EnvironmentAsia
The international journal published by the Thai Society of Higher Education Institutes on Environment

Available online at www.tshe.org/EA
EnvironmentAsia 3(1) (2010) 13-19

Characterization of Perylene in Tropical Environment: Comparison of New and Old Fungus Comb for Identifying Perylene Precursor in *Macrotermes gilvus* Termite Nests of Peninsular Malaysia

Alireza Riyahi Bakhtiari [a], Mohamad Pauzi Zakaria [a], Mohamad Ramin [b], Mohammad Ismail Yaziz [c], Mohamad Nordin Hj Lajis [d] and Xinhui Bi [e]

[a] *Center of Excellence in Environmental Forensics, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.*
[b] *Department of Animal Science, Faculty of Agriculture, UPM 43400, Serdang, Selangor, Malaysia.*
[c] *Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[d] *Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[e] *State Key Laboratory of Organic Geochemistry, Guangdong Key Laboratory of Environment and Resources, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, P.R. China.*

Abstract

This is the first record on the distribution of perylene in new and old fungus combs of termite nest (*Macrotermes gilvus*) in order to determine perylene source in tropical environment. Twenty four samples of new and old fungus combs, inner and outer nest walls, fresh and decomposed bark, decomposed stem, soil, and soil-wood interface were collected in order to test of two hypotheses; i) Perylene is produced in the termiteûs hindgut (*M. gilvus*) and ii) Perylene is present only in new fungus comb of *M. gilvus* termite nests. For one Station (Station A) the profile of perylene concentration was the following order: fungus comb > outer nest wall ≥ Soil-Wood interface ≥ decomposed stem ≥ decomposed bark ≥ Inner nest wall > Soil. For the other Station (i.e. B) the profile was new fungus comb > inner nest wall > old fungus comb ≥ outer nest wall ~ Soil. The perylene concentration was found up to 21-54 times higher in fungus comb as compare to the rest of the samples in Station A. whereas, the perylene concentration was 85-400 times higher in new fungus comb as compare to the remaining samples in Station B, this can be due to the production or accumulation of perylene in these nests. On the other hand, smaller termite nests (Stations C and E) no perylene was detected, due to the fact that the new fungus comb was not found in those nests. The results confirmed the following hypotheses; perylene occurs only in new fungus comb and may be attributed to the high concentrations of aromatic rings of lignin in new fungus comb.

Keywords: perylene; termite; new fungus comb; old fungus comb; *Macrotermes gilvus.*

1. Introduction

Fungus-growing termites (Isoptera; Termitidae; Macrotermitinae) are abundant in the African and Asian tropics (Wood and Sands, 1978; Abe and Matsumoto, 1979). *Macrotermes gilvus* is widely distributed in southern Asia, from Burma to Indonesia and the Philippines (Roonwal, 1970). Their enlarged hindguts have been thought of as anaerobic digesters in which a symbiotic gut microflora depolymerizes cellulose and hemicelluloses and ferments the resulting carbohydrates to short-chain fatty acids (Brune, 1998).

In the nest, young workers masticate and ingest the collected plant litter which passes rapidly through the termite gut without digestion. The resulting fecal pellets (primary feces) are pressed together to form a sponge-like structure (called fungus comb). The symbiotic fungi, genus *Termitomyces,* grow on the comb-like matrices of the fungus comb. They form mycelia and white round and asexual conidial structures called fungus nodules (Ohkuma, 2001). The fungus nodules are usually consumed by young workers, whereas the old senescent combs are consumed by old workers to produce final feces (Ohkuma, 2001).

Perylene is a five-ring nuclear polycyclic aromatic hydrocarbon (PAH) that has been found widely in sediments, peats, fossil crinoids and oil shales (Ishiwatari *et al.,* 1980; Wakeham *et al.,* 1980; Venkatesan, 1988).

