Temporal and spatial distribution of mutagenic index in PM_{10} collected at Bangkok, Thailand

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Abstract

Temporal and spatial distributions of mutagenicity index (MI) extracted from PM_{10} samples collected during 2006 from seven monitoring stations in an urban residential area of Bangkok were carefully investigated. During the dry season (i.e. November–February), MI values of Salmonella typhimurium TA 98 was higher than those collected during the wet season (i.e. May–July). The addition of S9 mixture resulted in a 9.8% increase in total mean of the bacterial mutagenicity index. For S. typhimurium TA 100 without S9 mix, the total mean of mutagenicity index was 7.4% higher than those of S. typhimurium TA 98 at the same condition. The addition of S9 mix was also 9.1% increased in the mutagenicity index of S. typhimurium TA 100. The correlation of mutagenicity index between S9 mix added and non-added of the S. typhimurium TA 98 was higher than S. typhimurium TA100. All studied stations showed MI values lower than the safety level of 2.0.
Keywords: PM_{10}, PAH, mutagenicity index, Bangkok, Thailand.

1 Introduction

Anthropogenic activities in any megacities are known to increase particulate matters in the atmospheric environment. Among them, the fine particles (particles size smaller than 10 micron, PM_{10}) have been shown their impacts on

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human's respiratory tract. According to the Pollution Control Department of Thailand (PCD) standard, the safety limit of PM_{10} in the ambient air monitoring for 24 hours is 120 μg m^{-3}. One of the most important components of the PM_{10} is polycyclic aromatic hydrocarbons (PAHs), which are originated from incomplete combustion of gasoline. Because of its property as a carcinogen, World Health Organization (WHO) set PAHs (expressed as a concentration of Benzo[a]pyrene), safety limit at 1 ng m^{-3} [1-4]. PAH concentrations on PM_{10} are varied according to the traffic condition, therefore, traffic jam could lead to cancer in human.

The Ames test is a biological assay to assess the mutagenic potential of chemical compounds [5]. A positive test indicates that the chemical might act as a carcinogen (although a number of false-positives and false-negatives are known) [6]. As cancer is often linked to DNA damage, the test also serves as a quick assay to estimate the carcinogenic potential of a compound since it is difficult to ascertain whether standard carcinogen assays on rodents were successful. The procedure is described in a series of papers from the early 1970s by Bruce Ames and his group at the University of California, Berkeley. The test uses several strains of the bacterium Salmonella typhimurium that carry mutations in genes involved in histidine synthesis, so that they require histidine for growth. The variable being tested is the mutagen's ability to cause a reversion to growth on a histidine-free medium. The tester strains are specially constructed to have both frameshift and point mutations in the genes required to synthesize histidine, which allows for the detection of mutagens acting via different mechanisms. Some compounds are quite specific, causing reversions in just one or two strains [7]. The tester strains also carry mutations in the genes responsible for lipopolysaccharide synthesis, making the cell wall of the bacteria more permeable, and in the excision repair system to make the test more sensitive [8, 9]. Rat liver extract is optionally added to stimulate the effect of metabolism, as some compounds, like benzo[a]pyrene, are not mutagenic themselves but their metabolic products are [10]. The bacteria are spread on an agar plate with a small amount of histidine. This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have the opportunity to mutate. When the histidine is depleted only bacteria that have mutated to gain the ability to produce its own histidine will survive. The plate is incubated for 48 hours. The mutagenicity of a substance is proportional to the number of colonies observed.

As Salmonella is a prokaryote, it is not a perfect model for humans. An adapted in vitro model has been made for eukaryotic cells, for example yeast structure. The original test also doesn't count for metabolites that are formed by in the hepatic system. Modified tests can include liver S9 to help recreate the system and observe whether the parent molecule's metabolites formed in the hepatic system are positive. Further tests would be needed to determine the specific metabolite that causes a positive Ames to further any drugs development. Drugs that contain the nitrate moiety sometimes come back positive for Ames when they are indeed safe. Nitroglycerin is an example that gives a positive Ames yet is still used in treatment today. The conditions of the Ames test are dosed at very high concentrations and with nitrate compounds that

can potentially generate nitric oxide (NO), an important signal molecule, will give a false positive. Long toxicology and outcome studies are needed with such compounds to disprove a positive Ames test. This study intends to determine mutagenic index extracted from PM_{10} collected PCD monitoring stations located in Bangkok residential area quantified by using Ames test.

