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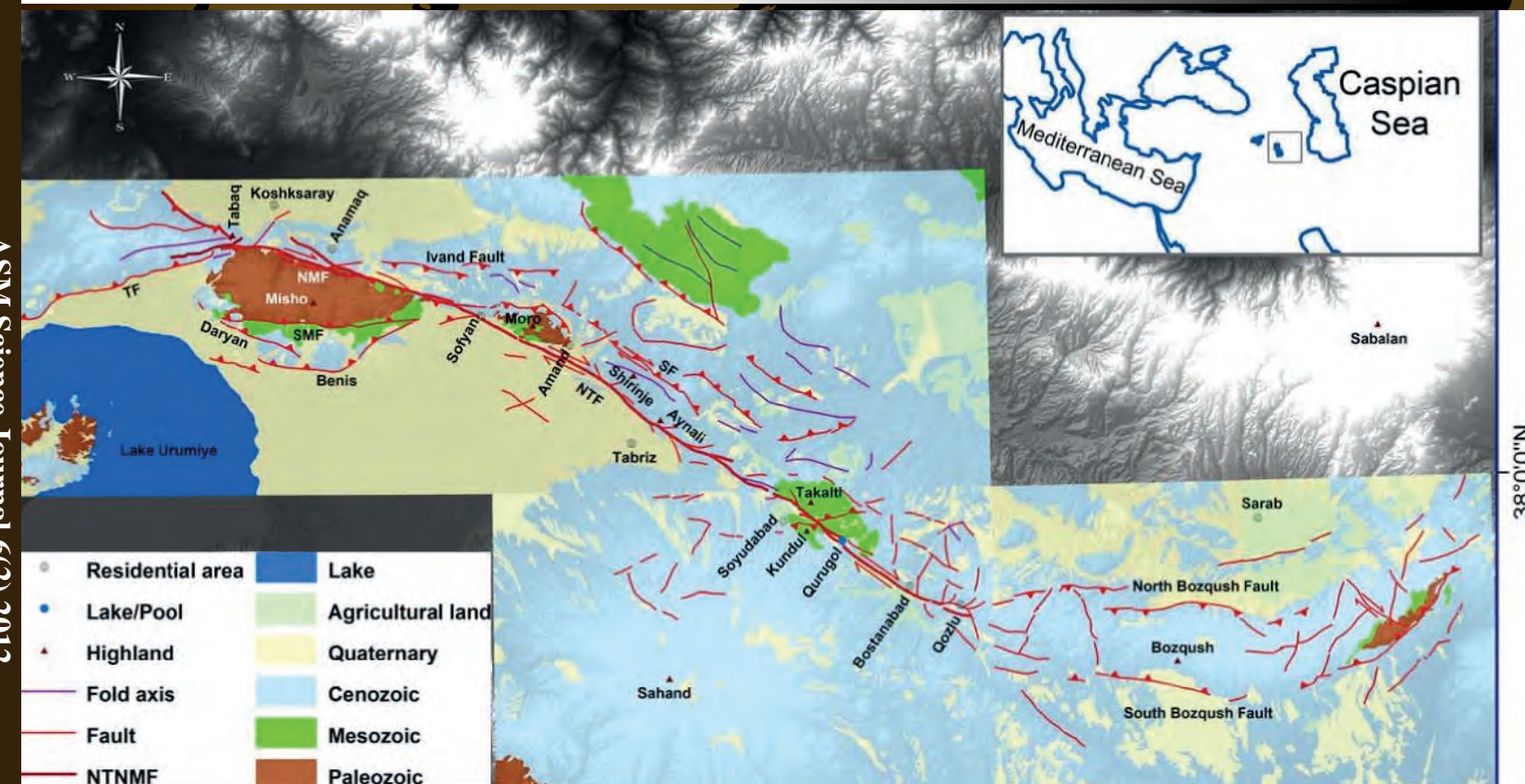


# ASM Science JOURNAL

In Pursuit of Excellence in Science

Vol. 6, No. 2, December 2012 • ISSN : 1823-6782

ASM Science Journal 6(2) 2012





**Price (2 Issues)**

Malaysia: RM100 (*Individual*)  
RM200 (*Institution*)

Other Countries: USD50 (*Individual*)  
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### Cover:

Both the figures/illustrations are depicted in the article (pp. 124–136) entitled *Longitudinal Profiles of Bedrock Rivers around North Tabriz and North Misho Faults: Implications for Geomorphic Fault Segmentation (Eastern Azerbaijan Province, Iran)*. The North Tabriz and North Misho faults (NTNMF) are major faults with transcurrent tectonics. The interactions of the different segments of the NTNMF with each other and with other major faults in the adjacent region have caused different patterns of deformation in this tectonic regime. The difference in the deformation patterns affects the morphology of streams. The correlation between the parameters of longitudinal profiles and lithostructural information were examined in this research article to investigate the effects of the NTNMF on the longitudinal profiles of streams.

The figure (pp. 109) on the upper section of the cover design is the shaded relief Digital Elevation Model (DEM) with lithologic units and geologic structures (fault and fold axes) compiled for this study. Figure in the lower section of the cover (Figure 7, pp. 116) shows the schematic model for the correlation between stream concavity index and structural regime along NTNMF.



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**ASM Science Journal is listed and indexed in Scopus.**

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- To act as a forum for maintaining awareness on the part of the Government of the significance of the role of science, engineering and technology in the development process of the nation and for bringing national development needs to the attention of the scientists, engineers and technologists
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# Anticonvulsant Activity of $\alpha$ -terpineol Isolated from *Myristica Fragrans* in GAERS Model of Absence Epilepsy

M.R. Islam<sup>1</sup>, S. Muthuraju<sup>1</sup>, C.H. Tarmizi<sup>1</sup>, M.M. Zulkifli<sup>1</sup>, H. Osman<sup>2</sup>,  
H. Mohamad<sup>3</sup> and J.M. Abdullah<sup>1\*</sup>

Epilepsy is a neurological disorder characterized by recurrent seizures resulting from excessive abnormal electrical discharges in the brain. Medicinal plants may play an invaluable role to discover the new antiepileptic drugs. The aim of the present study was to investigate the anticonvulsant activity of  $\alpha$ -terpineol isolated from *Myristica fragrans* Houtt. The  $\alpha$ -terpineol showed a significant inhibition of the seizure episodes and spikes in absence seizures model of Genetic Absence Epilepsy Rats from Strasbourg (GAERS) rats by using electroencephalography records. It showed dose-dependent anticonvulsant activity that was comparable to the known antiepileptic drug of diazepam. It showed a rapid onset and relatively short duration of anticonvulsant effects. The present findings suggest that  $\alpha$ -terpineol might possess antiepileptic activities against the partial seizures of human because it prevented seizures in well-established genetic absence seizure animal model of GAERS rats.

**Key words:** Epilepsy; electroencephalography; brain; plant extract; implantation; DSI telemetry

$\alpha$ -Terpineol (R-2-4-Methyl-3-cyclohexenyl isopropanol) is a constituent of *Myristica fragrans* Houtt. (Family: Myristicaceae) (Atta-ur-Rahman *et al.* 2000). The whole plant, nutmeg, in addition to its common use as a kitchen spice, alternatively it has been used as a stimulant, an antidiarrhoeal, carminative, stomachic, tonic, and as an aphrodisiac (Evans 1996; Nadkarni 1998). Nutmeg showed a wide array of pharmacological actions including being an analgesic (Sonavane *et al.* 2001), antifungal (Nadkarni 1998), antimicrobial (Takikawa *et al.* 2002), anti-inflammatory (Olajide *et al.* 1999) as well as having hepatoprotective (Morita *et al.* 2003) activities.

Numerous central nervous system activities have been reported for different methods of nutmeg extract. The chloroform extract of nutmeg possesses an analgesic effect against acetic acid-induced mice (Olajide *et al.* 1999), the trimyristin and the acetone insoluble fraction of the hexane extract of nutmeg seed demonstrated anxiogenic and antidepressant activity in mice (Sonavane *et al.* 2002; Dhingra & Sharma 2006). Recently, nutmeg oil showed the anticonvulsant activity against pentylenetetrazole (PTZ)-

induced seizure and hind limb tonic extension phase in the maximal electric shock (MES) seizure test (Wahab *et al.* 2009).

Medicinal plants have played and continue to play a valuable role in the drug discovery process (Abedin 1990; Heinrich 2000). Plant extracts can be an important source for the development of better and safer drugs for the treatment of epilepsy. Several plants that were reputed to possess antiepileptic properties in different folklore cultures have been found to exhibit anticonvulsant activities in different animal models (Raza *et al.* 2000). Epilepsy is the commonest neurological condition affecting people of all ages, race and social class. There are an estimated 50 million people with epilepsy in the world, of whom up to 75% live in resource-poor countries with little or no access to medical services or treatment (Meinardi *et al.* 2001; Bailer & White 2010). In general, the incidence of epilepsy in developed countries is taken to be around 40–70 per 100 000/year while the incidence of epilepsy in resource-poor countries is generally higher in the range of 100–190 per 100 000/year (Sander 2003; Sander & Shorvon 1996).

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The entire available antiepileptic drugs are synthetic molecules associated with side effects and approximately 30% of the patients continue to have seizures with this therapy (Poole *et al.* 2000; Bailer & White 2010). Several natural products including quinone derivatives, which are considered to screen better safety and efficacy profile, are known for their anticonvulsant effects (Hosseinzadeh and Parvardeh 2004). Recently we found that  $\alpha$ -terpineol is the most abundant compound of nutmeg oil isolated from *Myristica fragrans* exhibited the inhibitory effect on gamma amino butyric acid (GABA)<sub>A</sub> receptor *in vitro* (patent pending, Department of Neuroscience, University Sains Malaysia; Abdullah 2011). However,  $\alpha$ -terpineol has not been tested so far for *in vivo* anticonvulsant properties, though the plant extract is traditionally used against 'such disorders' (Wahab *et al.* 2009).

Greater insight into the pathophysiology underlying epilepsy can be achieved by the use and study of experimental animal models (Dedeurwaerders 2005). Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are strain of Wistar rats, originally bred in Strasbourg (Marescaux *et al.* 1992), and are generally accepted as an experimental animal model of human absence seizure, because GAERS share many clinical characteristics with typical human absence epilepsy (Danover *et al.* 1998). All GAERS show idiopathic (genetic) generalized seizures characterized by paroxysmal unresponsiveness to environmental stimuli and cessation of ongoing activity. During the epileptic episodes, the electroencephalography (EEG) of GAERS displays the bilateral and synchronous spike and wave discharges (SWDs), predominantly over the fronto-parietal cortex (Van Hese *et al.* 2003). The number, duration, and frequency of SWDs increase reaching a maximum at the age of four to six months by which time 100% of GAERS have developed SWDs (Pinault *et al.* 2001). GAERS represent a useful animal model for the evaluation and development of new antiepileptic drugs, as it is known that drugs that are effective against human absence seizures also suppress SWDs in GAERS dose-dependently (Large *et al.* 2012). Therefore, the present study aimed to investigate the anticonvulsant activity of  $\alpha$ -terpineol in GAERS rat model.

## MATERIALS AND METHODS

### Isolation of $\alpha$ -terpineol

The nutmegs were collected from Balik Pulau, Penang Island in Malaysia in July, 2009 (Figure 1). The dried kernels of the ripe seed of *Myristica fragrans* (nutmeg) about 8 kg were used in this study. The kernels of nutmeg were chopped into small pieces and placed carefully into the water distiller up to the appropriate level. The distilled water was poured into the water distiller up to half full. At the end of the opening part, a small vial filled with hexane solvent was connected into the distiller. The boiling process

took approximately 6 to 8 hours. Once the water distiller was cooled, the mixture of hexane solvent containing essential oils in a small vial was concentrated using rotary evaporator to remove the waste of hexane. Then the pure essential oil of nutmeg was stored in a refrigerator at 8°C. Structural elucidation of the compound was carried out by using gas chromatography-mass spectrometer (GC-MS; Agilent, Atlanta, GA, USA) with HP-5MS column (diameter 0.25 mm, length 30 m, film thickness 0.25  $\mu$ m) and helium gas as the carrier. The inlet temperature of gas chromatography was set to 280°C with splitless mode. 1  $\mu$ l of sample was injected into the GC-MS system. Gas flow was set to 1.2 ml/min. The initial oven temperature was 70°C, it was increased to 280°C and trap low temperature at -30°C, and the final temperature was maintained for 18 min. The automatic peak detection and mass spectrum was performed for a total runtime of 26 min (Goren *et al.* 2003). During this period, a total of 16 components were detected from nutmeg oil and identified as  $\alpha$ -pinene,  $\beta$ -terpinene,  $\alpha$ -phellandrene, 3-carene,  $\alpha$ -terpinene, *p*-cymene, limonene,  $\gamma$ -terpinene,  $\gamma$ -terpinolene, 2-norbornanol, borneol, 4-terpineol,  $\alpha$ -terpineol, myristicin and elemicin (Table 1). The chromatogram of identified compounds is shown in Figure 2. The most abundant compound was  $\alpha$ -terpineol (28.05%). The structure of  $\alpha$ -terpineol is depicted in Figure 3.

### Animals

A total 35 (thirty five) male GAERS rats, four to six months of age and weighing 187 g – 325 g were used in this study. The parent GAERS rats were gift a from Kyoto University, Japan. The animals were born and raised under environmentally controlled conditions (12 h light/dark cycles, 20°C–22°C in the animal facility house of the USM Health Campus, with food and water *ad libitum*. All animals were treated according to the guidelines approved by the Animal Ethics Committee of USM.

### Chemicals

Diazepam (Hospira, Malaysia),  $\alpha$ -terpineol (Department of Chemistry, USM) and 0.9% saline were used in this experiment.

### Surgery: Implantation of DSI telemetry

Prior to surgery (5 min – 15 min), the animals were anesthetized with ketamin and xylazine (80 mg/kg and 7.5 mg/kg, respectively, i.p.) and additional ketamine (5 mg/kg, i.p.) were given during surgery when a sensorial pain stimulus by squeezing the footpad, elicited motor reflexes (Dedeurwaerdere *et al.* 2005). After proper anesthesia, the fur on the head and back were clipped rostral to the medial canthus of the eyes to immediately cranial to the last cervical vertebra in a strip approximately 3 cm wide. The animals were placed on a heating pad and secured in



Figure 1. Collecting zone of nutmeg in Penang Island, Malaysia, latitude: +5.35 (5°19'36"N) and longitude: +100.233 (100°12'03"E).

