TREE BARKS AS BIOINDICATOR FOR ORGANIC AND INORGANIC POLLUTANTS: A PRELIMINARY STUDY

Muhamad Fares Ameran[1], *Zaini Yusoff [2], Non Daina Masdar[2], Mohamad Dzul Helmee Salmi[2], Mohd Lias Kamal[2], and Zaini Hamzah[1]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), 40450, Shah Alam, Selangor, Malaysia*
[2]*Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Perlis, 02600 Arau, Perlis, Malaysia*

e-mel: zaini.yusoff@perlis.uitm.edu.my

Abstract

A preliminary study was carried out to investigate the level of air pollution due to anthropogenic (human activities) sources using biomonitoring techniques. Tree barks from six sampling sites near a cement plant in Chuping Perlis were used to detect the level of PAHs and heavy metals emission in the area. Extraction of PAHs and heavy metals was done using Soxhlet extraction and hot block digestion technique respectively. PAHs content was analyzed using Gas Chromatography with Flame Ionization Detector (GC-FID) and compared with six low molecular weight (LMW) single PAHs standards while the heavy metals detection was analyzed by Inductively Coupled Plasma with Mass Spectrometry (ICP-MS). The result showed the presence of all six LMW PAHs with concentration of total PAHs ranging from 3.75 to 22.15 µg/kg. The result from heavy metal determination showed the presence of all the eight targeted elements in the tree bark samples. The concentration of Zn and Cu were found to be among the highest which were 7391.34 µg/kg and 7087.35 µg/kg respectively while Cd gave the lowest concentration at 62.25 µg/kg.

Keywords: Tree bark, Heavy metal, PAHs, Bioindicator

Introduction

Organic and inorganic pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals are common industrial pollutants that not only contribute to air pollution but also often relate to adverse health effect to mankind.

PAHs, the most stable form of hydrocarbons are classified as persistent organic pollutants (POPs) and are suspected to be carcinogenic and toxic to human (IARC, 1991). PAHs which consist of carbon and hydrogen in ring structure attached to at least two benzene rings can be divided into two types, low molecular weight (LMW) PAHs and high molecular weight (HMW) PAHs. Heavy metal refers to metal with an elemental density above 7 g cm^{-3} and poses threats to human health due to its toxicity effect (Hodson, 2004; Duffus, 2002). These two pollutants are generated from two main sources; from natural phenomena (volcanoes, forest fire, etc) and anthropogenic (human activities) sources.

Industrial emission is one of the major anthropogenic sources of PAHs and heavy metals contamination in the environment besides traffic and agriculture sources (Ravindra, *et al.,* 2008). The amounts and quantity of these pollutants released through the chimney from industrial stacks depend on several factors; type of input fuel, additives, the manufacturing process, etc. (Kaantee, *et al.,* 2004; Tsai, *et al.,* 2002; Manoli, *et al.,* 2004; Hawthorne, *et al.,* 2002). Once released to the atmosphere these pollutants are easily spread due to the atmospheric deposition i.e. sedimentation, impaction and interception (Addo, *et al.,* 2012). These dispersed airborne particles can be directly deposited onto any surfaces via dry deposition, wet deposition or through fall and will subsequently accumulate in soils and sediments (Maliszewska, *et al.,* 2003).

The degree of contamination of PAHs and heavy metal pollutants in the atmosphere can be quantified by biomonitoring technique. Biomonitoring is a technique whereby organisms or biomaterials are used to obtain information on certain characteristics of the biosphere. Animals, plants and sediment have been

widely utilized as bioindicators in determining the level of contamination cause by these pollutants.

Tree barks are among the most excellent biosorbent material and have been used as bioaccumulator of toxicants due to its porous surface, readily available, simple, reliable and cheap (Orecchio, *et al.,* 2008). Accumulation of airborne pollutants on tree barks depends on the barks texture, the presence of epiphytic organisms and the exposure time. Since the contaminants are deposited on the outer bark surface, the depth of sampling has an influence on the level of contamination. In this study the tree barks samples were collected near a cement plant in Chuping, Perlis. These tree barks sample act as bioindicators that can be used to demonstrate the level of PAHs and heavy metal contamination in the respective area.

