

Tracing the Untraceable
Fingerprinting Pollutants through
Environmental Forensics

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Prof. Dr. Mohamad Pauzi Zakaria



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Contents

ABSTRACT	1
INTRODUCTION	3
EVALUATION ON THE STATUS OF PETROLEUM HYDROCARBON POLLUTION RESEARCH IN MALAYSIA	4
DISTRIBUTION AND SOURCES OF PAHs IN RIVERS AND ESTUARIES IN MALAYSIA	13
PAHs POLLUTION IN MARINE ORGANISMS	23
PAHs IN MANGROVES ECOSYSTEM, ESTUARIES AND COASTAL SEDIMENTS	28
ATMOSPHERIC INPUT OF PAHs in MALAYSIA	31
THE WAY FORWARD – GLOBAL MONITORING OF POPs	34
INTERNATIONAL PELLET WATCH	42
ANTIBIOTICS MONITORING IN ASIAN WATERS	48
MONITORING OF OTHER POLLUTANTS OF HUMAN CONCERN IN MALAYSIA	50
PHARMACEUTICALS IN SURFACE WATER	54
SCIENTIFIC EXPEDITION TO ANTARCTICA AND THE ARCTIC	56
SUMMARY	60
REFERENCES	62
BIOGRAPHY	71
ACKNOWLEDGEMENT	73
LIST OF INAUGURAL LECTURES	75



ABSTRACT

Investigation of source, distribution and transport pathways of compound-specific petroleum hydrocarbon pollution is not well documented in Malaysia. In the beginning, research on petroleum hydrocarbon pollution was bulk analysis in nature. Bulk analysis of petroleum hydrocarbons gives a broad spectrum of total petroleum hydrocarbon pollution that may also include non-toxic biogenic (natural) sources. Since petroleum hydrocarbon consists of many classes of hydrocarbons and each of them has different characteristics and toxicities, compound-specific analysis of the pollutants is necessary. Therefore, in the late 1990's, intense research by our group on compound-specific petroleum hydrocarbon opened a new phase in Malaysia using environmental forensics and fingerprinting techniques. Intensive research on the source, distribution and transport pathways of compound-specific petroleum hydrocarbon pollution was conducted in terrestrial, atmospheric and aquatic environments in Malaysia. It was found that sediments from Malaysian rivers and estuaries are contaminated with petrogenic polycyclic aromatic hydrocarbons. Furthermore, Malaysian rivers and estuaries were found to be contaminated with PAHs originated from landfill leachates. Major contributor of land-based petroleum hydrocarbon pollution was that of the used-crank case oil. Malaysian coastal beaches were also contaminated with tar balls pollution originated from previous offshore oil spills by foreign oil tankers and domestic oil spillages. PAHs were also found in coastal marine organisms including fishes in aquaculture cages. Atmospheric transport of pyrogenic PAHs in Malaysia was influenced by mobile sources such as motor vehicles and biomass burning. Monitoring of PAHs using our techniques was applied to Asian countries and results showed that petroleum hydrocarbon pollution is widespread and pose significant human health threat. Our research covers land,

Tracing the Untraceable

atmospheric and sea-based sources of petroleum hydrocarbon pollution. Our research style and techniques are now applied to other contaminants of human concern such as pharmaceutical and personal care products (PCPPs), sewage pollution, PFOA and PFOS, PBDE, and heavy metals, among others. This lecture will take a closer look at the research and our major contribution to knowledge for scientific and general communities at large.

INTRODUCTION

Industrial Revolution saw rapid development of industry throughout the world. One of the most significant changes brought about by the Industrial Revolution was the invention of the internal combustion engine. Thus, the demand for fossil fuel to fire the industries has exponentially increased. Among the most important fossil fuel is petroleum hydrocarbons used in motor vehicles and electrical power generations. Petroleum fuel demand has increased tremendously and that led human to source the crude oil deposits in every corner of the globe. Consequently, large quantities of crude oils are released from oil wells into the environment either during production, storage, transport, use or ultimate disposal. The released oils enter the biogeochemical cycle where the routes of input and movement encompasses marine ecosystems, including both chemical and biochemical reactions (e.g., biodegradation, detoxification, metabolism). The participation of spilled oils in biogeochemical cycle also includes that of reservoirs of temporary (days to decades) and long-term (centuries or more) accumulation, regional and global atmospheric and ocean circulation processes. Human society has learnt to adapt in using petroleum hydrocarbons in powering their machines. Until human society is able to find cheap, reliable and practical alternative forms of renewable energy, petroleum will continue be part of and parcel and inseparable in human civilization. Therefore, continued dependence on petroleum hydrocarbons will continue well into our future. This scenario is not welcomed since petroleum hydrocarbons have afforded adverse impacts on ecosystems, when they are accidently spilled to the aquatic environment.

EVALUATION ON THE STATUS OF PETROLEUM HYDROCARBONS POLLUTION RESEARCH IN MALAYSIA

Historically, petroleum pollution studies in Malaysia had been poorly demonstrated and documented. Prior to this work, only bulk analysis of petroleum hydrocarbons was determined. Although Malaysia is under a constant threat of petroleum hydrocarbons pollution, very little accurate and realistic approach in dealing with petroleum hydrocarbons pollution in Malaysia has been conducted. This is especially significant when compound-specific levels of the petroleum hydrocarbons are determined. Compound-specific analysis is important since petroleum contains hundreds of species of hydrocarbons and each individual hydrocarbon physically, biologically, and chemically behaves differently. The exact chemical structures control the fate and the effects of petroleum hydrocarbons in the environment. The new shift from bulk analyses to compound-specific analysis of petroleum hydrocarbons will be addressed in the present research.

The author's doctoral thesis (Zakaria, 2002) and a number of publications that follow found four fundamental findings on compound-specific analysis of petroleum hydrocarbons in Malaysia waters – *Development of Analytical Method of Biomarkers for the identification of oil pollution sources in the straits of Malacca, the application of molecular markers for source identification of tar ball pollution, distribution and sources of PAHs in rivers and estuaries in Malaysia, and PAH pollution in Malaysia: application of LAB as molecular marker in the understanding of the distribution and transport pathway*. This section describes those four major findings.

Development of Analytical Method of Biomarkers for the Identification of Oil Pollution Sources in the Straits of Malacca

Domestic crude oil production in Malaysia is shown in Figure 1. Domestic crude oil production has increased significantly from 1980 to 2004 but eased off slightly. However, the number ship traffic in Malaysia waters saw an increase over the last few decades (Figure 2).

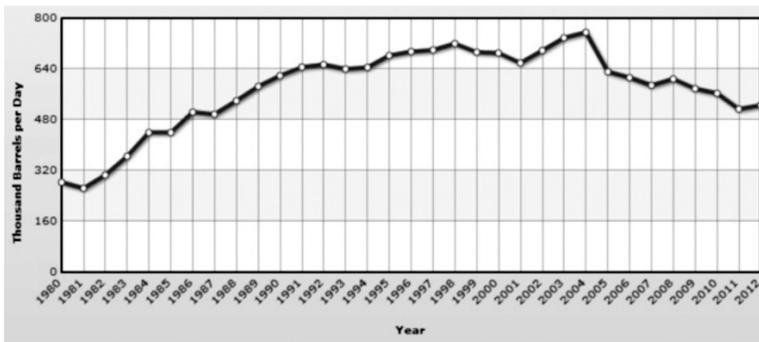


Figure 1 Malaysia crude oil production 1980 – 2012: Source United States Energy Information System

Crude Oil Definition: A mixture of hydrocarbons that exists in liquid phase in natural underground reservoirs and remains liquid at atmospheric pressure after passing through refineries. Depending upon the characteristics of the crude stream, it may also include small amounts of hydrocarbons that exist in gaseous phase in natural underground reservoirs but are liquid at atmospheric pressure after being recovered from oil well (casing head) gas in lease separators and are subsequently comingled with the crude stream without being separately measured. Lease condensate recovered as a liquid from natural gas wells in lease or field separation facilities and later mixed into the crude stream is also included. In addition, small

amounts of non-hydrocarbons produced with the oil, such as sulfur and various metals. Drip gases, and liquid hydrocarbons produced from tar sands, oil sands, gilsonite, and oil shale are also found in crude oil. Liquids produced at natural gas processing plants are excluded. Crude oil is refined to produce a wide array of petroleum products, including heating oils; gasoline, diesel and jet fuels; lubricants; asphalt; ethane, propane, and butane; and many other products used for their energy or chemical application.

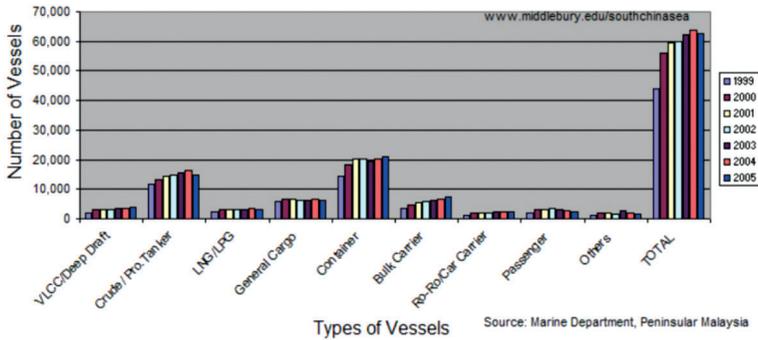


Figure 2 Number and type of vessels, Strait of Malacca 1999-2005 (Data: Marine Department)

Determination of the exact source of the spills is critically important. Our research group examined the utility of biomarker compounds, triterpanes, to identify the source of the oil spills. Middle East crude oils (MECO), South East Asian crude oils (SEACO), tarballs, sediments, and mussels were analyzed. The most distinctive compositional features for the crude oils are that 17R, 21 α (H)C29 norhopane and C31-C35 homohopanes, especially C35 homohopanes, are depleted in SEACO. These remarkably different hopane compositions can be explained by the fact that MECO and SEACO are derived from carbonate (marine) and lacustrine/deltaic shale (non-marine) source rocks, respectively. Two of the

eight tar balls samples we've collected on the coast of Peninsular Malaysia derived from Middle East petroleum based on their molecular markers. The results of oleanane analyses imply that a low concentration of oleanane does not always indicate a Middle East petroleum contribution although at high concentration oleanane can be a useful biomarker for South East Asian oil sources. When our group applied source-identifier to sediment and mussel samples, the results showed the Middle East oil signature. One possible explanation is that Middle East oil is used in formulating Malaysian lubricating oils that are the source of oil found in sediment and mussel samples. This is consistent with the analytical results for the lubricating oil used in Malaysia and the street dust samples.

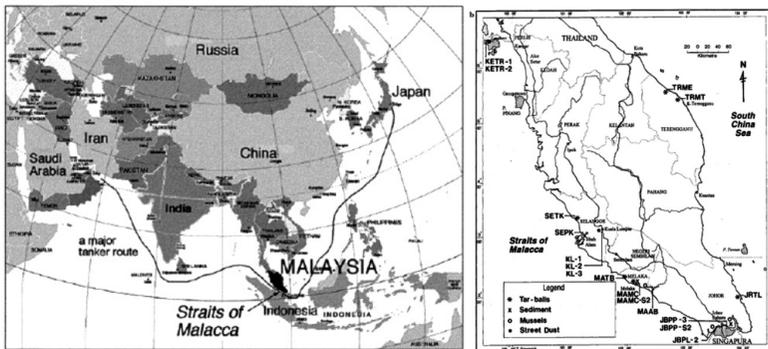


Figure 3 (a) Location of the Straits of Malacca. (b) Sampling locations for tar-ball, sediment, mussel, and street dust (Zakaria *et al.*, 2002)

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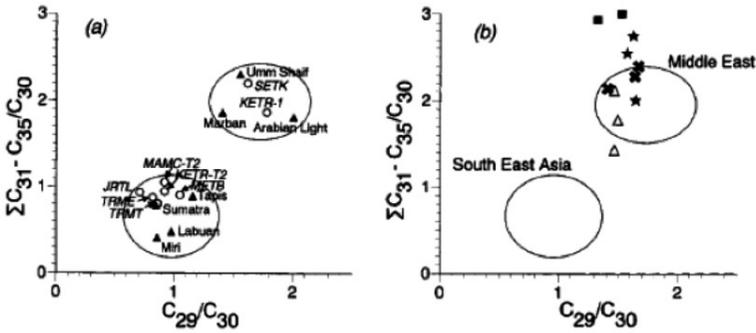


Figure 4 C₂₉/C₃₀ vs α C₃₁-C₃₅/C₃₀ diagrams: (a) the crude oil samples (closed triangle) and the tar ball samples (open circle) and (b) the sediment (cross), mussel (open triangle), street dust (star), and lubricating oil (closed square) samples. The circles are drawn based on the data for the crude oils in panel (a)

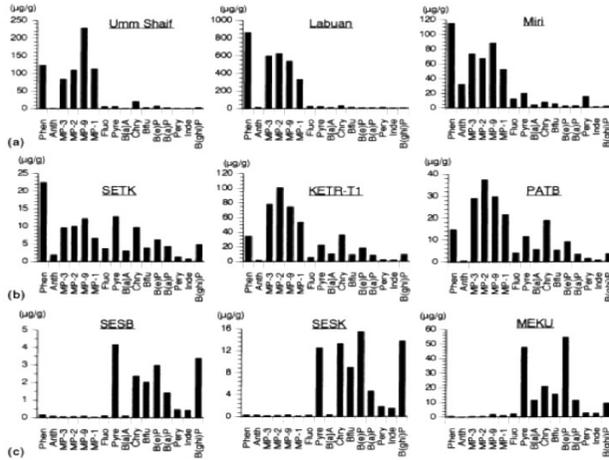
(Zakaria *et al.*, 2002)