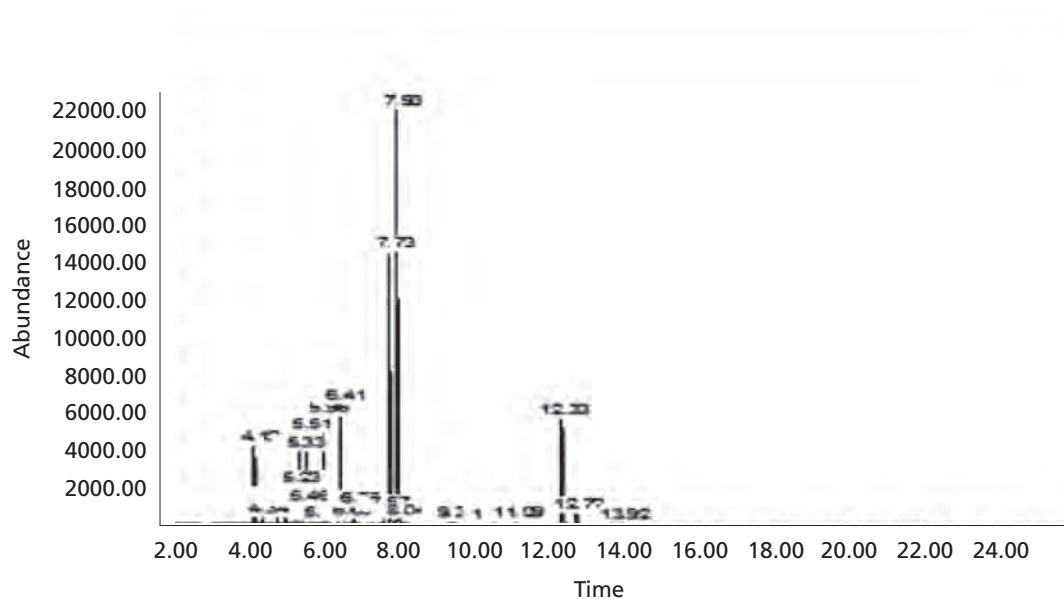


Figure 2. Chromatogram of the essential oil of nutmeg showing peaks of chemical substances.

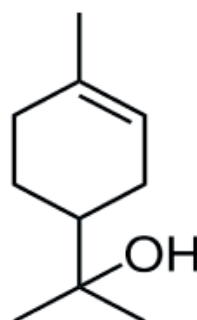


Figure 3. Structure of  $\alpha$ -terpineol.

Table 1. The constituents of nutmeg oil isolated from *Myristica fragrans* detected by GC-MS method.

Peak	RT (min)	Area %	Library/ID	Chemical formula
1	4.12	4.62	$\alpha$ -Pinene	C <sub>10</sub> H <sub>16</sub>
2	4.75	1.39	$\beta$ -Pinene	C <sub>10</sub> H <sub>16</sub>
3	4.95	1.31	$\beta$ -Terpinene	C <sub>10</sub> H <sub>16</sub>
4	5.15	1.53	$\alpha$ -Phellandrene	C <sub>10</sub> H <sub>16</sub>
5	5.24	2.50	3-Carene	C <sub>10</sub> H <sub>16</sub>
6	5.33	5.66	$\alpha$ -Terpinene	C <sub>10</sub> H <sub>16</sub>
7	5.46	1.19	<i>p</i> -Cymene	C <sub>10</sub> H <sub>14</sub>
8	5.51	7.41	Limonene	C <sub>10</sub> H <sub>16</sub>
9	5.96	7.06	$\alpha$ -Terpinene	C <sub>10</sub> H <sub>16</sub>
10	6.41	8.23	Terpinolene	C <sub>10</sub> H <sub>16</sub>
11	6.79	1.23	2-Norbornanol	C <sub>7</sub> H <sub>12</sub> O
12	7.57	1.03	Borneol	C <sub>10</sub> H <sub>18</sub> O
13	7.74	18.48	4-Terpineol	C <sub>10</sub> H <sub>18</sub> O
14	7.93	28.05	$\alpha$ -Terpineol	C <sub>10</sub> H <sub>18</sub> O
15	12.33	9.14	Myristicin	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub>
16	12.71	1.17	Elemicin	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>

Table 2. Effect of  $\alpha$ -terpineol on seizures of GAERS rat model.

Treatment group (mg/kg, i.p.)	No. of seizures (24 hrs) Mean $\pm$ S.E.M.	No. of spikes (24 hrs) Mean $\pm$ S.E.M.	% Protection of seizures Vs control		% Protection of spikes	
			First 6 h of injection	18 h of injection	First 6 h of injection	18 h of injection
0.9% Saline (300 $\mu$ l/kg)	1218.5 $\pm$ 22.4	23749 $\pm$ 498	0	0	0	0
$\alpha$ -terpineol (10 mg/kg)	1163.8 $\pm$ 20.3	23508 $\pm$ 475	7.15	5.40	0.81	0.62
$\alpha$ -terpineol (20 mg/kg)	1029.0 $\pm$ 16.8*	20281 $\pm$ 371*	53.41	20.12	54.42	18.95
$\alpha$ -terpineol (50 mg/kg)	917.2 $\pm$ 15.9*	17838 $\pm$ 343*	93.73	32.52	94.76	33.0
Diazepam (10 mg/kg)	863.8 $\pm$ 26.8*	16767 $\pm$ 301*	94.68	38.25	97.39	39.69

The data were expressed as mean  $\pm$  S.E.M., n = 7. The data analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. \*p<0.05 compared to the control group.

a stereotaxic apparatus (Stoelting Model 51600; Illinois, U.S.A.). The surgical site and surrounding area were swabbed with 70% ethyl alcohol and scrubbed with a 4% chlorohexidine solution. A 3 cm – 4 cm mid-sagittal incision was made on the scalp and the skin reflected with hemostats to expose the entire dorsal portion of the skull. The periosteum was removed and homeostasis achieved with sterile cotton-tip applicators. Bregma was marked and two holes were bored through the skull with drilling (# 105 drill bit). Stainless steel electrodes (DSI Model F40-EET; St. Paul, MN, U.S.A.) insulated, except at the tip were implanted bilaterally into the brain over the frontal and parietal cortex. The other two electrodes were placed in the neck muscle of EMG recording to compare with the EEG spikes. A EEG electrodes were fixed to the skull of the rat with dental acrylic. The radio telemetry unit was placed subcutaneously into the pocket over and caudal to the scapula. Using blunt-ended scissors, a subcutaneous pocket was made caudally from the incision by pushing aside connective tissue and

then the skin was sutured. The method of telemetry implantation was followed from White *et al.* (2006; 2010). The surgical procedures of our experiment were considered as minimum to mild pain scale according to the pain assessment, and it was managed by local anesthesia (Kohn *et al.* 2007).

### Spikes and Seizures Monitoring and Quantification

The EEG activity was acquired by DSI Dataquest telemetry software and analyzed off-line using DSI Neuro-Score software (St. Paul, MN, USA), configured for automatic detection and saving of spikes and seizures. The following was the setting for seizure detection: amplitude threshold, 3; seizures duration, > 2 s; detection threshold, 0; minimum frequency, 3 Hz; short burst detection, turned off; length of EEG kept before and after each seizure, 1 min. After implantation, baseline EEG was recorded for 1 week. Analysis of EEG was performed by a 'blinded' unbiased investigator. All seizure EEGs were revised manually.



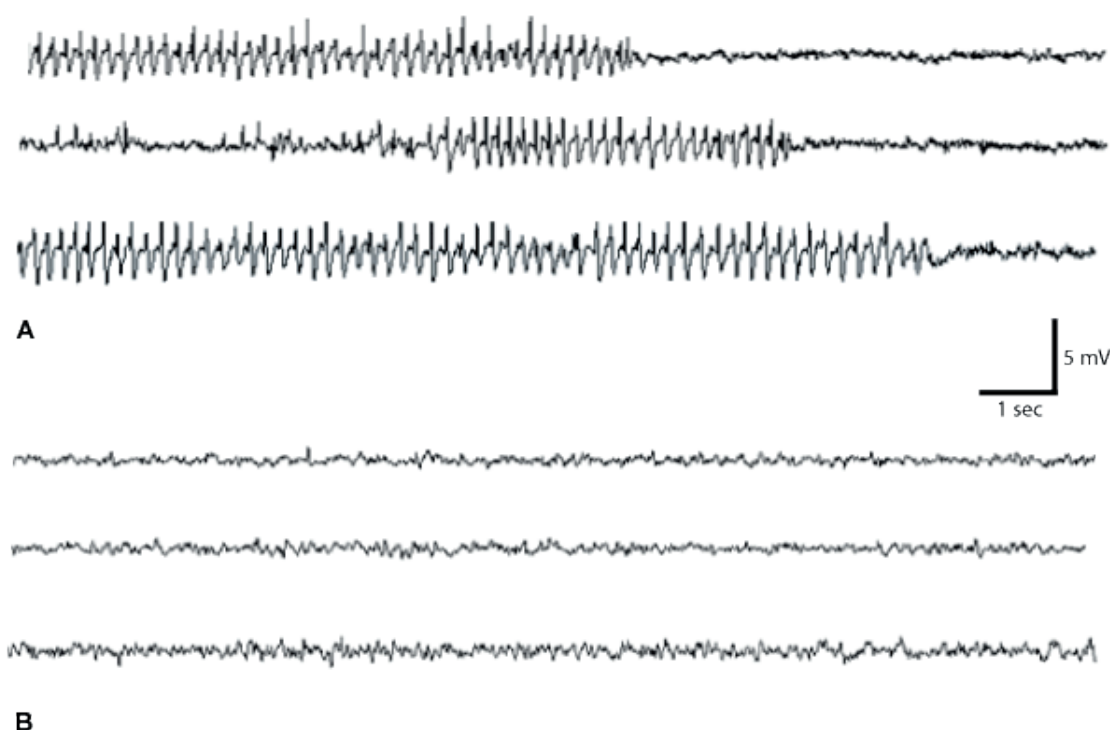


Figure 4. Electroencephalographic activity recorded before and after  $\alpha$ -terpineol treatment (50 mg/kg, i.p.) from the same GAERS rat. Panel A was recorded prior to treatment; shows spikes and wave discharges (SWDs) surrounded by normal background EEG. Panel B was recorded after  $\alpha$ -terpineol treatment; shows no SWDs in EEG.

The amplitude threshold for spike detection was set at 4. The spike bursts lasting less than 3 s were not counted as seizures.

### Statistical Analysis

The values were expressed as mean  $\pm$  S.E.M. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by multiple comparison test of Dunnett's t-test. P values  $< 0.05$  were considered as significant.

## RESULTS

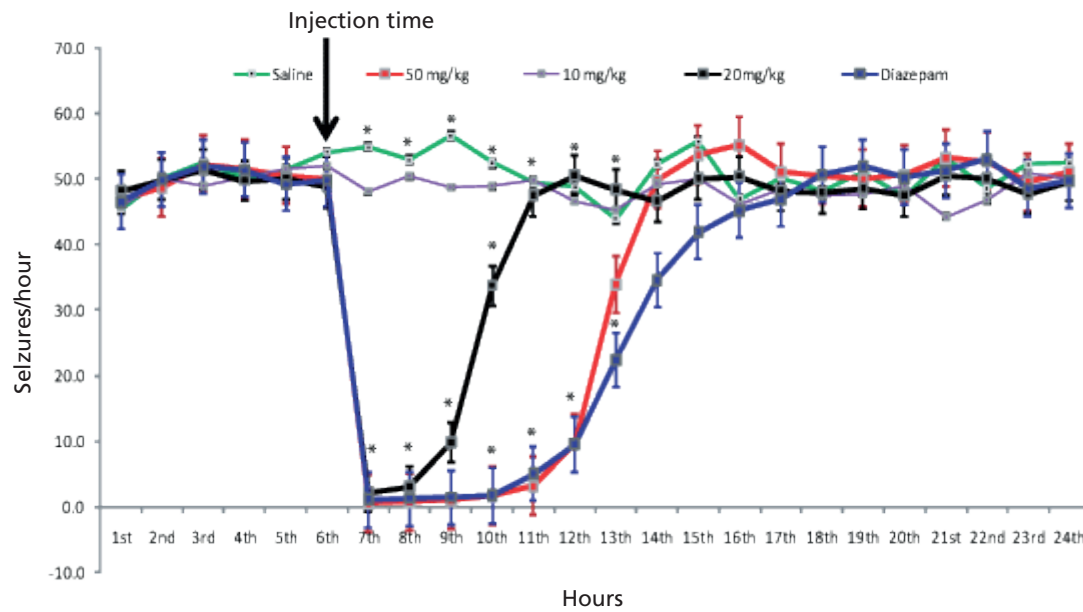
### Behavioural Effects

The injections of  $\alpha$ -terpineol isolated from *Myristica fragrans*, diazepam and 0.9% saline were given at day 6 after determining the baseline EEG. No mortality was observed in the treated animals. A dose of 10 mg/kg (i.p.)  $\alpha$ -terpineol induced no behavioural effects. A dose of 20 mg/kg showed the rearing and sleep after 6 m – 7 min of injection and lasted for 2 h. A dose of 50 mg/kg resulted in rearing, sleep, no stimuli reflex within 3 min – 4 min of injection, and lasted up to 5 h in GAERS rats compared

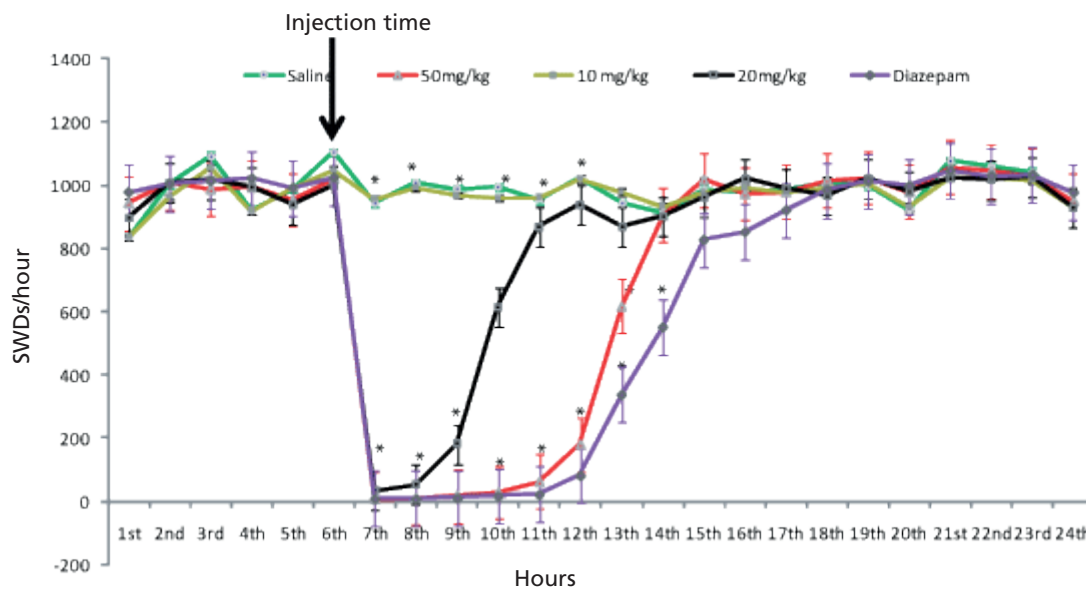
to control animals. Diazepam (dose of 10 mg/kg, i.p.) treatment showed similar behavioural effects but was lasting longer up to 6 hrs; whereas 0.9% of saline treated animals induced no behavioural symptoms.