It is believed that perylene produced in anoxic conditions and the conversion of perylenequinone to perylene results from a reduction reaction, due to the fact that quinine compounds are sensitive to oxidization. Therefore, its preservation in sediments

requires a reducing environment (Aizenshtat, 1973). A number of studies have found that high perylene concentrations in surface oxic sediments in tropical and subtropical environments (Zakaria *et al.,* 2002; Boonyatumanond *et al.,* 2003; Opuene *et al.,* 2007; Kosmehl *et al.,* 2008; Liu *et al.,* 2008). However, this is in contrast to results from several studies in other latitudes which showed that perylene is either absent or present only in minor concentrations in oxic surface sediments (*e.g.*; Unlu and Alpar, 2006). However, far too little attention has been paid to source of perylene. There are a few unanswered questions; first, "Why is perylene in abundance in surface oxic sediments in tropical and subtropical environments?" second, "What is the source of perylene in peninsular Malaysia?" third, "How high are the concentrations of perylene in new fungus comb as compared to old fungus comb of *M. gilvus* termite nests and what is the interpretation?"

In recent years, evidence was presented that there are biological sources of the PAHs naphthalene, phenanthrene, and perylene in the tropics, which may contribute significantly to the environmental load in rural background areas of Brazil (Wilcke *et al.,* 2000; 2003; 2004).

In this study, perylene concentrations in different compartments and surrounding soil and plants of *M. gilvus* termite nests in peninsular Malaysia were investigated in order to determine the source of perylene. The main focus of this study is to fill the gap in knowledge about differences between perylene concentrations in new fungus comb and old fungus comb of *M. gilvus* termite nests. The objectives of this study are to test the hypotheses (i) Perylene is produced in the termite's hindgut (*M. gilvus*), and (ii) Perylene is present only in new fungus comb of *M. gilvus* termite nests.

2. Materials and Methods

2.1. Collection of samples

In March and May 2007, we collected samples of the termite nests from four Stations in the campus of Universiti Putra Malaysia (UPM). The map of the sampling locations is shown in Fig. 1. As the termites belong to the wood and the soil-wood interface feeding genera, we also collected composite samples from the surrounding soils and plants. Overall, 4 soils, 1 soil-wood interface, 3 bark samples, 1 decomposed stem and 15 samples from 4 termite nests.

Soil samples were taken from the top 0-10 cm of the mineral soil after removal of the organic layer if present, near each termite nest. Each sample was bulked from five individual collections from a surface area of ca. 1 m^2.

The wood samples comprised fresh and partly decomposed stem material and bark of the dominating species next to the termite nests.

Combs of progressively increasing age were separated and defined as "fresh" comb. It was the top rim of the comb and freshly made; "old" comb was the bottom of the comb and eaten by the termite workers. Two separate samples of each type of comb and also inner and outer nest wall were taken from each colony. In Station B the size of mound was huge and it was placed in surface area of ca. 1 m^2. In Station C and E the nests were very small and in Station A nest had middle size (Fig. 2).

All samples were wrapped in clean aluminum foil, stored in closed plastic bags in a cool box and deep-frozen. In the laboratory, nest and soil samples were sieved to <2 mm. The wood samples were ground prior to the analyses.

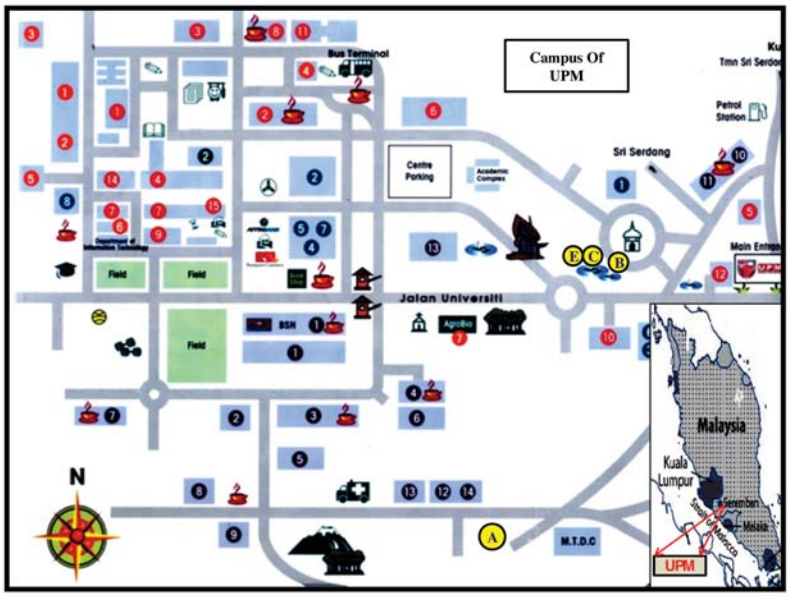

Figure 1. Sampling location for termite nest inside the campus of Universiti Putra Malaysia (UPM) in Selangor state