Polycyclic Aromatic Hydrocarbon (PAHs) and Hopanes in Stranded Tar-balls on the Coasts of Peninsular Malaysia: Applications of Biomarkers for Identifying Sources of Oil Pollution

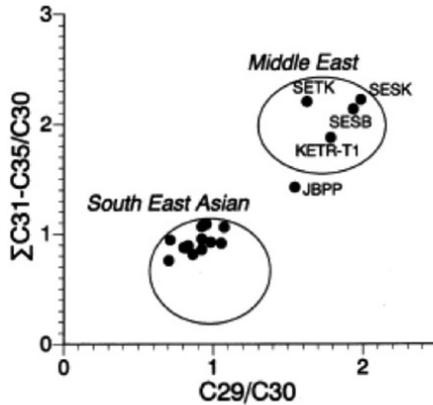
Malaysian coasts are subjected to constant threats of petroleum pollution such as routine and accidental oil spill from tankers, spillage of crude oils from inland and offshore oil fields, and run-off from land-based human activities. The strategic location of the Straits of Malacca serves as a major shipping lane. In this research, we expand the utility of biomarker compounds, hopanes, in identifying the source of tar-balls stranded on selected Malaysian coasts. Twenty tar ball samples collected from the east and west coast were analyzed for hopanes and polycyclic aromatic hydrocarbons (PAHs). Four of the 13 tar-ball samples we collected from the west coast of Peninsular Malaysia were identified as the Middle East

crude oil (MECO) based on their molecular markers. This result indicated that tanker-derived sources significantly contributing the petroleum pollution in the Straits of Malacca. Using molecular marker technique, tar-balls found on the east coast originated from the offshore oil platforms in the South China Sea. The presence of South East Asian crude oil (SEACO) tar-balls on the west coast offers several plausible explanations. A few the tar-balls could have been transported via sea currents from the east coast. The tankers carrying SEACO to other countries could have spilt the oil as well. Furthermore, discharge of tank washings and ballast water from the tankers were suggested based on the abundance in higher molecular weight n-alkanes and the absence of unresolved complex mixture (UCM) in the tar-ball samples. Other possibilities are that the tar-balls may have been originated from the Sumatran oil fields and spillage from nearby oil refineries in Port Dickson and Malacca. The results of PAHs analysis suggest that all the tar-ball samples have undergone various extent of weathering through evaporation, dissolution and photo-oxidation.

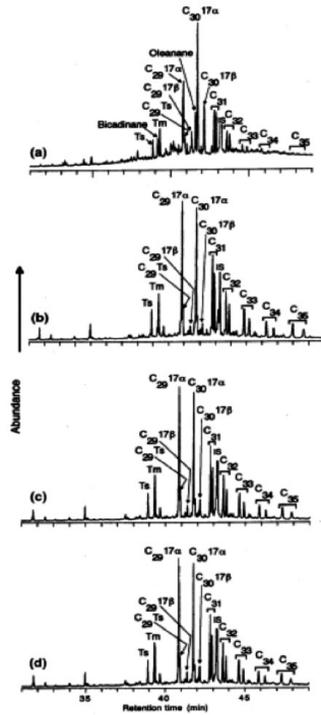
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PAH concentrations in crude oil samples (a) and tar-ball samples collected in the coasts of Peninsular Malaysia ((b) and (c)). Weathered and fresh signature of tar-ball samples is shown in the (b) and (c), respectively. Phen: phenanthrene; Anth: anthracene; MP-3: 3-methylphenanthrene; MP-2: 2-methylphenanthrene; MP-9: 9-methylphenanthrene; MP-1: 1-methylphenanthrene; fluo: fluoranthene; Pyre: pyrene; B[a]A: benz[a]anthracene; Chry: chrysene; Bflu: benzo[fluoranthene]; B[e]P: benzo[e]pyrene; B[a]P: benzo[a]pyrene; Pery: perylene; Ind: indeno[1,2,3-cd]pyrene; B[ghi]P: benzo[ghi]perylene.



C_{29}/C_{30} vs $\Sigma C_{31}-C_{35}/C_{30}$ diagram for tar-ball samples. The circles indicating both MECO and SEACO categories were established through the analysis of crude oils in Zakaria *et al.* (2000). The names of the tar-ball samples grouped into MECO category and JBPP are indicated, while those for SEACO category are not indicated because they would be so crowded.



Gas chromatograms of triterpanes for: (a) MESM, (b) SETK, (c) SESB, and (d) SESK. Ts: 18 α (H), 21 β (H), 22,29,30-trisnorhopane; Tm: 17 α (H), 21 β (H), 22,29,30-trisnorhopane; C₂₉ 17 α : 17 α (H), 21 β (H)-norhopane; C₂₉Ts: 18 α (H)-30-nor-neohopane; C₂₉ 17 β : 17 β (H), 21 α (H)-norhopane; C₃₀ 17 α : 17 α (H), 21 β (H)-hopane; C₃₀ 17 β : 17 β (H), 21 α (H)-hopane; C₃₁ to C₃₅: homohopanes consisting of C₃₁ to C₃₅ carbons with S and R stereoisomer at 22 carbon, respectively. IS: Internal Injection Standard (17 β (H),21 β (H)-hopane).

Figure 5 Snapshots of research findings (adapted from Zakaria *et al.*, 2001)

In order to understand petroleum pollution from tar balls extensively, our group expanded the study in 2009. Results from our previous studies conducted in 2001 indicated that 30% of tar ball samples originated from MECO and the rest came from SEACO. Our group focused our research in the east coast of peninsular Malaysia since it faces the South China Sea and is vulnerable to

oil pollution because of intense petroleum production activities in the area. The South China Sea serves as a marine superhighway for supertankers carrying crude oil from the Middle East to the Northeast Asian countries. Consequently, any oil spills can cause petroleum hydrocarbon pollution and contamination to the surrounding areas. Residual oil spills from tanker traffic and petroleum production stranded on coastal beaches usually end up as tar-balls. Elucidating the sources of tar-balls using a fingerprinting technique becomes essential in assessing environmental impacts and perhaps settling legal liabilities for affected stakeholders. This research utilizes a multimodal molecular marker approach through the use of diagnostic ratios of alkanes, hopanes, and polycyclic aromatic hydrocarbons (PAHs) to determine the source, distribution and weathering of tar-balls. Hopane ratios (e.g., C_{29}/C_{30} , and $\sum C_{31}$ s(-) C_{35} / C_{30} ratios) were used to identify the sources of tar-balls. The weathering effects were also conducted by using alkanes, namely the unresolved complex mixture (UCM) and low molecular weight/high molecular weight (L/H) ratios. Similarly, PAHs were also used for the determination of weathering processes undergone by the tar-balls. The multimodal molecular marker gave strong indication of the sources of tar-balls in this study. A case in point, 16 out of 17 samples originated from South East Asian Crude Oil (SEACO) with one sample from Merang, Terengganu actually has its origin from North Sea Oil (Troll). The TRME-2 sample may have originated from a supertanker's ballast water discharge. The second possibility is that the tar ball may have been transported via ocean currents. All 'weathered' sample characterizations were based on the presence of UCM and other ratios. The multimodal molecular marker approach applied in this research has enabled our group to partially understand the transport behavior of tar-balls in the marine

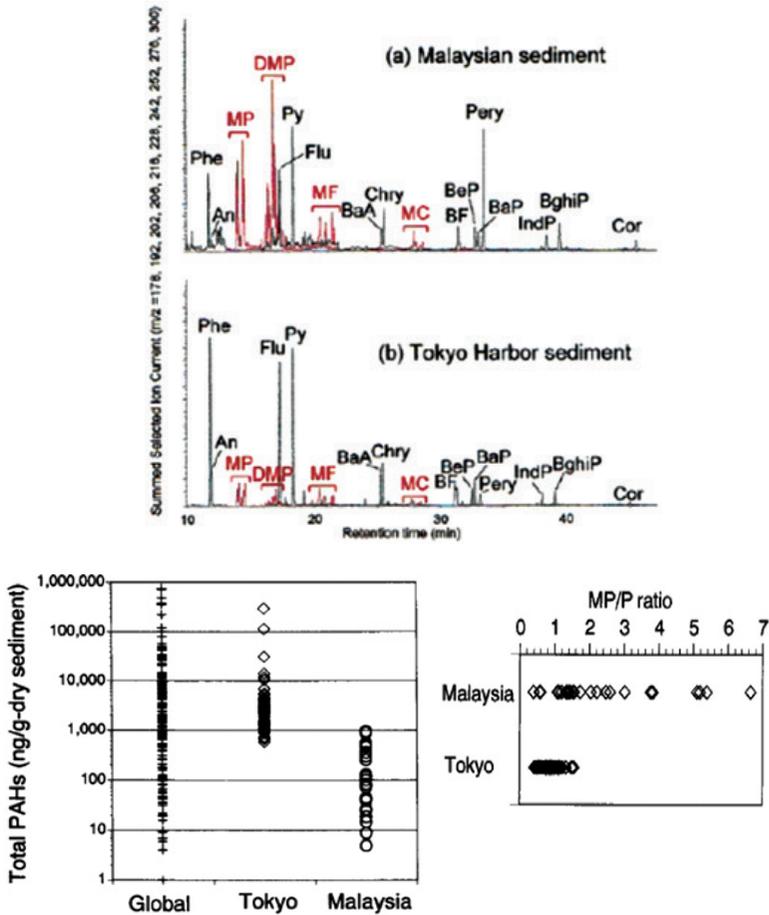
environment and has revealed insights into the weathering process of tar-balls (Chandru *et al.*, 2009).

DISTRIBUTION AND SOURCES OF PAHs IN RIVERS AND ESTUARIES IN MALAYSIA

In this section our group described distribution of PAHs in selected rivers and estuaries in Malaysia. The study was the first report on the distribution and sources of polycyclic aromatic hydrocarbons (PAHs) in riverine and coastal sediments in South East Asia. Rapid transfer of land-based pollutants into aquatic environments by heavy rainfall and runoff waters is of great concern. Twenty-nine Malaysian riverine and coastal sediments were analyzed for PAHs (3-7 rings) by gas chromatography mass spectrometry. Total PAHs concentrations in the sediment ranged from 4 to 924 ng/g. Alkylated homologues were abundant for all sediment samples. The ratio of the sum of methylphenanthrenes to phenanthrene (MP/P), an index of petrogenic PAHs contribution, was more than unity for 26 sediment samples and more than 3 for seven samples for urban rivers covering a broad range of locations. The MP/P ratio showed a strong correlation with the total PAHs concentrations, with an r^2 value of 0.74. This ratio and all other compositional features indicated that Malaysian urban sediments are heavily impacted by petrogenic PAHs. This finding is unique to other studies reported in many industrialized countries where PAHs are mostly of pyrogenic origin. The MP/P ratio was also significantly correlated with higher molecular weight PAHs such as benzo[a]pyrene, suggesting unique PAHs source in Malaysia which contains both petrogenic PAHs and pyrogenic PAHs. As for PAHs and hopanes, their fingerprints indicated that used crankcase oil is one of the major contributors of the sedimentary PAHs. Two major possible routes of inputs to aquatic environments can be identified: (1) spillage and dumping of

Tracing the Untraceable

waste crankcase oil and (2) leakage of crankcase oils from vehicles onto road surfaces, with the subsequent washout by street runoff. N-Cyclohexyl-2-benzothiazolamine (NCBA), a molecular marker of street dust was detected in the polluted sediments. NCBA and other biomarker profiles confirmed our hypothesis of the input from street dust contained the leaked crankcase oil. The fingerprints excluded crude oil, fresh lubricating oil, asphalt, and tire-particles as major contributors.



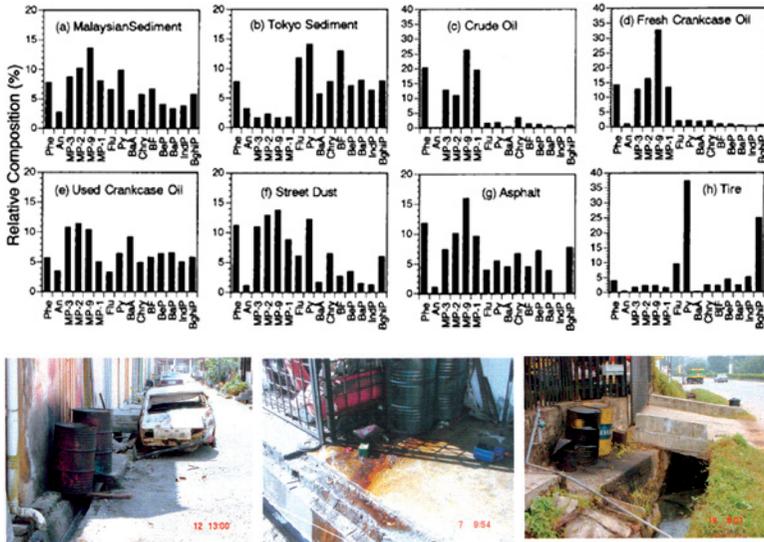


Figure 6 Snapshots of major findings for the above study (adapted from Zakaria *et al.*, 2002)

Landfill Leachate as a Source of Polycyclic Aromatic Hydrocarbons (PAHs) to Malaysian Waters