### Antiepileptic Effects

An intraperitoneal dose of 10 mg/kg  $\alpha$ -terpineol was less effective to control seizures and spike and wave discharges (SWDs) in GAERS rats (Table 2 and Figure 5), but 20 mg/kg and 50 mg/kg (i.p.) decreased the number of seizure episodes and number of SWDs (Table 2; Figures 4 and 5), and almost completely stopped seizures for 2 h and 5 h, respectively. Then the number of seizure episodes and SWDs gradually increase to non-treated control animals (Figure 5). Although 10 mg/kg was ineffective, however 50 mg/kg reduced seizure episodes and SWDs to 93.73% and 94.76%, respectively in first 6 h, and 32.52 and 33.0%, respectively after 18 h of injection in comparison to the control animals. The known antiepileptic drug diazepam (10 mg/kg, i.p.) showed the similar results and the seizures episodes and SWDs were reduced to 94.68% and 97.39%, respectively in first 6 h, and 38.25 and 39.69%, respectively at 18 h of injection in GAERS compared to the control group of animals. The saline treated animals showed no changes before and after treatments (Figure 5).



A



B

Figure 5. Time course of seizure frequency and effects of treatment on seizures and SWDs. A: Significantly lower seizure episodes were observed after treatment with 20 and 50 mg/kg, i.p. of  $\alpha$ -terpineol compared to the control group. B: Effects of treatment on SWDs distribution; SWDs were also significantly reduced after treatment compared to the control group. The data were shown as mean  $\pm$  S.E.M., \*p<0.05 vs. control and n = 7.

## DISCUSSION

The observations with of the present study indicated that  $\alpha$ -terpineol possesses the dose-dependent anticonvulsant activity against the GAERS rats. GAERS are generally accepted as an experimental animal model of human absence epilepsy due to GAERS share many clinical characteristics with typical human absence epilepsy (Danober *et al.* 1998). GAERS is a useful animal model for the evaluation and development of new antiepileptic drugs, since it is known that drugs effective against human absence seizures also suppress SWDs in GAERS dose dependently. The quantification of the occurrence of SWDs in GAERS is an important tool to assess the efficacy of new antiepileptic therapy for human absence epilepsy (Shaw 2007). Recently, one of our co-investigator found that the  $\alpha$ -terpineol extracted from *Myristica fragrans* exhibited the inhibitory effect on GABA<sub>A</sub> receptors *in vitro* on *Xenopus* oocyte. The conventional standard antiepileptic drugs (benzodiazepines, phenobarbital, valproate, vigabatrin, gabapentin) generally enhance GABA<sub>A</sub> receptor inhibition (Loscher & Schemidt 1994; Czapiński *et al.* 2005). The novel antiepileptic drugs (tiagabine, vigabatrin, gabapentin) also act selectively through the GABA-ergic system by the inhibition of GABA-aminotransferase or reducing the neuronal and glial uptake of GABA (Czapiński *et al.* 2005).

Nutmeg oil of *Myristica fragrans* exhibited anticonvulsant activity against PTZ-induced seizures, MES test, lithium-pilocarpine-induced status epilepticus in mouse and rat models, and also showed an acceptable safety profile by acute toxicity and acute neurotoxicity tests (Sonavane *et al.* 2002; Wahab *et al.* 2009). Previous studies on the constituents of nutmeg oils revealed several compounds such as linalool and pinene analogues have anticonvulsant activities in seizure susceptible mouse (Consroe *et al.* 1981; Elesabetsky & Brum 2003). Moreira *et al.* (2001) reported  $\alpha$ -terpineol, another major constituent of nutmeg oil, induced the dose-dependent and reversible blockade of the compound action potential propagation of the rat sciatic nerve that supports our present finding. In conclusion, the present study confirmed that  $\alpha$ -terpineol was an important constituent of nutmeg oil and provided scientific evidence for traditional use for the treatment of epilepsy. Thus, it might be effective for the treatment of partial seizures in humans and  $\alpha$ -terpineol was a possible candidate of new antiepileptic drugs for human absence seizures.

## ACKNOWLEDGEMENTS

This work was supported by an institutional grant from U.S.M. Research University Individual grant (No. 1001/PSKBP/812019), Universiti Sains Malaysia, Malaysia. Dr Rafiqul Islam (a TWAS-USM Post-doctorate Fellow from the Department of Anatomy and Histology, Bangladesh

Agricultural University, Mymensingh-2202, Bangladesh) is acknowledged for his work related mainly in conducting the experiment and analysis. We also wish to show our appreciation to the distributor of the GAERS rats used in this experiments, i.e. the Institute of Laboratory Animal, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Japan.

*Date of submission: December 2011*

*Date of acceptance: July 2012*

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# An Improved Estimator for the Population Mean Using Additional Information

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An improved estimator that made use of information on auxiliary variables and variables of interest which was not drawn up yet was proposed to estimate the population mean of the variable under study. Under simple random sampling without replacement, the mean square error up to the first order of approximation was derived. An empirical study was also carried out to show the properties and efficiency of the proposed estimator.

**Key words:** Auxiliary variable; ratio-type; product-type estimators; efficiency comparison

Consider a finite population  $U = U_1, U_2, \dots, U_N$  of  $N$  units. Let  $y$  and  $x$  stand for the variable under study and auxiliary variable respectively. Let  $(y_i, x_i)$ ,  $i = 1, 2, 3, \dots, n$  denote the values of the units included in a sample  $s_n$  of size  $n$  drawn by simple random sampling without replacement (SRSWOR). In order to have a survey estimate of the population mean  $\bar{Y}$  of the study character  $y$ , assuming the knowledge of the population mean  $\bar{X}$  of the auxiliary character  $x$ , the well known ratio estimator is:

$$t_r = \bar{y} \left( \frac{\bar{x}}{\bar{X}} \right) \quad (1)$$

and the conventional product estimator is given by:

$$t_p = \bar{y} \left( \frac{\bar{x}}{\bar{X}} \right) \quad (2)$$

The mean square error (MSE) equations of these estimators are given by:

$$MSE(\bar{y}_r) = \frac{1-f}{n} \bar{Y}^2 [C_y^2 + C_x^2 - 2\rho C_y C_x] \quad (3)$$

$$MSE(\bar{y}_p) = \frac{1-f}{n} \bar{Y}^2 [C_y^2 + C_x^2 + 2\rho C_y C_x] \quad (4)$$

where,  $f = \frac{n}{N}$   $C_y = \frac{s_y}{\bar{Y}}$   $C_x = \frac{s_x}{\bar{X}}$

Using information on the auxiliary variable and the variable of interest not yet drawn, Adewara (2006) proposed the following estimators:

$$\bar{y}_{mr} = \frac{\bar{y}^*}{\bar{x}^*} \bar{X} \quad (5)$$

$$\text{and, } \bar{y}_{mp} = \frac{\bar{y}^* \bar{x}^*}{\bar{x}} \quad (6)$$

where,  $\bar{x}$  and  $\bar{y}$  are the means of the auxiliary variables and variable of interest yet to be drawn.

The relationship between  $\bar{X}$ ,  $\bar{x}$  and  $\bar{x}^*$  is:

$$\bar{X} = f \bar{x} + (1-f) \bar{x}^* \quad (7)$$

While that between  $\bar{Y}$ ,  $\bar{y}$  and  $\bar{y}^*$  is:

$$\bar{Y} = f \bar{y} + (1-f) \bar{y}^* \quad (8)$$

see Srivenkataramana and Srinath (1976).

The MSE expressions of  $\bar{y}_{mr}$  and  $\bar{y}_{mp}$ , respectively, are:

$$MSE(\bar{y}_{mr}) = \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 [C_y^2 + C_x^2 - 2\rho C_y C_x] \quad (9)$$

$$MSE(\bar{y}_{mp}) = \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 [C_y^2 + C_x^2 + 2\rho C_y C_x] \quad (10)$$

Singh and Agnihotri (2008) defined a family of ratio product estimators of population mean  $\bar{Y}$  as:

$$t_{rp} = \delta \bar{y} \left( \frac{a\bar{x}+b}{a\bar{x}+b} \right) + (1-\delta) \bar{y} \left( \frac{a\bar{x}+b}{a\bar{x}+b} \right) \quad (11)$$

where,  $a$  and  $b$  are known characterizing positive scalars and  $\delta$  is a real constant to be determined such that the MSE of  $t_{rp}$  is minimum.

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### Proposed Estimator

Adapting the Adewara (2006) method to Singh and Agnihotri (2008) estimator, we propose an improved estimator as:

$$t_{mrp} = \delta \bar{y}^* \left( \frac{a\bar{x}+b}{a\bar{x}^*+b} \right) + (1-\delta) \bar{y}^* \left( \frac{a\bar{x}^*+b}{a\bar{x}+b} \right) \quad (12)$$

where,  $a$  and  $b$  are either real numbers or the functions of the known parameters of the auxiliary variable,  $x$ , such as coefficient of variation ( $C_x$ ), kurtosis [ $\beta_2(x)$ ] or correlation coefficient ( $\rho$ ). For details see Khosnevisan *et al.* (2007) and Singh *et al.* (2007).

Let,

$$\bar{x}^* = \bar{X} \left[ 1 - \left( \frac{n}{N-n} \right) e_1 \right]$$

$$\bar{y}^* = \bar{Y} \left[ 1 - \left( \frac{n}{N-n} \right) e_0 \right],$$

$$\bar{x} = \bar{X}(1 + e_1) \text{ and } \bar{y} = \bar{Y}(1 + e_0)$$

Such that:

$$E(e_0) = E(e_1) = 0$$

$$\text{and } E(e_1^2) = \frac{N-n}{nN} C_x^2, \quad E(e_0^2) = \frac{N-n}{nN} C_y^2,$$

$$E(e_0 e_1) = \frac{N-n}{nN} \rho C_y C_x$$

Expressing in terms of  $e$ 's, we have:

$$t_{mrp} = \bar{Y} \left\{ 1 + \left( \frac{n}{N-n} \right) e_0 \right\} \left[ \delta \left[ \frac{a\bar{x}+b}{a\bar{x} \left\{ \left( 1 - \frac{n}{N-n} \right) e_1 \right\} + b} \right] + (1-\delta) \frac{a\bar{x} \left\{ \left( 1 - \frac{n}{N-n} \right) e_1 \right\} + b}{a\bar{x}+b} \right] \right. \\ \left. \bar{Y} \left\{ 1 + \left( \frac{n}{N-n} \right) e_0 \right\} \left[ \delta \left\{ 1 - \left( \frac{n}{N-n} \right) \theta e_1 \right\}^{-1} + (1-\delta) \left\{ 1 - \left( \frac{n}{N-n} \right) \theta e_1 \right\} \right] \right] \quad (13)$$

$$\text{where, } \theta = \frac{a\bar{X}}{(a\bar{X}+b)}$$

We assume that  $|\theta e_1| < 1$  so that  $(1 - \theta e_1)^{-1}$  is expandable. From Equation 13 to the first order of approximation we have:

$$t_{mrp} \cong \bar{Y} \left( \frac{n}{N-n} \right) [1 + e_0 + e_1 \theta (2\delta - 1)] \quad (14)$$

Squaring both side of Equation 14 and neglecting terms of  $e$  having power greater than two we have:

$$(t_{mrp} - \bar{Y})^2 = \bar{Y}^2 \left( \frac{n}{N-n} \right)^2 [e_0^2 + e_1^2 \theta^2 (2\delta - 1)^2 + 2\theta e_0 e_1 (2\delta - 1)] \quad (15)$$

Taking expectation of both sides of Equation 15 we get the MSE of  $t_{mrp}$  to the first degree of approximation as:

$$MSE(t_{mrp}) = \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 [C_y^2 + C_x^2 \theta^2 (2\delta - 1)^2 + 2\theta \rho C_y C_x (2\delta - 1)] \quad (16)$$

This is minimised for:

$$\delta = \frac{1}{2} \left( 1 + \frac{k}{\theta} \right) = \delta_0 \text{ (say)} \quad (17)$$

Substitution of Equation 17 in Equation 16 yields the minimum MSE of  $t_{mrp}$  as:

$$MSE(t_{mrp})_{\min} = \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 C_y^2 (1 - \rho^2) \quad (18)$$

**Remark 1:** If we take  $a=1, b=0$  and  $\delta = 1$  in Equation 12, we get the estimator  $\bar{y}_{mr}$  as proposed by Adewara (2006). If we also take  $a = 1, b = 0$  and  $\delta_0$  in Equation 12, we get the estimator  $\bar{y}_{mp}$  proposed by Adewara (2006).

### Efficiency Comparison

We have compared the efficiencies of the proposed estimator under optimum conditions with the estimators  $\bar{y}$ ,  $\bar{y}_r$ ,  $\bar{y}_p$ ,  $\bar{y}_{mr}$  and  $\bar{y}_{mp}$ .

First we compared the MSE of the proposed estimator under optimum conditions with the sample mean  $\bar{y}$ ,  $MSE(t_{mrp})_{\min} < MSE(\bar{y})$

if,

$$\bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 C_y^2 (1 - \rho^2) < \bar{Y}^2 \left( \frac{N-n}{nN} \right) C_y^2$$

On rearranging the terms, we got:

$$\left( \frac{n}{N-n} \right)^2 (1 - \rho^2) < 1 \quad (19)$$

This was always true, for any value of  $\rho, n$  and  $N$ .

Next we compared MSE of the proposed estimator under optimum conditions with the MSE's of  $\bar{y}_{mr}$  and  $\bar{y}_{mp}$ .

$$MSE(t_{mrp})_{min} < MSE(\bar{y}_{mr})$$

$$\begin{aligned} \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 C_y^2 (1-\rho^2) < \\ \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 [C_y^2 + C_x^2 - 2\rho C_y C_x] \\ \left( \rho - \frac{C_x}{C_y} \right)^2 > 0 \end{aligned} \quad (20)$$

This was always true.

Similarly,

$$MSE(t_{mrp})_{min} < MSE(\bar{y}_{mr})$$

$$\begin{aligned} \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 C_y^2 (1-\rho^2) < \\ \bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 [C_y^2 + C_x^2 + 2\rho C_y C_x] \\ \left( \rho + \frac{C_x}{C_y} \right)^2 > 0. \end{aligned} \quad (21)$$

This was also always true.

Finally we compared the MSE of the proposed estimator under optimum conditions with the MSE of  $\bar{y}_r$  and  $\bar{y}_p$ ,

$$MSE(t_{mrp})_{min} < MSE(\bar{y}_r)$$

$$\bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 C_y^2 (1-\rho^2) < \bar{Y}^2 \left( \frac{N-n}{nN} \right) [C_y^2 + C_x^2 - 2\rho C_y C_x] \quad (22)$$

As we have already shown that  $MSE(t_{mrp})_{min} < MSE(\bar{y}_{mr})$  and Adewara (2006) has shown that  $MSE(\bar{y}_{mr}) < MSE(\bar{y}_r)$ , hence it is obvious that  $MSE(t_{mrp})_{min}$  would be less than  $MSE(\bar{y}_r)$ .

