 g (dry weight) of I. myosuroides and H. cupressiforme, respectively.	(Foan & Simon, 2012)
Passive	Sphagnum magellanicum	<ol> <li>The samples were pooled together from various sampling points and homogenized.</li> <li>O.1 g of moss was then placed in a glass vessel and mix with in situ microbes. A total of 12 vessels were incubated under a controlled chamber with 26 °C and 60% relative humidity for 120 days exposure. They were collected after 0, 30, 40, 60, and 120 days, respectively.</li> <li>All samples were dried and passed through a 0.25-mm sieve before subjected for analysis.</li> </ol>	(Wang et al., 2018)
Passive	Dicranum scoparium, Hylocomium splendens, and Racomitrium lanuginosum	All mosses were sampled in triplicate across five different time localities.	(Martinez- Swatson <i>et al.</i> , 2020a)
Passive	Sanionia uncinate and Warnstorfia sarmentosa	The samples were obtained at various locations and intervals. These are a result of the fire catastrophe, the cleanup of debris, and environmental changes around the Brazilian Antarctic Station. The samples were kept frozen at 20 °C in metal containers for future analysis.	(Colabuono <i>et al.</i> , 2015)

	Compound (IUPAC Name)	Chemical formula	Molecular weight	Structure
1	Naphthalene (Nap)	$C_{10}H_8$	128.2	
2	Acenaphthylene (Acpy)	$C_{12}H_8$	152.2	
3	Acenaphthene (Ace)	C12H10	154.2	
4	Fluorene (Flt)	$C_{13}H_{10}$	166.2	
5	Phenanthrene (Phe)	C14H10	178.2	
6	Anthracene (Ant)	C14H10	178.2	
7	Fluoranthene (Flt)	C16H10	202.3	
8	Pyrene (Pyr)	C16H10	202.3	
9	Chrysene (Chry)	C <sub>18</sub> H <sub>12</sub>	228.3	

10	Benzo(a)anthracene (BaA)	C18H12	228.3	
11	Benzo(b)fluoranthene (BbFL)	$C_{20}H_{12}$	252.3	
12	Benzo(k)fluoranthene (BkFL)	C20H12	252.3	
13	Benzo(a)pyrene (BaP)	C <sub>20</sub> H <sub>12</sub>	252.3	
14	Dibenz(ah)anthracene (DahA)	C22H14	278.4	
15	Indeno(1,2,3-cd)pyrene (InP)	C22H12	276.3	
16	Benzo(ghi)perylene (BghiP)	C22H12	276.3	

Seventeen PAHs have become a concern among health personnel due to their carcinogenic characteristics (Table 2) and are commonly found in waste sites. Besides these 17 PAHs, alkylated PAHs including 7,12-dimethylbenzo(a)anthracene; 1-methylphenanthrene; 2,3,5-trimethylnaphthalene; 1 methylnaphthalene; 2-methylnaphthalene and 2,6-dimethylnaphthalene.

Between diesel-fuel type and gasoline, the PAHs emission rate is higher from diesel compared to gasoline; however, the effects are less toxic than those emitted from gasoline type. Flt and Pyr are emitted from petrol and diesel vehicles, with additional IP from petrol vehicles and Chry, BbFL, BkFL from diesel powered vehicles. The PAH profile of diesel bus emissions has also shown a large predominance of phenanthrene and small amounts of chrysene and benzo(e)pyrene. The sum of the concentration of nine major combustions PAH (CPAH) (Flt, Pyr, BaA, Chry, B(b+k)F, BaP, BeP, InP and BghiP) in these samples (excluding BeP) accounted for 73% of the total PAH mass. The ratio of CPAH/TPAH was 0.73. The value of CPAH/TPAH ratio has been calculated for non–catalyst (0.41) and catalyst (0.51) automobiles and heavy duty diesel trucks (0.30). Higher CPAH/TPAH ratios indicate more extensive combustion activities in the city. A less efficient emission control system in the vehicle fleet may also account for this high CPAH/TPAH ratio. In India, vehicles are not equipped with a catalytic converter. According to Rogge *et al.* (1993), non– catalyst vehicles emit 27 times more PAH, especially HMW PAH, than catalyst equipped vehicles (Rajput & Lakhani, 2010). Also, increased PAH emissions in vehicles under cold start have been observed, especially for low-speed cars (Zheng *et al.*, 2018). Valle-Hernández *et al.* (2013) reported that gasoline vehicles had emitted HMW PAHs such as BkFL, BaP, InP and BghiP during the non-Olympic summer period; the three main sources found a total of 93% of the PAHs emission along the road.

The largest PAHs fraction was 42% produced from diesel vehicles, followed by 36% from gasoline vehicles and coal combustion recorded 15% as low coal consumption produced during non-Olympic summer. Hence, the roadside site favoured the direct emissions from vehicle exhaust. Furthermore, they observed that the BaA and Chry emissions were produced by diesel emissions, while BaP was from vehicles with and without catalytic converters. Catalytic converters are used in automobiles as an emission control method to reduce toxic by-products pollutants (such as hydrocarbons, CO and NO<sub>x</sub>) and oxidation process to less harmless substances like CO2, water vapour and nitrogen gaseous (Xin & Pinzon, 2014). Old design cars powered by diesel and petrol with an outmoded catalytic converter produced 5-10 times higher PAH emissions than modern design cars. Soot formation is linked with the existence of hydrocarbons from most combustion processes, as hydrocarbons become a precursor for the soot particles in the atmosphere. The condensation of volatile particles could happen in the atmosphere in two ways: nucleating to form new particles or condensing on the existing particle surfaces. They

first develop a coating layer on the non-volatile particles surface when precursor species condense. However, condensation of PAHs can occur several feet behind a vehicle by adsorption on existing particles, thereby allowing some mixing of exhaust plumes from different sources of pollution (Wang *et al.*, (2019). Black carbon particle results from incomplete carbon-based fuel combustion like diesel and directly contributes to global warming simply because they could absorb sunlight.

#### 4. The fate of polyaromatic hydrocarbon

PAHs are widespread and easy to access into the environment through various pathways; hence the toxicity impacts on organisms could also happen through various actions. These compounds' fate is moderately persistent in the environment, including air, soil, water, plant, food and human.

#### 4.1 Fate on air

The PAHs are commonly found via gaseous and particulate phases (Lammel, 2015). Air is one of the major pathways for the environmental distribution and transboundary deposition of PAHs (Hussain *et al.*, 2019). Long-range PAHs airborne transportation is deposited to the air via evaporation from the earth's surface.