In our previous studies, PAHs were found to present in Malaysian aquatic environment. Our group suspected that landfills could be a major source of PAHs since combustion of wastes materials in landfills may produce pyrogenic PAHs. During our research period, disposing of wastes in open dumpsites was a common method for the disposal of municipal solid wastes in Malaysia. In 1990, there were about 230 official municipal dumpsites in Malaysia. In 1994, the amount of wastes generated and collected in Kuala Lumpur was around 3 million kg/day. In year 2000, the amount of wastes generated has increased to about 7.9 million kg/day and was expected to increase to more than 11 million kg/day in 2010. This study focused on 2 landfill sites in Malaysia: Taman Beringin

and Ulu Maasop landfills. The Taman Beringin landfill is located in the city of Kuala Lumpur (representing an urban area) while the Ulu Maasop landfill is located in Negeri Sembilan (representing a rural area). Taman Beringin landfill receives a mixture of municipal, commercial, agricultural, recreational, domestic and mixed industrial wastes originating from households and industrial premises while the Ulu Maasop landfill receives mostly domestic waste. Leachate from both landfills are directly discharged into the adjacent rivers. The objectives of this research were to understand the composition and sources of compound-specific PAHs in the landfill leachates and to understand their transport pathways. Leachate, river water and sediment samples were collected at the landfill sites in 2003. The leachate samples were subjected to liquid-liquid extraction using a mixture of 1:1 toluene:methanol and re-extracted twice with hexane. Water samples were filtered and the filtrate containing particulate matter were freeze-dried and soxhlet-extracted. Sediment samples were freeze-dried and soxhlet-extracted with dichloromethane. All the extracts were purified and fractionated by a method described by Zakaria *et al.*, 2002. PAHs were analyzed by gas chromatography mass spectrometry (GC-MS). This is the first report of compound-specific PAHs in landfill leachates in Malaysia. High concentrations of PAHs were detected in the leachate, river particulate and sediment samples for both landfills sites. Total PAHs concentrations in the Taman Beringin leachate, river particulates and sediments ranged from 9.9-575.6 ng/g dry wt, 3.9-5.0 ng/g, and 1.5×10^5 ng/g, respectively while PAHs concentrations for the Ulu Maasop leachate, river particulates and sediments range from 11.8-2836 ng/g dry wt., 3783-5904 ng/g, and 1.31×10^4 - 3.06×10^5 ng/g, respectively (Zakaria *et al.*, 2005). River-borne particulate matter is an important carrier of land-based contaminants to the ocean. Particulate matter adsorbs various

organic and inorganic pollutants such as PAHs and heavy metals. These contaminants along with them and are finally deposited in coastal and open oceans. Landfill sites in Malaysia are normally situated near major towns and rivers. Direct discharge of leachates to these rivers will enhance the transport of organic contaminants such as PAHs to the coastal environments and open oceans. The research has demonstrated that PAHs in leachate pose potential PAH pollution in Malaysian waters. Additional inputs of PAHs from landfill leachate must therefore be controlled to preserve the coastal ecosystems in Malaysia.

Mahat *et al.* (2006) investigated the sources and concentration of polycyclic aromatic hydrocarbon (PAHs) in the sediments in the Langat Estuary. Six stations were chosen, beginning at the estuary and end at the upstream of Langat river. The determination of concentration and compound of PAHs were analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS). The samples were calculated using methylphenanthrene/ phenanthrene (MP/P) ratio to determine the sources. The value of less than 1 is the pyrogenic sources and more than 1, consist of petrogenic sources. Total PAHs for sediment samples of Station 1 to Station 6 ranged from 322 ng/g to 2480 ng/g (dry weight). Furthermore, MP/P ratios for sediment samples had values ranging from 0.88 to 1.27, while L/H ratios for the same samples ranged from 0.25 to 26.96. There were 17 compounds of PAHs detected in the samples. Sediment samples from Station 1 until Station 6 showed an MP/P ratio value of less than 1, with the exception of Station 4 and Station 6. We concluded that this area is dominated by pyrogenic sources.

In order to continuously monitor petroleum hydrocarbon input, our group conducted another PAHs survey in Langat River basin has seen rapid developments in industrialization, urbanization and dramatic population increases since the last time we sampled

the river (Bakhtiari *et al.*, 2009). The composition and sources of polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbon (n-alkanes) concentrations were determined in surface sediments (SS) and suspended particulate matter (SPM) collected from six locations in the Langat River. The total n-alkanes concentrations ranged from 5900 to 23000 g/g in SPM and 1700 to 8600 g/g in SS samples. Total PAHs concentrations varied from 306 to 7968 ng/g in SPM and 558 to 980 ng/g in SS. PAHs and n-alkanes were dominated by higher molecular weight compounds in SS and low to medium molecular weight compounds in SPM. Carbon preference index (CPI) values for n-alkanes in ranges C₂₅₋₃₃, C₁₅₋₃₅ and C₂₅₋₃₅ varied from 0.95 to 2.49 in SS and close to unity in SPM. The CPIs values indicated multiple n-alkanes sources (petrogenic and natural). PAHs isomer pairs ratios indicated multiple (petrogenic and pyrogenic) with predominance of pyrogenic PAH sources. Analysis of the possible source of PAHs and n-alkanes indicated a complicated, combined PAHs and n-alkanes source in the Langat River. Therefore, over a three-year period, the river became increasingly polluted.

After we discovered that Langat River was highly polluted, our group shifted our attention to Prai Strait in Penang (Sakari *et al.*, 2008). The multiple land use hinterland of the Strait of Prai is located in the Northwest of Peninsular Malaysia plays an important economic role in the Southeast Asia. Twenty surface sediment samples were collected to measure the concentration and determine the characterization, sources and origins of the aliphatic hydrocarbons. Samples (top 4 cm) were extracted with Soxhlet, treated with activated copper and subjected to 2-steps column chromatography for purification and fractionation. Alkane fraction injected into Gas Chromatography–Flame Ionization Detector (GC-FID) for instrumental analysis. The results showed

that total n-alkane concentrations are ranging from 512 to 10770 ng/mg d.w. Carbon Preferences Index (CPI) revealed an extreme widespread anthropogenic input and naturally derived (CPI= 0 to 4.88) hydrocarbons in the study area. The ratio of C31 /C19 indicated that natural hydrocarbons are generating from terrestrial vascular plants and transfer of hydrocarbons via riverine input. The characteristics of major hydrocarbons provided evidences that oil and its derivatives either fresh or degraded are the major contributors of the pollution in the study area. Statistical approaches also confirmed that 85% of study area affected by oil sources of pollution. Our group concluded that aliphatic hydrocarbons were mostly transferred by lateral input to the marine environment as compared to the atmospheric input.

In the same year of 2008, our group surveyed PAHs study in the east coast (Sakari *et al.*, 2008). The east coasts undergo less rapid development and thus we presumed that PAHs pollution in the area is less significant. However, baseline information on PAHs for the east coast is not well documented. A total of 14 surface sediment samples were collected from the Kelantan, Besut and Terengganu rivers. The samples were dried, soxhlet extracted, fractionated and injected into GC-FID and GC-MS for alkanes and polycyclic aromatic hydrocarbons (PAHs), respectively. Elevated concentrations hydrocarbons were found in all stations located in the vicinity of urban runoffs for all rivers. The concentrations of aliphatic hydrocarbons range from 1000 to 7168000 ng/g (dry wt.) and PAHs concentration ranged from 58 to 1689 ng/g (dry wt.) for the rivers. The PAHs patterns in urbanized stations suggest the dominance of low molecular weight PAH components (2.4 rings). Based on MP/P ratio, the source of hydrocarbons was influenced by the city runoffs are mostly petrogenic origin. The sources of hydrocarbons on the upstream and downstream stations have been

to be of pyrogenic origin. The concentrations of PAHs and alkanes for a number of stations in this study are comparable to polluted sites in Malaysia. In conclusion, all three rivers showed low to moderate petroleum hydrocarbon contamination. These results suggested that immediate remedial measure in preventing further contamination has to be implemented by local, state and federal agencies.

In 2010, we conducted a detail study in Klang river that passed transect the city of Kuala Lumpur (Bakhtiari *et al.*, 2010). Klang River is of one the most important rivers in Malaysia. Surface sediment samples were collected from five locations at the downstream of the river. A primary purpose of our research was to examine the spatial distribution, composition, and sources of 19 parent polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbon (n-alkanes). The total concentrations of the 19 PAHs in the sediments were found to range from 1304 to 2187 ng/g (dry wt.). Meanwhile, total concentrations of n-alkanes ranged from 17008 to 2-7116 µg/g (dry wt.). The concentration of n-alkanes in the sediment was significantly correlated ($r = 0.991, p = 0.001$) with the content of sediment organic carbon. In this research, all the sediments exhibited phenanthrene/anthracene (PHE/ANT;15) fluoranthene/(fluorantene+pyrene) (FLT/FLT+PYR;0.4), methylphenanthrenes/ phenanthrene (MP/P;1), combustion PAHs/ total PAHs (CombPAH/ 19PAH;0.3), terrigenous/aquatic ratio for hydrocarbons greater than 23. The data showed that petrogenic and natural inputs were predominant at all the locations investigated. Multiple sources of n-alkanes and PAHs in the river sediments were also explained by low carbon preference index (CPI) values, different ratios of diploptene/ C_n-alkanes, poor correlation between diploptene and C-C, average chain length (ACL) of 29.54 ± 0.09 , correlation between CPI and ACL ($r = 0.847, p = 0.035$), and high ratio of naphthalene/ total PAHs.

Historical profiles of Polycyclic Aromatic Hydrocarbons (PAHs), Sources and Origins in Dated Sediment Cores, Straits of Malacca, Malaysia

After our group successful completion of Klang river study, we shifted our focus to reconstruction the PAHs pollution history in the same river (Sakari *et al.*, 2010). Polycyclic aromatic hydrocarbons and pentacyclic triterpanes (hopanes) were determined in 2 sediment cores collected from offshore of Klang River estuary and Old Port of Klang City, located in Straits of Malacca, Malaysia. Strait of Malacca is among the world's busiest waterways and Port Klang is one of the busiest, premier and largest ports in the Straits. The port has been an important trading post since the colonial era of the Portuguese, Dutch and the British Straits Settlements. Beside massive shipping traffic, old part of Port Klang is heavily influenced by urbanization and industrialization. The highest concentration of polycyclic aromatic hydrocarbons was found in Old Port Klang with PAHs from 34 to 2426 ng/g while offshore Klang showed total PAHs concentration from 7.37 to 32.97 ng/g. Also, these concentrations are significantly elevated in upper layers in both cores. Sediment core collected in Old Klang port carries mixture of petrogenic and pyrogenic signatures with methylphenanthrene to phenanthrene (MP/P) ratio range from 0.63 to 1.11 while the core collected from an offshore station of the Klang river estuary revealed a highly pyrogenic signature as evidenced from the MP/P ratio of 0.22 to 0.83 indicating atmospheric transport and non-conservative behavior of the pollutant as the distance from the source increases. All sediment intervals showed PAHs concentration below the effects range-low (ER-L) sediment toxicity threshold values (total PAHs < 4000 ng/g) but indicative of polluted environment (> 1000 ng/g). Although results from pentacyclic triterpanes in this research revealed that hopane is not a conventional molecular marker for

source identification of pyrogenic sources of PAHs, but apparently showed no significant of oil spills occurred in the study area. Results from the analysis of other alkyl substitute of PAHs such as ratio of Methyl Pyrene to Pyrene (MPy/Py), parent PAHs and hopanes as well as ratios such as Fl/Py and Ph/An indicated mostly pyrogenic source originated from street dust and asphalt in Klang valley including Kuala Lumpur. This indicates that the sedimentary environment around Port Klang mostly receives short and medium range transport materials via rivers and atmosphere, although heavy ship traffic and urban runoffs could easily be thought of receiving petrogenic input.

Using the above technique, reconstruction of PAHs pollution history was also conducted in the Strait of Johor. Reconstruction of PAH history from two sedimentary cores of the Strait of Johor in peninsular Malaysia showed predominance of urban over marine-based sources of oil pollution. Laboratory analysis of the cores found oil pollution from city-sourced material since 1910 and 1940 in the near-shore and offshore samples, respectively. Using hopane as molecular biomarker of petroleum pollution, this research found a predominance of Southeast Asian-originated oil and its derivatives. This research concludes that crankcase oil, asphalt, street dust and urban sediments considerably contribute to oil pollution of the study area (Sakari *et al.*, 2010).

In 2012, our group published another major work on the reconstruction of PAHs pollution history in Tebrau Straits (Sakari *et al.*, 2012). In the last century, application of fossil fuel as the primary source of energy caused environmental pollution in many countries including Malaysia. Two sediment cores were collected from the Tebrau Strait at the southern part of Peninsular Malaysia near the border line to Singapore, where entering into the South China Sea. The samples were sliced in intervals, extracted with

dichloromethane in Soxhlet apparatus, cleaned and fractionated in 2-steps column chromatography, and analyzed in Gas Chromatography – Mass Spectrometry. The results showed that PAHs input were started soon after World War II and exponentially increased from 1980 onward by 310 ng/g d.w., in comparison it was negligible and probably nature derived during 18th and 19th century. The application of compound-specific ratios and pentacyclic triterpanes suggested the vicinity of sources that atmospherically transported to the sampling locations. They were originated from combusted oil of Southeast Asian and the Middle East, polluting urban sediment and street dusts prior to final deposition. Biomass burning appeared historically as a predominant minor background pollution of both cores. Remarkably, crankcase oil was not traced in this study while it was reported as a predominant source in Malaysia. This research suggested ocean-going ships and Singapore International Airport as the main sources of petroleum pollution in recent decades since there was insignificant rural development surrounding the studied area.