Similarly we can write expressions for:

$$MSE(t_{mrp})_{min} < MSE(\bar{y}_p)$$

$$\bar{Y}^2 \left( \frac{N-n}{nN} \right) \left( \frac{n}{N-n} \right)^2 C_y^2 (1-\rho^2) < \bar{Y}^2 \left( \frac{N-n}{nN} \right) [C_y^2 + C_x^2 + 2\rho C_y C_x] \quad (23)$$

### Data Statistics

In this section, we tried to show that theoretical findings are correct by using two populations (1 and 2) earlier used by Adewara (2006) and Khoshnevisan *et al.* (2007).

Table 1. Data used for the analysis.

Population	N	n	Y	X	Cy	Cx	$\rho$
Population 1	2010	100	26.30	117.28	0.9468	0.7443	0.67
Population 2	20	8	19.55	18.8	0.3553	0.3943	-0.92

Table 2. Showing estimators with corresponding MSE's.

Estimator	Population 1	Population 2
$\bar{y}$	5.8915	3.6175
$\bar{y}_r$	3.3263	—
$\bar{y}_p$	—	0.6871
$\bar{y}_{mr}$	0.0092	—
$\bar{y}_{mp}$	—	0.3054
$(t_{mrp})_{min}$	0.0089	0.2473

## CONCLUSION

From Table 2, we concluded that the proposed estimator performed better than other competing estimators discussed in the paper.

*Date of submission: October 2011*

*Date of acceptance: May 2012*

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# Longitudinal Profiles of Bedrock Rivers around North Tabriz and North Misho Faults: Implications for Geomorphic Fault Segmentation (Eastern Azerbaijan Province, Iran)

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The North Tabriz and North Misho faults (NTNMF) comprise a dextral strike-slip fault system in a compressional-extensional regime of complex geometry, the displacements along the different segments of the main and subsidiary faults affect the longitudinal profiles of streams. This study presents detailed observations of longitudinal profiles that have been affected by deformation. Two domains are distinguishable along the NTNMF, as indicated by the response of the longitudinal profiles to related vertical displacements. The domain that surrounds the North Misho fault is characterised by concave longitudinal profiles and high  $H^*$  values (the difference between the estimated elevation and the actual elevation of the intersection point on the profile). The domain that surrounds the North Tabriz fault is characterized by generally less concave or even slightly convex longitudinal profiles and either no knickpoint or low  $H^*$  values. Sub-domains of each domain can also be recognised. The NTNMF affects the longitudinal profiles of streams by three mechanisms: (i) crushing the fault zone and lowering the base level; (ii) creating local, sometimes temporary, compressional-extensional or compressional-contractional regimes through subsidiary faults or interaction with other faults; and (iii) dividing major, probably more independent, deformational zones. This paper shows that within the study region, the stream responses to geological forces can be effectively controlled through lithostructural transitions in the tectonic regimes where flow occurs and through spatial relationships between the streams and geologic structures (such as faults, folds, fault-induced scarps, and landslides).

**Key words:** Strike-slip fault; longitudinal profiles; lithostructural transition; concavity index;  $H^*$  values; stream-fault bearing; deformation patterns; tectonics

Stream channels have been subjected to many studies that have aimed to decipher tectonics. Some have examined the relationship between a general tectonic regime, a drainage area, and the stream behaviour (Duvall *et al.* 2004; Carretier *et al.* 2006; Chen *et al.* 2006; Robel *et al.* 2008), while others have examined streams flowing on landscapes around specific faults (Kendrick *et al.* 2002; Dorsey & Roering, 2006; Whittacker *et al.* 2008). The longitudinal profile is a direct tool used for the representation of streams. Conventional analysis of longitudinal profiles (i.e. analysis of elevation versus distance and slope versus distance) are used by many researchers for landscape evaluation and interpretation of tectonic activities (Hack 1957, Hack 1973; Keller and Rockwell 1984; Reed 1981; Demoulin 1998; Bishop & Goldrick 2000; Bishop *et al.* 2005; Chen *et al.* 2006;

Goldrick & Bishop 2007; Phillips & Lutz 2008; Rehak *et al.* 2008). Recently, a method based on the slope area relationship has become common in longitudinal profile analysis. This method is used to assess the response of drainage areas to tectonic signals (Snyder *et al.* 2000; Kirby & Whipple 2001; Kirby *et al.* 2003; Duvall *et al.* 2004; Carretier *et al.* 2006; Wobus *et al.* 2006; Robl *et al.* 2008; Whittacker *et al.* 2008) and, more specifically, the effects of faults on drainage areas and landscape evolution (Dorsey & Roering 2006; Demnsmore *et al.* 2007). In this study, the conventional analysis is chosen because of the available DEM's precision (SRTM 90 m) and in consideration of the remarks by Snyder *et al.* (2000) and Goldrick and Bishop (2007) who reported scatter in slopes as a result of low resolution of DEMs, even for USGS 30 m DEM.

The North Tabriz and North Misho faults (NTNMF) are strike-slip faults within a transcurrent tectonic regime. The physiography of the adjacent region is strongly controlled by the activity of these faults and tectonomagmatic history of the region. This in turn, affects the characteristics of drainage networks and in the case of this study, stream longitudinal profiles. The study of longitudinal profiles along the strike of the faults can provide information about the patterns of vertical relative displacement. The DS form of the longitudinal profile can be used to distinguish the equilibrium profile from the disequilibrium profile. One is a steepening response to a more resistant lithology, whereas the other is a transient channel steepening response due to knickpoint propagation because of vertical relative displacement. Long-profile disequilibrium is resolved over time by the upstream passage of a wave of incision (a knickpoint). While resistant lithologies reduce the rate of knickpoint propagation, non-resistant lithologies allow rejuvenation to be readily propagated headwards and for graded (equilibrium) long profiles to be re-established (Bishop & Goldrick 2010). Moreover, the study of correlations between the parameters of longitudinal profiles and lithostructural information can provide estimates about the effect of deformation on the longitudinal profiles.

This study aims to distinguish the effects of different deformation patterns along the strike of the NTNMF on stream longitudinal profiles. In this regard, the following were determined: (i) the concavity index and  $H^*$  values (see "Database and Method" section); (ii) the relationship between concavity and stream-fault bearing; and (iii) the correlation between the results obtained in steps (i) and (ii) and the lithostructural information.

### Geologic and Climatic Setting

The North Tabriz fault is a major tectonic structure in a geologically complex domain, which is situated in the Alpine-Himalayan fold-thrust fault belt between the Caspian Sea and the Black Sea. This region is included as a part of the east Anatolian-Iranian plateau (Koçyiğit *et al.* 2001), as well as the Lesser Caucasus and the Talish block (Golonka 2002). The Pliocene, this region southeast of the Black Sea and southwest of the Caspian Sea has been affected by three tectonic processes: (i) a force applies in a roughly northward direction because of counterclockwise rotation and convergence of the Arabian plate with respect to Eurasia (Talebian & Jackson 2002; Vernant *et al.* 2004); (ii) a relative relaxation in the westward direction as a result of dextral North Anatolian and sinistral East Anatolian transform faults, with the consequent WSW escape of the Anatolian Platelet (Koçyiğit *et al.* 2001); and (iii) the collision of the Indian plate and Lut block with Eurasia, resulting in the formation of a system of NW-SE-trending transform faults (Golonka 2004) that imposes limits in the south and east directions. As a result, this region has been undergoing transcurrent deformation.

### Lithostructure along NTNMF

According to Eftekharnajad (1975), the North Tabriz fault (NTF) is old and during the Early Devonian Ages, it had been dividing the Azerbaijan region into two different blocks. Geometrically, the North Tabriz and North Misho faults, also known collectively as the North Tabriz fault, generally extend in the NW-SE direction (Figure 1). They consist of different segments with slightly varying azimuths and dips. Their azimuth increases in the clockwise direction from  $\sim 101^\circ$  to  $\sim 123^\circ$  from the northwest, where the central part of North Misho fault (NMF) intersects with the Tasuj fault (TF) towards the southeast, at the west end of the Bozqush mountain range. Their general dip has been suggested as vertical (Berberian & Arshadi 1976; Aghanabati 2006); however according to Moradi *et al.* (2008), their vergence varies along the strike. The displacements along the different segments of this fault zone are combinations of right-lateral and reverse components (Berberian & Yeats 1999; Vernant *et al.* 2004; Hesami *et al.* 2003; Karakhanian *et al.* 2004). These faults, owing to their transcurrent deformation and complex geometry, and through the interactions of various segments with other major faults or high-order faults and the interactions among themselves, have produced several areas of upthrow and subsidence on different scales. Thus, these faults control the lithology of the adjacent region.

The NMF and South Misho fault (SMF), together with their subsidiary faults, have upthrown the heights of the Misho mountain range (Figure 1). The NMF divides older Proterozoic Mesozoic lithologies, which are mainly predominated by pelletic micaceous shale with fine grained sandstone, carbonate rocks, and embedded intrusive rocks from younger Cenozoic Quaternary lithologies, which mostly consist of marl, sandstone, volcanic breccia, conglomerate and Quaternary sediments. The northwest half of the NTF divides younger Quaternary sediments from generally older Mesozoic-Cenozoic lithologies, which are predominated by marl, sandstone, and carbonate rocks. Near the northwest end of this northwest half, Precambrian-Mesozoic and Quaternary units have been juxtaposed owing to the raising of the Moro Mountain by the displacements along this fault and the presence of a subsidiary arc-shaped reverse fault to its northeast. Here, the lithologies mainly consist of carbonate rocks and conglomerate, with few outcrops of intrusive rocks. The southeast half of the NTF separates older Cenozoic lithologies, which consist mainly of red sandstone with marl, and red conglomerate from Plio-Quaternary pumice, volcanic ash, and conglomerate units formed by volcanic activity at Sahand Mountain. Further, at the southwest side of this southeast half there are outcrops of plutonic-subvolcanic rocks that were emplaced during the Tertiary. Along the central part of this southeast half of the NTF, sandy and marly limestone, calcareous shale, and

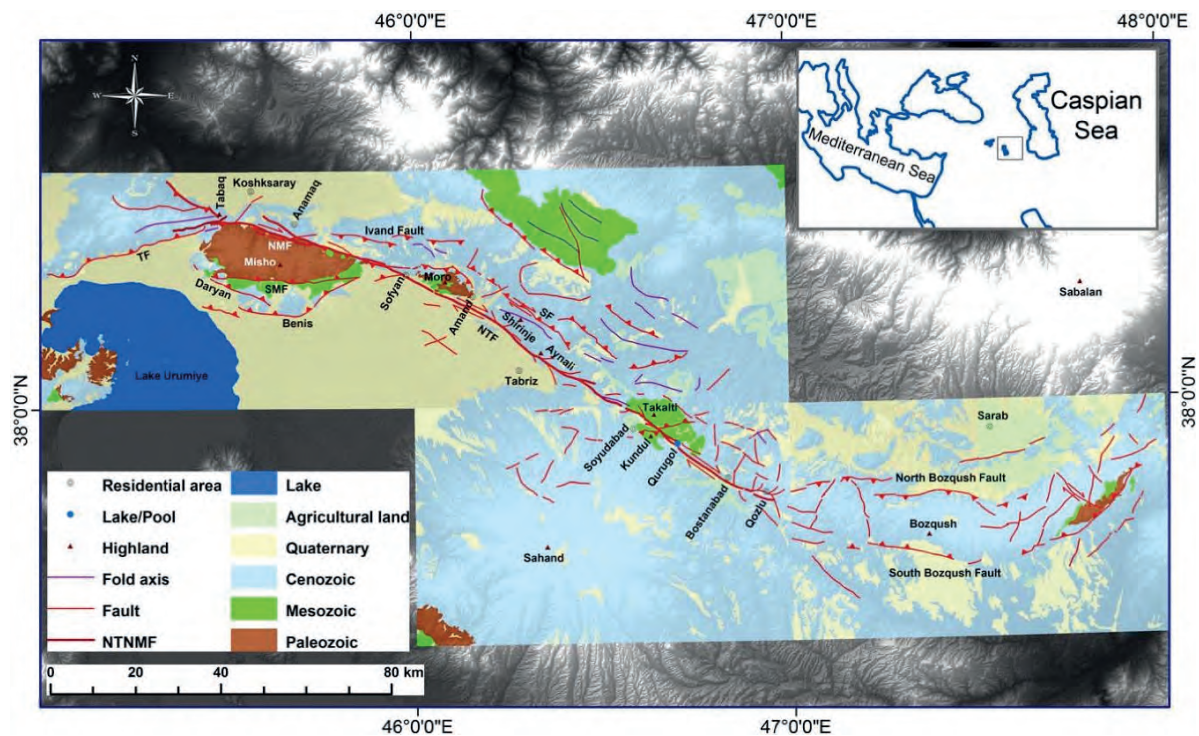


Figure 1. Shaded relief DEM with lithologic units and geologic structures (fault and fold axes) compiled for this study. Iranian 1:100 000 geologic maps were digitized. Shown are the North Misho fault (NMF), North Tabriz fault (NTF), Shirinje fault (SF), South Misho fault (SMF), and Tasuj fault (TF).

conglomerate have been exposed because of interactions of different NTF segments.

### Climate

The annual amount of precipitation in northwest Iran (West Azerbaijan, East Azerbaijan, and Ardabil provinces) ranges from 194 mm and 866 mm. However, the precipitation in the area drained by the selected streams only ranges from 194 mm to 336 mm. The northwest part of the study region generally receives more than 284 mm, i.e. between 293 mm and 308 mm. In the central part of the study region, the precipitation in the area drained by the selected streams ranges from 269 mm to 336 mm. The Moro Mountain, however, receives only 284 mm – 293 mm. In the southeast part of the study region, the annual precipitation ranges from 269 mm to 308 mm. In short, most of the streams drain the area with annual precipitation of less than 293 mm, while the highest annual precipitation occurs in the northwest and northeast parts of the study region.

### Database and Method

Longitudinal profiles were derived from SRTM 90-m digital elevation modelling (DEM) data. A routine method was applied to extract the stream longitudinal profile data by routing flow on a stream network in an ArcInfo

environment. Then, using scripts in MATLAB, repeated elevations in a profile were filtered so that each vertex in a profile had a unique elevation. Finally, the elevation data was processed by cubic interpolation to obtain vertices with a constant 10-m elevation interval along the profile. As usual, elevation and downstream distance were the parameters correlated to construct the longitudinal profile, while slope and downstream distance were the parameters used in the logarithmic DS plot.

The intersection points of lithologic boundaries and faults with streams were extracted by digitizing and using geological maps of the study region (Asadian 1993; Asadian *et al.* 1993; Asadian *et al.* 1994; Khodabandeh & Amini-fazl 1993; Khodabandeh & Amini-fazl 1995; Behrouzi *et al.* 1997; Faridi & Hagh-farshi 2006). The routes of 153 channels were selected by considering the traces of the NTNMF and their subsidiary faults as well as physiographic landscapes (Figure 2). The lengths of the profiles ranged from 2 km to 264 km. The criteria for the selection of channels were as follows: (i) whether the NTNMF was crossed or not; (ii) the position of the stream divide and/or the outlet around the NTNMF; (iii) the presence of landscapes formed by the NTNMF and their subsidiary faults (i.e. relay zones, fault damage zones etc.); and (iv) the azimuth of the stream route.