The occurrence of airborne PAHs in the atmosphere as gaseous and absorbed to particulate phases depends on the molecular weight, reaction with other air components, volatility, temperature, relative humidity, precipitation and type of fine particles that exist in the atmosphere, which can influence

				Sources		
Polyaromatic hydrocarbons	Cigarettes/ e-cigarettes	Diesel fuels	Grilled /charred food	Petroleum products, coal tar, coal- burning	Wildfire/ agricultural smoke	Health concern(s)
Acenaphthene (Acp)	/	/	/	/	/	Irritant
Acenaphthylene (Acy)	/	/	/	/	/	-
Anthracene (Ant)	/	/	/	/	/	-
Benzo(a)anthracene (BaA)	/	/	/	/	/	Cancer risk
Benzo(a)pyrene (BaP)	/	/		/	/	Cancer risk
Benzo(e)pyrene (BeP)	/		/	/	/	-
Benzo(b)fluoranthene (BbFL)	1	/	/	1	/	Cancer risk
Benzo(j)fluoranthene (BjFL)	/	/	/	/	/	Cancer risk
Benzo(g,h,i)perylene (BghiP)	/		/	/	/	-
Benzo(k)fluoranthene (BkFL)	/	/	/	/	/	Cancer risk
Chrysene (Chry)	/	/	/	/	/	-
Dibenz(ah)anthracene (DahA)	/		/	/		Lung irritant
Fluoranthene (Flt)	/	/	/	/	/	Cancer risk
Fluorene (Flu)	/	/	/	/	/	Irritant
Naphthalene (Nap)		/	/	/	/	Irritant
Indeno(l,2,3- cd)pyrene (InP)	/	/	/	/	/	Cancer risk
Phenanthrene (Phe)	/	/	/	/	/	Irritant
Pyrene (Pyr)	/	/	/	/	/	Irritant

Table 3: Sources of PAHs

partitioning (Amato-Lourenco *et al.*, 2017; Ravindra *et al.*, 2008). Meteorological conditions, including wind direction, wind speed, solar radiation and temperature, influence the presence of particle number and distribution. These factors determined the particles physical and chemical modification (Dominick *et al.*, 2018).

PAHs with more than five aromatic rings are often absorbed by particles and undergo a high condensation temperature. While PAHs with 2 to 3 aromatic rings were found mainly in the gaseous phase, their dispersal globally and dominant during winter or preferential in Arctic regions (Abdel-Shafy & Mansour, 2016). The reactions of PAHs with nitrogen oxides which are simultaneously emitted, can result in the conversion of inactive PAHs to nitroarenes, compounds of potent carcinogenic activity. Photochemical reactions under ambient conditions can also form nitroarenes. Many experimental studies have shown that PAH can react readily with ozone at ambient concentrations; quinones and epoxides are possible products of such reactions. PAHs and NPAHs appeared to be coated with a single or a mixture of PAH because individual congeners are produced similarly.

Atmospheric residence time is rendered by particulate matter's dynamic behaviour (Shilla & Routh, 2018).

This residence time also is an essential parameter in discussions of the natural cycles of such compounds and is, therefore, of significant importance whenever we wish to consider the atmosphere as a link in geochemical and ecological systems. It has been established that both coarse particles (larger than 3 to 5  $\mu$ m) and nuclei range particles (below 0.1 µm) are similarly limited in their atmospheric residence times and, consequently, in their effects, although their removal mechanisms are different. The removal mechanism between the coarse particles and nuclei range particles are different, but both share similar limited residence times. Nuclei range particles are formed by fossil fuel combustion, whereas coarse particles are mainly generated by mechanical wear processes such as abrasion of automobile tires, roadway asphalts, and soil resuspension. Size ranges of between 0.1 to 3 µm diffuse more slowly and are expected to stay longer in the atmospheric because they can be transported over long distances upon weather and barely removed by rainwater. Pollution survey shows the PAHs concentration differs based on seasonal variations. Their concentrations during the winter are more complex than in summer (Škrdlíková et al., 2011). Contrasting with global emission scenarios and threats, PAHs concentration in the Arctic regions does not show declination trends, and PAHs are identified as chemicals of emerging concern in the Arctic (Yu et al., 2019).

Moreover, snow efficiently soaks atmospheric pollutants because individual snowfall renegades are mostly dendritic snow crystals that cl together once they collide Over the past decades, studies suggested that snow and ice interact physically and chemically with the atmosphere to form a large surface area and a medium for adsorption and dissolution of pollutants and physicochemical reactions. The interaction involves the oxidation potential and gas-to-particle partitioning McNeill *et al.*, (2012). Snowpack can serve as a reaction medium and a temporary or permanent sink for air pollutants and their potential emission source (Błaś *et al.*, 2010; Nazarenko *et al.*, 2016).

Being a semi-volatile compound under atmospheric conditions allows them to move between the earth's surface in repeated temperature-driven deposition cycles and volatilisation (Igwe & Ukaogo, 2015). PAHs are persistent pollutants that are deposited at far distances from their origin and are mostly found everywhere in the environment, which are in the air, vegetation, soils, sediments, inland and seawaters (Augusto *et al.*, 2013; Edokpayi *et al.*, 2016; G. P. Vuković, 2015). PAHs are deposited into the atmosphere in two ways: natural petroleum, volcano eruptions, forest fires and erosion of ancient sediment, and anthropogenic processes.

However, PAH contamination along the roadside and industrial levels in urban areas is higher than in rural areas. Actually, they also contaminate indoor spaces without realizing the effect of PAHs. The indoor air quality is even worse and worse than outdoor air quality. Humans tend to bring outdoor pollution to their houses.

Subsequently, the presence of these organic compounds caused the droplets, rainwater or storm water to become saturated, eventually, wash away to the water bodies and land.

#### 4.2 Fate on soil/water

Responding to low biodegradability, some PAHs are present in the environment (Olayinka *et al.*, 2018). By comparing the water residence time between LMW-PAHs and HMW-PAHs, LMW-PAHs have a shorter duration in the water column. They tend to be rapidly volatile and degraded; meanwhile, the HMW-PAHs accounted with high soluble in lipids and more hydrophobic, which makes them insoluble in the water and tend to accumulate on surfaces, settle on the sea floor, potentially enter the food chain or in non-polar matrices (X. Wang & Wang, 2006; Nasher *et al.*, 2013).

The petroleum industry has also greatly impacted many degrees of biota life in coastal waters, between pyrogenic (high temperature between 100°C and 300°C and short duration from incomplete combustion) and petrogenic (related to oil-derived samples) (Sinaei & Mashinchian, 2014). Similarly, the by-product petrogenic is closely related to oil-polluted; meanwhile, pyrogenic PAHs are characterized for samples from industrial fields. The pyrogenic hydrocarbons mainly result from maritime transportation and harbour activity. The exhaust products released from ships are composed of elemental, organic and inorganic carbon, sulphate, and ash, as well as nitrates—meanwhile, the type of fuel used by maritime transport influences the PAHs concentration produced.

The coastal environment suffered from the disposal of used lubrication oils, gas flaring, leakages from ship vessels, runoff from crude oil tanks, offshore drilling, and production operations. Though oil is a major energy source globally, it is a mixture of hydrocarbon compounds, and once they spill into the sea, they gradually mix with water or drift to the bottom and decay marine life.

Biotransformation and biodegradation by benthic organisms are why PAHs settle in sediments.