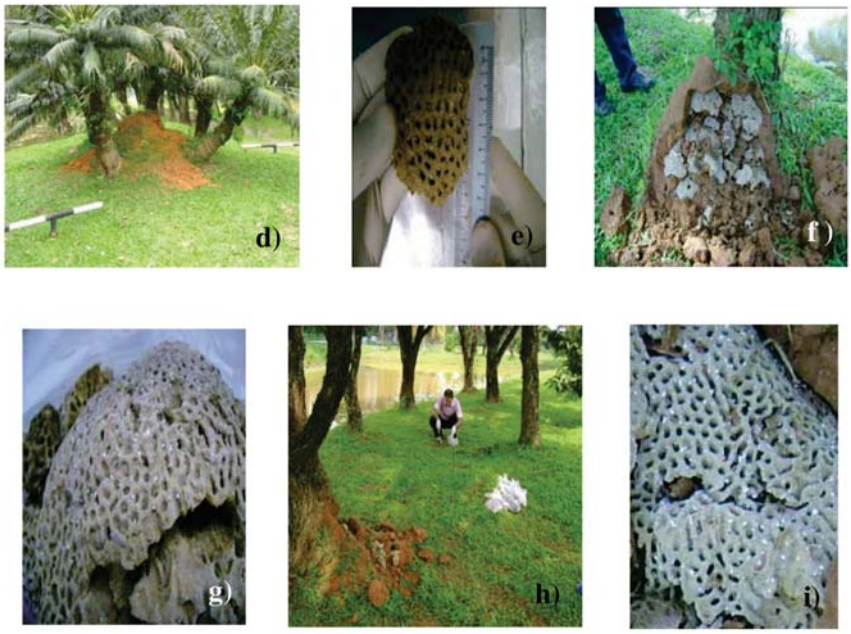

Figure 2. d) Huge termite nest in Station B, e) fungus comb in Station B, f) and h) small termite nests in Station C and E, respectively. g), and i) fungus combs in Stations C and E, which only contains old fungus comb, suggesting that in combs of progressively increasing age (Station B), combs are thick and new and old fungus combs are easily distinguishable

2.2. Chemical analysis

2.2.1. Analytical procedure for PAHs

The samples were purified and fractionated by a method described elsewhere (Zakaria *et al.,* 2002). This method relies upon extraction, two-step silica gel chromatography and gas chromatography-mass spectrometry (GC-MS). All solvents were distilled before use. All glassware was rinsed with methanol, dichloromethane (DCM), and distilled hexane several times to remove organic contamination. Sodium sulfate anhydrous, disposable Pasteur pipettes and screw-top vials were baked for 2-3 h at 500˚C prior to use to remove organic contamination.

The amount of each sample that is representative of 5 g of sample dry weight was precisely weighed and homogenized using mortar and pestle in sodium sulfate to remove any water.