Material and method

Reagents and standards

All reagents used were obtained from the laboratory of Universiti Teknologi Mara Perlis (UiTM Perlis). 65 % nitric acid, HNO_3, 30 % hydrogen peroxide, H_2O_2, and dichloromethane, CH_2Cl_2, used were all analytical reagent grade. Distilled and deionised water used were from the Milli-Q water purification system (Millipore, USA). All six standard single PAHs (naphthalene, pyrene, fluoronthrene, anthrecene, fluorene and phenenthrene) were purchased from Sigma Aldrich, while analytical multi-element standard 3 solutions were supplied by Perkin Elmer.

Sampling sites

The cement industry in Perlis is located at Bukit Keteri which is about 20 km from Kangar, the capital city of Perlis. This cement industry is the third largest cement manufacturer in Malaysia, which approximately contributes 18 % of the country's cement market share. Tree barks were collected from six stations located within 0.5-2 km radius from cement plant in December 2010. At each station 20-30 g of the barks from three (3) selected trees aged between 20-30 years were collected. The exact location of the selected tree samples are shown on the map in Figure 1. The detail information and characteristics of the six stations are described in Table 1.

Figure 1: Point location for each station

Table 1: Detail description and characteristic sampling stations

Station	Coordinate	Distance from centre of cement plant	Specific activities
1	6° 30.9736' N 100°15.4448' E	0.5 km	Nearby quarry & moderate traffic volume
2	6° 30.4957' N 100°15.6635' E	0.5 km	Heavy traffic activities (main road) & weekly night market.
3	6° 30.5621' N, 100°15.2615' E	0.5 km	Nearby local resident with moderate traffic activities in early morning & late evening.
4	6° 31.7832' N 100° 15.5017'E	2 km	Close to main road. Road users are heavy vehicles moving to and from quarries and cement industry.
5	6° 29.6890' N 100° 15.9995'E	2 km	Low traffic activities. Surrounded by paddy fields.
6	6° 30.4383' N 100° 14.4277'E	2 km	Close to the main road and medium population of local resident.

Sampling and sampling technique

The external surface of the tree bark, at a height of 1-1.5 m above the ground and about 2 mm in depth was removed using a hard steel knife. The height of 1-1.5 m was chosen as suggested by Barnes, (1976) who mentioned that high concentration of airborne

pollutants could be found deposited at 1-2 m height from the ground and to differentiate from contamination from the top soil. About 5-10 g of the bark was collected from each tree. Samples from three (3) barks from the same sampling sites (same location) were mixed or combined as a single sample. The collected samples were refrigerated at 4 ^{o}C to avoid exposure to light and heat (Orecchio, *et al.,* 2008).

Extraction method

PAHs

Samples of tree barks were dried for about a week after which they were pulverized into uniform size with a mortar and pestle. The mortar and pestle was carefully cleaned and dried after each grinding to avoid contamination. In PAHs extraction, a triplicate of 5 g of the powdered tree bark sample was extracted in Soxhlet extractor using 200 mL of dichloromethane for 24 hours. Sodium sulfate anhydrous, Na_2SO_4 was added to the extract to remove water or moisture content in the sample. The extract was filtered into a round bottom flask using 45 mm Whatman filter paper and concentrated to 10 mL using rotary evaporator at 20 ^{o}C. The extract was again filtered using Milipore syringe filter and placed into a vial for analysis.

Heavy Metals

Heavy metals were extracted using hot block digestion method. 0.25 g of dried ground bark sample was mixed with 5 mL of concentrated HNO_3 acid and 1 mL of hydrogen peroxide. This digestion process involved heating the sample in the acid reagent mixture at 150 ^{o}C for 120 min. The digested samples were allowed to cool to room temperature and then filtered using 0.25 mm filter size to eliminate the 'cloud' samples which can cause problem to instruments. 1 mL of the digested sample was then drawn into a 10 mL or 15 mL centrifugal tube and diluted to 10 mL with Milli-Q water (Melaku, *et al.,* 2005). The digested sample was then filtered using 0.45µm Polytetrafluoroethylene (PTFE) syringe filter before proceed to ICP-MS analysis.