2 Methodology

2.1 Sampling site descriptions and monitoring period

To investigate the influence of traffic emissions and industrial activities on the genotoxicity of Bangkok atmosphere, PM_{10} samples were collected at the Community Housing Klongchan, Nonsi Witthaya School, Singharat Pittayakhom School, Thon Buri Electricity Sub, Chok Chai 4 Police Station, Dindang Community Housing and Bodindecha (Sing Singhaseni). For all monitoring sites, each sample covered a period of 24 hours taken at a normal weekday every month from January-December 2006 forming a database of 84 individual air samples (i.e. 12 x 7 = 84).

Air Quality Monitoring Stations Operated by Pollution Control Department, Bangkok, Thailand

Code	Sampling Site
54T	DHA (Dindang Housing Authority)
15T	WHS (Watsing High School)
53T	CPS (Chokchai 4 Police Station)
12T	NHS (Nonsree High School)
52T	EGAT (Electricity Generating Authority of Thailand)
10T	KHA (Klongchan Housing Authority)
61T	BHS (Badindecha High School)

Figure 1: PCD monitoring stations in Bangkok, Thailand.

2.2 Materials and methods

2.2.1 Microorganisms and culture medium

- *Salmonella typhimurium* strain TA 98 and TA 100.
- Oxoid nutrient broth NO.2 (Oxoid, England).

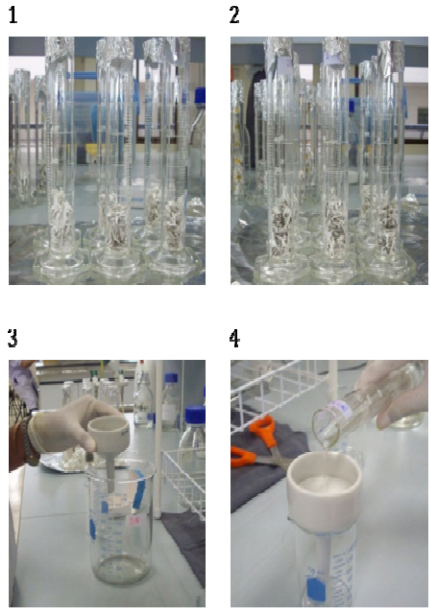

Figure 2: Extraction of mutagenic substances from PM_{10}.

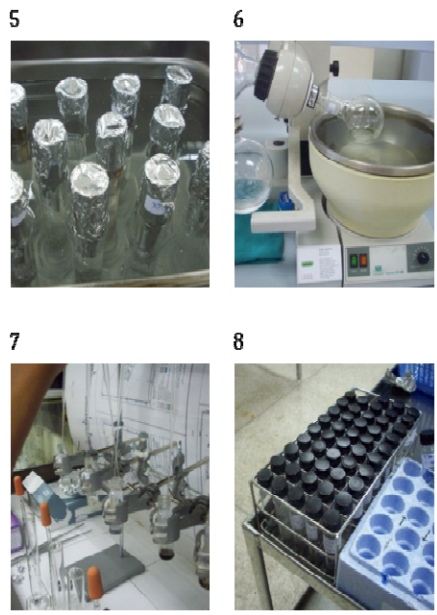

Figure 3: Evaporation of mutagenic substances.

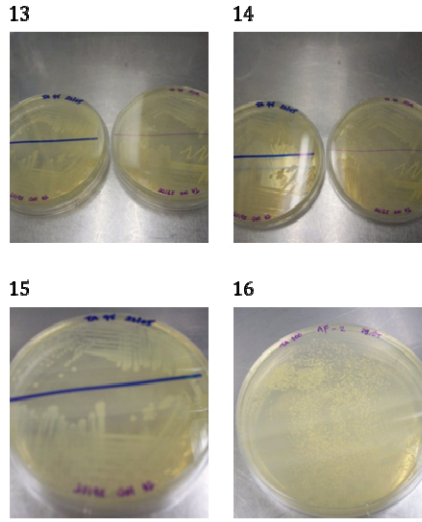

Figure 4: Microbiological plates.