PAHs have two or more aromatic rings causing them to have a stable molecular structure. PAHs with heavier weights have lower vapour pressures, are less water-soluble, and are carcinogenic and mutagenic (Gupte *et al.*, 2016). On the other hand, PAHs with low weight are considered less carcinogenic as they are more soluble in water and remain in the gaseous

phase than heavier weight in the atmospheric particulate phase (Srivastava *et al.*, 2017). However, PAHs with low molecular weight are less persistent but much more abundant and can react with other pollutants, such as O3 and NOx, to create highly toxic nitrated and oxy-PAH compounds (Lammel, 2015).

#### 4.3 Fate on plant/food/human

Foods like vegetation and fruit are also not excluded from being contaminated by PAHs, through the air, water, and soil from long-distance airborne transportation of contaminated particles (Adeyeye, 2019; Hamidi *et al.*, 2016; Zelinkova & Wenzl, 2015). Also, the cooking activities contribute to the exposure of food to the PAHs, which this paper does not discuss. The presence of PAHs in foods can be found in different food ranges, such as fruits and vegetables, cereal products, poultry, dairy products, oils, infant-based formulations, nuts and spices. The PAH levels found in unprocessed foods in rural areas reflect the background contamination that could originate from long-distance airborne transportation.

Compared to meat, the number of PAHs produced by fruits and vegetables is smaller because they are likely to be consumed raw and contain lower lipid content (Paris, Ledauphin, Lopez, *et al.*, 2018). Trace exposure to PAHs gets into a human via ingestion, as they are consumed in large consumption in their diet intake and uptake from the surrounding area of the crops (soils), polluted air on crops, or food processing steps. Furthermore, PAHs also naturally contaminate vegetables through gaseous deposition and emission from incomplete burning of fossil fuels to the fruits or vegetables and proximity grown nearby roadways, heavily travelled roads or in industrial areas (Abou-Arab *et al.*, 2014; Mohammad W. Ashraf *et al.*, 2013) and size of the spatial distribution pattern (Petrová *et al.*, 2017).

Daily intake consumption among nine vegetables studied in King Fahd Teaching Hospital, Saudi Arabia, revealed the potato as the primary source of PAHs exposure (0.85 µg p-1d-1), followed by carrot (58g µg p-1d-1) and cabbage (50g µg p-1d-1). Meanwhile, PAHs concentration on all vegetable parts revealed peel is contaminated more than the core. In the same study, the maximum B(a)A concentration was found in turnip cores (2.21  $\pm$ 1.75 µg kg<sup>-1</sup>), and the highest B(e)P concentration was found in potato (2.90 ±1.10 µg kg-1) (Muhammad Waqar Ashraf & Salam, 2012). The concentrations of Shanghai green cabbage and Chinese cabbage grown near industrial areas of Shanghai detected almost 16 PAHs congeners. PAH intake via ingestion through green cabbage had the most significant contribution (6.97e14.06 ng p-1d-1) among sampled vegetables in the study (Jia et al., 2018). In another study, 4.2 to 15.6  $\mu$ g/kg of B(a)P was observed in kale in different locations in Western Germany, while in comparison to a different location, 0.19 to 0.34  $\mu$ g B(a)P/kg was observed in wheat samples from rural area whereas, 0.72 to 3.52 µg B(a)P/kg was proximity to industrial power plants (Paris, Ledauphin, Poinot, et al., 2018). Bivalves or bottom dwelling-filter feeders like oysters, mussels, and clams filter out water, and low metabolic activity allows the pollutants to concentrate within their tissues, making them reliable pollution bio-indicators. On their basis, it is possible to determine the ecological condition of the sea and its response to human activity. A high number of PAHs are bonded to particles that fall out from air deposition or mix with those on the sea floor. (Nwaichi & Ntorgbo, 2016).

Dreadful hydrocarbon pollution greatly impacts fisheries, molluscs and crustaceans subject to odour and flavour. However, this situation may suppress growth and impair reproduction. It has been discovered that exposure to PAHs occurs in fish through the food chain to fish and diffusion through gills and skin. Sediment-dwelling biota (crabs and molluscs) and bottom dwelling-filter feeders are inevitably exposed to contamination (Lamichhane *et al.*, 2016; Purcaro *et al.*, 2015). PAH is not significantly present in the marine food web because most organisms have degradative potential. The most important degradative processes for PAHs in aquatic systems are photooxidation, chemical oxidation, and biological transformation by bacteria and animals (Abdel-Shafy & Mansour, 2016).

More than hundreds of PAHs are formed; only 16 PAHs are listed in the list of priority pollutants by the U.S. Environmental Protection Agency (USEPA) in 1976 of the U.S. Clean Water Act as tabled in Table 3 (Edokpayi et al., 2016; Kuppusamy et al., 2017). These 16 compounds are often called parent PAHs and have become a primary environmental concern due to their persistence, the chance of exposure, ubiquitously in the environment and exert toxicity in humans and organisms and levels in the environmental samples. The UNECE Convention on Long-range Transboundary Air Pollution (LRTAP) has designated PAHs as persistent organic pollutants (POPs) based on their persistence, toxicity and anthropogenic emission. Also, in the OSPAR convention, PAHs have been added as hazardous substances (Balmer et al., 2019). Most foreign and national agencies have agreed on PAHs as the most potent mutagens and carcinogenic that rapidly and widely spread in the human environment (Abdel-Shafy & Mansour, 2016; Gad & Gad, 2014).

The International Agency for Research on Cancer (IARC) classified Benzo[a]Pyrene (BaP) as one of the most carcinogenic compounds (Group 1 carcinogen) and has been extensively studied on its impact on human health (IARC, 2010b). In 2018, the European Food Safety Authority (EFSA) CONTAM agreed to conclude that BaP was not a suitable indicator for total PAHs in food contents. They decided that benz[a]anthracene,  $\Sigma PAH4$ (benzo[a]pyrene, benzo[b]fluoranthene and chrysene) and ΣPAH8 (benzo[ghi] perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3cd]pyrene) were suitable markers to estimate PAHs contamination in food. Following the release of this EFSA opinion, Commission Regulation (EC) No 1881/2006 was substituted by Commission Regulation (EU) No835/2011 (European Commission, 2011), setting maximum limits for both benzo[a]pyrene and PAH4 (Ingenbleek et al., 2019).

European Union agreed and highly recommends that PAHs exposure in food products be extensively studied in a wide range of food matrices. Based on the discussion above, it can be summarised that the more significant contributor for PAHs is greatly influenced by cooking temperature, method, and type of fuel or oil used for cooking and storage. Other routes to get to food are air, soil, and water. The emission rate for BaP emitted by cooking was significantly higher than those emitted from traffic sources, accounting for 675 kg/year and 61.4 kg/year. This was mainly attributed to cooking may release and regard as a significant source of PAHs more than traffic from carcinogenic potency (Abdullahi *et al.*, 2013).