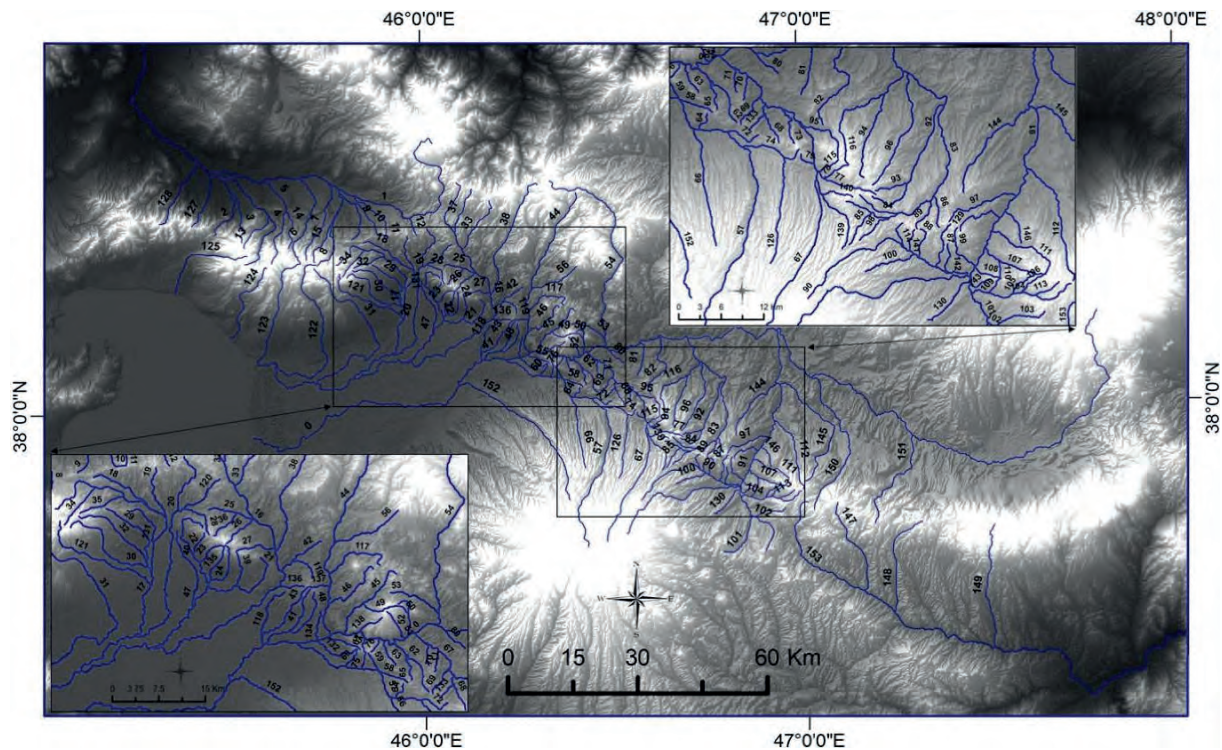


Figure 2. Index map of streams examined in this study, with shaded relief map in background. Labels are parallel to streams.

Following Bishop and Goldrick (2000) and Goldrick and Bishop (2007), the concavities of the longitudinal profiles were determined from  $\lambda$ . The constants  $\lambda$  and  $\gamma$  were determined from a linear regression of slope versus distance on a log-log plot (DS plot). Positive and negative  $\lambda$  values indicate concave and convex profiles, respectively. Using the DS plot, the knickpoints (disequilibrium steepening) along profiles crossing the NTNMF were determined. Generally following Bishop *et al.* (2005), two or three long profile vertices upstream of the knickpoint were projected linearly onto the intersection point of the fault and channel. Next, the elevation of the downstream projection of the reach above the knickpoint was estimated at the point where the NTNMF crosses the channel. Then, the difference between the estimated elevation and the actual elevation of the intersection point on the profile (i.e.  $H^*$ ) was calculated (Figure 3). In these profiles, the first knickpoint upstream of the NTNMF was considered as uplift triggered by the youngest activity of the NTNMF. The values of  $\lambda$  and  $H^*$  are illustrated in Figure 4.

## RESULTS

To facilitate recollection and visualization, the study region was divided into three zones along the strike of the NTNMF. The first zone (Z1) included the north and south slopes of the Misho mountain range. The second zone (Z2) included the area between the east boundary of the Misho

mountain range and Aji-chay River (channel 0). The third zone (Z3) extended from Aji-chay River to the west end of the Bozqush mountain range (Figure 4).

### Correlation between $\lambda$ , $H^*$ , and lithostructures

*Misho mountain range (Z1).* On the north slope of the Misho mountain range (northern Z1), considering the fairly constant, moderate gradient of the downstream part of longitudinal profiles that included Quaternary sediments and the presence of knickpoints with fairly high values of  $H^*$  (Figures 4 and 5a) and in view of the lithostructural setting along the NMF (i.e. coexistence of faults and lithologic boundaries), the concavity of the profiles was attributed to (i) the enhanced but constant uplift whose rate increased towards the divide (Robl *et al.* 2008) and (ii) the presence of more resistant units upstream and less competent units downstream (Duvall *et al.* 2004).

Interactions of the NTF, NMF, and Ivand faults east of the Misho mountain range (eastern Z1) had produced compression north of the NTF, where the northwestward change in the downstream direction of some streams was a manifestation of eastward uplift escalation. The northward extension of compression was a probable reason for the increased concavity, where this extension produced compensated vertical displacement around the NTF and thus steady state conditions along the profiles.

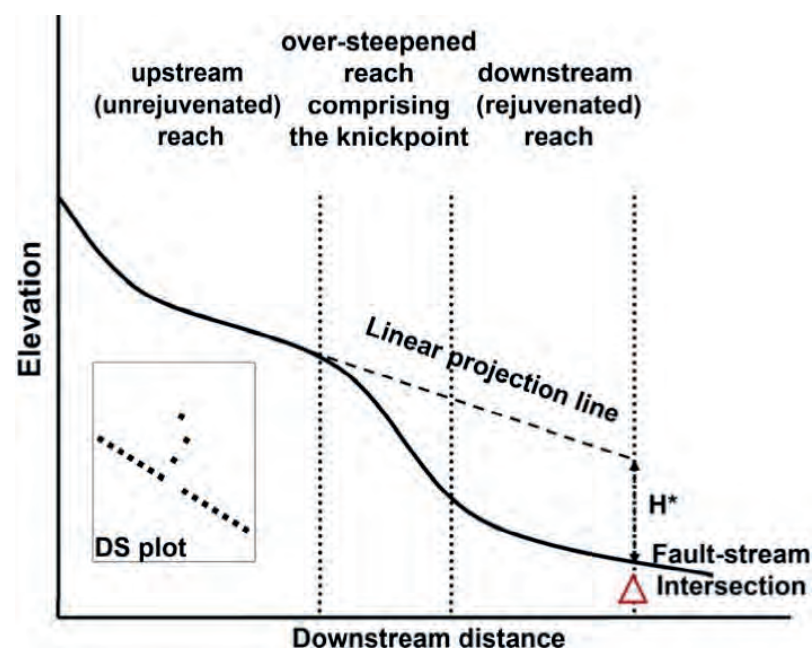


Figure 3. Longitudinal profile with disequilibrium steepening (knickpoint) caused by vertical relative displacements of fault, propagating headwards. Disequilibrium steepening appears as a disordered outlier on the DS plot.

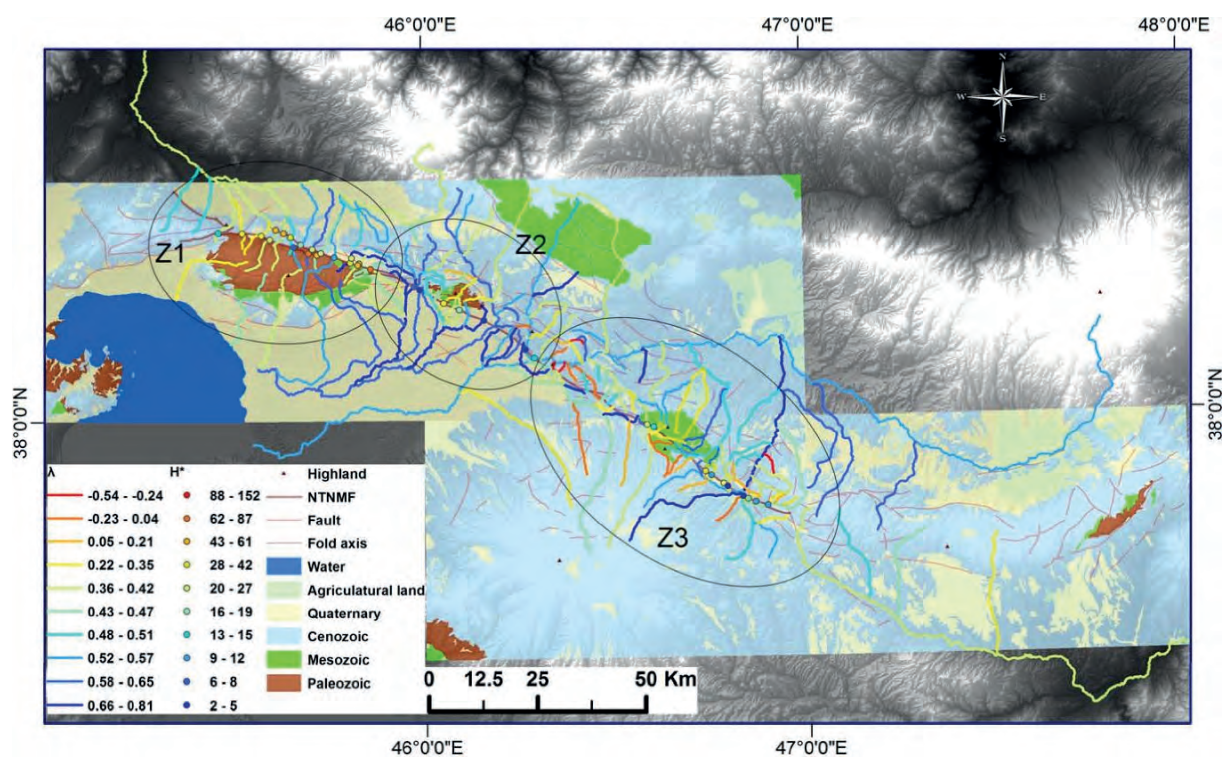


Figure 4. Map of concavity index ( $\lambda$ ) and difference ( $H^*$ ) between elevation predicted by linear projection and actual elevation at the fault-stream intersection point, as determined for streams in neighbourhood of NTNMF. The background consists of shaded relief and lithologic maps.



The lesser concavity of the central and western parts in comparison with the eastern part could be explained by a wider, less smooth, and gentler steepening zone. This could be partly ascribed to not only the presence of a lithologic boundary (i.e. granite-pelletic micaceous shale with fine-grained sandstone) (channels 4 and 5) but also the interactions of central and western segments of the NMF with the Tasuj fault as well as the interaction of the central and southeast segments of the NMF that increased the rate and extent of uplift (channels 2, 3, and 13).

The presence of both long and short longitudinal profiles along the NMF allowed a comparison of the concavity for different lengths. The longer streams had a greater concavity in comparison with the shorter ones. Considering that, lithologically, the changing parameter was shorter flow on less resistant Plio-Quaternary lithologies (channels 7 and 15), the predominance of structural factors could be inferred from the decreased concavity near the NMF, where the deformation was more intensive due to displacements along the NMF and its subsidiary faults and uplift was more effective with respect to erosion.

There was no remarkable difference between the longitudinal profiles of the south and north slopes (Figures 4 and 5b). On the south slope of the mountain range (southern Z1), the relatively high concavity of some profiles could be attributed to (i) the enhanced uplift of upstream parts where major vertical displacements occurred north of major faults (i.e. SMF, Benis, and Daryan) and (ii) the very low gradation of downstream parts in the case of streams that flowed longer distances but away from active major faults and under more stable conditions (Wobus *et al.* 2006). The elevated uplift around the conjunction of the NMF, SMF, and NTF (eastern Z1) as a result of their interaction, where the mountain front progressed southward and Quaternary sediments were elevating, was responsible for less concave profiles (Figure 4, channels 29, 30, 32, 34, and 35).

*Moro and Shirinje Mountains (Z2).* On the southeast slope of the Moro Mountain (central Z2), the NTF bisected most profiles, so their divides and outlets lay not far away from it. In that regard, the creation of less concave profiles with a constant, relatively high gradation (Figure 5c), as well as the presence of knickpoints with moderate values of  $H^*$ , could be explained by (i) more resistant lithologies with dense faulting upstream and (ii) ongoing uplift on both sides of the NTF, where Quaternary sediments were elevating and the mountain front progressed southwestward because of transpressional uplift (Figure 4, channels 23, 24, 47, and 135).

On the northeast slope of the mountain the presence of upstream steepening in some profiles and the prevalence of constant, moderate downstream gradation in nearly all profiles (Figure 5d) could be attributed to lithostructural

factors. For the reason that faults were generally also lithologic boundaries, dense faulting in the area with resistant lithologies which experienced a compressional regime would postpone the morphotectonic transition from a high-relief compressional regime to a low-relief stable or extensional regime along the longitudinal profiles (Figure 4, channels 25, 26, and 27).

Towards the southeast on the southwest slope of Shirinje Mountain (southeast Z2), the increase in concavity was probably a result of a compressional regime that was caused by interactions between the NTF and the Shirinje fault. There, the upstream uplift of moderately resistant lithologies, which had mainly been established on those faults and on a fold between them, was the main reason for the increase in concavity (Figures 4 and 5e). The elevated Quaternary sediments along the southwest side of the NTF indicated that uplift had been experienced there. This explained the rough, moderate graded reaches around the NTF as well as the presence of some streams with high values of concavity and onsets along the southwest side of the NTF (Figure 4, channels 43 and 118). Conversely, these profiles to some extent gave an estimate of the width of the fault zone. Nevertheless, the reduced occurrence and smaller values of  $H^*$  were consistent with the obvious right-lateral deflection of some stream channels in this part of the NTF.

*Aji-chay River- west end of Bozqush mountain range (Z3).*

#### Northeast slope of Aynali Mountain (northwest Z3).

Major fault positions and lithology were factors that caused quite a decrease in the concavity values on the northeast and interior slopes of Aynali Mountain. There existed a restraining relay zone between the NTF and the Shirinje fault which had similar settings in terms of dip slip and strike-slip components. This zone together with high-order faults of the NTF created a compressional regime that enhanced the uplift. A moderately resistant lithology, with low dip layering ( $5^{\circ}$ – $25^{\circ}$  northeast), probably had a delaying effect on the erosion process (Figures 4 and 5g).

Along the southwest slopes of the highlands that faced the NTF, between Aji-chay River and the west end of the Bozqush mountain range (Z3), draining tributaries were generally perpendicular or sub-perpendicular to the fault and did not leave the deformation zones formed by its segments and their high-order faults. The concavity values had decreased along this part of the NTF (Figures 4, 5f, 5i, and 5j).