PAHs POLLUTION IN MARINE ORGANISMS

Our group had not only focused our attention to PAHs in sediments and atmosphere but we had also surveyed the distribution, sources and transport pathways of PAHs in biota (Shahbazi *et al.*, 2010). Marine shellfish is an important food source to humans. PAHs pollution in marine organisms therefore became top priority for our group's research. Distribution of polycyclic aromatic hydrocarbons (PAHs) was determined in green mussels (*Perna viridis*) from various sites in coastal waters of Peninsular Malaysia between August 2004 and January 2007, in order to assess contamination by petroleum hydrocarbons. The range of Σ PAHs detected in mussels was from 766 to 110500 (ng/g lipid wt.). High concentrations of

PAHs were found in mussel tissues collected near Penang Bridge. The ratios of methyl phenanthrenes to phenanthrene (Σ MP/P ratio) for Penang, Kg. Pasir Puteh and Tebing Runtuh (Johore Straits) were greater than 2, indicating extensive input of petrogenic PAHs. The results indicated that male individuals elevated more considerable concentrations of PAHs in their soft tissues in comparison to female individuals. The results of independent sample T-test showed that there were no significant differences ($p > 0.05$) between male and female mussels analysed in the Pasir Panjang station. Negative significant correlations ($r = -0.890, p < 0.01$) and ($r = -0.0655, p < 0.05$), were found between weight and total of PAHs in female and male species, respectively. This indicated that body weight of each individual was not affected by the PAHs concentrations. The present study proposes the use of soft tissue of *Perna viridis* as a biomonitor of perylene bioavailability and contamination in coastal waters of Peninsular Malaysia.

The concentration of polycyclic aromatic hydrocarbon (PAH) in three fish species with different feeding habits and habitat including Lolong (Selar boops), Kerisi (*Nemipterus peronii*) dan Mengkarong (*Trachinocephalus myops*) from offshore of Perhentian Island, Malaysia was also determined. Three individuals from each species were taken at random and the PAHs contents were determined in the muscles. Ten PAH compounds, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene and dibenzo(a,h)anthracene were determined. PAH in fish tissues was extracted using Soxhlet method and detected using gas chromatography – mass spectrometry (GC-MS). The level of PAH in fish tissue ranged from 17.89 – 42.18 ng/g wet weight and 393.98 – 511.07 ng/g lipid weight. The order of PAH concentration in wet weight was Kerisi (511.07 ng/g) > Mengkarong (409.50

ng/g)>Lolong (393.98 ng/g) but in terms of lipid weight, the order was Kerisi (511.07 ng/g)> Mengkarong (409.50 ng/g)> Lolong (393.98 ng/g). Kerisi has the lowest lipid content of 3.5% compared to Lolong (6.5 %) and Mengkarong (10.3%). No obvious significant difference ($p>0.05$) of PAH levels in three fish species was observed (ANOVA, $p>0.05$). There was no significant relationship between lipid content and PAH accumulation in fish. Based on fish consumption rate of 142.2 g/day, the Potency Equivalent Concentration (PEC), which is a carcinogenic potency value for PAH, was found to be ranged from 0.41 – 0.63 ng/g wet weight in all three species of fish. This value is below the limit set by USEPA, which is 0.67 ng/g wet weight for human consumption.

Our focused was not only to understand the distribution and sources of PAHs in the marine organism but also to investigate whether selected organisms can be excellent bioindicator organisms. For the first time in 2010, the distributions of polycyclic aromatic hydrocarbons (PAHs) were studied in the soft tissues (STs) (mantle, gills, foot, gonad, muscle, byssus, and remaining soft tissues) of the green-lipped mussel *Perna viridis*, collected from eight geographic locations along the coastal waters of peninsular Malaysia. The STs of the mussels collected from the eastern part of the Johore Straits indicated higher bioavailability of and contamination by PAHs than from other areas. The results also indicated a significantly higher concentration of the lower molecular weight (LMW) PAHs in tissues compared to that of the higher molecular weight (HMW) PAHs, perhaps due to the greater bioavailability of the more water-soluble LMW PAHs or related to a partial biotransformation of the higher molecular weight PAHs. The results also suggest that the differences found in the contents of PAHs in various STs of *P. viridis* were mainly due to differences between individual PAHs volatility and solubility in water, as well as the mechanism of PAH accumulation

by mussels. The gonad was shown to contain the highest levels of PAHs, but it is not a potential biomonitoring organ because it is highly influenced by spawning conditions. Isomeric PAH ratios were used to differentiate pollution sources. The findings of the research suggest that STs of *P. viridis* are good biomonitors of the bioavailability and contamination with PAHs in Malaysia and other tropical coastal waters (Shahbazi *et al.*, 2010).

After a successful investigation of using tissues of bivalve as bioindicator, our group had also investigated risk assessment of eating cockles. The concentration of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) present in the sediment and water of Peninsular Malaysia as well as in the cockle *Anadara granosa* was investigated. As usual, samples were extracted and analysed with GCMS. The concentrations of total carcinogenic polycyclic aromatic hydrocarbons (t-PAHs) were measured between 0.80_ 0.04 to 162.96_ 14.74 ng/g wet weight (ww) in sediment, between 21.85_ 2.18 to 76.2_ 10.82 ng/L in water samples and between 3.34_ 0.77 to 46.85_ 5.50 ng/g ww in the cockle tissue. The risk assessment of probable human carcinogens in the Group B2 PAHs was calculated and assessed in accordance with the standards of the United States Environmental Protection Agency (USEPA). Case I in the toxicity assessment analysed the cancer risk to consumers of Malaysian blood cockle. Case II assessed the risk of cancer from exposure to PAHs from multiple pathways. The average cancer risk of Case I and Case II were found to be classifiable as unsafe according to the USEPA standard. The cancer risk due to PAHs acquired by the ingestion of blood cockle was (8.8 ± 0.54) _ to (2.67 ± 0.06) , higher than the US EPA risk management criterion. The non-cancer risks associated with multiple pathways in Kuala Gula, Kuala Juru and Kuala Perlis were higher than the USEPA safe level, but the non-

cancer risk for eating blood cockle was below the level of USEPA concern (Seideh *et al.*, 2011).

Our group had also investigated PAHs pollution in aquaculture fish. Manan *et al.* (2011) conducted a study at selected locations along the Straits of Malacca. Twelve aquaculture fish samples were collected to determine the level of hydrocarbon pollution in the fishes. The homogenized fish tissues were extracted using Soxhlet, fractionated and analyzed by using GCMS. Hopanes and PAHs were detected and were found in the range of 57.59 to 9610.31 ng/g (dry weight). Ratios C29/C30 and C31-C35/C30 have been used for recognizing the sources of oil pollution. Two samples were identified as Sumatera originated while the source for other samples cannot be determined. Additionally, MP/P ratio was used to determine the anthropogenic PAHs sources. Six stations were polluted with petrogenic sources while the other six stations were from pyrogenic sources. The MP/P ratio shows strong positive correlation with total PAHs with an r^2 value of 0.79. Further analysis is needed in order to identify the sources of oil pollution in ten fish samples with unidentified oil sources.

After a successful completion of PAHs in aquaculture fish, we further improved our study in 2013 (Retnam *et al.*, 2013) to elucidate the source of the pollutants in aquaculture sediments. Using diagnostic ratios, chemometric and multivariate techniques, our group investigated polycyclic aromatic hydrocarbons (PAHs) pollution in surface sediments within aquaculture areas in Peninsular Malaysia. The samples were analysed using Soxhlet extraction, silica gel column clean-up and GCMS. The total PAH concentrations ranged from 20 to 1841 ng/g with a mean of 363 ng/g dw. The application of chemometric techniques enabled clustering and discrimination of the aquaculture sediments into

four groups according to the contamination levels. A combination of chemometric and molecular indices revealed that the source of petroleum hydrocarbon be attributed to vehicle emissions, oil combustion and biomass combustion. Source apportionment using absolute principle component scores–multiple linear regression showed that the main sources of PAHs are vehicle emissions 54%, oil 37% and biomass combustion 9%. Land-based pollution from vehicle emissions is the predominant contributor of PAHs in the aquaculture sediments of Peninsular Malaysia.

PAHs IN MANGROVES ECOSYSTEM AND ESTUARIES AND COASTAL SEDIMENTS

Recently, we continue our investigation to understand the composition and source identification of polycyclic aromatic hydrocarbons in mangrove sediments. Mangrove ecosystem is a very important ecosystem for marine organism. The ecosystem is breeding and spawning grounds for fish and shrimps. Therefore, special attention must be paid to these fragile ecosystems. We embarked a comprehensive study of the composition, origin and sources of specific polycyclic aromatic hydrocarbons (PAHs) in sediments of mangrove estuary in the western part of Peninsular Malaysia. Mangrove sediments were analyzed for 17 PAHs by gas chromatography–mass spectrometry. Total PAH concentrations in the sediments ranged from 20 to 112 ng/g on a dry-weight basis. High molecular weight PAHs were abundant in the sediments. Parent PAH ratios revealed that pyrogenic input has important contribution to the sedimentary PAHs. Ratios of alkylated PAHs indicate that the sedimentary PAHs were influenced by petrogenic PAHs, which implies that petrogenic input has contribution to the sedimentary PAHs but that it is not a major factor in distribution of PAHs within the estuary. Combustion-derived PAHs show a positive

and very strong correlation with total PAHs ($R^2 = 0.926, p < 0.05$). Total methylphenanthrenes show very weak correlation with total PAHs ($R^2 = 0.0928, p < 0.05$). The PAH concentrations were found to increase with distance from the upstream of the estuary to the coastal area of the Straits of Malacca. For the assessment of sediment contamination using biological thresholds, none of the individual studied PAH compounds exceeded the values of the effect range low–effect range median guideline and the threshold effects level–probable effects level guideline. This research demonstrates that the sediments of the mangrove ecosystem facing the Straits of Malacca and Sumatra are influenced by anthropogenic PAH inputs as a result of human activities such as biomass burning, vehicle emissions and boating activities (Raza *et al.*, 2013).

Characterization of Hopanes in Surface Sediments from Southwest Corner of South China Sea: Application of PCA Utilizing of Individual Hopanes

Ismail *et al.* (2013) investigated hydrocarbon pollution in the South China Sea. Oil pollution has been introduced into the aquatic environment of the South China Sea through anthropogenic activities such as discharging of oil during extraction, transportation and consumption. In order to determine the source of oil pollution in South China Sea, 30 surface sediment samples were collected in 2008 and were analyzed for pentacyclic triterpanes (hopane) by gas chromatography mass spectrometer (GCMS) with m/z 191. This study investigates the utility and limitation of individual hopane in tracing the sources of oil pollution by using Principle Component Analysis (PCA). We had characterized the utility of hopane in the determination of the source of oil pollution was done by integrating diagnostic ratios of C_{29}/C_{30} and $\Sigma C_{31}-C_{35}/C_{30}$ with PCA. PCA has classified all target components into 3 major principle

components prior to determine their importance in identifying the source of oil pollution. From the rotated component pattern, first principle component (63.24% of variability) loaded by 9 target compounds which namely Ts, Tm, 17 α (H), 21 β (H)- norhopane, 17 α (H), 21 β (H)- hopane, 22S, 17 α (H), 21 β (H)- homohopane, 22S, 17 α (H), 21 β (H)- bishomohopane, 22S, 17 α (H), 21 β (H)- trishomohopane, 22S, 17 α (H), 21 β (H)- tetrakishomohopane and 22S, 17 α (H) and 21 β (H)- pentakishopane. Those individual hopanes listed in PC1 were excellent in tracing both oil pollution as all of them degrade less and generally unaffected by even severe biodegradation. This findings give better understanding for non-consideration of both 17 β (H), 21 α (H)- norhopane, 17 β (H), 21 α (H)- hopane in determining the origin of oil spill.

Updated Studies of PAHs in Malaysian Rivers

Beginning 2014, our group began to pay more attention to hydrocarbon pollution in small but important urban rivers around the country. For example, we chose a small river in Kota Bharu, Kelantan as our study area. Pengkalan Chepa River is located in the urban area that receives pollution loads from surrounding area, sewage treatment and industrial garages and workshops. This research focuses on the determination of PAHs in suspended solid in Pengkalan Chepa River during rainy day, rainy season and sunny day. Total concentrations of PAHs in suspended solid in rainy day ranged from 323.5 to 1,153.1 ng/g while 7,421.2–1,786.71 ng/g in rainy season and 353.7–560.8 ng/g in sunny day (Seyedreza *et al.*, 2014)

Our continuing effort in the understanding of petroleum hydrocarbon pollution extended to a study conducted at selected locations along the Kuala Selangor River, Malaysia. Seven surface sediment samples were collected to determine the level

of hydrocarbon pollution in the sediments. The homogenized sediments were extracted using soxhlet, fractionated and analyzed by using GCMS. PAHs were detected and were found in the range of 563–1,037 ng/g (dry weight). Ratio MP/P was used to determine the anthropogenic PAHs sources where seven stations were polluted by petrogenic sources. Furthermore, MP/P ratios for sediment samples had values ranging from 1.752 to 18.6, while L/H ratios for the same samples ranged from 0.2978 to 1.393. There were 26 compounds of PAHs detected in the samples. Further analysis and more data would be needed in order to identify the sources of oil pollution in seven sediment samples with unidentified oil sources (Masood *et al.*, 2014)

ATMOSPHERIC INPUT OF PAHs IN MALAYSIA

All of the above reports so far dealt with riverine inputs of PAHs. Since there was an episodic event of haze in Malaysia, we figured that atmospheric PAHs must be looked at. Thus, in 2002, we report measurements of molecular and carbon isotopic compositions of Malaysian atmospheric polycyclic aromatic hydrocarbons (PAHs) in smoke haze from the 1997 Indonesian forest fire. Comparison of the carbon isotopic compositions ($\delta^{13}\text{C}$) of individual PAHs from the smoke haze, with those from other PAHs sources (soot collected from gasoline and diesel vehicle muffler, wood burning smoke), enables us to discriminate among the diverse sources of atmospheric PAHs. Soot PAHs extracted from gasoline and diesel vehicles show heavy isotopic signatures with a large inter-species $\delta^{13}\text{C}$ variation from -12.9‰ to -26.6‰ , compared to soot PAHs extracted from wood burning smoke that are isotopically light, and have a small inter-species $\delta^{13}\text{C}$ variation from -26.8‰ to -31.6‰ . Values from -17.7‰ to -27.9‰ were obtained for the corresponding PAHs extracted from the smoke haze, indicating

Tracing the Untraceable

that they are derived mainly from automotive exhaust. Molecular and isotopic compositions of PAHs extracted from smoke haze were similar to those extracted from non-haze aerosol. Quantitative estimation shows that wood burning contribution to Malaysian atmospheric PAHs ranges from 25% to 35% with no relation to haze intensity, while automotive contribution ranges from 65% to 75%. These results suggest that the major contributor of PAHs in Malaysian air is automotive exhaust whether smoke haze is observed or not.