Studies showed that the mechanism of harmful actions is closely associated with the function of membrane fluidity

(cellular membrane) and enzyme systems. Because of this, PAHs serve as potent immune suppressants and are confirmed as carcinogenic and mutagenic to all organisms. According to Gupte et al. (2016), several aspects also influence the rapidness of toxicity mechanisms, such as chemical oxidation, microbial degradation, visualisation, water and lipid solubility, sorption and atmospheric photolysis. The ability of PAHs to bind with DNA and proteins leads to carcinogenesis and mutation effects. Studies have shown evidence between PAH-DNA adduct covalently bond in PAH doses and different organs (Ewa & Danuta, 2017). The quick absorption of PAHs by aquatic organisms is because of their high lipophilicity and low water solubility. Along with the increase in molecular weight, the PAHs vapour pressure and water solubility continue to decline, and lipophilicity increases, making the PAHs structure more recalcitrant.

This is because pyrogenic sources produce more thermodynamic stable and noxious PAHs than petrogenic sources. For example, when benzo[a]pyrene undergoes photolysis. An increase in hydrophobicity endows these chemicals with high octanol: water partition coefficients ( $k_{ow}$ ), as a result of which they tend to adsorb to soil organic matter, are less bioavailable and therefore are not easily degraded, giving rise to greater persistence in soil; thus it becomes naturally recalcitrant in soil (Okere, 2011).

Furthermore, all household activities elevate indoor pollution through cooking (frying, heating, and roasting in particular), cleaning products, fuel-burning combustion appliances, tobacco products and heating, cooling, or humidification devices. Other factors that contribute to the arose of nitrogen dioxide, carbon monoxide and fine particles from poorly installed wood-burning or gas. The EPA reported that indoor air pollutants could reach up to 2 to 5 times and occasionally become 100 times worse than outdoor levels (Van Tran et al., 2020). To put it another way, the air quality inside can be more toxic than the air from outside. After such activities, pollutant concentrations can persist in the air for a long time. Indoor air pollution is synonymous with cooking and heating, as mentioned before. The widespread ability of PAHs to transport long distances and their presence influence their occurrence in foods depending on their physical and chemical properties.

The significant pathways for PAHs entry into plants are through plant roots (Bisht et al., 2015; Kang et al., 2010). In addition, surface absorption of volatile PAHs from the air can be regarded as another pathway concentrated into leaves through a wet and dry particle-bound deposition. These pollutants move through the cuticle and adsorb to plant cell walls. The influencing factors in retaining PAHs depend on leaf morphology (species, leaf, branch) and the abundance of stomata, trichomes, and cuticular wax. Studies showed that the coated wax on leaf surfaces and stomata aids plants in battling dust and airborne elements attached to or trapped in (Muhammad Waqar Ashraf & Salam, 2012; Liang et al., 2017). Although PAHs accumulate mainly on the products surface, due to their lipophilic nature, some diffusion can occur to inner layers (Alagić et al., 2015), where water activity and fat content have a determinant role in migration rate (Gomes et al., 2013).

The aquatic environment often represents a particulate matter sink, receiving and accumulating; only approximately 33% are in dissolved form (Baali & Yahyaoui, 2020). As for marine organisms, they are intoxicated by PAHs from both point sources, such as direct discharge from waste and oil spills, and non-point sources, such as runoff from various land type usage and atmospheric deposition.

The most abundant environmental PAHs are low molecular weight even though they show less carcinogenic and mutagenic properties and become more toxic when they react with other pollutants (Barrán-Berdón et al., 2012; Jamhari et al., 2014). The LMW-PAHs existence in the environment samples emerges as petrogenic pollution, direct input from petroleum or its product, such as naphthalene, fluorine and acenaphthene. In contrast, the HMW-PAHs such as benzo[a]anthracene, benzo[a]pyrene, chrysene, and dibenzo[a,h] have high carcinogenic properties, which indicate pyrogenic sources and fewer LMW PAHs implies pyrolytic sources (Jamhari et al., 2014; Lias et al., 2014). The occurrence and biological activity of the large PAHs seem to be a continuation of the small PAHs. They are mostly present as combustion products but at lower levels than the small PAHs because of the kinetic limitation of their formation through the addition of successive rings. Specifically, the more isomers possible for larger PAHs, the lower the occurrence of specific structures. Naphthalene, consisting of two coplanar sixmembered rings sharing an edge, is another aromatic hydrocarbon. It is not a true PAH by formal convention, though it is referred to as a bicyclic aromatic hydrocarbon (Malhat et al., 2019). HMW-PAHs, due to their low vapour pressure, become resistant to surface to air exchange after deposition (Majumdar *et al.*, 2017).

#### 5 Analysis of polyaromatic hydrocarbon from mosses

#### 5.1 Moss sampling preparation and storage

Sample preparation may target the matrix of the sample, the analytes, or both. As general rules, there are general protocols involved for analysing organic pollutants in biological and environmental. Ambient air is sampled by collecting suspended particulate matter on glass-fibre, polytetrafluoroethylene, or quartz-fibre filters employing high-volume or passive samplers. Filters may not retain most PAH as they are volatile. Thus, they are commonly trapped by adsorption on polyurethane foam. Despite these filter instrumental methods, ambient PAH also can be trapped using green solutions; leaves have been widely used in biomonitoring studies. Concha-Graña et al. (2015) collected Pseudoscleropodium purum from unpolluted wood in Galicia (NW Spain) away from urban and industrial areas and cleansed using bidistilled water before being oven-dried at 45°C and ground it into a homogenous powder in a mill. Pleurozium schreberi was collected from a forest 10 to 100 meters from the southern roadside. Next, the samples were wrapped in aluminium foil, labelled, and transported in a cool box. Samples were frozen at -26°C in the lab. During the hand-cleaning process, only 2.5 year old green parts were chosen as a bio-monitor for the study in accordance with the protocol established by the UNECE ICP Vegetation International. Sucharová & Holá, (2014b) used two drying processes for the samples: half was air-dried in between filter paper sheets at ambient temperature up to 25°C, and the other half used a freeze dryer for 17 hours. The grounding processes were carried out in a rotor-speed grinder and vibratory ball micro grinder. Moss samples Hypnum plumaeforme, widespread distribution in urban areas. Only the green and brown-green tips of moss samples were collected for subsequent analysis. Plant samples were wrapped in aluminium foil, air-dried at ambient temperature, and stored in paper and sealed polyethylene bags in the dark at room temperature until PAH analysis. To obtain a number of large samples, moss samples from multiple locations were combined for the next procedure (Concha-Graña *et al.*, 2015; Sucharová & Holá, 2014b)

To ensure sampling activity complies with the requirement of the scope of study while acknowledging the operational and economic constraints, Fernández et al. (2015) classified sampling types for mosses based on their research aims: (1) to characterize pollution derived from focal sites of emission (i.e., industrial facilities, mining areas, etc.); and (2) to characterize pollution distribution in a wide area (i.e., at regional, national or transnational levels). For aim (1), by following a logarithmic grid, moss samples are gathered around the focal site, and the number of sampling sites is reduced as the distance from the source increases. The extent of the pollution-affected area will determine the number of sampling sites. According to Fernández et al. (2015), mosses sampling is recommended to be carried out from 20 sampling sites for road and industrial areas and 20 - 49 sampling sites for urban areas within 6 to 40 km in the circumference of the focal site. For aim (2), various studies proposed cost effective sampling grid approaches such as follows: (a) 30 × 30 km sampling grid (Boquete *et al.*, 2009); (b)  $25 \times 25$  km sampling grids (Pesch *et al.*, 2008); and (c) 32  $\times$  32 km sampling grids Ferna'ndez *et al.* (2007). Hence, this manuscript suggests that the sampling grids between 25 x 25 km until 32 km x 32 km would be the cost-effective range of the sampling grid without compromising the sample representativeness. This range is equivalent to sampling 1 site/1000 km2 to 4 sites/1000 km2.