#### Sahand Mountain (southwest Z3).

In this part, the stream profiles were controlled by two different morphotectonic mechanisms. The upstream parts of the profiles were controlled by radial lineaments formed by volcanic activities at Sahand Mountain 12 to



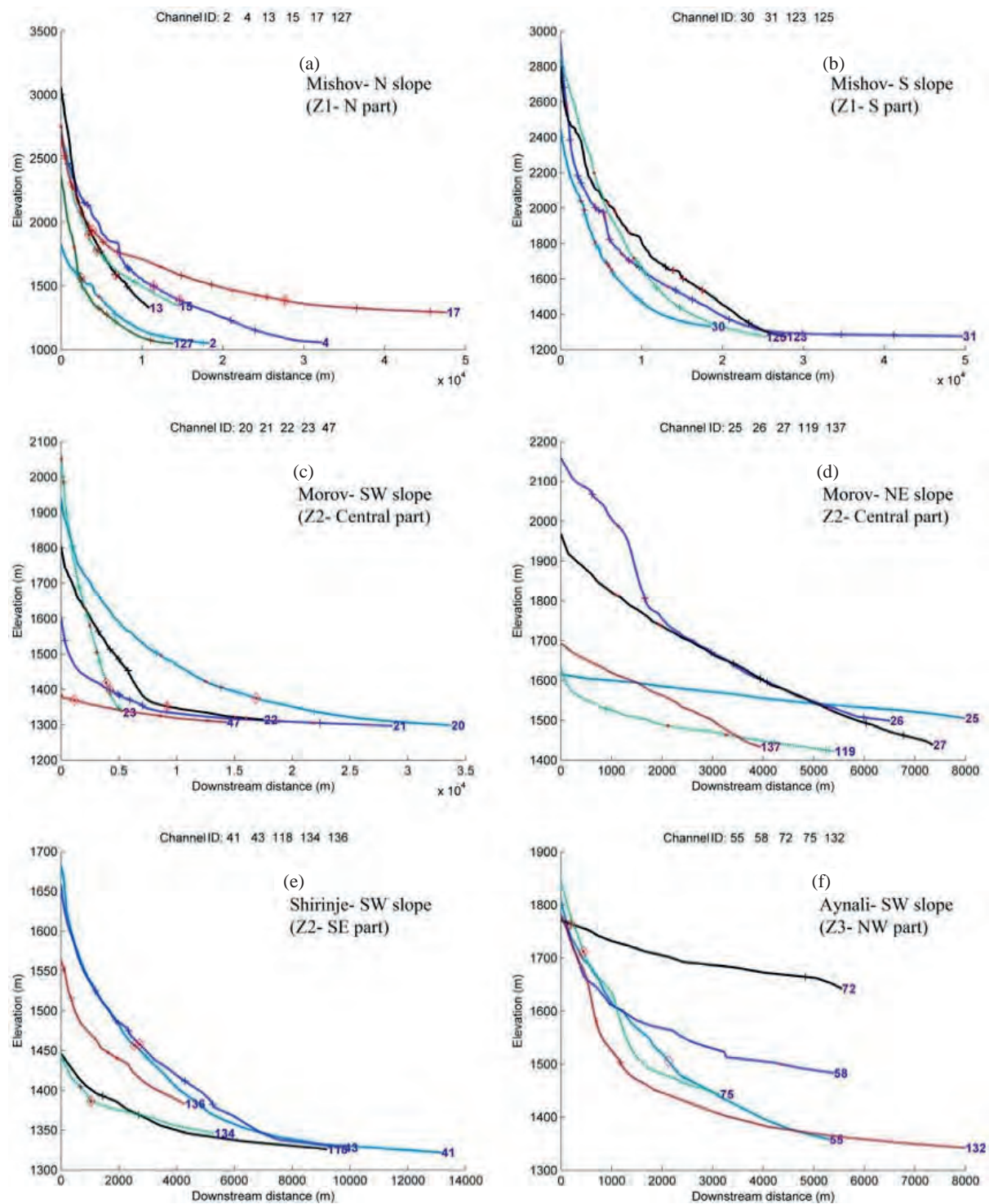


Figure 5. Stream longitudinal profiles for different slopes along NTNMF. To avoid confusion, similar profiles are excluded from the plots. In addition, marginal profiles are included for individual slopes. The diamond: intersection points of the stream channel with the NTNMF; dot: intersection points of the stream channel with fault; plus: intersection points of the stream channel with lithologic boundary.



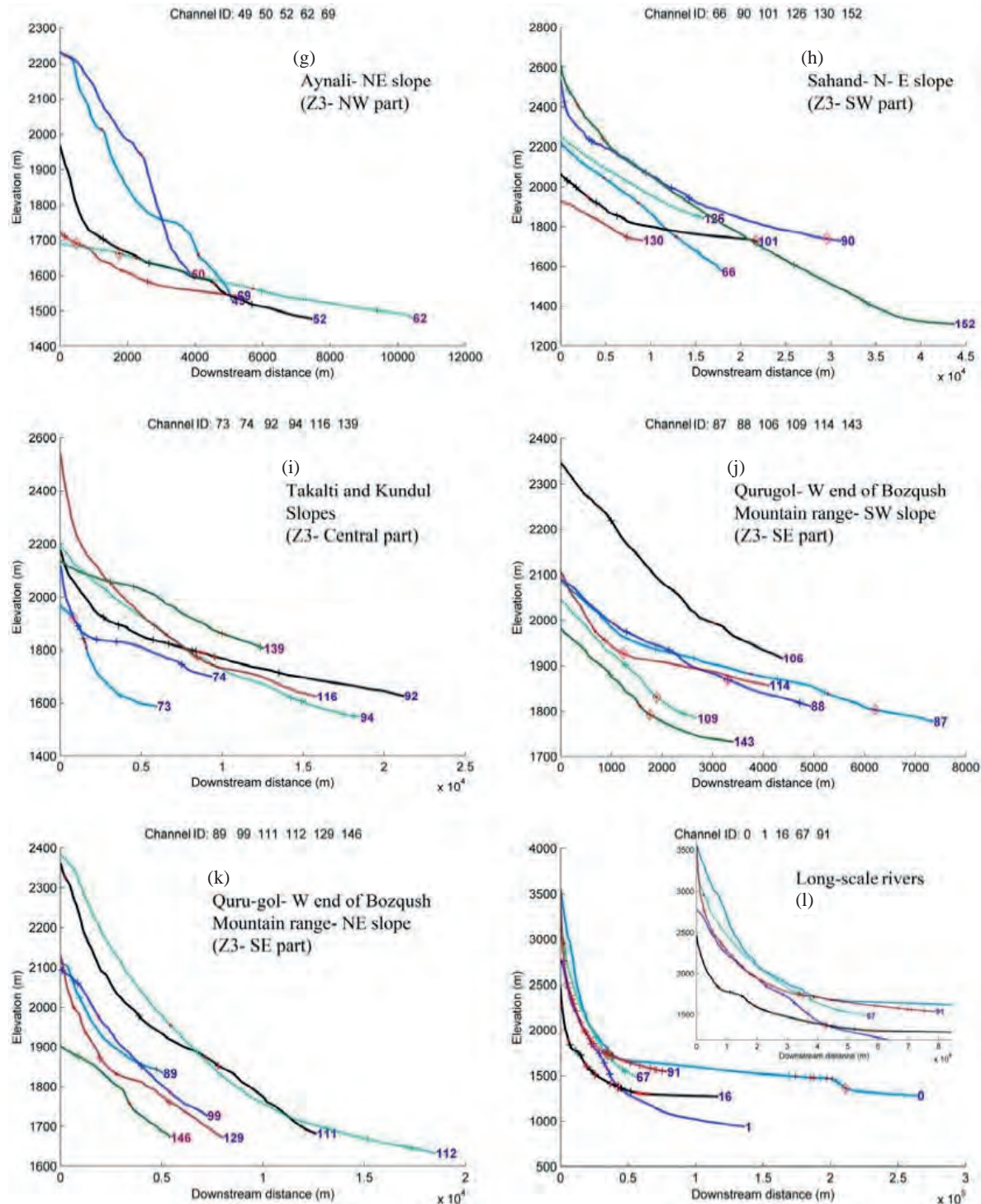


Figure 5 (cont.). Stream longitudinal profiles for different slopes along NTNMF. To avoid confusion, similar profiles are excluded from the plots. In addition, marginal profiles are included for individual slopes. The diamond: intersection points of the stream channel with the NTNMF; dot: intersection points of the stream channel with fault; plus: intersection points of the stream channel with lithologic boundary.



0.14 Ma ago. The lithostructural inheritances of these activities (i.e. the radial lineaments as well as highly resistant and uncomplicated lithologic units) caused little fluctuation in each channel along either individual reaches or the entire channel. The downstream parts of the profiles, however, were controlled by the strike-slip regime of the NTF zone. The creation of small-scale relaxing relay zones (i.e. pull-apart basins) had been causing subsidence and deposition between the highlands formed by escalated uplift at the northeast side of the fault and the elevations of Sahand Mountain. These mechanisms gave plausible reasons for the two differing behaviours of the profiles, wherein the streams had downstream parts that were either parallel (E-SW slopes) or not parallel (NW-E slopes) to the NTF. In this regard, the parallel profiles had greater concavity in comparison with those that were not parallel (Figures 4 and 5h).

The effect of the transcurrent deformation of the NTF could also be seen in the responses of the two rivers. In spite of the similarity in terms of length and bedrock lithologies, river 67 was notably less concave than river 91. This could be attributed to greater engagement with the NTF and its subsidiary faults by the downstream part of river 67, so unstable conditions along the profile increased because of more intensive compressional deformation near the NTF.

#### Takalti and Kundul Mountains (central Z3).

The Takalti and Kundul Mountains were a result of a contraction in the fault tip zones with compressional regimes, where older lithologic units were elevated above younger ones while subvolcanic and plutonic rocks were emplaced at different times. The combination of escalated uplift and highly resistant lithologies was probably responsible for the creation of generally unstable (less concave to convex) profiles (Figure 4, channels 68, 73, 94, 96, 139, and 140).

The more concave profiles on the north and southeast slopes of Takalti were similar in their greater lengths of moderate gradation along the downstream side (Figure 5i). The concavity described could be attributed to not only an unbalanced enhanced uplift in the upstream areas because of igneous complex emplacement and fault-tip related compression (channels 92, 95, and 116) but also the presence of relatively less compressional downstream areas (channels 92 and 95).

Along the northeast side of the NTF on the northwest slope of Takalti, the presence of scarp caused sharp steepening that decreased concavity. This scarp was formed by displacements in strike-slip faults with reverse and normal components as well as the consequent rock mass instability. From the Aji-chay River to the Kundul Mountain (northwest to central Z3), the NTF almost

traversed a divide that, in turn, caused no NTF-related knickpoints to appear on the profiles (Figure 4).

#### Qurugol to west end of Bozqush mountain range (southeast Z3)

On the southwest slope between the Qurugol pool and the west end of the Bozqush mountain range, the following factors were considered to explain the moderate decrease in concavity: (i) escalated uplift at the northeast side of the NTF because of displacements along it and minor parallel faults with dextral and reverse or thrust components along its northeast side as well as high-order faults with a compressional regime in the fault tip damage zone along its southeast end, (ii) rapid erosion along the NTF zone (Dorsey & Roering 2006), and (iii) emplacement of igneous rocks southeast of Qurugol (the northwest end of this part) (Figures 4 and 5j).

On the northeast and interior slopes, the streams flowed on similar lithology towards the southeast, they showed a slight decrease in concavity (Figure 4). The high fault density, high dip layering, and elevational decoupling of the longitudinal profiles at the fault tip damage zone were indications of the more intensive or extensive compressional deformation in this area. These conditions were considered reasons for the above-mentioned decrease. The downstream steepening and nearly straight nature of it in some profiles coincided with their fault line tracing in a parallel or sub-parallel manner (Figure 5k).

There was little difference between the  $H^*$  values along the central part of this segment and those closer to its tips. The  $H^*$  values were lower along its central part than at its tips (Figure 4). The frequent occurrence of knickpoint creation was also a manifestation of intensive deformation along this part of the NTF.

#### **Correlation of Concavity with Stream-fault Bearings and Lithologic Boundaries**

Four observations could be made by considering the distribution of concavity values as well as the spatial relationships of the streams, faults, and lithologic boundaries. Firstly, within the longitudinal profiles studied, the nearly straight and convex profiles mainly had azimuths that ranged from  $320^\circ$  to  $110^\circ$  in the clockwise direction (Figure 6). This corresponded with streams that drained the Sahand Mountain and northeast interior slopes of the highlands that extended along the NTF. Secondly, almost all streams whose downstream flows were parallel to the NTF had concavity values of more than 0.5. Thirdly, among the streams that traced the lithologic boundary-fault traces, the ones that flowed on lithologies with a relatively high resistance in the fault tip damage zone of the NTF has straight longitudinal profiles (channel 140), whereas



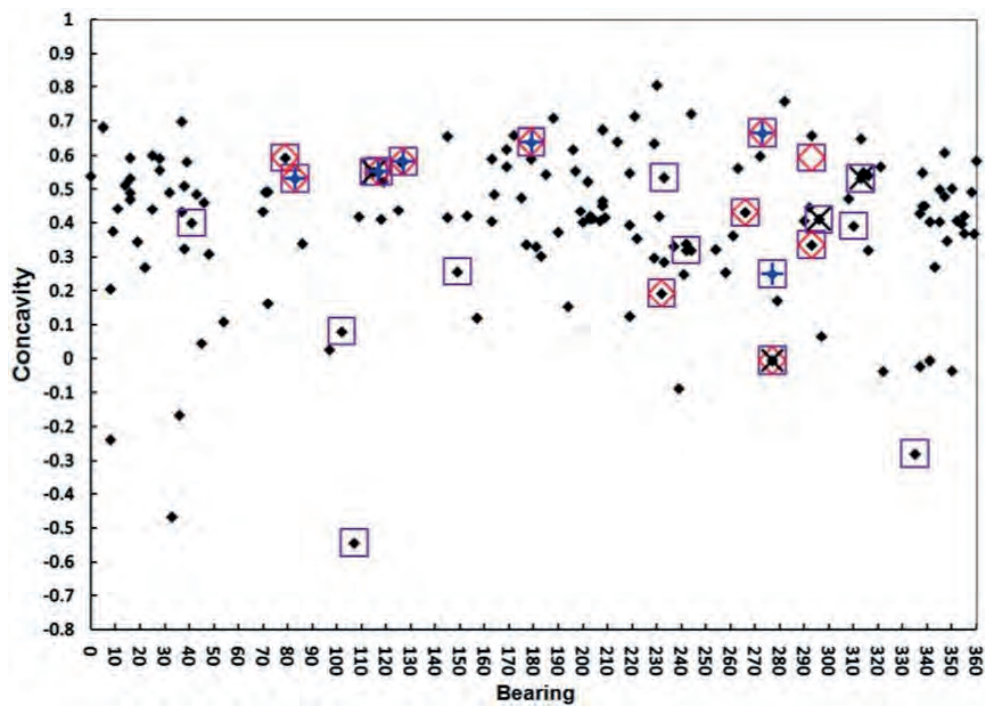


Figure 6. Plot of concavity index ( $\lambda$ ) as function of stream bearing for different lithostructural situations. Solid point: stream; square: a stream that follows a fault trace; diamond: a stream that follows the NTF or its high-order faults; plus: a stream that exhibits downstream parallelism with the NTF; cross: a stream that follows a lithologic boundary-fault trace.