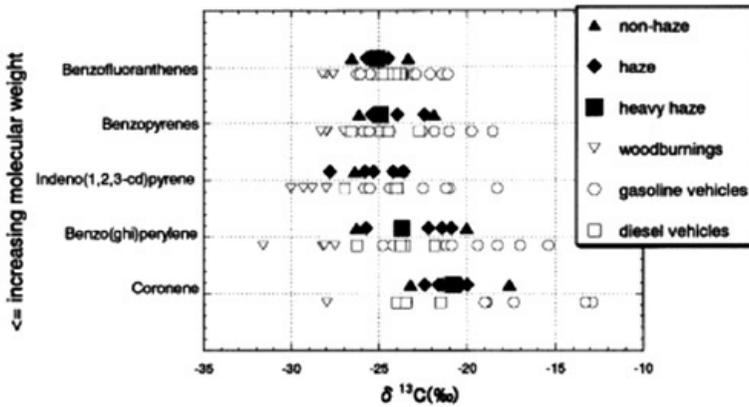


Figure 7 Results of PAHs extracted from Malaysian aerosols (upper and filled plots; triangles are non-haze, diamonds are haze, and square is heavy haze) and several combustions sources (lower and open plots; triangles are woodburnings, circles are gasoline-vehicle exhausts, and squares are diesel-vehicle exhausts). PAHs are sorted in the order of molecular weights (Okuda *et al.*, 2002)

In order to continue our effort to understand the input of atmospheric PAHs, we conducted a study on the atmospheric transport of the pollutant. Atmosphere has long been known as free way for the transport of particle reactive chemicals far distances.

Polycyclic aromatic hydrocarbons (PAHs) are priority pollutants in the environment where anthropogenic sources such as petroleum are studied. Chemical characterization of deposited PAH in peninsular Malaysia showed that compounds resulting from combustion contribute more to atmospherically transported compounds than do un-combusted materials, which usually follow the lateral transport, local oil discharge and in-situ natural production. The composition and characterization of PAH demonstrated a higher abundance of parent compounds than of alkyl substitutes. These include pyrene, benzo(e)pyrene, benzo(a)pyrene and benzo(k)fluorethene. Parent compounds represent a higher proportion of combusted organic materials as well as of petroleum that transfers via atmospheric movement rather than laterally via, for example, rivers and run-off discharges. Diagnostic chemical ratios of chemical compounds such as phenanthrene to anthracene and fluorethene to pyrene showed that combusted materials are dominant that are transferred by atmosphere movement, either over short or long distances, in the studied area (Sakari *et al.*, 2009).

In 2009, we continue our research to further understand atmospheric PAHs by expanding our sampling areas throughout peninsular Malaysia. There were 18 individual PAHs and 16 individual hopanes detected in the samples. Total PAH concentrations ranged from 0.58 to 10.49 ng/m³, and hopanes ranged from 1.62 to 10.49 ng/m³. Source identification using diagnostic ratios suggests that the biomass burning and vehicular emissions are the main sources of PAHs in the samples. Additionally, the strong influence of crankcase oil incorporated in street dust was identified pointing out a significant source of hopanes in the study area (Bahry *et al.*, 2009).

THE WAY FORWARD – GLOBAL MONITORING OF POPS

Sewage Pollution and Sewage Markers

Our group embarked the project on sewage pollution using sterols as molecular markers. Traditionally, fecal coliforms bacteria are used as indicator of fecal contamination. There are several disadvantages using coliforms bacteria sewage indicators. Fecal coliforms survival decreases as salinity increases. Thus, in the marine and estuarine waters, coliform bacteria test has less utility due to die-off of the bacteria. Therefore, chemical test using fecal sterols are more reliable for the monitoring of fecal contamination. Our group was the first to report on fecal pollution using molecular markers in Southeast Asia where serious sewage pollution has occurred. A simple and sensitive analytical method using gas chromatography-mass spectrometry for 10 sterols in various environmental samples was developed to monitor extensive areas of tropical Asia. First, the method was applied to wastewater to confirm that >95% of sterols existed in the particulate phase. Then the approach was applied to a tropical Asian region, Malaysia and Vietnam, with a selection of 59 sampling stations in total. River water and sediment samples were collected and analyzed for chemical markers (coprostanol and other sterols) and microbiological markers (fecal coliforms and fecal streptococci). Particulate coprostanol concentrations ranged from <0.0001 to 13.47 $\mu\text{g/L}$ in tropical river and estuarine waters, indicating severe fecal pollution in populous areas. Coprostanol concentrations in the sediments ranged from 0.005 to 15.5 $\mu\text{g/g-dry}$. The sedimentary coprostanol concentrations were lower than those reported in some urban areas of industrialized countries. This is probably because frequent heavy rain induces intensive input of eroded soil, which dilutes fecal material in river sediments. The relationship between the concentrations of fecal sterols and bacterial

indicators was examined in an attempt to develop public health criteria for coprostanol levels applicable to the tropical region. Coprostanol concentrations of 30-100 ng/L or percent coprostanol levels of 2% corresponded to \approx 1000 fecal coliforms per 100 mL, which is set for secondary contact limit in many countries (Isobe *et al.*, 2002).

Our group has not only used fecal sterols as markers for sewage but we also use alkylbenzenes. Alkylbenzenes are used as raw material in the production of anionic surfactants. Residual linear alkylbenzenes (LABs) from leftover of Friedel-Crafts reactions is released to the aquatic environments during cleaning and washings. LABs are hydrophobic and partitioned into sewage particles released from homes. LABs can also be used to measure the efficiency of sewage treatments plants using I/E ratios. In our research molecular markers of sewage, were measured in 34 green mussels collected from India, Indonesia, Malaysia, Thailand, Cambodia, Vietnam, and the Philippines together with blue mussels collected from Tokyo Bay, Japan. Linear alkylbenzene (LAB) concentrations in South and South East Asian countries ranged from 10 to 1640 ng- Σ LAB/g-dry tissue. In some populous cities, LAB concentrations were similar or higher than those found in northern Tokyo Bay that is heavily impacted by sewage effluents. I/E ratios (a ratio of internal to external isomers of LABs) in the South and South East Asian countries (1–3) were much lower than those in Tokyo Bay (3–8), indicating sewage discharged in the coastal zone is poorly treated (e.g., raw sewage and/or primary effluents). Alkylbenzenes with branched alkyl chains, tetrapropylene-based alkylbenzenes, were also detected in mussels from Indonesia and Philippines. This “tell-tale” sign indicates that poorly degradable detergents are still in use in this area, although they have long been phased-out in many industrialized countries (Tsutsumi, 2002).

After a successful application of LABs as markers for sewage pollution in 2004, we have applied our research to monitor sewage pollution in more countries in Southeast Asian countries. This paper reports the result of sewage pollution monitoring conducted in South and Southeast Asia during 1998–2003 using linear alkylbenzenes (LABs) as molecular tracers of sewage contamination. Eighty-nine water samples collected from Malaysia, Vietnam, and Japan (Tokyo), and 161 surface sediment samples collected from Tokyo, Thailand, Malaysia, Philippines, Vietnam, Cambodia, Indonesia, and India were analyzed for alkylbenzenes. The concentration range of Σ LABs in river water particles in Southeast Asia (<0.005 – 0.913 $\mu\text{g/L}$) was comparable to or higher than those found in Tokyo (<0.005 – 0.638 $\mu\text{g/L}$). I/E ratios (a ratio of internal to external isomers of LABs) in tropical Asian waters were close to the value of LABs in raw sewage (~ 1) and much lower than those in secondary effluents (3–5). This suggests that untreated or inadequately treated sewage is discharged into the water. Σ LABs concentrations in sediments from South and Southeast Asia ranged from <0.002 – 42.6 $\mu\text{g/g-dry}$ with the highest concentration occurring at several populous cities. Low I/E ratios of the sediments with high Σ LABs concentrations suggest a heavy load of untreated sewage. Clearly in view of the current data and evidence of the implications of sewage pollution, this paper highlights the necessity of the continuation of water treatment system improvement in tropical Asia (Isobe *et al.*, 2004).

Monitoring of EDCs in Asia

Isobe *et al.* (2007) had conducted a comprehensive monitoring survey for polycyclic aromatic hydrocarbons (PAHs) and phenolic endocrine disrupting chemicals (EDCs) utilizing mussels as sentinel organisms was conducted in South and Southeast Asia as

a part of the Asian Mussel Watch project. Green mussel (*Perna viridis*) samples collected from a total of 48 locations in India, Indonesia, Singapore, Malaysia, Thailand, Cambodia, Vietnam, and the Philippines during 1994-1999 were analyzed for PAHs, EDCs including nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA), and linear alkylbenzenes (LABs) as molecular markers for sewage. Concentrations of NP ranged from 18 to 643 ng/g-dry tissue. The highest levels of NP in Malaysia, Singapore, the Philippines, and Indonesia were comparable to those observed in Tokyo Bay. Elevated concentrations of EDCs were not observed in Vietnam and Cambodia, probably due to the lower extent of industrialization in these regions. No consistent relationship between concentrations of phenolic EDCs and LABs were found, suggesting that sewage is not a major source of EDCs. Concentrations of PAHs ranged from 11 to 1,133 ng/g-dry, which were categorized as “low to moderate” levels of pollution. The ratio of methylphenanthrenes to phenanthrene (MP/P ratio) was >1.0 in 20 out of 25 locations, indicating extensive input of petrogenic PAHs. This study provides a bench-mark for data on the distribution of anthropogenic contaminants in this region, which is essential in evaluating temporal and spatial variation and effect of future regulatory measures.

International Mussel Watch had been developed in the United States in 1970's to monitor POPs using mussels. The International Mussel Watch (IMW) Program was undertaken under the auspices of the United Nations Educational, Scientific and Cultural Organization (UNESCO) Intergovernmental Oceanographic Commission, and the United Nations Environment Program (UNEP) Ocean and Coastal Areas Program, to assess the extent of chemical contamination, primarily in the equatorial and subequatorial areas of the southern hemisphere, with particular attention to coastal areas of developing countries. The project was a complete success and many countries

across the globe participated in the program. There are several attributes of using mussels as sentinel organisms:

1. A correlation exists between the pollutant content of the organism and the average pollutant concentration in the surrounding habitat; contaminant concentration factors of many-fold over seawater concentrations are common.
2. Bivalves are cosmopolitan, minimizing the inherent problems that arise when comparing data from markedly different species; this issue will be more important in tropical areas.
3. Bivalves have reasonably high tolerance to many types of pollution and can exist in habitats contaminated within much of the known range of pollution.
4. Bivalves are sedentary and better representative of the study area than mobile species.
5. Bivalves often are abundant in relatively stable populations that can be sampled repeatedly throughout the study region.
6. Many bivalve species are sufficiently long-lived to allow the sampling of more than one year-class, if desired.
7. Bivalves are often of a reasonable size, providing adequate tissue for analysis.
8. Bivalves are easy to sample and hardy enough to survive in the laboratory, allowing defecation before analysis (if desired) and laboratory studies of pollutant uptake.
9. Several bivalve species tolerate a range of salinity and other environmental conditions, making them adaptable for transplantation experiments.
10. Bivalves are generally metabolically passive to the contaminants in question and not alter the chemical after uptake; uptake by

the organism provides an assessment of bioavailability from environmental compartments

11. Bivalves are commercially valuable seafood and a measure of chemical contamination is of public health interest.

Ramu *et al.* (2007), collected mussel samples to measure the levels of polybrominated diphenyl ethers (PBDEs) and organochlorines (OCs) in the coastal waters of Asian countries like Cambodia, China, Hong Kong, India, Indonesia, Japan, Korea, Malaysia, the Philippines, and Vietnam. PBDE are organobromine compounds tused as flame retardant. Like other brominated flame retardants, PBDEs have been used in many industrial and commercial applications such as building materials, electronics, furnishings, motor vehicles, airplanes, plastics, polyurethane foams and textiles. In our research, PBDEs were detected in all the samples analyzed, and the concentrations ranged from 0.66 to 440 ng/g lipid wt. Higher concentrations of PBDEs were found in mussels from the coastal waters of Korea, Hong Kong, China, and the Philippines, which suggests that significant sources of these chemicals exist in and around this region. With regard to the composition of PBDE congeners, BDE-47, BDE-99, and BDE-100 were the dominant congeners in most of the samples. Among the OCs concentrations of DDTs, the highest followed by PCBs > CHLs > HCHs > HCB. Total concentrations of DDTs, PCBs, CHLs, and HCHs in mussel samples ranged from 21 to 58 000, 3.8 to 2000, 0.93 to 900, and 0.90 to 230 ng/g lipid wt., respectively.