## 5.2 Analysis of polyaromatic hydrocarbons in mosses

The solvent selection is crucial for solvent extraction to ensure the excellent separation between samples and desired analytes. According to the theory of miscible and intermiscibility, "like dissolves like", the more similar polarity of the solvent to the solute will perform better extraction (Zhang et al., 2018). A compound that separates solid materials into liquid form is called solid-liquid extraction, while liquid removal to another liquid is called liquid-liquid extraction. Several aspects must be considered when selecting solvents based on selectivity, solubility, safety, and cost. The selected solvent must have high extraction capacity, be safe to use, non-explosive, and offer lowprice options. In addition, as much as possible, the solvent must not react chemically with the sample extract, low viscosity, high recovery, and low temperature to avoid degradation to the sample. The high temperature rapidly causes the loss of solvent and causes impurities in the matrices. For plant matrices extraction, the plant type, the part of the plant to extract, and the nature of bioactive compounds must also be highlighted (Alternimi et al., 2017). The successful and effective extraction depends on the particle size of sample matrices, ease, and high penetration of solvents to the sample (Tzanova et al., 2020). The extraction efficiency and quality depend on the period consumed in the extraction process. However, a more extended period will not affect the extraction after the solute equilibrium is reached inside and outside the solid material. The greater the solvent-to-solid ratio is, the higher the extraction yield is; however, a solvent-to-solid ratio that is too high will cause excessive extraction solvent and requires a long time for concentration (Zhang et al., 2018). They mentioned in phytochemical studies that alcohols (EtOH and MeOH) are the most used solvents in the extraction process (Zhang et al., 2018).

#### 5.2.1 Extraction of polyaromatic hydrocarbon

Extraction is the prime step for accurate identification and subsequent analysis of atmospheric PAHs in the environment.

#### 5.2.2 Solvents of extraction

Different solvents have different applications; polar solvents meet polar compounds, while nonpolar solvents meet nonpolar compounds. To begin with any extraction process, solvent selectivity is among the vital key in terms of volatility, cost, efficiency and environmental health impact (Płotka-Wasylka *et al.*, 2017). rephrase methanol, ethanol, acetonitrile, acetone, dichloromethane, toluene, cyclohexane and n-hexane (Abubakar & Haque, 2020).

#### 5.2.3 Extraction method

Soxhlet extraction and ultrasonication are the two most prevalent traditional extraction techniques. Other alternative extraction methods, such as Pressurized Liquid Extraction (PLE) or Accelerated Solvent Extraction (ASE), Microwave-Assisted Extraction (MAE), Membrane Assisted Solvent Extraction (MASE), extraction methods that use liquid water as the extractant, such as Pressurized hot water extraction (PHWE), and Supercritical fluid extraction (SFE), which uses supercritical fluids as the extracting solvent, are also utilized. Different extraction methods use various organic solvents to improve extraction yield (Frantz *et al.*, 2015; Mukhopadhyay *et al.*, 2020; Yamaguchi & Lee, 2010). Modified extraction techniques with low or no solvent consumption have also been developed for improved extraction efficiency. More details on the method of extraction are shown in Table 4.

## 5.2.4 Analysis of the distribution of polyaromatic hydrocarbon in mosses

One of the difficulties associated with detecting PAHs in environmental samples is the complexity of the PAH mixture in these samples. Even after a thorough cleaning, trim levels of PAHs and hundreds of other compounds may still be present. Analytical procedures with strong chromatographic resolving power and detector selectivity are typically required to assess specific chemicals in such combinations. The three-step basic process for the analysis and PAHs determination in environmental samples are as follows (Guimarães et al., 2013): (1) extraction and isolation from the sample matrices; (2) clean-up and a fraction of the PAHs mixtures into subgroups or layers to purify the samples; and (3) identification and quantitative determination of the individual components in each of these subgroups or layers. There have been a variety of analytical methods used to determine trace amounts of PAHs in environmental samples over the years, including gas chromatography (GC) and high-performance liquid chromatography (HPLC) equipped with various types of detectors, as well as thin-layer chromatography (TLC) equipped with fluorimetric detectors (Adeniji et al., 2018). The most common analytical procedures for measuring PAH concentrations in environmental samples are GC/MS and HPLC with UV or spectrofluorimetric detectors. GC is one of the earliest chromatographic separation techniques to be invented and has retained its significance. The popularity of gas chromatography is due to a desirable combination of very high selectivity and resolution, good accuracy and precision, a wide dynamic concentration range, and high sensitivity. The gas chromatograph-mass spectrometer (GC-MS) is a powerful tool

Table 4: N	Method of	extractions
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Sample preparation	Sample weight	Extract method & description	Solvent information	Evaporation after extraction	Reference
NM	NM	Falc Sonicator: Twice	25 mL of dichloromethane, each for 20 minutes.	Nitrogen used to remove solvent	Capozzi <i>et al.</i> , (2017)
NM	NM	Matrix solid phase dispersion: C <sub>18</sub> (octadecyl functionalized silica Supelclean- Envi 18 and hexane	Dichloromethane:hexane (20:80)	NM	Carrieri <i>et al</i> ., 2021
<ol> <li>The homogenized samples were dried for 48 hours under 40 °C</li> <li>The samples were kept in glass vials in the dark and at room temperature.</li> </ol>	NM	FexIKA® vario control extractor: 5 hours	Hexane:dichloromethane (1:1)	NM	Ciesielczuk et al., (2012)
The samples were stored at 20°C until drying, homogenisation and further analysis.	3g of moss	Soxhlet extraction	Acetone	Shaken out with n,n-dimethylformic amide and cyclohexane.	Dreyer <i>et al.</i> , 2018
The homogenized samples were dried at 40 °C to a constant weight.	0.5 g	Ultrasonic bath	Dichloromethane	<ol> <li>Nitrogen used to remove solvent.</li> <li>Evaporate to dryness at a temperature of water bath of 30±5°C</li> </ol>	Hajiyeva <i>et al</i> ., 2017
NM	0.38–1.64 g	Pressurized liquid extraction	N- pentane:dichloromethane (90:10), Program: pressure 1,500 psi, preheat time of 2 min, static time of 5 min, 70% flush volume, 60 s purge time, 2 static cycles, 100°C	<ol> <li>Evaporated to less than 5 mL.</li> <li>The samples were reconstituted to 5 mL with n- pentane: dichloromethane (90:10) after 8μg ml<sup>-1</sup> of recovery standard mix.</li> </ol>	(Martinez-Swatson <i>et al.</i> , 2020b)
<ol> <li>The samples were dried in an oven 40 °C.</li> <li>The samples were kept in PE bags at 4 °C until analysis.</li> </ol>	NM	Sonication-assisted solvent extraction (DSASE)	Dichloromethane: hexane (6:4)	Concentrated under a nitrogen stream to 0.5 mL from 2 mL.	Domeño et al., 2012
The samples were dried using	1.5 g	Pressurized liquid extraction	Acetonitrile, cyclohexane,	1) Concentrated	Foan & Simon, (2012)