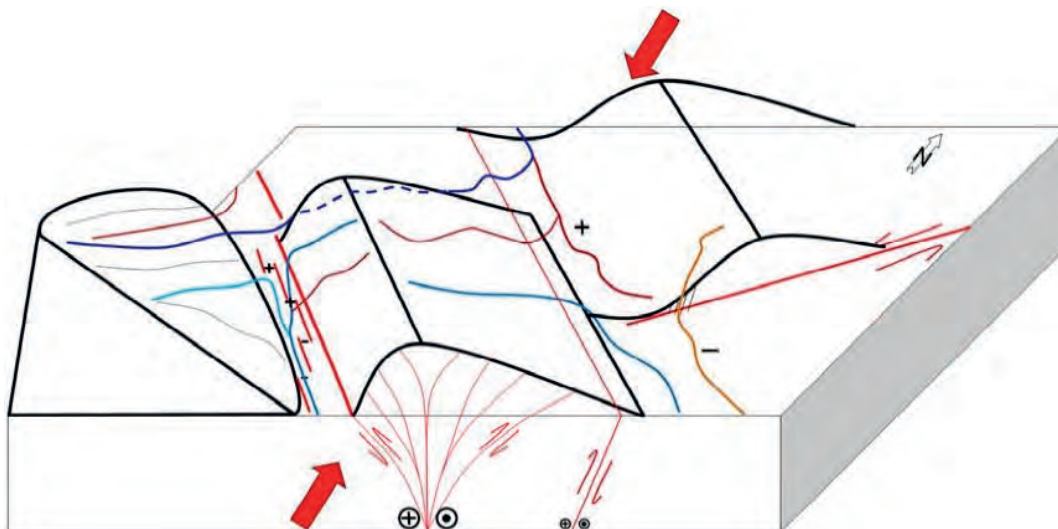


Figure 7. Schematic model for the correlation between stream concavity index and structural regime along NTNMF. Coloured curves denote streams and their concavity (it increases and decreases towards blue and red, respectively). The straight red lines: faults traces; thin hatch lines: scarp; dotted curves: lineaments; plus/minus signs: local compressional/extensional regimes; red arrows: maximum far-field compressional stress.



those flowing on lithologies with a relatively low resistance have concavity values greater than 0.4 (channels 53, 80, and 107). In this regard, besides the effect of the lithologic factor, the high rate of compressional deformation along the NTF zone could account for a low value of concavity. Fourthly, streams with concavity values between 0.3 and 0.6 showed frequent occurrences.

## DISCUSSION

### Patterns of Concavity Index and Vertical Relative Displacements along NTNMF

The calculated values of the concavity index and their distribution revealed that along the northwest half of the NTF, and east of Misho (eastern Z1, and Z2) in comparison to other areas, the profiles generally showed larger values of  $\lambda$ . The calculated values of  $H^*$  and their distribution implied that different patterns of relative uplift existed along the NTNMF. In other words, the areas around the NMF, northwest NTF, and southeast NTF (Z1, Z2, and Z3) were characterized respectively by streams that exhibited frequent occurrences of disequilibrium steepening with large  $H^*$  values, infrequent occurrences of disequilibrium steepening with small  $H^*$  values, and moderate occurrences of disequilibrium steepening with small  $H^*$  values.

Two domains are distinguishable along the NTNMF, as indicated by the response of the longitudinal profiles to related vertical displacements. The domain that surrounded the NMF (Z1) was characterized by concave longitudinal profiles and high  $H^*$  values. The domain that surrounded the NTF (Z2 and Z3) was characterized by generally less concave or even slightly convex longitudinal profiles and either no knickpoint or low  $H^*$  values. Sub-domains of each domain could also be recognised (Figure 4).

Evidently, the extent and duration of uplifting processes around the NMF were greater than those around the NTF. This inference was based on not only the profile concavities and measured  $H^*$  values of the selected streams along the NTNMF but also the relationship between the tectonic and landscape developments in terms of the correlations between deformation and time and between channel gradient and rock uplift (Burbank & Anderson 2008; Wobus *et al.* 2006). In this regard, the inference could be partly explained in two ways: (i) by the interaction between the NMF on the north slope and the SMF, Daryan, and Benis faults on the south slope and (ii) by the predominance of the lateral-slip component along the different segments of the NTF, which constantly modified its geometry and consequently the geomorphology of the hosting area. Moyyed and Mojarad (2007) suggested SMF as a candidate for the location of the primary Paleo-Tethys suture zone (Hercynian structural stage). The long-term deformation due to the above-mentioned weakness probably provided stability to the

uplifting process. The lower values of concavity on the south slope of the highlands that faced the NTF could also be attributed to the second explanation, in that approaches to equilibrium conditions were prevented by interchange between the predominance of uplifting and erosional processes due to compressional-extensional switching.

Overall, the frequent occurrence of streams with concavity values between 0.3 and 0.6 was a manifestation of the young and active deformation that surrounded the NTNMF. These values were consistent with those in a report on active orogens by Dorsey and Roering (2006) as well as those referenced therein (Whipple 2004).

### NTNMF Effect on Stream Longitudinal Profiles

The NTNMF affected the profiles of streams within the adjacent regions in three ways as shown in Figure 7 (1) By lowering the fault valley and enhancing the uplift rate in adjacent hillslopes: this, consequently, caused two profile types. First, the tributaries that were restricted to hillslopes and did not flow into the fault valley had lesser concavity. Their disequilibrium conditions because of tectonic activities were deduced from calculated  $H^*$  values. Second, the tributaries that flowed downstream into the fault valley had greater concavity ( $\lambda > 0.5$ ). The correlation of stream-fault bearing indicated that fault activities accelerated, as expected, the approach to greater concavity for streams that followed fault traces. In general, the co-existence of these types of profiles was an indication of enhanced ongoing vertical relative displacements around the faults; (2) By dividing the areas with a relatively less compressional or extensional regime from those that generally experienced more intensive compression; the co-existence of great concavity with knickpoints indicated that the transition probably occurred under steady-state conditions rather than equilibrium conditions; (3) By fault interactions, in terms of the spatial distribution of faults and the type of deformation caused by their interactions. The coexistence of some features was an indication of perturbations from the equilibrium or steady-state conditions of streams by fault interactions and intensified compressional deformation. These features were the lower concavity values, the frequent occurrence of knickpoints or greater  $H^*$  values, major faults conjunctions (i.e. Tasuj fault, northwest and central segments of the NMF, also NMF-SMF-NTF), fault tip damage zones (i.e. southeast end of the NTF), and relay zones between fault segments (i.e. Kundul and Takalti).

### Correlation between Lithostructural Transition and Concavity

The correlation between lithostructural information and profile concavity indicated that the streams that experienced a relative transition between different tectonic regimes presumably exhibited different concavity values, depending on the location and continuity (i.e. whether the transition



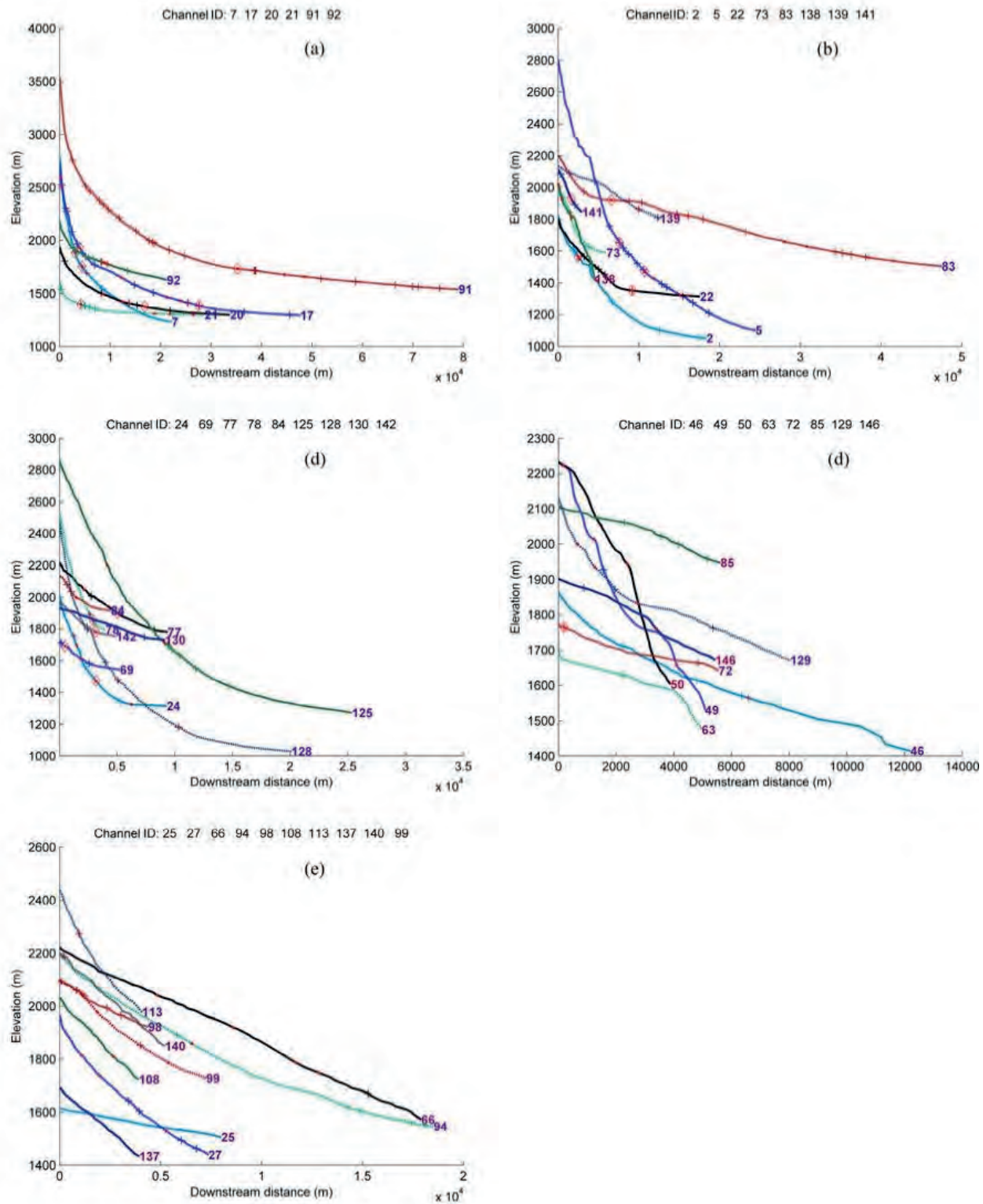


Figure 8. Stream longitudinal profiles for different types of lithostructural transitions recognised in the study region.



takes places in a steady-state condition). The streams that experienced a similar tectonic regime had concavities that were usually confined to lesser values (slightly concave to slightly convex). In this regard, within the dataset used, the transitions have been categorised into the following five types: (1) The transition was located upstream and occurred under steady-state conditions (Figure 8a). (0.53, 0.81, 0.26); (2) The transition was located upstream and occurred under unsteady-state conditions (Figure 8b). (0.38, 0.688, -0.089); (3) The transition was located downstream and occurred under steady-state conditions (Figure 8c). (0.31, 0.55, -0.47); (4) The transition was located downstream and occurred under unsteady-state conditions (Figure 8d). (0.11, 0.56, -0.54); No transition was experienced (Figure 8e). (0.23, 0.53, -0.04).

The values within parentheses were the average, maximum, and minimum concavity of the selected profiles that satisfied the conditions of each type, respectively. All types, except the first, had relatively small values of concavity.

Overall, within the study region, the stream responses to geologic forces were effectively controlled through lithostructural transitions in tectonic regimes where flow occurred and through spatial relationships between streams and geologic structures (such as faults, folds, fault-induced scarps, and landslides).

## CONCLUSION

The North Tabriz and North Misho faults were major faults with transcurrent tectonics. The interactions of the different segments of the NTNMF with each other and with other major faults in the adjacent region had caused different patterns of deformation in this tectonic regime. The difference in the deformation patterns affected the morphology of streams. The correlation between the parameters of longitudinal profiles (i.e.  $\lambda$  and  $H^*$ ) and lithostructural information had been examined to investigate the effects of the NTNMF on the longitudinal profiles of streams. The observations revealed the following: (1) Two domains were distinguishable along the NTNMF, as indicated by the response of the longitudinal profiles to related vertical displacements. The domain that surrounded the NMF was characterized by concave longitudinal profiles and high  $H^*$  values. The domain that surrounded the NTF was characterized by generally less concave or even slightly convex longitudinal profiles and either no knickpoint or low  $H^*$  values. Sub-domains of each domain could also be recognised. The reasons for these differences were the more stable tectonic evolution and greater contractional deformations around the NMF in comparison with the NTF. In general, young and active deformations around the NTNMF manifested itself as frequent occurrence of less concave profiles; (2) The NTNMF affected the longitudinal

profiles of streams by three mechanisms: (i) by crushing the fault zone and lowering the base level; (ii) by creating local, sometimes temporary, compressional-extensional or compressional-contractional regimes through subsidiary faults or interaction with other faults; and (iii) by dividing major, probably more independent, deformational zones; and (3) The stream responses to geologic forces were effectively controlled through lithostructural transitions in tectonic regimes where flow occurred and through spatial relationships between streams and geologic structures (such as faults, folds, fault-induced scarps, and landslides).

## ACKNOWLEDGMENT

I would like to thank P. Bishop for reviewing an earlier version of the manuscript and M. Faridi for providing insightful comments on the first draft of the manuscript.

*Date of submission: September 2011*

*Date of acceptance: May 2012*

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# Fast Fabrication of Polyimide Membrane on Silicon Wafer

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Since its invention, polyimide (PI) has been widely used in micro-electro-mechanical system (MEMS) devices. For fabrication, the PI membrane, PI-2723 HD-Microsystems was used as the membrane material due to its Young's modulus of 2.7 GPa and its film thickness could easily be controlled by changing the speed of the spin coater system. The application PI as membrane structure on silicon wafers therefore gave a much better mechanical performance than conventional membranes made of silicon dioxide ( $\text{SiO}_2$ ) or silicon nitride ( $\text{Si}_3\text{N}_4$ ) layers. The fabrication of PI membrane was the same as for  $\text{SiO}_2$  and  $\text{Si}_3\text{N}_4$  membranes; the basic step was to etch a side of the silicon wafer  $\langle 100 \rangle$  using wet anisotropic etching. This paper proposes an effective process for fabrication of PI membrane with fast and little supervision. In this process, a dual step process was wet anisotropic etching of single crystal silicon  $\langle 100 \rangle$  using potassium hydroxyl (KOH) with different concentrations and temperature processes. For the first process, 45% KOH under boiling temperature was used to etch at least 90%–95% of the silicon. In the second process, the silicon was submerged in 45% KOH with temperature at  $70^\circ\text{C}$ – $80^\circ\text{C}$  to etch away the residual silicon until a clean and transparent PI membrane was achieved. Using this method, the fabrication of PI membrane could be generated fast.