Teuten *et al.* (2009), conducted a massive study to investigate the transport and release of chemicals from plastics to the environment and wildlife. Plastics debris in the marine environment, including resin pellets, fragments and microscopic plastic fragments, contain organic contaminants, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, petroleum

hydrocarbons, organochlorine pesticides (2,2'-bis(*p*-chlorophenyl)-1,1,1-trichloroethane, hexachlorinated hexanes), polybrominated diphenylethers, alkylphenols and bisphenol A, at concentrations from sub ng g⁻¹ to µg g⁻¹. Some of these compounds are added during plastics manufacture, while others adsorb from the surrounding seawater. Concentrations of hydrophobic contaminants adsorbed on plastics showed distinct spatial variations reflecting global pollution patterns. Model calculations and experimental observations consistently show that polyethylene accumulates more organic contaminants than other plastics such as polypropylene and polyvinyl chloride. Both a mathematical model using equilibrium partitioning and experimental data have demonstrated the transfer of contaminants from plastic to organisms. A feeding experiment indicated that PCBs could transfer from contaminated plastics to streaked shearwater chicks. Plasticizers, other plastics additives and constitutional monomers also present potential threats in terrestrial environments because they can leach from waste disposal sites into groundwater and/or surface waters. Leaching and degradation of plasticizers and polymers are complex phenomena dependent on environmental conditions in the landfill and the chemical properties of each additive. Bisphenol A concentrations in leachates from municipal waste disposal sites in tropical Asia ranged from sub µg l⁻¹ to mg l⁻¹ and were correlated with the level of economic development.

Sources of sedimentary PAHs in Tropical Asian Waters: Differentiation Between Pyrogenic and Petrogenic Sources by Alkyl Homolog Abundance

In the year 2009, our group made a comprehensive survey of petroleum hydrocarbon pollution in several Asian countries. We collected surface sediment samples from 174 locations in

India, Indonesia, Malaysia, Thailand, Vietnam, Cambodia, Laos, and the Philippines and analyzed them for polycyclic aromatic hydrocarbons (PAHs) and hopanes. We found that PAHs were widely distributed in the sediments, with comparatively higher concentrations in urban areas (Σ PAHs: ~ 1000 to $\sim 100\ 000$ ng/g-dry) than in rural areas (~ 10 to ~ 100 g-dry), indicating large sources of PAHs in urban areas. To distinguish petrogenic and pyrogenic sources of PAHs, we calculated the ratios of alkyl PAHs to parent PAHs: methylphenanthrenes to phenanthrene (MP/P), methylpyrenes + methylfluoranthenes to pyrene + fluoranthene (MPy/Py), and methylchrysenes + methylbenz[a]anthracenes to chrysene + benz[a]anthracene (MC/C). Analysis of source materials (crude oil, automobile exhaust, and coal and wood combustion products) gave thresholds of MP/P = 0.4, MPy/Py = 0.5, and MC/C = 1.0 for exclusive combustion origin. All the combustion product samples had the ratios of alkyl PAHs to parent PAHs below these threshold values. Contributions of petrogenic and pyrogenic sources to the sedimentary PAHs were uneven among the homologs: the phenanthrene series had a greater petrogenic contribution, whereas the chrysene series had a greater pyrogenic contribution. All the Indian sediments showed a strong pyrogenic signature with MP/P ≈ 0.5 , MPy/Py ≈ 0.1 , and MC/C ≈ 0.2 , together with depletion of hopanes indicating intensive inputs of combustion products of coal and/or wood, probably due to the heavy dependence on these fuels as sources of energy. In contrast, sedimentary PAHs from all other tropical Asian cities were abundant in alkylated PAHs with MP/P $\approx 1-4$, MPy/Py $\approx 0.3-1$, and MC/C $\approx 0.2-1.0$, suggesting a ubiquitous input of petrogenic PAHs. Petrogenic contributions to PAH homologs varied among the countries: largest in Malaysia whereas inferior in Laos. The higher abundance of alkylated PAHs together with constant hopane profiles suggests widespread inputs

of automobile-derived petrogenic PAHs to Asian waters (Saha *et al.*, 2009).

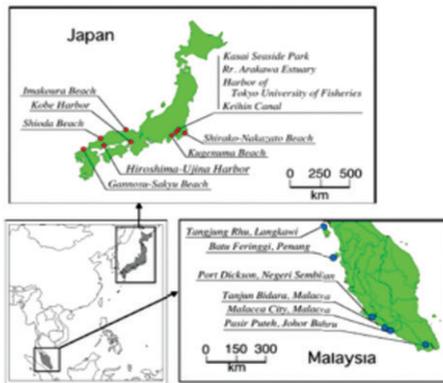
INTERNATIONAL PELLET WATCH

International Pellet Watch: Global Monitoring of Persistent Organic Pollutants (POPs) in Coastal Waters. 1. Initial Phase Data on PCBs, DDTs, and HCHs

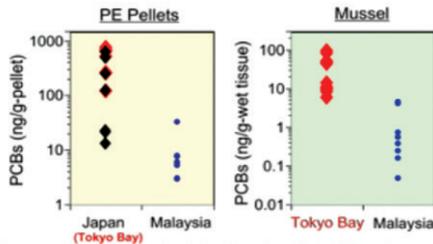
Ogata *et al.* (2009) conducted a comprehensive research on global monitoring of POPs using plastic resin pellets. Plastic resin pellets are small granules generally with shape of a cylinder or a disk with a diameter of a few mm. These plastic particles are industrial raw material transported to manufacturing sites where “user plastics” are made by re-melting and molding into the final products. Resin pellets can be unintentionally released to the environment, both during manufacturing and transport. The released resin pellets are carried by surface run-off, stream, and river waters eventually to the ocean. Resin pellets can also be directly introduced to the ocean through accidental spills during shipping. Because of their environmental persistence, they are distributed widely in the ocean and found on beaches and on water surfaces all over the world.

Samples of polyethylene pellets were collected at 30 beaches from 17 countries and analyzed for organochlorine compounds. PCB concentrations in the pellets were highest on US coasts, followed by western Europe and Japan, and were lower in tropical Asia, southern Africa and Australia. This spatial pattern reflected regional differences in the usage of PCBs and was positively correlated with data from Mussel Watch, another monitoring approach. DDTs showed high concentrations on the US west coast and in Vietnam. In Vietnam, DDT was predominant over its metabolites

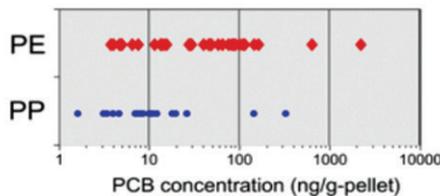
(DDE and DDD), suggesting the principal source may be current usage of the pesticide for malaria control. High concentrations of pesticide HCHs were detected in the pellets from southern Africa, suggesting current usage of the pesticides in southern Africa. This study demonstrates the utility and feasibility of the International Pellet Watch approach to monitor POPs at a global scale.



Sampling locations of plastic resin pellets and mussels in 1998 - 2000



PCB concentrations in beached plastic resin pellets (left) and mussels (right). Red diamonds : Tokyo Bay, black diamonds : the other Japanese locations than Tokyo Bay, blue circles : Malaysia



Tracing the Untraceable

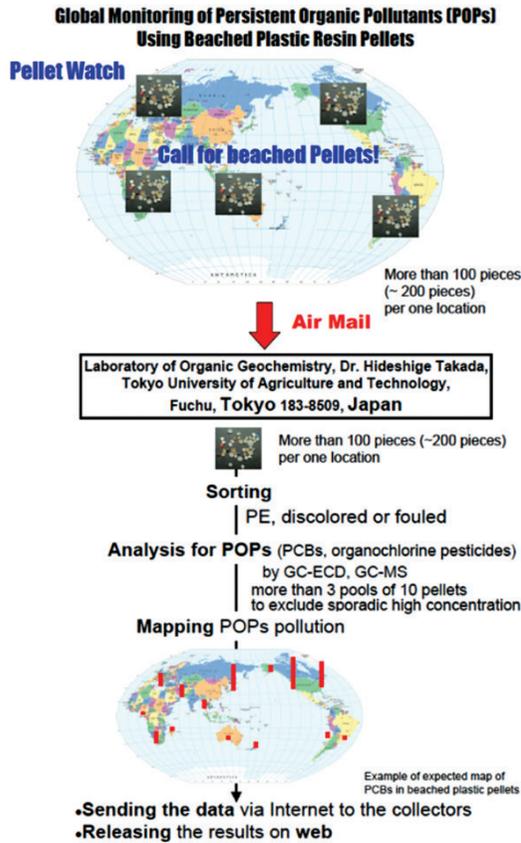
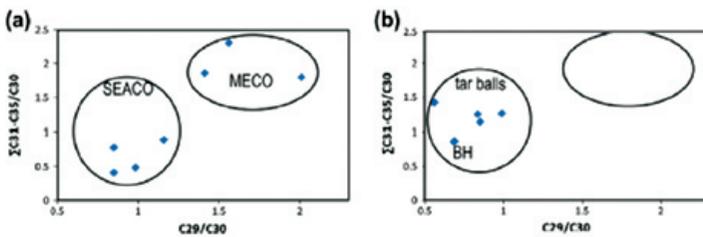


Figure 8 Snapshots of the activities and results of IPW

Identification of Sources of Tar Balls Deposited Along The Goa Coast, India, using Fingerprinting Techniques

After a decade of successful research in tar balls research, our group expanded our studies in other parts of the world (Suneel *et al.*, 2013). Since oil spills can occur in where oil production is active, the identifications of such spills become paramount. There

are very few reported studies in Indian subcontinent insofar as oil pollution is concerned. Eastern and western seaboard of Indian subcontinent are major routes for oil tankers carrying crude oil from the Middle East countries to Northeast Asian countries. Therefore, we embarked a study in Goa, India to have a baseline on the oil pollution status in this region. Deposition of tar balls along the coast of Goa, India is a common phenomenon during the southwest monsoon. Representative tar ball samples collected from various beaches of Goa and one Bombay High (BH) crude oil sample were subjected to fingerprint analysis based on diagnostic ratios of n-alkane, biomarkers of pentacyclic triterpanes and compound specific stable carbon isotope ($\delta^{13}\text{C}$) analysis to confirm the source. The results were compared with the published data of Middle East Crude Oil (MECO) and South East Asian Crude Oil (SEACO). The results revealed that the tar balls were from tanker-wash derived spills. The study also confirmed that the source is not the BH, but SEACO. The present study suggests that the biomarkers of alkanes and hopanes coupled with stable carbon isotope analysis act as a powerful tool for tracing the source of tar balls, particularly when the source specific biomarkers fail to distinguish the source.



(a) Cross-plot diagram of MECO and SEACO data (Zakaria et al., 2000); (b) cross-plot diagram for the present analysis of four tar balls and BH.

Figure 9

PCBs and DDTs in Surface Mangrove Sediments from the South of Iran

Our group's monitoring tasks are now expanded to Middle Eastern countries. Compound-specific studies of POPs in Iran are not well documented. Iran Mangrove sediments were collected during wet and dry seasons from nine stations in Khamir, Laft and natural reservoir mangrove-dense areas of Hormozgan province in the south of Iran. Σ PCBs ranged from 5.33 to 15.5 ng/g dry weight and the dominant congener was no.153. Average Σ DDTs for Khamir and Laft mangroves were 16.58 ± 1.51 and 18.8 ± 9.98 ng/g dry weight. DDT was more abundant than DDE and DDD isomers, which indicated the input of DDT. The sediment quality guideline shows that the concentration of Σ PCBs were below the ER-M guideline, whereas levels of Σ DDTs were between ER-L and ER-M. The mangrove ecosystem in Hormozgan province is suffering from urban and industrial development (Nozar *et al.*, 2013).

Polycyclic Aromatic Hydrocarbons Identification and Source Discrimination in Rural Soil of the Northern Persian Gulf Coast

Recently, Kakki *et al.* (2014) focused on PAHs in the Persian Gulf. Due to strategic situation of the Persian Gulf, identifying the petroleum pollution level and source is an important issue. Therefore, this research enhanced fingerprinting method of applying biomarkers Polycyclic Aromatic Hydrocarbons (PAHs) in identifying source and distribution of oil spills of the exposed areas. 10 soil samples collected from the northern coasts of the Persian Gulf along three provinces in the south of Iran. PAH concentrations in the soil ranged from 42.76 to 5596.49 ng.g⁻¹. In

the present research, the distribution of 3 ringed PAHs was much higher than the other PAHs. Phenanthrene and alkylated derivatives of phenanthrene such as 3-methyl, 2-methyl, 9-methyl and 1-methyl phenanthrene were distinctively higher than the other components. PAHs concentration can be considered as no or little risk of toxicity for the organisms living in soil except for fluoranthene, comparing LD_{50} . Applying marker ratios revealed that in most of the sampling sites showed perogenic sources and it emphasizes on the direct impact of oil and petroleum products to the lands due to oil well exploitation and transferring pipelines.

Oil Biodegradation

Contamination due to the spillage of petroleum or petroleum-derived hydrocarbons on different ecosystems has become critical issues to environment and living things. The use of native bacteria as an environmental friendly treatment of hydrocarbons pollution is very promising. The objective of the study is to identify the native hydrocarbon-degraders isolated from oil refinery. Bacteria from crude oil were isolated and cultured in enrichment media. The isolated strains were then cultured in different oil concentration. The isolated bacterial strains were Gram-stained and further identified via 16S rRNA sequencing. The resultant sequences were then aligned with the available online database for identification of the bacteria. The isolated bacteria belonged to families of *Pseudomonadaceae* and *Moraxellaceae* (*Acinetobacter* genus). The bacteria show high degradation of hydrocarbon and can be used to remediate polluted soils in tropical environment (Alavijeh *et al.*, 2014).