A Soxhlet extractor was used for the extraction of lipid from the samples using 270 ml of distilled dichloromethane for 10 hours. About 100 µl of the PAH surrogate internal injection standard mixture where 10 ppm of each component, containing naphthalene-d_8, anthracene-d_{10}, chrysene-d_{12} and perylene-d_{12} was added for quality control of the PAH analyses. The eluate was purified and fractionated using two-step silica gel column chromatography. First step silica gel chromatography was accomplished through 5% H_2O deactivated silica gel column (1 cm i.d. 9 cm, ~6 g, 100-200 mesh; F.C.923, Davison Chemical, Columbia, MD, USA). Hydrocarbons ranged from n-alkanes to PAHs were eluted with 20 ml of dichloromethane/hexane (1:3 v/v). Second step column chromatography was a fully activated silica gel (0.47 cm i.d.18 cm, ~3 g, 60-200 mesh; Sil-A-200; Sigma, Kuala Lumpur, Malaysia). PAHs were eluted with 14 ml of dichloromethane/hexane (1:3, v/v). The PAH fraction was evaporated to approximately 1 ml transferred to a 1.5 ml amber and evaporated to dryness under a gentle stream of nitrogen and re-dissolved in 100 µl of isooctane containing *p*-terphenyl-d_{14} as an internal injection standard (IISTD) for PAHs analysis. PAHs analyses were conducted using an Agilent Technology 5973A quadrupole mass spectrometer integrated with an Agilent 6890 gas chromatograph. A 30 m fused silica capillary column of 0.25 mm i.d. and 0.25 µm film thickness was used in the analysis. Carrier gas was helium. GC-MS operating conditions were 70eV ionization potential with the source at 200˚C and electron multiplier voltage at ~2000eV. The injection port was maintained at 310˚C and the sample was injected with splitless mode followed by purge 1 min after the injection. Column temperature was held at 70˚C for 2 min, then programmed at 30˚C/min to 150˚C, and then 4˚C/min to 310˚C and held for 10 min. A selected ion monitoring method was employed after a delay of 3 min. Identification and quantification were achieved using ChemStation software based on internal standard method.

Quality control for the PAHs analyses was carried out by monitoring the recovery of surrogate internal injection standard (naphthalene-d_8, anthracene-d_{10},

chrysene-d_{12} and perylene-d_{12}) spiked just after extraction. The PAH concentrations are corrected against the spiked surrogates. Recoveries ranged from 87% to 103%. P-terphenyl-d_{14} as Internal Injection Standard (IIS) was used for quantification and eliminates errors due to variation in sample injection. In addition, the procedure of blank was used to demonstrate all solvents, reagents, glassware, and other sample processing hardware to be free from interferences under the conditions of analysis.

2.2.2. Analytical procedure for total organic carbon (TOC)

The analytical procedure used in this study was described by Nelson and Sommers, (1996). Briefly, the samples were dried overnight at 60°C in oven and then homogenized using mortar and pestle. Sample preparation was carried out to eliminate inorganic carbon. For this purpose, 1-1.5 g of the sample was mixed with 1-2 ml of 1 M HCl to eliminate all carbonates and dried for about 10 hours at 100-105°C to eliminate residual HCl. The TOC% was then determined using a LECO CR-412 Carbon Analyzer with a furnace temperature of 1350°C and an oxygen boost time of 60s.

3. Results and Discussion

3.1. First hypothesis; Perylene is produced in the termiteûs hindgut (M. gilvus)

All perylene concentrations and TOC patterns are given in Table. 1. Perylene was present in the 0-10 cm soil layer of A, B, C and E Stations in concentrations 0.0- 2 ng/g, (*n= 4*). The highest perylene concentrations were observed in new fungus comb of termite nest in Station B (397 ng/g) and then in fungus comb of termite nest in Station A (123 ng/g), (Table 1). In termite nests of Stations A and B, the central part had larger perylene concentrations than its walls (4-6 ng/g) in Inner nest walls and (0.0- 6 ng/g) in Outer nest wall, respectively. In Station A, perylene concentrations tended to increase in the order fungus comb > outer nest wall ≥ Soil-Wood interface ≥ decomposed stem ≥ decomposed bark ≥ Inner nest wall > Soil in, It was up to 21-54 times higher in fungus comb than its in outer nest wall, decomposed stem, decomposed bark, Inner nest wall, and soil. In Station B, perylene concentrations tended to increase in the order new fungus comb > inner nest wall ≥ old fungus comb > outer nest wall ~Soil, It was up to 85 - 400 times higher in new fungus comb than its in inner nest wall, old fungus comb, outer nest wall, and soil, suggesting that this compound had been produced within the nests.