Instrumentation

Analysis of PAHs

The PAH extracts from the tree bark were analyzed by Gas Chromatography coupled to Flame Ionization Detector (GC-FID). Six of known concentration of PAHs standard consists of naphthalene, pyrene, fluoronthrene, anthrecene, fluorene and phenenthrene

were prepared using dichloromethane as solvent. All samples and standard PAHs were analyzed by HP5890 II GC with split/spiritless capillary injection and an HP7673A auto sampler Hewlett-Packard at suitable condition. Control of instrument and data manipulation was performed by a VG Lab base data system. Conditions of analyses were referred to a study done by Wu, *et al.,* (2001). Silica capillary column (HP-5, 30 m x 0.32 mm x 0.25 µm) was used to separate individual compounds. The column was programmed from 80 ^{o}C to 290 ^{o}C at 6 ^{o}C min^{-1}. The injector temperature was kept at 280 ^{o}C with detector temperature of 300 ^{o}C and flow rate of carrier gas (N_2) was 1.3 mL min^{-1}. Identification of the PAH compounds was achieved by comparing GC retention time with those of authentic standards.

Analysis of heavy metals

The instrument used was a Perkin Elmer NexION® 300X Inductively Coupled Plasma with Mass Spectrometry (ICP-MS). In the initial stage of ICP-MS analysis, daily performance program using Perkin Elmer Smart Tune Solution was carried to optimize the instrument before analysis. A calibration curve with 0.999 correlation was obtained from five known concentrations prepared with Milli-Q water ranging from 0.5, 1, 3, 7, and 10 ppb using of Multi-element standard of heavy metals (Perkin Elmer Standard 3).

Results

PAHs

All samples indicated the presence of all the six standard single PAHs. The identification of the single PAH component in the sample was carried out by comparing with the retention times of each single PAHs standard under the same experimental conditions. The comparison of the retention time is shown in Table 2. The result indicates the presence of PAHs in sample of tree barks taken from all the six sampling sites with concentration of total PAHs ranging from 3.75 to 22.15 µg/kg of dry matrix. The highest concentration of total PAHs is detected in station 2 (22.15 µg/kg) followed by station 3 (13.48 µg/kg).

Heavy Metals

Figure 2 shows the result of the average concentration of heavy metal detected in samples from 6 stations surround cement plant in Chuping, Perlis. The heavy metals distribution in the studied area indicated a high concentration of Zn, Cu, Ni, Cr and Pb but low in concentration of Li, Cd and Co element. The average

concentration for Zn, Cu, Ni, Cr and Pb were 7391.34, 7087.35, 4657.35, 3158.25 and 2396.55 µg/kg respectively which are considered highly polluted.

Table 2: Comparison retention time, t_R (min) of PAHs standard and samples

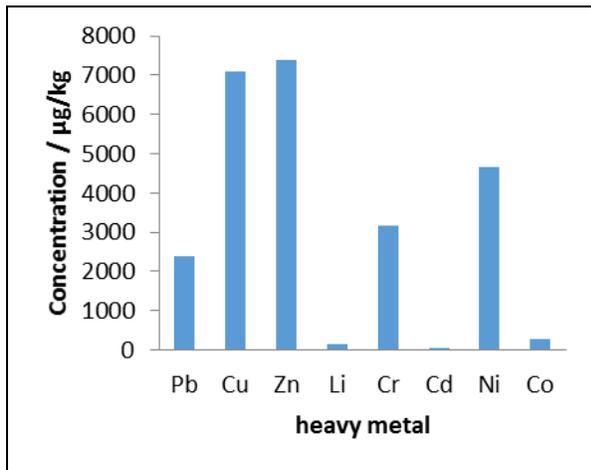| Analytes	Retention time of standard PAHs	Retention time detected in sample (average)
Naphthalene	8.673	8.683
Fluorene	17.344	17.375
Phenanthrene	21.321	21.374
Anthracene	21.490	21.433
Fluoranthene	26.437	26.452
Pyrene	27.347	27.359

Figure 2. Average concentration of heavy metal detected.

Discussions

PAHs study

In this study, naphthalene, phenanthrene, fluorene, anthracene, and pyrene were selected as the analyte representing the low molecular weight of PAHs. PAHs exert both acute and chronic toxic effects. However, only PAHs with higher molecular weight exert chronic toxic effect (which is potentially carcinogenic) whereas low molecular weights PAHs are classified as having acute toxicity. PAHs themselves are not carcinogens but can be converted into carcinogenic derivatives when metabolized (Fisher, *et al.,* 2011).

A toxic equivalence factor (TEF) has been developed for a number of these potentially carcinogenic PAHs. TEF values represent the potential level that these single PAHs are suspected toxic in environment. (Fisher, *et al.,* 2011; Schneider, *et al.,* 2002).The factor expresses the potency of each PAHs relative to benzo[a]pyrene, a compound which has TEF value of unity. The closer the TEF value to unity, the greater potential of the compound to be carcinogenic. Table 3 gives the chemical structure and TEF values of each of single PAHs. The listed TEF values (Table 3) were obtained from studies done by other researchers on the same selected PAHs.