freeze dryer and homogenized using stainless steel mill.		(PLE) and Soxtec extraction followed by solid-phase extraction (SPE)	dichloromethane (DCM) and n-hexane	under a nitrogen stream to 0.1 mL. 2) 1 mL of ACN was added before filtered with PTFE syringe filters (I.D. 13 mm, 45 µm)	
NM	5 g	Accelerated Solvent Extraction (ASE)	Dichloromethane: acetone (1:1)	Concentrated under air stream and added 1 mL of ACN	Godzik <i>et al.</i> , 2014
The samples were air-dried for 72 hours at room temperature of $22^{\circ}$ C and homogenized afterward.	3 g	Ultrasonic bath	n-hexane: dichloromethane (1:1)	The extracts were left evaporated for 24 hours until concentrated to 1 mL.	AM. Rotaru <i>et al.</i> , (2017)
The samples were air-dried at room temperature and kept in the dark room.	3.1 g	Soxhlet extraction	Toluene	NM	Oishi, 2013
The samples were oven-dried at 45 °C and homogenized afterward.	0.5 g	Syncore Analyst evaporator	Hexane: acetone (90:10)	Concentrated in the Syncore to 0.3 mL and 1 mL of hexane was added.	Concha-Graña, Piñeiro- Iglesias, <i>et al.</i> , 2015

### Table 4.1: Method of detections

Equipment	Preparation	Column (Length x inner diameter x film thickness)	Flow/ Gas	Program	Injection/ Mode	Internal Standard (IS)	Reference
GC/HRMS	The homogenized samples were extracted with Soxhlet and purified with alkaline silica gel.	HP-5 capillary Size: 30 m x 0.25 mm x 0.25 μm	NM	<ol> <li>1) Started at 50°C for 1 min;</li> <li>2) increased by 14°C min<sup>-1</sup> to 220°C;</li> <li>3) increased by 7°C min<sup>-1</sup> to 300°C;</li> <li>4) held for 40 min at 300°C.</li> </ol>	Splitless/SIM	NM	Oishi, 2013
PTV-GC-EI- MS-MS	1) The extracts were eluted with hexane:mixnexane/dichloromethane(10:20:80)usingaVisiprep	Size: 60 m×0.25 mm, 0.25 μm	NM	NM	NM	Anthracene D10	Vingiani <i>et</i> <i>al.</i> , 2015

	vacuum. 2) 20 µL of internal standard was added into the eluate after concentrated with Syncore.						
GC-MS	<ol> <li>The extracts were concentrated to 4 mL with a rotary evaporator and dried under a gentle nitrogen stream.</li> <li>The samples were added with 0.4 mL of mixture of ISs.</li> </ol>	HP-5MS Size: 30m x 0.25mm x 0.25µm	1.11 mL min <sup>-1</sup> , Helium	1) Started with 70°C; 2) up by 20°C min <sup>-1</sup> to 280°C; 3) held for 24min	SIM	Acenaphthyle ne D8, fluoranthene D10, benzo(k) fluoranthene D12, benzo(g,h,i) perylene D12	De Nicola et al., 2013
GC	<ol> <li>The samples were subjected to cleaned up using gel permeation chromatography (GPC).</li> <li>Dried under gentle nitrogen stream.</li> <li>Eluted with 10 mL of cyclohexane: ethylacetate (1:1).</li> <li>Cleaned up to 5 mL by GPC and concentrated using rotary evaporator to 0.5 mL.</li> <li>Cleaned up through adsorption chromatography (AC) with silica gel using mixture of petrolether and dichloromethane (4:1).</li> <li>The eluates were mixed with propan-2-ol before final purification on a SephadexÒ LH- 20 column and concentrated again under nitrogen stream.</li> <li>Dissolved in 0.5 mL cyclohexane.</li> </ol>	HP-5MS Size: 30 m x 0.25 µm fused-silica capillary column.	1 mL min <sup>-1</sup> , ultra- pure Helium	1) Started at 90 °C for 4 min; 2) increased to 100 °C at a rate of 10 °C min <sup>-1</sup> ; 3) raised to 290 °C by 3 °C min <sup>-1</sup> ; 4) held at 290 °C for 22 min.	SIM	Deuterated acenaphthene, benzo(a) pyrene, and phenanthrene	Rodriguez <i>et</i> <i>al.</i> , 2010
GCeMSD	<ol> <li>The extracts were purified through activated silica gel;</li> <li>Dried under a nitrogen stream to 200 mL.</li> </ol>	VF-17MS column	1.3 mL min <sup>-1</sup> , Helium	<ol> <li>Started at 50 °C; 2) ramped to 30 °C by min<sup>-1</sup>;</li> <li>raised to 350 °C;</li> <li>held for 9 min.</li> </ol>	SIM	Naphthalene D8, acenaphthene D10, phenanthrene D10, chrysene D12, and perylene D12	Capozzi <i>et al.</i> , 2017