Table 1. Perylene concentrations (ng/g) and total organic carbon (mg/g) in termite nest, soil & plant samples in campus of UPM

Station	Name of samples	Perylene	TOC	Description of site
A	Soil	2.3	16	Remote area
	Fungus Comb	123	374	Termite nest size was large
	Inner nest wall	4	12	Date of sampling
	Outer nest wall	6	12	03 March 2007
	Decomposed bark	4	379	
	Decomposed stem	4	430	
	Soil-wood interface	5	19	
B	Soil	N.D	18	Near of artificial Lake
	New Fungus comb	397	471	Termite nest size was huge and
	Old Fungus comb	5*	449	surrounded between four trees
	Inner nest wall	6	30	Date of sampling
	Outer nest wall	N.D	18	01 May 2007
C	Soil	N.D	18	Near of artificial Lake
	New Fungus comb	17*	460	Termite nest size was small
	Old Fungus comb	N.D	445	Date of sampling
	Inner nest wall	N.D	11	01 May 2007
	Outer nest wall	N.D	13	
	Fresh bark	3*	540	
E	Soil	N.D	14	Near of artificial Lake
	New Fungus comb	5*	450	Termite nest size was small
	Old Fungus comb	0.6*	436	Date of sampling
	Inner nest wall	0.5*	18	01 May 2007
	Outer nest wall	0.5*	8	
	Fresh bark	1*	496	

N.D= non detected

***** It is not perylene concentratios. It can be its isomer (different retention time but close to perylene retention time with same molecular weight)

The result demonstrates that perylene in nests may result from termite activity. It is generally believed that the perylene is formed in anoxic conditions, due to the fact that the conversion of perylenequinone to perylene is a reduction reaction (Aizenshtat, 1973). Meanwhile, recent investigations employing micro sensor techniques have demonstrated that the metabolic activity of the gut micro biota maintains steep oxygen and hydrogen gradients within the gut lumen. Only the centre of the dilated gut regions is rendered anoxic (Brune, 1998). Termite hindguts have been thought of as anaerobic digesters in which a symbiotic gut microflora de-polymerizes cellulose and hemicelluloses and ferments the resulting carbohydrates to short-chain fatty acids (Brune, 1998).

3.2. Total Organic carbon (TOC)

Total organic carbon concentration in organic and inorganic samples ranged from 374 to 430 mg/g and 12 to 19 mg/g in Station A, respectively (Table 1).

A cluster analysis based on the contributions of perylene concentrations and TOC revealed that the samples may be classified into two main groups (organic and inorganic) and six subgroups, according to their perylene concentrations and TOC patterns (Fig. 3). The clusters have been chosen to maximize the differences among cases in different cluster. At the highest level, Inner nest wall and Outer nest wall (subgroup 1) were separated from all the other samples because of their contributions to perylene concentrations and TOC. At the stage two, decomposed bark and decomposed stem (subgroup 2) were separated from the remaining samples. At stage three were Inner nest wall and soil-wood interface (subgroup 3). At the stage four were soil and Inner nest wall (subgroup 4). At the stage five were Fungus comb and decomposed bark (subgroup 5) because of their equivalent contributions of TOC were separated. At the last stage, Fungus comb and soil (subgroup 6) were separated because of their very diverse contribution to perylene concentrations and TOC. This gave rise to the assumption that perylene is produced in the hindgut of termites. This may explain why perylene was found in abundance in fungus comb. The results of cluster analysis also confirmed the first hypothesis.

3.3. Second hypothesis; Perylene is found only in new fungus comb of M. gilvus termite nests.

In Station B, the perylene concentration was 397 ng/g in new fungus comb of termite nest. The perylene concentration tended to increase in the order new fungus comb > inner nest wall ≥ old fungus comb > outer nest wall ~Soil.