Distribution, Sources Identification, and Ecological Risk of PAHs and PCBs in Coastal Surface Sediments from the Northern Persian Gulf

Ecological risks assessment is critical in places where petroleum hydrocarbon pollution is significant. Our research group conducted a study in the Northern Persian Gulf. Hormozgan Province plays a vital role in fishery, petroleum, and industrial activities in southern Iran. However, no comprehensive studies on organic pollution have been performed. PCBs and PAHs were analyzed in surface sediments from areas receiving industrial (nine sites), river (one site), and urban (two sites) effluents. The sediment samples were collected in March and September 2010 (in dry and wet seasons) at the highest tidal time. The overall pollution level of PCBs ranged from 2.5 ± 0.8 to 462.0 ± 206.7 ng/g dry weight. CB153 congener dominated in most of the sediment samples. Congener profiles of PCBs showed close similarity with formulations of commercial products such as Aroclor 1260 and 1254g. A wide range of 55.3 to 1231.6 ng/g dry weight was detected for Σ PAHs. Results of PCA and PCA-MLR tests confirmed both petrogenic and pyrogenic origins for PAH pollution. The higher means of Σ PAHs and Σ PCBs in industrial and urban wastewaters were found near the shore, evidencing the role of these wastewaters in the PAH and PCB contamination in Hormozgan sediment. The concentrations of PAHs and PCBs in detected hotspots exceed the U.S. NOAA sediment quality guidelines (Seyedeh *et al.*, 2014)

ANTIBIOTICS MONITORING IN ASIAN WATERS

Seven sulfonamides, trimethoprim, five macrolides, lincomycin and three tetracyclines were measured in 150 water samples of sewage, livestock and aquaculture wastewater, and river and coastal waters,

in five tropical Asian countries. The sum of the concentrations of the target antibiotics in sewage and heavily sewage-impacted waters were at sub- to low-ppb levels. The most abundant antibiotic was sulfamethoxazole (SMX), followed by lincomycin and sulfathiazole. The average concentration of SMX in sewage or heavily sewage-impacted waters was 1720 ng/L in Vietnam (Hanoi, Ho Chi Minh, Can Tho; n = 15), 802 ng/L in the Philippines (Manila; n = 4), 538 ng/L in India (Kolkata; n = 4), 282 ng/L in Indonesia (Jakarta; n = 10), and 76 ng/L in Malaysia (Kuala Lumpur; n = 6). These concentrations were higher than those in Japan, China, Europe, the US and Canada. A predominance of sulfonamides, especially SMX, is notable in these tropical countries. The higher average concentrations, and the predominance of SMX, can be ascribed to the lower cost of the antibiotics. Both the concentration and composition of antibiotics in livestock and aquaculture wastewater varied widely. In many cases, sulfamethazine (SMT), oxytetracycline (OTC), lincomycin, and SMX were predominant in livestock and aquaculture wastewater. Both human and animal antibiotics were widely distributed in the respective receiving waters (i.e., the Mekong River and Manila Bay). SMT/SMX ratios indicate a significant contribution from livestock wastewater to the Mekong River and nearby canals, with an estimated ~ 10% of river water SMX derived from such wastewater. Mass flow calculations estimate that 12 tons of SMX is discharged annually from the Mekong River into the South China Sea. Riverine inputs of antibiotics may significantly increase the concentration of such antibiotics in the coastal waters.



Figure 10 Map of South Asia and Southeast Asia showing sampling sites (closed circles) (Shizumi, et al. 2014)

MONITORING OF OTHER POLLUTANTS OF HUMAN CONCERN IN MALAYSIA

Persistent Organic Chemicals in Malaysian Waters

A critical review of the levels of POPs from 1980 to 2002 was conducted by our group. We focused the review on the detection of several toxic substances and estimate their potential danger for the health of both marine life and humans. The compounds include PAHs, TBTs and pesticides. In summary, we found that the levels of some selected POPs is lower than that of the levels in some countries in Southeast Asia (Somchit *et al.*, 2009).

Antibiotics and Hormones

Simultaneous determination of veterinary antibiotics and hormone in broiler manure, soil and manure compost by liquid chromatography–tandem mass spectrometry

In this research, a multi-residue analytical method was developed to quantify nine antibiotics and one hormone in soil, broiler manure and manure compost (Ho, 2012). The developed method was based on ultrasonic extraction with MeOH:ACN:EDTA:McIvaine buffer, solid phase extraction (SPE) using HLB (3 cc/60 mg) cartridge, followed by instrumental analysis using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with 25 min total run time. It was validated and tested on soil, broiler manure and manure compost samples and showed that the method is able to simultaneously detect and quantify the target analytes with good selectivity and sensitivity. The developed method was linear in a concentration range from its instrumental quantification limit (IQL) to 500 ng/mL, with correlation coefficients higher than 0.999. The overall method performance was good for the majority of the analytes, with recoveries range from 63% to 121% in all the sample matrices. The method quantification limit (MQL) for the 10 target analytes in the soil, broiler manure and manure compost samples were 2–10, 3–16 and 5–15 µg/kg dry weight (DW), respectively. The method has also included tilmicosin, an antibiotic known to be reported in the environment for the first time. The developed method was then applied on broiler manure samples and its relative manure amended agricultural soil samples to identify and quantify veterinary antibiotic and hormone residues in the environment. These analytes were detected in broiler manure and soil samples, with maximum concentrations reaching up to 78516.1 µg/kg DW (doxycycline) and 1331.4 µg/kg DW (flumequine), respectively.

The results showed that the method can potentially be adopted for the analysis of veterinary antibiotic and hormone wastes in solid environmental matrices.

Fecal Sterols

Composition and sources of sterols in Pulau Tinggi, Johor, Malaysia

As mentioned previously, fecal coliforms bacteria are used as indicator of fecal contamination. There are several disadvantages using coliforms bacteria as sewage indicators. For example, fecal coliforms survival decreases as salinity increases. Thus, in the marine and estuarine waters, coliform bacteria test has less utility due to quick die-off of the bacteria. Therefore, chemical test using fecal sterols are more reliable for the monitoring of fecal contamination. Our group was the first to report on fecal pollution using molecular markers in Malaysia and Southeast Asia where serious sewage pollution has occurred. This research explores the role of sterols as lipid biomarkers to indicate their input that originates from various sources in the marine environment. Sterols and their ratios were investigated in sediments taken from sixteen sampling stations at Pulau Tinggi, Johor in order to assess the sources of organic matter. The compounds extracted from the sediments were quantified using a gas chromatography-mass spectrometry (GC-MS). The distributions of sterols indicated that organic matter at all sampling stations originated from a mixture of marine source and terrestrial origins at different proportions (Ali, 2011). A total of eleven sterols were quantified, with the major compounds being phytosterols (44% of total sterols), cholesterol (11%), brassicasterol (11%) and fecal sterols (12%).

Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)

Perfluorinated compounds (PFCs) are not degradable and persistent compounds, bioaccumulative and possess toxic characteristics. The compounds have been produced in large quantities and then applied and disposed without proper monitoring and regulation within the past half-century. Although the PFCs productions were to end today, the compounds would continue to persist in the environment for many years to come. Currently in Malaysia no studies have addressed perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) and no regulations has been developed to control these compounds. The analytical method used in this study involves solid phase extraction together with liquid chromatography coupled with mass spectrometry (LC/MS/MS). S# is referring to sampling station. Results from the water samples from Langat River, Malaysia, indicated that relatively high concentration of PFOS can be found in the eastern of the river, one of the most populated areas within the basin (S16–S35) with the highest concentration in station S35 (43.5 ng/mL) (Zainuddin *et al.*, 2012). Reason for this concentration is that the compound was highly used in industry as well as in manufacturing processes and consumer products. Relatively high concentration of PFOA can be found in station S6 (5.94 ng/mL) because this station is the station receiving effluent from landfill site. Further studies on the PFCs compounds could provide more data set and larger view of current state of PFCs contamination in Malaysian aquatic environments.

PHARMACEUTICALS IN SURFACE WATER

The growing interest in the environmental occurrence of veterinary and human pharmaceuticals is essentially due to their possible health implications to humans and ecosystem. This study assesses the occurrence of human pharmaceuticals in a Malaysian tropical aquatic environment taking a chemometric approach using cluster analysis, discriminant analysis and principal component analysis. Water samples were collected from seven sampling stations along the heavily populated Langat River basin on the west coast of peninsular Malaysia and its main tributaries. Water samples were extracted using solid-phase extraction and analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for 18 pharmaceuticals and one metabolite, which cover a range of six therapeutic classes widely consumed in Malaysia. Cluster analysis was applied to group both pharmaceutical pollutants and sampling stations. Cluster analysis successfully clustered sampling stations and pollutants into three major clusters. Discriminant analysis was applied to identify those pollutants which had a significant impact in the definition of clusters. Finally, principal component analysis using a three-component model determined the constitution and data variance explained by each of the three main principal components.

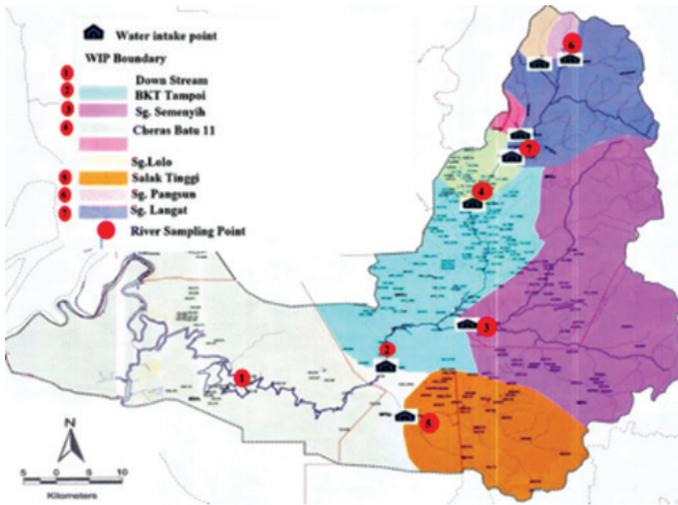


Figure 11 Map of Langat Rivers showing the sampling sites (Al-Odaini *et al.*, 2012)

Heavy Metals and PAHs

In this research, the ranges of pollutants found in the soft tissues of *Perna viridis* collected from Kg. Masai and Kg. Sg. Melayu, both located in the Straits of Johore, were 0.85–1.58 $\mu\text{g/g}$ dry weight (dw) for Cd, 5.52–12.2 $\mu\text{g/g}$ dw for Cu, 5.66–8.93 $\mu\text{g/g}$ dw for Ni and 63.4–72.3 $\mu\text{g/g}$ dw for Zn, and 36.4–244 ng/g dry weight for ΣPAHs . Significantly ($p < 0.05$) higher concentrations of Cd, Cu, Ni, Zn and ΣPAHs in the mussels were found in the water of a seaport site at Kg. Masai than a non-seaport site at Kg. Sg. Melayu population. The ratios of low molecular weight/high molecular weight hydrocarbons (2.94–3.42) and fluoranthene/pyrene (0.43–0.45) in mussels from both sites indicated the origin of the PAHs to be mainly petrogenic. This study has demonstrated the utility of using the soft tissues of *P. viridis* as a biomonitor of

PAH contamination and bioavailability in the coastal waters of Peninsular Malaysia (Yap *et al.*, 2012).

In addition, Hg levels in eight parts of *Asystasia gangetica* (L.) T. Anderson collected from 14 sites were determined using a Mercury Atomizer MA-1S and a Mercury Detector MD-1. It was found that the ranges for all the parts were 3.21-18.2 µg/kg dry weight for flowers, 1.29-11.2 µg/kg dry weight for stalks, 0.32- 29.4 µg/kg dry weight for seeds, 0.87-10.2 µg/kg dry weight for pericarps, 1.45-18.1 µg/kg dry weight for remainders, 11.8-68.2 µg/kg dry weight for leaves, 0.73-20.9 µg/kg dry weight for stems, and 3.40-33.1 µg/kg dry weight for roots. The overall Hg accumulation pattern in decreasing concentrations was leaf > root > flower > flower remains > stalk > stem > pericarp > seed. This study provided the background levels of Hg in this non-native invasive weed species in Malaysia. However, further studies are needed to confirm it as a biomonitors of Hg in this region (Chew, 2012).

SCIENTIFIC EXPEDITION TO ANTARCTICA AND THE ARCTIC

In 2008 the author has joined a research trip to Antarctica to fingerprint oil sources. In the year 2011, the author has the opportunity to join Arctic Scientific Expedition for the same research topic. The following snapshots are the moments of valuable experience in scientific research in the poles for him.