2.2.2 Chemicals

- 2-(2-Furyl-3-(5-nitro-2-furyl) acrylamide) (AF-2) (Wako, Japan).
- β-D-Glucose-6-phosphate sodium salt (Sigma, England), β-Nicotinamide adenide di-nucleotide (β-NADP) (Sigma, USA.), Ampicillin (Sigma, USA.), Citric acid, monohydrate (Sigma, Australia), D-Biotin (Sigma, Hong Kong), Dimethyl sulfoxide (DMSO) (Sigma, USA.), Glucose anhydrous (Sigma, China), Glycerol (Sigma, USA) L-Histidine.HCl.H_2O (Sigma, USA.) NaCl (Sigma, USA.), NaOH (Sigma, Sweden) and $NH_4H_2PO_4$ (Sigma, Japan).
- Acetone (BDH, England).
- Dichloromethane (DCM), Na_2HPO_4 ,$Na_2HPO_4.H_2O$ and $MgSO_4.7H_2O$ (Merck, Germany).
- K_2HPO_4 anhydrous (Ajax, Australia).
- $MgCl_2.6H_2O$ (Fluka, Germany).

2.2.3 Analytical methods

PM_{10} collection and sample preservation were done according to the US-EPA compendium method IO-2.2 standard methods [11]. For each sample, mutagenic extraction was done twice at room temperature by using 40 ml. of dichloromethane per sample with ultra-sonication for 15 minutes at a time. The extracts were filtered through Whatman filter paper No. 42. Then the filtrates were dried before being dissolved in dimethyl sulfoxide (DMSO). Solutions were kept at -20˚C until used. The modified protocol of Aems test (Vinitketkumnuen et.al., 2002) using *Salmonella typhimurium* strain TA 98 and TA 100 was used for mutagenicity index determination. DMSO and AF-2 were used as negative and positive control respectively. Each test was done in six replications. The mutagenicity index was calculated as shown below.

Mutagenicity index = Colony counts on the test plate/Average counts on the negative control plates.

The ratio of ≥ 2 is considered as positive.

3 Results and discussion

The mutagenicity of extracts of the samples was compared in the Salmonella according to standard AMES test method. The dependence of the effects on sampling time and on sampling location was investigated with the aid of a calculation of mutagenic index (MI). This MI was used to estimate the increase in mutagenicity above background levels at the seven monitoring sites in urban area of Bangkok due to anthropogenic emissions within that area. Mutagenicity of the dry season PM_{10} extract of each station was higher than from the wet season. The similar results between the test without (a) and with S9 addition (b) were found (Figure 5). This could be the result of higher PM_{10} in dry season, which is normal. Addition of S9 resulted in 9% higher total mean of the mutagenicity index. This indicated the possible health hazard effect on human. Because S9 is imitated the function of liver that react with PHA. The higher mutagenicity index means the possible of causing cancer after entering into human. The different trend was found in *Salmonella typhimurium* TA 100 (Figure 6). Three peaks of mutagenicity index were found in the test without S9 addition (fig 6 a). They were in February July and September. There were no coincident to explain this observation. But it occurred in most of the stations. Addition of S9, 9.1% increased of TA100 mutagenicity index was observed. TA100 showed higher mutagenicity index than TA98 in the test both without and with S9 addition (7.4 and 6.7% respectively). Different mutagenicity index pattern of the two strains suggest for different chemicals that cause mutation on the bacteria. None of the test could reach the index value of 2, hence the PM_{10} extracts could not be stated as mutagen. Moreover, the correlation between "with" and "without" S9 addition are relatively low (table 1). Therefore, the PM_{10} extract of these stations can be considered as not harmful to public health.

In addition, applications of the AMES method showed that the average MI of PM_{10} collected at all sampling sites were 1.37 ± 0.10 (TA98; +S9), 1.24 ± 0.08 (TA98; -S9), 1.45 ± 0.10 (TA100; +S9) and 1.30 ± 0.09 (TA100; -S9) with relatively less variations. Comparison of the results obtained with the different AMES-test variants (i.e. +S9 and –S9) reconfirms that the particulate mutagenic concentrations measured at residential areas of Bangkok are moderately low in comparison with previous results observed in other countries.