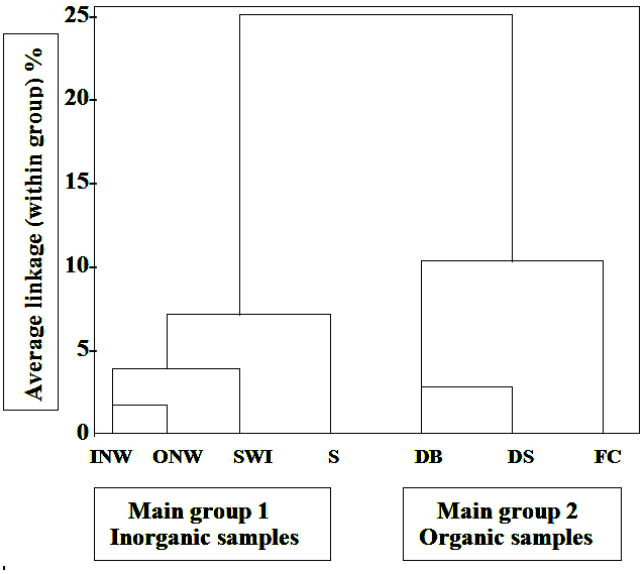

Figure 3. Dendrogram for samples from Station A, inner nest wall (INW), outer nest wall (ONW), soil-wood interface (SWI), Soil (S), decomposed bark (DB), decomposed stem (DS) and fungus comb (FC), according to their perylene concentration and TOC pattern after hierarchical cluster analysis. Inner nest wall and outer nest wall (subgroup 1), decomposed bark and decomposed stem (subgroup 2), inner nest wall and soil-wood interface (subgroup 3), soil and inner nest wall (subgroup 4), decomposed bark and fungus comb (subgroup 5), soil and fungus comb (subgroup 6)

Total organic carbon concentrations in organic and inorganic samples ranged from to 450 to 470 mg/g and 17 to 31 mg/g in Station B, respectively (Table 1).

A cluster analysis based on the contributions of perylene concentrations and TOC indicated that the samples may be classified into four groups, according to their perylene concentrations and TOC patterns (Fig. 4). The clusters have been chosen to maximize the differences among cases in different cluster. At the highest level, soil and outer nest wall (group 1), were separated from all the other samples because of their contributions to perylene concentrations and TOC. At the stage two, soil and old fungus comb (group 2), were separated from the remaining samples. At stage three were soil and inner nest wall (group 3), and at stage four, soil and new fungus comb (group 4). Their very diverse contribution to perylene concentrations and TOC, we suggest that in combs of progressively increasing age, perylene concentrations are significantly higher in new fungus comb than old fungus comb. Meanwhile, the results obtained from *n*-alkane concentrations in this station confirmed the increasing age of comb and significant difference between new and old fungus comb. This is attributed to different concentrations of chitin and *n*-alkanes in the new fungus comb and old fungus comb in huge

nest (Riyahi Bakhtiari *et al.,* full article in preparation for publication). This may be explained to the fact that when the plant materials are consumed by young workers and passes through gut, in anaerobic condition, it does not undergo degradation process and digestion resulting formation of perylene in new fungus comb. Hence, high concentrations of perylene can only be found in new fungus comb. In addition, the new fungus comb is found to be an ideal condition for *Termitomyces* sp to grow and they become an agent for further break-down of lignin. As the new fungus comb is consumed by young workers, a minor concentration of existing perylene is broken down to derivatives of perylene in the form of isomer. This is reflected in the isomeric composition of perylene in old fungus comb. Results obtained from total ion chromatograms of fungus comb in Station C and E confirmed this. Perylene was obviously absent in bottom part of comb called 'old fungus comb', and also in top part of comb called 'new fungus comb'. Interestingly, the isomer of perylene was found in two parts of combs. Therefore, in Stations of C and E there was no new fungus comb at top part of combs. This can be related to colony population size that is different in small and big nests (Adams, 1998; Tschinkel *et al.,* 1995).