Fisher, *et al.,* (2011) argued that these six single PAHs were categorized as low molecular weight PAHs due to the number of benzene rings in their structures (2 to 4 benzene rings), thus they are not carcinogen as high molecular weight PAHs but only acute toxicity. These factors represent toxicity equivalence factors (TEF) (Orecchio, *et al.,* 2008).The result in Figure 3 shows that all the six low molecular weight PAHs were detected in all the stations except for phenanthrene and anthracene which were not detected in station 4 and 5 respectively. The absence of these two PAHs could be due to the very small concentration and can be trace using spiking or standard addition method analysis. Fisher, *et al.,* (2011) state that the 2 to 4 rings type of PAHs exist primarily in gas phase in atmosphere and easily lost during atmospheric deposition (wet or dry deposition).

Table 3: Single PAHs descriptions and TEF values from other studies regarding their carcinogenic potential

Name	Structure[a]	MW[b]	TEF[c] (a)	TEF[c] (b)
Napthalene		128.19	-	-
Fluorene		166.21	-	0.001
Phenanthrene		178.22	0.0005	0.001
Anthracene		178.22	0.0005	0.01
Fluoranthene		202.26	0.05	0.001
Pyrene		202.26	0.005	0.001

[a,b]Ravindra, *et al.,* (2008); [b]Molecular weight (MW)
[c]Orecchio, *et al.,* (2008);
[c]TEF (a) : Larsen and Larsen, (1998)
[c]TEF (b) : Schneider, *et al.,* (2002)

Station 2 is located about 0.5 km from the center of the cement plant. Traffic activities such as lorries, trailer trucks and bulldozers entering and leaving the plant and the nearby quarry may contribute to the high concentration of PAHs. The distribution pattern of the six single PAHs is shown in Figure 4. Since the types of PAHs presence in these stations were of low molecular weight PAHs, it may be assumed that the cement plant is the major contributor to emission of PAHs. Wang, *et al*., (2010) reported that cement plants in China that used coal and tires as raw material during manufacturing process (coal combustion) contributed the large sources of LMW PAHs into the environment. Other possible sources of PAHs in the area are the open burning activities from the nearby Chuping sugarcane plantation as well as the emission from traffic activities.
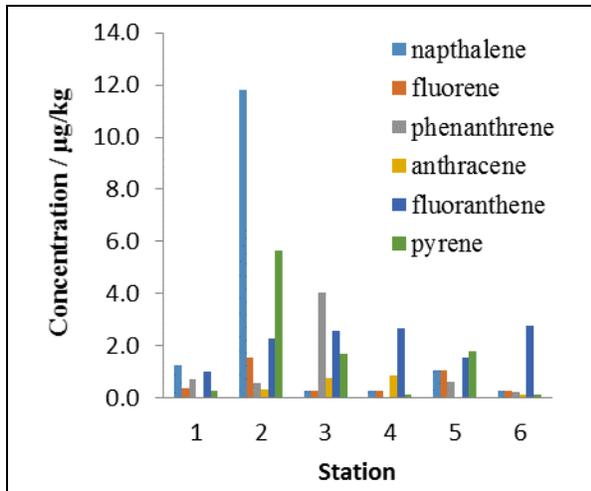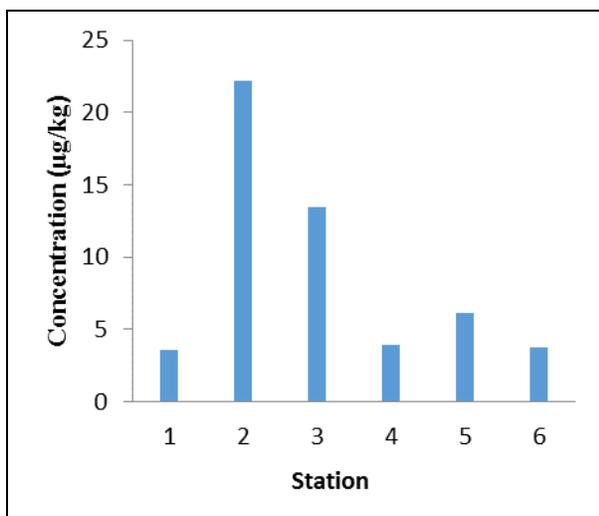

Figure 3. Distribution pattern of six single PAHs

Figure 4. Distribution of total PAHs in all six sampling stations.