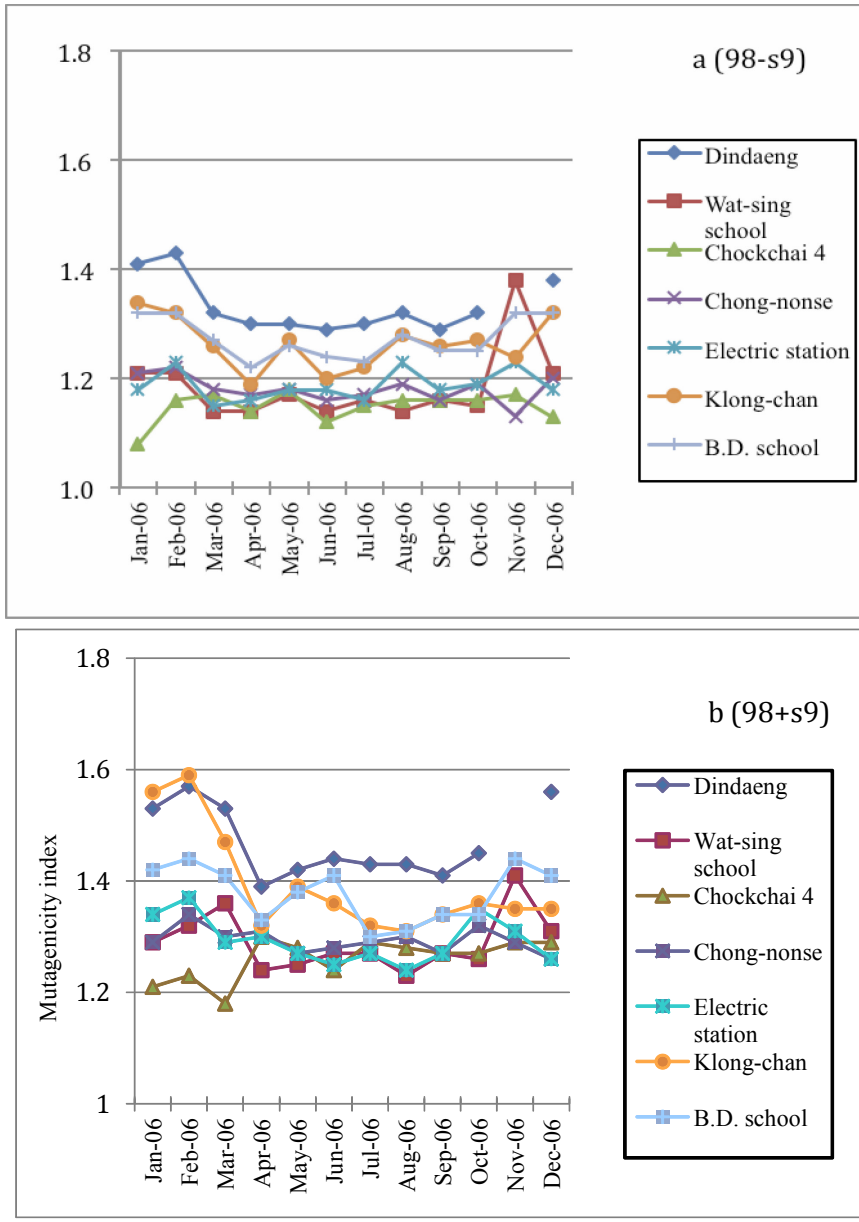

Figure 5: The profile of mutagenicity index of *Salmonella typhimurium* TA 98 with (b) and without (a) S9 addition.