GC with masses the selection detector (MSD)	<ol> <li>The extracts were concentrated with a rotary evaporator at a water bath temperature of 30±5°C to 2 mL.</li> <li>Dried under a gentle nitrogen stream to 1 mL before injected to samplers.</li> </ol>	Column ZB Size: 60 m x 0.25 mm x 0.25 μm.	Flow rate: NM Helium	NM	SCAN	NM	Hajiyeva et al., 2017
GC equipped with a triple quadrupole MS detector	The samples were extracted with 10 mL of methanol for 24 hours via lab shaker.	SCION 5 MS Size: 30 m × 0.25 mm × 0.25 μm	2 mL min <sup>-1</sup> , Helium 5.0	<ol> <li>1) Started at 70 °C, 2) raised 200 °C by 15 °C min<sup>-1</sup>;</li> <li>3) held for 5 min;</li> <li>4) raised to 320 °C by 8 °C min<sup>-1</sup>,</li> <li>5) kept for 5 min.</li> </ol>	NA	Naphthalene D8, phenanthrene D10, chrysene D12 and perylene D12	Świsłowski et al., 2021
GC-MS	<ol> <li>After sonicated for 4 hours with 3 x 40 mL of dichloromethane, the extracts then were concentrated to 1 mL using rotary evaporator.</li> <li>Purified with concentrated tetraoxosulphate (VI) acid: silica gel (1:8).</li> <li>The extracts were firstly eluted with 100 mL of hexane followed by 200 mL of dichloromethane.</li> </ol>	Column coated with 10 µm porous silica with C <sub>8</sub> -bonded Size: 15 cm x 4.6 mm	1.5 mL <sup>-1</sup> , Purifie d helium	NM	Splitless mode	NM	Adie et al., 2021
Trace GC Temperature vaporization- gas chromatograph y-tandem mass spectrometry	The homogenized dried samples were kept for storage in glass vials in the dark under room temperature before analysis.	DB-XLB column Size: 60 m x 0.25 mm x 0.25 μm	1 ml min <sup>-1</sup> , Helium	<ol> <li>1) Started at 50 °C and held for 3 min;</li> <li>2) raised by 4 °C min<sup>-1</sup> to 325 °C and held for 20 min</li> </ol>		Dibenz(a,h)an thracene D14 (for Dibenz(a,h)an thracene, Indeno(1,2,3- cd)pyrene, and Benzo(ghi)per ylene) and anthracene D10 (for the other PAHs)	Carrieri <i>et al.</i> , 2021
APGC-Q-TOF- MS	<ol> <li>1) The 2 mL extracts were purified with SPE filled with dichloromethane: hexane (6:3).</li> <li>2) The extracts were then eluted with a mixture of hexane:</li> </ol>	HP-5MS Size: 30 m x 0.25 x 0.25 μm	1 mL <sup>-1</sup> , Helium	<ol> <li>1) Started at 50 °C and held for 2 min;</li> <li>2) raised to 280 °C by 10 °C min<sup>-1</sup>;</li> <li>3) held for 12 min.</li> </ol>	APGC	1- nitronaphthle ne, 2- nitrofluorene, 1,5-	Domeño et al., 2012

	dichloromethane (65:35).					dinitronaphth alene, 2- methyl-1- nitronaphthal ene, 6- nitopyrene and 2- nitrofluoranth e and acenaphthene D10.	
GC-MS	The samples were cleaned up with activated silica.	Size: 60 m x 0.25 mm x 0.25 μm	1.1 ml min <sup>-1</sup> .	<ol> <li>Started at 40°C and held for 2 min,</li> <li>raised to 315°C by 5°C min<sup>-1</sup> and held for 14 min.</li> </ol>	SIM	NM	Martinez- Swatson <i>et</i> <i>al.</i> , 2020a
Trace GC chromatograph equipped with a GC Combi-PAL autosampler	20 mL of internal standard was added into 1 mL filled up with Hexane.	DB-XLB column Size: 60 m x 0.25 mm x 0.25 μm	1 ml min <sup>-1</sup> , Helium	<ol> <li>1) Started at 50 °C, held for 3 min;</li> <li>2) increased to 325 °C, and held for 20 min.</li> </ol>	NM	Anthracene D10	Concha- Graña, Muniategui- Lorenzo, <i>et</i> <i>al.</i> , 2015
Gas chromatograph y – tandem mass spectrometry	<ol> <li>The samples were extracted with an ultrasonic clearner for 15 min and Soxhlet for 24 hours.</li> <li>Both extracts later were concentrated using rotary evaporator to 5 mL;</li> <li>1 mL of filtrate and 10 μL of internal standard were subjected for analysis.</li> </ol>	AB-5MS Size: 30 m × 0.25 mm × 0.25 μm	1.0 mL min <sup>-1</sup> , Helium	<ol> <li>Started at 70°C, a ramp and hold for 20 min;</li> <li>increased to 300°C at 10°C min<sup>-1</sup>;</li> <li>kept for 7 min.</li> </ol>	Splitless	Naphthalene D8, acenaphthene D10, phenanthrene D10, chrysene D12, and perylene D12.	(R. Wu et al., 2017)
HPLC	<ol> <li>1) 1 mL of acetonitrile was added into 0.1 mL of concentrated extracts.</li> <li>2) The solvent was reconstituted by 80 ng of internal standard FTN d<sub>10</sub>.</li> </ol>	1) LC-PAH C18 column (particle size 5 $\mu$ m) Size: 250 mm x 4.6mm Supelcosil <sup>TM</sup> Size: 20 mm x 4.6 mm precolumn (particle size	1.5 mL min <sup>-1</sup>	<ol> <li>60% acetonitrile         <ul> <li>(ACN) at was             maintained for 5 min;</li> <li>2) linear ramped to             100% ACN by 25 min;</li> <li>3)10min plateau at             100% ACN.</li> </ul> </li> </ol>	Selected fluorescence wavelengths	Anthracene D10 and Benzo(a) pyrene D12	Foan & Simon, 2012

		5µm)						
HPLC	The solvent after clean-up through semipermeable membranes for times was concentrated and re- dissolved in 1 mL acetonitrile.	201TP5415	1 mL min <sup>-1</sup> ,	Mobile phases were acetonitrile (ACN) and water. The gradient was 50-100% of ACN for 45 min.	(Certified reference	NM	(Godzik al., 2014)	et

for detecting air, water, and soil (Fair *et al.*, 2010). It is beneficial for quantifying volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs), polychlorinated biphenyls (PCBs), organochlorinated pesticides, brominated flame retardants and polycyclic aromatic hydrocarbons (PAHs). More details on the method of detections are shown at Table 4.1.

#### 6. Current works worldwide

Air pollution remains one of the world's most severe human threats and urban environmental issues. The World Health Organization highlighted that more than 91% of city dwellers deal with low air quality exceeding recommended limits. Rapid industrialization and urbanization in most developing countries significantly impact the increased emissions of air pollutants. The deposited particle-bound polycyclic aromatic hydrocarbons (PAHs) impose human health risk factors for diseases like skin, heart attack, lung cancer, and reproductive, respiratory, cardiovascular and cardiopulmonary system. Effective long-term air pollution mitigation solutions must be designed and executed to address these issues. Moss is an excellent alternative for addressing air quality issues because of its pollution-removing properties and its robust capacity to tolerate all kinds of temporal conditions and low maintenance requirements. Few actions have been taken to elevate mosses as an advanced tool for atmospheric biomonitoring. Even though moss cannot measure air pollution, it has the potential to be a useful screening tool. It could aid in identifying the most polluted areas, from which a government monitor could assess the severity of the problem.