Mohamad Pauzi Zakaria



Figure 12 Arrival at Antractic Peninsula Airport. The author is standing on extreme left



Figure 13 The author is surrounded by friendly penguins chicks while conducting research in Antarctica

ARCTIC SCIENTIFIC EXPEDITION:



(a)

(b)



(c)

Figure 14 (a) Norwegian Arctic outpost in Svalbard Island (b) Collecting scientific data along the coastal beach in Poland Arctic Station (c) Snapshot with a polish scientist at Svalbard Museum

Awards

1. National Academic Award 2010 (Journal Publication)
2. PRPI 2010 Gold Medal – Application of hopanes as biomarkers in identifying the source of oil pollution: A case study in surface sediments from South China Sea, UPM, 2010
3. PRPI 2010 Silver Medal – Application of chemometric technique in identifying source apportionment of polycyclic aromatic hydrocarbons (PAHs) in sediment samples collected from South China Sea, UPM, 2010
4. PRPI 2010 Silver Medal – Determination of heavy metals in aquaculture fish from selected locations in the Straits of Malacca, UPM, 2010
5. Awarded a 5-Star and Role Model Supervisor, UPM 2013

Patent Filings

Patent Filing No. 1

Method for Simultaneous Quantification of Veterinary Antibiotics and Hormones in Soil and Biosolids

IP INFORMATION

IP Status	:	Pending Patent
Filed	:	2012-02-15
Application	:	PI2012000685
Country	:	Malaysia
Applicant	:	Universiti Putra Malaysia
Granted Date	:	0000-00-00

Patent Filing No. 2

Method for the quantification of Pharmaceuticals and Personal Care Products

IP INFORMATION

IP Status	:	Pending Patent
Filed	:	2012-06-15
Application No.	:	PI2012002704
Country	:	Malaysia
Applicant	:	Universiti Putra Malaysia
Granted Date	:	0000-00-00

SUMMARY

A major class of petroleum contaminant is groups of compounds consist of two or more fused benzene rings called polycyclic aromatic hydrocarbons (PAHs) that are carcinogenic, mutagenic and toxic. Source identification of petroleum pollution is necessary to prevent pollution entry into the environment. Our forensic investigations of petroleum hydrocarbon pollution in Malaysia and other neighboring countries revealed that the sources, distribution and transport pathways come from both lateral and vertical transport. The origins of the terrestrial based hydrocarbon pollutants mainly originated from automobile sources and biomass burning. The most significant input was from used crankcase oil. Our research had also concluded that sea-based hydrocarbon pollution originated both from foreign sources and domestic sources. One of the most important aspects of our research is that we are able to reconstruct hydrocarbon pollution history. This method enables us

to map the hydrocarbon pollution from the time when industrial and urbanization activities started and can be extrapolated into the future. This attribute is important to devise planning in the monitoring and pollution abatement strategies. Research methodologies in petroleum hydrocarbons had also enabled us to trace down other pollutants of human concern in Malaysia and elsewhere. Our research had also contributed significantly in global monitoring of Persistent Organic Pollutants (POPs). Petroleum pollution is known as point and non-point source of contaminations in the environment. Most of the studied locations showed high contribution of PAHs from combusted fuel, coal, biomasses and wood materials except for the southern part of Peninsular Malaysia in which revealed dominance of petroleum products. The findings indicate that PAHs are delivered from different intermediate materials such as asphalt, street dust, vehicle emission and crankcase oil. However, there has been a decline of PAHs input into the marine environment in recent years. Petroleum is shown to be a significant cause of marine pollution since the second quarter of 20th century. An overview on sourced materials of petroleum pollution indicates multi-approach necessity toward pollution control, regardless of concentration and possible degradation processes. Various sectors both governmental and non-governmental are needed for prevention and control of petroleum pollution where different sources apparently contribute to the pollution generation process.

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BIOGRAPHY

Mohamad Pauzi Zakaria was born in Sep 25 1957 in Besut, Terengganu. He got his early primary and lower secondary education in Besut, Terengganu. He went to Sekolah Menengah Sains Kelantan (now Faris Petra) in 1974 and obtained Grade 1, Malaysia Certificate of Education (MCE) in 1975. He was offered MARA Scholarship to do Bachelor's Degree in Monmouth College, Illinois, USA. The following year he joined Western Michigan University and graduated with a degree in Biology in 1980. He obtained masters degree from Florida Institute of Technology and University of Massachusetts, USA. Mohamad Pauzi Zakaria obtained his doctorate degree in Organic Geochemistry from Tokyo University of Agriculture and Technology, Japan in 2002. He joined UPM in 1980 as a tutor and therefore he has served UPM for 34 years!

Mohamad Pauzi Zakaria is a known figure in research especially in the field of Environmental Forensics to determine the source of environmental pollution. His research has won numerous prestigious awards from UPM (PRPI), national (ITEX) and international (Geneva Palexpo, 2006). He was the Head of UPM Research Delegation Exhibitions to Geneva, Switzerland in 2006. He received scholarship from RONPAKU Japanese Society for the Promotion of Science (JSPS) for PhD studies. He also won 'Anugerah Akademik Negara' for 2010, the most prestigious award for academic achievement in Malaysia. Results from recent research were published in high impact international journal of Journal of Chromatography A entitled "Multi-residue Analytical Method for Human Pharmaceutical and Synthetic Hormones in River Water and Sewage Effluents by Soil-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry". The research has developed a method and application for the analysis of 23 pharmaceutical waste and synthetic hormones simultaneously

in water samples including river water and wastewater sewage treatment plant (STP). Results confirmed the applicability of environmental monitoring. So far, there is no single method capable of measuring all pharmaceutical waste. The method has been developed is recent, rapid and can be solved in just 30 minutes. The method developed by Mohamad Pauzi Zakaria and his group is very sensitive to a variety of pharmaceutical waste and hormone use in Malaysia. He successfully supervised 10 Ph.D students. In addition, he also has produced more than 80 publications in world's top journals including a book and four chapters in the book including a chapter published by Elsevier. He is among the world's top 20 researchers in hydrocarbon analysis. Also, Mohamad Pauzi Zakaria is a member of the American Chemical Society, International Society of Environmental Forensics, and the Vice President of the Malaysian Muslim Scientists Association (PERINTIS), AMETEC Steering Committee (APEC Marine and Environmental Training Center), Korean Ocean Research and Development Institute. At present, he is a Deputy Dean, Faculty of Environmental Studies, Universiti Putra Malaysia. He is also in the Editorial Board for an international journal. Mohamad Pauzi Zakaria has travelled to many countries around the world to conduct teaching, research and presenting papers. He went to the North and South Pole (Arctic and Antarctic) to conduct research in collaboration with many scientists around the world.

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His family has provided inspiration throughout his research career and therefore special thanks to his wife (Associate Professor Dr. Nor Azan Mat Zin) and 8 children (Ammar, Dr. Jinan, Ir. Maryam, Abrar, Aishah, Ahmad, Abdullah and Amjad). Last but not least a loving memory of his demised parents who would certainly would have enjoyed the fruit of success for their beloved son.



LIST OF INAUGURAL LECTURES

1. Prof. Dr. Sulaiman M. Yassin
*The Challenge to Communication
Research in Extension*
22 July 1989
2. Prof. Ir. Abang Abdullah Abang Ali
*Indigenous Materials and Technology
for Low Cost Housing*
30 August 1990
3. Prof. Dr. Abdul Rahman Abdul Razak
*Plant Parasitic Nematodes, Lesser
Known Pests of Agricultural Crops*
30 January 1993
4. Prof. Dr. Mohamed Suleiman
*Numerical Solution of Ordinary
Differential Equations: A Historical
Perspective*
11 December 1993
5. Prof. Dr. Mohd. Ariff Hussein
*Changing Roles of Agricultural
Economics*
5 March 1994
6. Prof. Dr. Mohd. Ismail Ahmad
*Marketing Management: Prospects
and Challenges for Agriculture*
6 April 1994
7. Prof. Dr. Mohamed Mahyuddin Mohd.
Dahan
*The Changing Demand for Livestock
Products*
20 April 1994
8. Prof. Dr. Ruth Kiew
*Plant Taxonomy, Biodiversity and
Conservation*
11 May 1994
9. Prof. Ir. Dr. Mohd. Zohadie Bardaie
*Engineering Technological
Developments Propelling Agriculture
into the 21st Century*
28 May 1994
10. Prof. Dr. Shamsuddin Jusop
Rock, Mineral and Soil
18 June 1994
11. Prof. Dr. Abdul Salam Abdullah
*Natural Toxicants Affecting Animal
Health and Production*
29 June 1994
12. Prof. Dr. Mohd. Yusof Hussein
*Pest Control: A Challenge in Applied
Ecology*
9 July 1994
13. Prof. Dr. Kapt. Mohd. Ibrahim Haji
Mohamed
*Managing Challenges in Fisheries
Development through Science and
Technology*
23 July 1994
14. Prof. Dr. Hj. Amat Juhari Moain
Sejarah Keagungan Bahasa Melayu
6 Ogos 1994
15. Prof. Dr. Law Ah Theem
Oil Pollution in the Malaysian Seas
24 September 1994
16. Prof. Dr. Md. Nordin Hj. Lajis
*Fine Chemicals from Biological
Resources: The Wealth from Nature*
21 January 1995
17. Prof. Dr. Sheikh Omar Abdul Rahman
*Health, Disease and Death in
Creatures Great and Small*
25 February 1995

Tracing the Untraceable

18. Prof. Dr. Mohamed Shariff Mohamed Din
Fish Health: An Odyssey through the Asia - Pacific Region
25 March 1995
19. Prof. Dr. Tengku Azmi Tengku Ibrahim
Chromosome Distribution and Production Performance of Water Buffaloes
6 May 1995
20. Prof. Dr. Abdul Hamid Mahmood
Bahasa Melayu sebagai Bahasa Ilmu-Cabaran dan Harapan
10 Jun 1995
21. Prof. Dr. Rahim Md. Sail
Extension Education for Industrialising Malaysia: Trends, Priorities and Emerging Issues
22 July 1995
22. Prof. Dr. Nik Muhammad Nik Abd. Majid
The Diminishing Tropical Rain Forest: Causes, Symptoms and Cure
19 August 1995
23. Prof. Dr. Ang Kok Jee
The Evolution of an Environmentally Friendly Hatchery Technology for Udang Galah, the King of Freshwater Prawns and a Glimpse into the Future of Aquaculture in the 21st Century
14 October 1995
24. Prof. Dr. Sharifuddin Haji Abdul Hamid
Management of Highly Weathered Acid Soils for Sustainable Crop Production
28 October 1995
25. Prof. Dr. Yu Swee Yean
Fish Processing and Preservation: Recent Advances and Future Directions
9 December 1995
26. Prof. Dr. Rosli Mohamad
Pesticide Usage: Concern and Options
10 February 1996
27. Prof. Dr. Mohamed Ismail Abdul Karim
Microbial Fermentation and Utilization of Agricultural Bioresources and Wastes in Malaysia
2 March 1996
28. Prof. Dr. Wan Sulaiman Wan Harun
Soil Physics: From Glass Beads to Precision Agriculture
16 March 1996
29. Prof. Dr. Abdul Aziz Abdul Rahman
Sustained Growth and Sustainable Development: Is there a Trade-Off 1 or Malaysia
13 April 1996
30. Prof. Dr. Chew Tek Ann
Sharecropping in Perfectly Competitive Markets: A Contradiction in Terms
27 April 1996
31. Prof. Dr. Mohd. Yusuf Sulaiman
Back to the Future with the Sun
18 May 1996
32. Prof. Dr. Abu Bakar Salleh
Enzyme Technology: The Basis for Biotechnological Development
8 June 1996
33. Prof. Dr. Kamel Ariffin Mohd. Atan
The Fascinating Numbers
29 June 1996
34. Prof. Dr. Ho Yin Wan
Fungi: Friends or Foes
27 July 1996
35. Prof. Dr. Tan Soon Guan
Genetic Diversity of Some Southeast Asian Animals: Of Buffaloes and Goats and Fishes Too
10 August 1996

Mohamad Pauzi Zakaria

36. Prof. Dr. Nazaruddin Mohd. Jali
*Will Rural Sociology Remain Relevant
in the 21st Century?*
21 September 1996
37. Prof. Dr. Abdul Rani Bahaman
*Leptospirosis-A Model for
Epidemiology, Diagnosis and Control
of Infectious Diseases*
16 November 1996
38. Prof. Dr. Marziah Mahmood
*Plant Biotechnology - Strategies for
Commercialization*
21 December 1996
39. Prof. Dr. Ishak Hj. Omar
*Market Relationships in the Malaysian
Fish Trade: Theory and Application*
22 March 1997
40. Prof. Dr. Suhaila Mohamad
Food and Its Healing Power
12 April 1997
41. Prof. Dr. Malay Raj Mukerjee
*A Distributed Collaborative
Environment for Distance Learning
Applications*
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42. Prof. Dr. Wong Kai Choo
*Advancing the Fruit Industry in
Malaysia: A Need to Shift Research
Emphasis*
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43. Prof. Dr. Aini Ideris
*Avian Respiratory and
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Attraction*
10 July 1999
44. Prof. Dr. Sariah Meon
*Biological Control of Plant Pathogens:
Harnessing the Richness of Microbial
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14 August 1999
45. Prof. Dr. Azizah Hashim
*The Endomycorrhiza: A Futile
Investment?*
23 Oktober 1999
46. Prof. Dr. Noraini Abdul Samad
*Molecular Plant Virology: The Way
Forward*
2 February 2000
47. Prof. Dr. Muhamad Awang
*Do We Have Enough Clean Air to
Breathe?*
7 April 2000
48. Prof. Dr. Lee Chnoong Kheng
Green Environment, Clean Power
24 June 2000
49. Prof. Dr. Mohd. Ghazali Mohayidin
*Managing Change in the Agriculture
Sector: The Need for Innovative
Educational Initiatives*
12 January 2002
50. Prof. Dr. Fatimah Mohd. Arshad
*Analisis Pemasaran Pertanian
di Malaysia: Keperluan Agenda
Pembaharuan*
26 Januari 2002
51. Prof. Dr. Nik Mustapha R. Abdullah
*Fisheries Co-Management: An
Institutional Innovation Towards
Sustainable Fisheries Industry*
28 February 2002
52. Prof. Dr. Gulam Rusul Rahmat Ali
*Food Safety: Perspectives and
Challenges*
23 March 2002
53. Prof. Dr. Zaharah A. Rahman
*Nutrient Management Strategies for
Sustainable Crop Production in Acid
Soils: The Role of Research Using
Isotopes*
13 April 2002

Tracing the Untraceable

54. Prof. Dr. Maisom Abdullah
Productivity Driven Growth: Problems & Possibilities
27 April 2002
55. Prof. Dr. Wan Omar Abdullah
Immunodiagnosis and Vaccination for Brugian Filariasis: Direct Rewards from Research Investments
6 June 2002
56. Prof. Dr. Syed Tajuddin Syed Hassan
Agro-ento Bioinformation: Towards the Edge of Reality
22 June 2002
57. Prof. Dr. Dahlan Ismail
Sustainability of Tropical Animal-Agricultural Production Systems: Integration of Dynamic Complex Systems
27 June 2002
58. Prof. Dr. Ahmad Zubaidi Baharumshah
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59. Prof. Dr. Shaik Md. Noor Alam S.M. Hussain
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