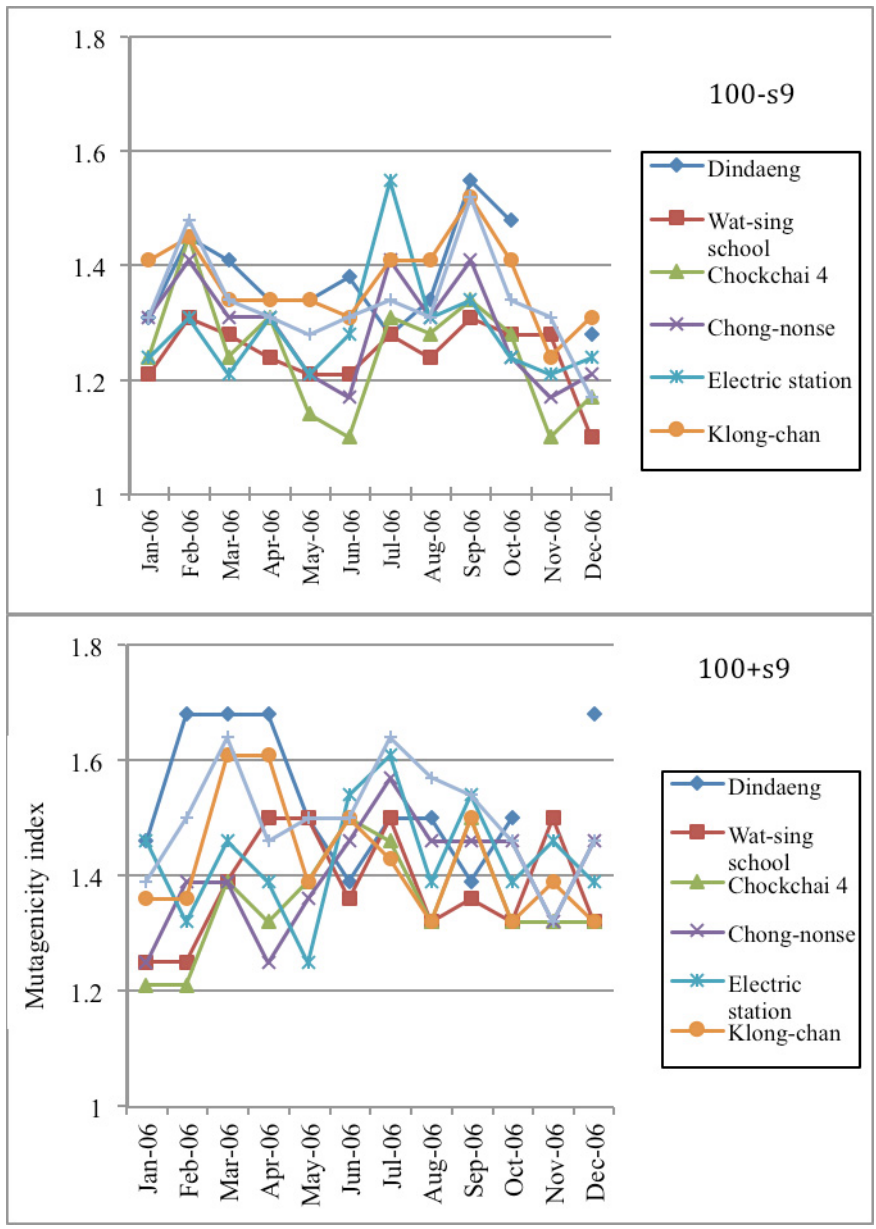

Figure 6: The profile of mutagenicity index of *Salmonella typhimurium* TA 100 with (b) and without (a) S9 addition.

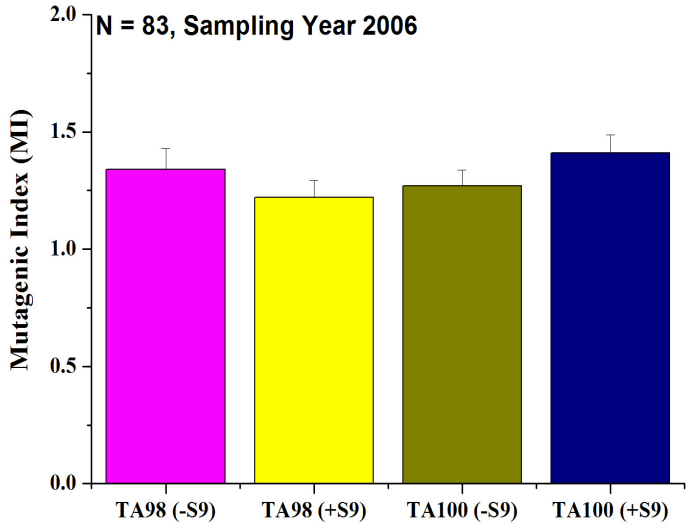

Figure 7: Comparison of MI values detected by TA98 & TA100 with and without S9.

Table 1: The correlation of mutagenicity index between the test without and with S9 addition.

	Correlation of mutagenicity index	
Stations	**TA98 (-/+ S9)**	**TA100 (-/+ S9)**
Dindaeng	0.86	-0.22
Wat-sing school	0.76	0.10
Chockchai 4	0.25	-0.27
Chong-nonse	0.36	0.20
Electric station	0.23	0.57
Klong-chan	0.64	-0.097
B.D. school	0.72	0.24

4 Conclusions

PAHs extract from PM_{10} samples show low degree of mutagenicity on both TA98 and TA 100 of *S. typhimurium*. The low mutagenicity index could suggest the safety of air in Bangkok in this aspect. The season dependent of the correlation between S9 -/+ on TA 98 suggested the changing of PAH composition in the extract of each season. The source of PAH could have been changed by some unknown reason.

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