## 6.1 The citytree by green city solutions, Germany

The CityTree technology is developed by Green City Solutions in Germany using various plants, including mosses and lichen, like natural air filters against harmful air pollutants (CITYTREE, 2019). According to the company's four cofounders, the idea is to choose species with a giant leaf surface area, allowing plants to absorb more toxins faster. A co-founder of Green City Solutions says moss is ideally suited to capture more pollutants because the leaf surface is substantially more prominent than any other plant (Tristan, 2017). He continues that the sensors are embedded within the structure to check its efficacy in filtering the air. Data collection, analyses and visualisation are the three main features of the CityTree. The developed technology is suggested as the world's first intelligent living air filter. The company focused on air quality challenges, particularly in an urban area that has worsened dramatically. They combine embedded mosses vertically on a wall with Internet-of-Things (IoT) technology and are engineered with an automatic water supply system to maintain the moisture and nutrients required for plant growth. The wall is a convenient, recyclable, living, breathing air filter that can be placed in cities to measure the level and types of pollution while actively clearing the air using a selected combination of plants that operate as effectively as a small forest. The goal of having CityTree as public furniture is to make it look like an urban forest (Chris, 2017). This public furniture structure is tailored to suit urban settings where mosses can hardly survive because of the lack of consistent moisture and shade (urbanNext, 2018). Incorporated into the structure are an irrigation water system that collects rainwater and pumps it into the soil and solar panels designed to generate electricity for the structure. Besides water, the irrigation system also

alleviates the urban heat island effect by creating a cooling effect in response to its surroundings. By reducing particulate matter, nitrogen dioxide, and ozone profile at 240 tonnes per year, each CityTree is beneficial in pollution hotspots and locations where people spend much time. The system's performance and efficiency, the quality of air, as well as its health requirement for mosses (such as temperature, water quality and soil humidity) are monitored and analysed remotely (IMNOVATION, 2021). The remote technology merges the moss purifying factor with improving airflow through the moss. With this, cleaner air and high moisture content can be achieved. Hence filtration could happen increasingly varies on levels of pollution at different times of the day. It is shown that the structure is easily measurable. CityTree is a revolutionary technology that cleans the air 275 times better than a single tree while taking up only 1% of the space that real trees do (Kfw, 2021). Besides serving as an outdoor air purifier, CityTree also gives digital and visual information using technologies such as Wi-Fi, NFC and digital screens. CityTrees have already been installed in 50 locations worldwide, including Germany, the UK, Norway, Belgium, France, Macedonia and even Hong Kong (PSO, 2019). In 2020, the EU-sponsored Green City Solutions project placed 15 more at polluting hotspots across Berlin.

The company's objective is to integrate its technology into the construction of buildings all over the world. According to independent research, the moss filters 82% of fine dust in the air and cools the air by  $2.5^{\circ}$ C. The reduced fine particulate pollution of up to 53% was removed significantly in its place nearby. Every hour, the CityTree filters 3500 m<sup>3</sup> of air, equivalent to the breathing volume of around 7000 individuals (CITYTREE, 2019). The CityTree does not need daily manual watering as it already has an installed irrigation system. To some extent, Citytrees can be adjusted. They can be purchased with or without an attached bench and with or without vandalism protection.

- 1. London Borough of Wandsworth (CITYTREE, 2019). After ten weeks of installation, the CityTree has successfully filtered 1.3 million m3 of air and 65 grams of PM0.1 (ultrafine particles). These pollutant amounts were 14400 kilometres from mobile exhaust emissions, and 5400 burned smoke cigarettes.
- Berlin (CITYTREE, 2019). After a year of installation, the CityTree has successfully filtered 6.3 million m<sup>3</sup> of air and 46.5 grams of PM0.1 (ultrafine particles). These pollutant amounts were 1.435 kilometres from mobile exhaust emissions, and 3.874 burned smoke cigarettes.
- 3. The Klima Pavilion, Hammersmith, Hampstead Hill Pre-Preparatory and Nursery School, and Berlin Charlottenburg are among the other pollution hotspots that feature the CityTree (CITYTREE, 2019).

### 6.2 MOSSpheres by MOSSclone Research Consortium

A new biotechnological tool keeps the moss inside the device and is placed at desired pollution hotspots as passive pollutant sensors. This project is a collaboration between partners from 5 universities, 5 SMEs in 5 EU countries (Germany, France, Ireland, Italy and Spain) sponsored by the European Union in the Seventh Programme (FP7) for "Eco-Innovation!" (Christian, 2015). The idea is to cultivate the moss clone in a controlled environment free of pollution, fungi, and bacteria

(Tepzz et al., 2016). An axenic devitalized moss clone is used in the passive contaminant sensor devices of the present invention. As used here, the term axenic describes a state of a culture in which only one species, variety, or strain of organism is present, and all other contaminating organisms are absent. The moss must be treated to stop its metabolic activity, guaranteeing that the moss metabolism has no devitalizing effect. Following devitalization, freezing the moss's chemical composition and characteristics are required. To ensure that all plants have the same character and identity, they are all grown from the exact moss clone. Thus, it is crucial to ensure that the first grown are pollution-free (Christian, 2015). The device is called a moss bag or MOSSpheres, in a spherical shape. Mosses are heated to nearly 120°C and inactivated before being put into the ball (Tepzz et al., 2016). Large contaminants have collected on the moss surfaces, they discovered. It demonstrated that pollution accumulation occurs whether they are alive or not. The upscaling of moss clones was carried out in massive bioreactors owned by Bovia, a Spanish company. The concept of the moss bag began with simple tea bags, upgraded in size and geometry optimized MOSSpheres (Christian, 2015). The moss-bag technique was developed to address one of the most common issues in biomonitoring surveys: a shortage of native moss material, particularly in places with poor environmental states (in this case, because there is a high level of air pollution). The moss bags are made of mosses, a substance that traps or accumulates air pollutants, and are sealed in a container format that protects the moss from vandalism, leaching, or being blown away on a suitable carrier. It could take a net shape with a precise mesh size that allows pollution molecules to pass through while larger particles are retained. The accumulation kinetics are influenced by the density of material inside the bag, the ratio between the outside surface and substance mass, and the surface exposed for rain interception. The European Patent Office already patents the design.

#### 7. Current works in Malaysia

In Malaysia, awareness of moss species is minimal, and the hidden treasures receive little attention. Studies on moss biomonitoring of ambient air have tended to focus on metals and rarely on PAHs. Researchers in Malaysia should broaden studies on PAHs if different varieties of moss are available. Local mosses are employed as a bio-indicator for air quality monitoring since they can offer results suited to our local environment.

#### 8. Conclusion

In conclusion, this review highlights the use of mosses to measure polycyclic aromatic hydrocarbons (PAHs) in the environment and presents useful analytical procedures for analysing PAHs in moss samples. Many studies have proven that mosses are powerful tools for monitoring PAHs in the atmosphere. PAH in the atmosphere is predominantly of anthropogenic origin. Once the major atmospheric sources of PAH have been identified, a number of other variables must also be considered. Combustion conditions, fuel composition, geographical location, climate and emission rates affect PAH concentrations in the atmosphere. Trends in the atmospheric levels of the various PAH compounds have been observed both in the long and short term. After PAH compounds are released into the atmosphere, they undergo various processes, namely removal, transport and degradation (reaction). The degradation of PAH can produce compounds which can be more or less mutagenic and carcinogenic than the parent PAH. A method for biomonitoring air quality in environments can be proposed based on the following steps: (1) Selection of the monitoring sites as a function of pollution grade, (2) Selection of plant species considering both availability of different species and capability of discriminate and bioaccumulate the pollutants of interest and (3) Sampling and analysis.

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#### **Conflict of interest statement**

We declare no conflict of interest.

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