3.4. Probably precursor of perylene

A number of studies have revealed that the lignin content decreases in old fungus comb of *M. ukuzii*, *M. natalensis* and *M. gilvus* (Rohrmann 1978; Hyodo *et al.* 2000). Grassé and Noirot (1958) proposed the 'lignin degradation hypothesis' which suggests that the symbiotic fungi have the ability to degrade lignin,

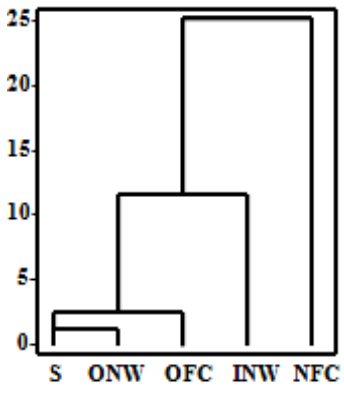

Figure 4. Dendrogram for samples from Station B, inner nest wall (INW), outer nest wall (ONW), Soil (S), old fungus comb (OFC) and new fungus comb (NFC) according to their perylene concentration and TOC pattern after hierarchical cluster analysis. Soil and outer nest wall (group 1), soil and old fungus comb (group 2), soil and inner nest wall (group 3) and soil and new fungus comb (group 4)

which makes cellulose more easily attacked by the termites' own cellulose. Meanwhile, the 'lignin degradation hypothesis' was examined by Hyodo *et al.* (2000) using CP/MAS 13C NMR to characterize lignin and to estimate an estimation of the in vitro digestibility of cellulose in fungus combs from the Southeast Asian fungus-growing termite, *M. gilvus.* They found that lignin, neutral carbohydrates and chitin concentrations showed significant differences among the different age combs: lignin concentration clearly decreased in old fungus comb with an accompanying increase of neutral carbohydrates and chitin. The results of CP/MAS 13C NMR revealed that signals around 110-160 ppm, especially at 154 ppm, corresponding to aromatic-C in lignin, decreased from new fungus comb towards old fungus comb. Therefore, it can be inferred that aromatic carbon rings in lignin play a major role as precursor of perylene. This assumption is based on their chemical structure and similar distribution pattern in new and old fungus comb, although further investigations are required in this field.

4. Conclusions

Our results support increasing evidence for large biological source of perylene in tropical environment that is associated with wide distribution of *M. gilvus* in southern Asia.

Perylene shows elevated concentration in fungus comb especially in new fungus comb and low concentration in old fungus comb and remaining samples. This can be concluded that perylene is produced inside the nest and in the hindgut of termite where exist anaerobic condition. Secondly, exist of biological source can be related to activity of termites and woody plants. Thirdly, it is inferred that due to high concentration of aromatic carbon rings of lignin in new fungus comb and also chemical structure of lignin it may be linked as precursor of perylene. Nevertheless, more research on this issue needs to be undertaken before the association between perylene and aromatic rings of lignin can be more clearly understood.

References

Abe T, Matsumoto T. Studies on the distribution and ecological role of termites in a lowland rain forest of west Malaysia. 3. Distribution and abundance of termites in Pasoh Forest Reserve. Japanese Journal of Ecology 1979; 29: 337-51.

Adams ES. Territory size and shape in fire ants: a model based on neighborhood interactions. Ecology 1998; 79: 1125-34.

Aizenshtat Z. Perylene and its geochemical significance. Geochimica et Cosmochimica Acta 1973; 37(3): 559-67.

Boonyatumanond R, Takada H, Toko A. The concentration of polycyclic aromatic hydrocarbon in surface sediment from canal, river, and coastal area in Thailand. 2nd Asian Pacific International Conference on Pollutants Analysis and Control. Ho Chi Minh City, Vietnam. 2003.

Brune A. Termites guts: the world's smallest bioreactors. Tibtech 1998; 16: 16-21.

Grassé P-P, Noirot C. Le meule des termites champignonnistes et sa signification symbiotique. Annales des Sciences Naturelles. Zoologie et Biologie Animale 1958; 11: 113-28.

Hyodo F, Inoue T, Azuma J-I, Tayasu I, Abe T. Role of the mutualistic fungus in lignin degradation in the fungus-growing termite Macrotermes gilvus (Isoptera; Macrotermitinae). Soil Biology & Biochemistry 2000; 32: 653-58.

Ishiwatari R, Ogura K, Horie S. Organic geochemistry of a lacustrine sediment (Lake Haruna, Japan). Chemical Geology 1980; 29: 261-80.