Heavy metal study

Detection of heavy metals in tree barks sample proved that the bark is able to act as biomonitor of inorganic material such as heavy metal in addition to organic pollutants such as PAHs (Schulz, *et al.,* 1999). The result on the distribution of heavy metals represents the distribution or the accumulation of the pollutants in one year period since the outer bark layer will be naturally replaced by new layer as part of growing process. Average concentration of heavy metal pollutants detected in Perlis cement plant are shown in Table 4.

High concentrations of heavy metals detected in the area probably come from anthropogenic sources such as activities from cement industries and traffic. Al-Alawi, *et al.,* (2007) stated that the cement industry was the major sources of atmospheric pollutants with large quantities of particulate matter generated at all stages during production process. The above statement was strengthen by Bermudez, *et al.,* (2010) who argued that cement plants emit metal and metalloid such as As, Cd, Ca, Co, Cr, Cu, Ni, Pb and Zn as atmospheric pollutants. The high concentration of Pb (2396.55 µg/kg) detected in this study indicated an agreement with the above arguments.

Table 4. Average concentration of heavy metal pollutant in Perlis cement plant.

Element	Concentration (µg/kg)
Pb	2396.55
Cu	7087.35
Zn	7391.34
Li	146.70
Cr	3158.25
Cd	62.25
Ni	4657.35
Co	277.80

High concentration of heavy metals may also originate from motor vehicle activities in the surrounding studied area. Nearly 70 % of road users surrounding the cement plant in the Chuping area are heavy vehicles such as bulldozers, lorries and trucks trafficking to and from the plant as well as the nearby quarry. In their study, Duong and Lee, (2011) concluded that the contamination level of heavy metals in the atmosphere was highly dependent on the traffic volume and atmospheric dispersion from traffic rotaries. For example Cu distribution are linked to the usage of copper brake particles which contribute the metal to the environment (Franky, *et al.,* 2011).

The heavy metals found in the atmosphere were in the form of airborne particulate matter. The ability of airborne to fly far from its sources is beyond

control. There are several meteorology factors that affect the distribution of the pollutants in the atmosphere i.e. wind direction, wind speed, humidity, and rainfall rate. This is because the pollutants are sensitive with the changes of atmospheric condition (Lee, and Hieu, 2011). Besides that, Jóźwiak, M. A and Jóźwiak, M., (2009) also inferred that meteorological condition during the season played a major part in carrying heavy metals from dust particle in atmosphere.

The heavy metals concentrations obtained in this study were compared to the minimal risk level (MRL) in atmosphere obtained from Agency for Toxic Substances and Disease Registry (ATSDR, 2013) as summarized in Table 5. The MRL was for chronic level (i.e. for duration of 1 year and above). The results indicate that the concentrations of selected heavy metals are higher than that stated in the MRL. This may be due to the accumulation of the airborne metal pollutant for a long period of time.

Table 5. Minimum Risk Levels concentration compared with obtained concentration

Elements	MRL (mg/kg/day)	Result concentration (mg/kg)
Pb	N/A	2.396
Cu	0.01	7.087
Zn	0.3	7.391
Li	N/A	0.146
Cr	0.005	3.158
Cd	0.005	0.062
Ni	0.002	4.657
Co	0.01	0.277

Conclusion

Polycyclic aromatic hydrocarbons and heavy metals were detected in sample of tree barks collected around cement plant in Bukit Keteri, Perlis. The extracted PAHs was analysed using GC-FID and compared to six single PAHs standards. The result indicates the presence of all the six single PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene). The concentrations of the six single PAHs are 20.07, 5.63, 6.20, 2.09, 12.86 and 12.91 µg/kg respectively. The highest concentration of PAHs detected is naphthalene. Since all the detected PAHs are the LMW PAHs, it may be assumed that the cement plant which utilizes coal combustion is one of the main contributors to the emission of PAHs in the area.

The heavy metals distribution in the studied area indicates a high concentration of Zn, Cu, Ni, Cr and Pb but low in concentration of Li, Cd and Co element. The average concentration for Zn, Cu, Ni, Cr and Pb were 7391.34, 7087.35, 4657.35, 3158.25 and 2396.55 µg/kg respectively. The results from this study proved that tree barks can act as bioindicators and biomonitors for assessing the quality of air in a specific area.

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