Kosmehl T, Hallare AV, Braunbeck T, Hollert H. DNA damage induced by genotoxicants in zebrafish (*Danio rerio*) embryos after contact exposure to freeze-dried sediment and sediment extracts from Laguna Lake (The Philippines) as measured by the comet assay. Mutation Research 2008; 650: 1-14.

Liu Y, Chen L, Jianfu Z, Qinghui H, Zhiliang Z, Hongwen G. Distribution and sources of polycyclic aromatic hydrocarbons in surface sediments of rivers and an estuary in Shanghai, China. Environmental Pollution 2008; 154: 298-305.

Nelson DW, Sommers LE. Total carbon, organic carbon, and organic matter. *In:* Methods of soil analysis, Part 3, Chemical methods (*Eds:* Sparks DL. *et al*.). SSSA, Madison, WI. 1996: 961-1010.

Ohkuma M, Maeda Y, Johjima T, Kudo T. Lignin degradation and roles of white rot fungi: study on an efficient symbiotic system in fungus-growing termites and its application to bioremediation. RIKEN Review 2001; 42: 39-42.

Opuene K, Agbozu IE, Ekeh LE. Identification of perylene in sediments: Occurrence and diagenetic evolution. Environental Science and Technology 2007; 4 (4): 457-62.

Rohrmann GF. The origin, structure and nutritional importance of the comb in two species of Macrotermitinae (Insecta, Isoptera). Pedobiologia 1978; 18: 89-98.

Roonwal ML. Termites of the Oriental region. *In:* Biology of Termites (*Eds*: Krishna K, Weesner FM). Academic Press London, London, Vol 2. 1970; 315-91.

Tschinkel WR, Adams ES, Macom T. Territory area and colony size in the fire ant, *Solenopsis invicta*. Journal of Animal Ecology 1995; 64: 473-80.

Unlu S, Alpar B. Distribution and sources of hydrocarbons in surface sediments of Gemlik Bay (Marmara Sea, Turkey). Chemosphere 2006; 64: 764-77.

Venkatesan MI. Occurrence and possible sources of perylene in marine sediments-a review. Marine Chemistry 1988; 25: 1-27.

Wakeham SG, Achaffner C, Glger G. Polycyclic aromatic hydrocarbons in recent lake sediments: II. Compounds derived from biogenic precursors during early diagenesis. Geochimica et Cosmochimica Acta 1980; 44: 415-29.

Wilcke W, Amelung W, Martius C, Garcia MVB, Zech W. Biological sources of polycyclic aromatic hydrocarbons (PAHs) in the Amazonian rain forest. Journal of Plant Nutrition and Soil Science 2000; 163: 27-30.

Wilcke W, Amelung W, Krauss M, Martius C, Bandeira A, Garcia MVB. Polycyclic aromatic hydrocarbon (PAH) patterns in climatically different ecological zones of Brazil. Organic Geochemistry 2003; 34: 1405-17.

Wilcke W, Krauss M, Lilienfein J, Amelung W. Polycyclic aromatic hydrocarbon storage in a typical Cerrado of the Brazilian savanna. Journal of Environmental Quality 2004; 33: 946-55.

Wood TG, Sands WA. The role of termites in ecosystems. *In:* Production ecology of ants and termites (*Ed*: Brian MV). Cambridge University Press, Cambridge, 1978: 254-92.

Zakaria MP, Takada H, Tsutsumi S, Ohno K, Yamada J, Kouno E, Kumata H. Distribution of polycyclic aromatic hydrocarbons (PAHs) in rivers and estuaries in Malaysia: a widespread input of petrogenic PAHs. Environmental Science and Technology 2002; 36: 1907-18.

Received 12 January 2009
Accepted 26 May 2009

Correspondence to
Dr. Mohamad Pauzi Zakaria
Center of Excellence in Environmental Forensics,
Faculty of Environmental Studies,
Universiti Putra Malaysia,
43400 Serdang, Selangor,
Malaysia
Tel: 60389466738
Fax: 60389468109
E-mail: mpauzi@env.upm.edu.my