**Key words:** MEMS; PI-2723 HD-Microsystems; polyimide membrane; anisotropic wet etching; potassium hydroxyl; silicon dioxide; microfabrication

In the fabrication of microelectromechanical system (MEMS) devices, such as microphone and microspeaker (Cheng *et al.* 2004; Horng *et al.* 2010), pressure sensor, gas sensor (R. Schellin *et al.* 1994; M. Aslam *et al.* 2004) or microfabrication of flat or corrugated membranes (Soin & Burhanuddin 2006), the usage of wet anisotropic etching is an important processing step. Potassium hydroxide (KOH), ethylene diamine pyrocatechol water (EDP) and tetramethyl ammonium hydroxide (TMAH) are anisotropic wet etchant solutions which are most commonly used to microfabricate membranes. However, KOH is most popularly used as an anisotropic wet etchant solution due to its non-toxic, unexpensive, easy to use and excellent anisotropic etching profile (Madou 2002; Zubel *et al.* 2001; Iosub *et al.* 2002).

Typically, silicon nitride and silicon oxide that have been deposited on silicon wafer as a mechanical support frame have been used as thin film membrane. In an application microspeaker, flexible membrane plays an important part to produce large deflections with low power consumption. Due to the silicon, silicon nitride and silicon oxide membranes are relatively stiff and do not produce a large displacement, polyimide (PI) with smaller Young's modulus and exhibiting good mechanical and thermal

properties is a good candidate for a MEMS microspeaker (Fatima & Burhanuddin 2009). It is also easy to fabricate and control layer thickness as compared to silicon nitride and oxide. Although cured, it exhibits good mechanical, thermal and electrical properties, but it can be attacked by strong bases and acid such as KOH and fuming nitric acid (HD Microsystems 1998). Therefore, selective protection materials and step processes for fabrication of thin film PI membrane must be considered.

In this paper, we focus on process fabrication of robust thin film polyimide membrane using silicon nitride as mask and etch stop material. The membrane will be applied for a MEMS microspeaker, sized  $2.5 \text{ mm}^2 \times 2.5 \text{ mm}^2$  and  $2 \text{ }\mu\text{m}$  thick. This is anisotropic wet etching, using KOH solution with two steps and different temperatures to etch away silicon until clean and transparent silicon nitride PI membrane is achieved.

## MATERIALS AND METHODS

The structure of a MEMS microspeaker when electro-magnetically actuated is shown in Figure 1.

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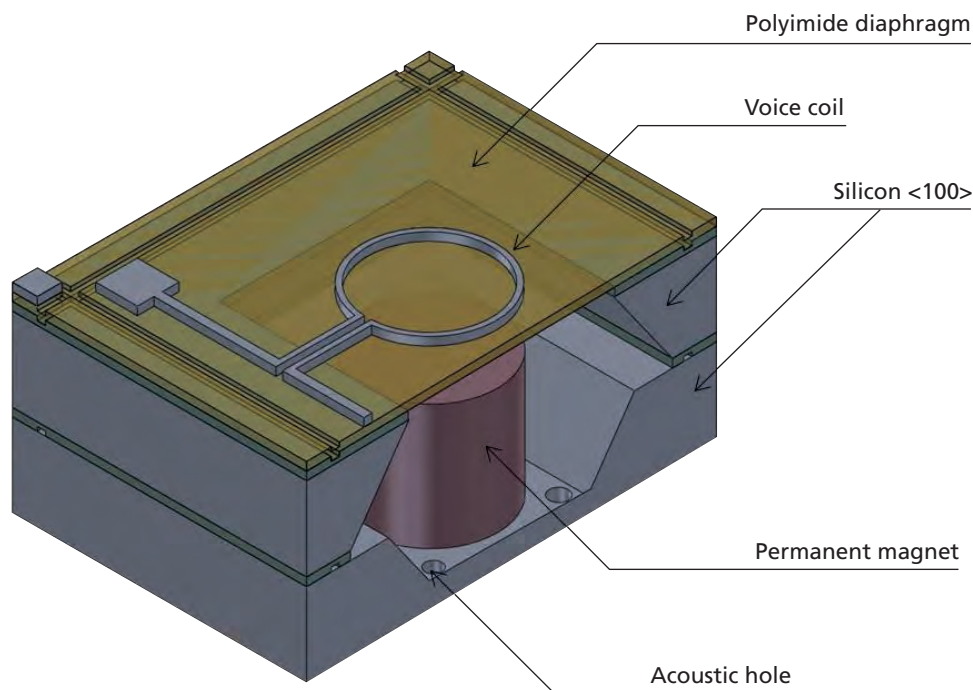


Figure 1. The structured 3D cross-section view of the MEMS microspeaker.

The microspeaker was constructed from two components. The first part was an upper frame silicon wafer used as substrate for the supporting membrane. PI-2723 HD-Microsystems were used as membrane material. The second part of the silicon frame, the lower frame, was used for laying a permanent magnet NdFeB disc (D101-N52, K&J Magnetics, Inc.).

The wet anisotropic etching experiment was performed in fabrication PI membrane, on single sided, polished (100) single crystal silicon 650  $\mu\text{m}$  thick wafers. This silicon wafer was double-sidedly coated by low pressure chemical vapor deposition (LPCVD) of silicon nitride ( $\text{Si}_3\text{N}_4$ ) as mask and with a thickness of 200 nm for the etch-stop layer.

Major steps in the fabrication process of polyimide membrane are illustrated in Figure 2. Firstly in Figure 2a,  $\text{Si}_3\text{N}_4$  coated silicon wafer was cleaned by standard wafer cleaning process system and then in Figure 2b, photoresist AZ4620 was coated onto the silicon wafer using spinner and it was patterned by photolithography (Karl-Suss MJB-3). The alignment mark was patterned in the top. This mark enabled visual alignment in subsequent steps to work without exposing underlying the silicon substrate as shown in Figure 2c. Next in the back, square windows were patterned to result in a targeted  $2.5\text{ mm}^2 \times 2.5\text{ mm}^2$  window on the front side. Etched  $\text{Si}_3\text{N}_4$  using deep reactive

ion etching (DRIE) for 30 seconds is shown in Figure 2d. In the next step of the process, the back was etched using 45% wt. of KOH solution at a boiling temperature ( $125^\circ\text{C}$ – $130^\circ\text{C}$ ) with an etching rate of  $7\text{--}8\text{ }\mu\text{m}/\text{min}$  until a thin layer of silicon, about  $50\text{ }\mu\text{m}$  was left (Figure 2e) after 1.5 h of etching time.

After etching the silicon at the back, the PI was spun at 4000 r.p.m. and baked at  $75^\circ\text{C}$  for 4 minutes as shown in Figure 2f. The baked PI was then cured in a nitrogen atmosphere at temperatures  $350^\circ\text{C}$ – $400^\circ\text{C}$  for 60 minutes.

Finally, an anisotropic wet etched silicon was prepared using the same solution with a difference of temperature i.e.  $70^\circ\text{C}$ – $80^\circ\text{C}$  until the silicon was etched through (Figure 2g). The remaining  $\text{Si}_3\text{N}_4$  on the front was removed by using DRIE for 30 second. The PI membrane was then released (Figure 2h).

## RESULTS AND DISCUSSION

The experiment had successfully fabricated PI membrane on a silicon substrate. The job had been carried out by two step process involving anisotropic wet etching with different temperature settings. Table 1 shows anisotropic wet etching using 45% KOH with different temperature settings.



Figure 2. Step process fabrication of polyimide membrane.

Table 1. Anisotropic wet etching characteristics of KOH.

Material	Temperature	Etch rate $\mu\text{m}/\text{min}$
45% KOH	Boiling	7 – 8
45% KOH	70°C–80°C	0.7 – 1.0

The etching rate of KOH at boiling temperature is higher than at lower boiling temperatures (Tanaka *et al.* 2004). It should be noted however, that generating thin film polyimide membrane onto a  $\text{Si}_3\text{N}_4$  layer would not be successful with just a single etching at boiling temperature. It is caused by a big hydrogen bubble generated on the silicon surface and this could be broken by a thin  $\text{Si}_3\text{N}_4$  layer before the whole membrane is released, as shown in

Figure 3. In addition, the big bubbles can block the silicon surface, so that the etching would not be uniform.

In addition, we observed that anisotropic wet etching using 45% KOH did not tend to etch silicon uniformly. Figure 4 shows that etching silicon at the membrane edge proceeds faster than at the centre. To avoid the membrane edge becoming thinner in the final stage, we continued to etch the membrane in the same solution at temperatures of 70°C–80°C to remove the remaining thin silicon layer. Figure 5 shows clearly that the thin remaining silicon layer at the membrane edge was removed faster than at the centre. After the remaining silicon layer was removed, the thin film silicon nitride as an etch stopper layer under the polyimide was removed by DRIE process with  $\text{CF}_4$  for 30 sec as shown in Figure 6.

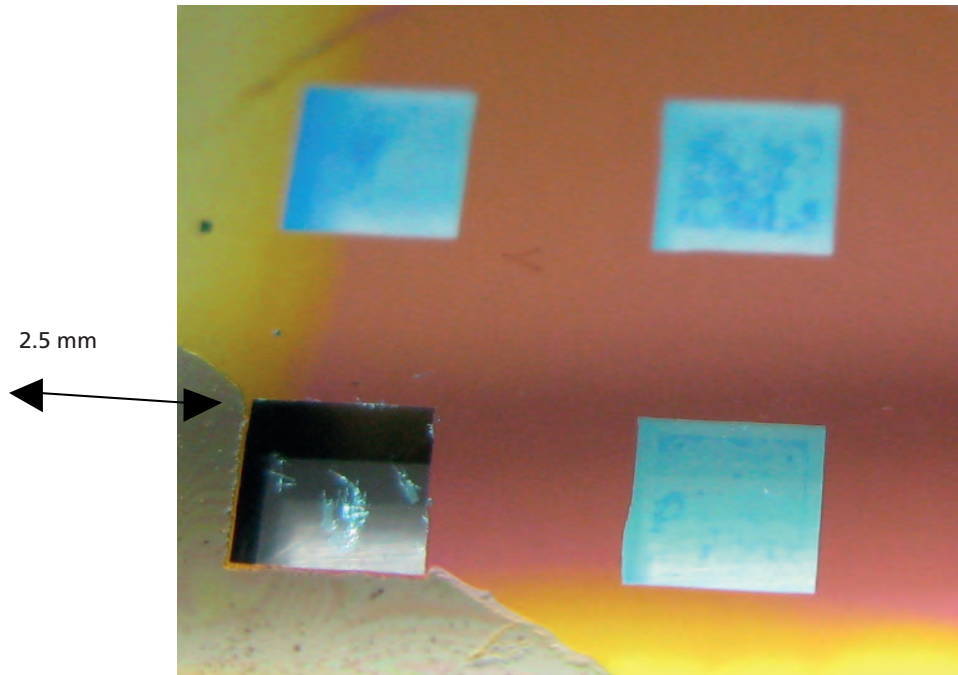


Figure 3. Silicon nitride membrane after etching through silicon.

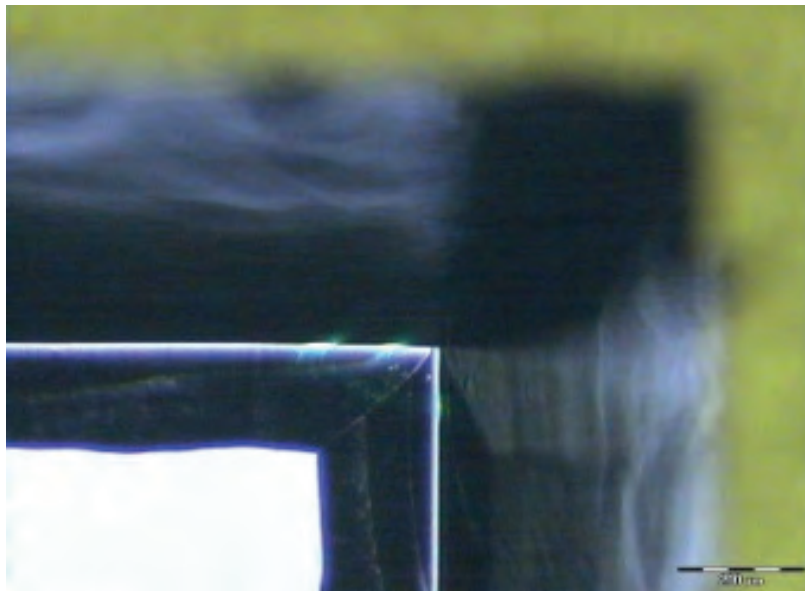


Figure 4. A microscope photograph showing the etching characteristics of 45% KOH at boiling temperature for 1.5 h of etching time.



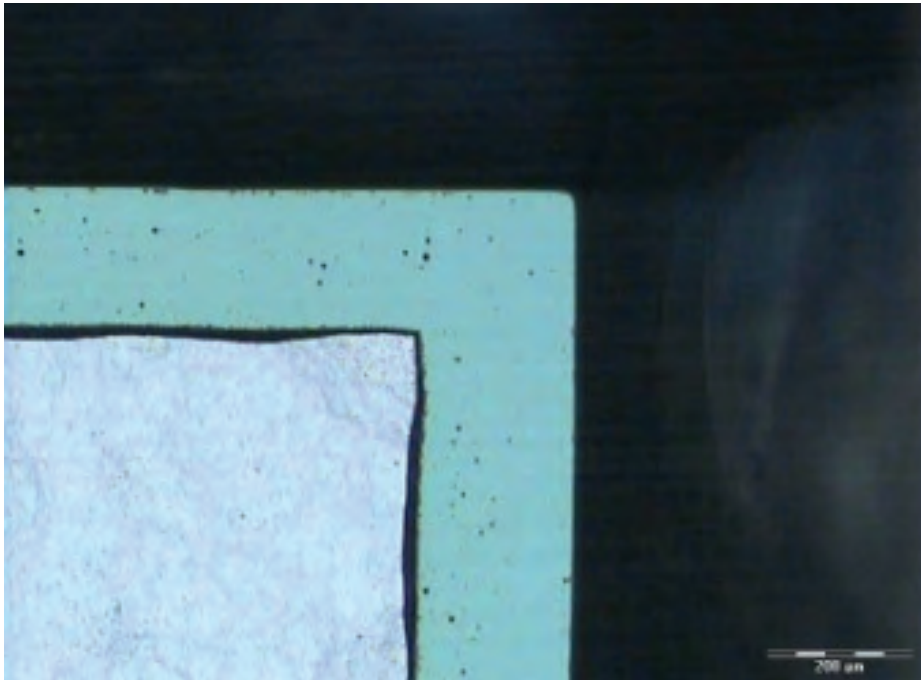


Figure 5. A microscope photograph showing characteristic 45% KOH at 80°C for the second step process.

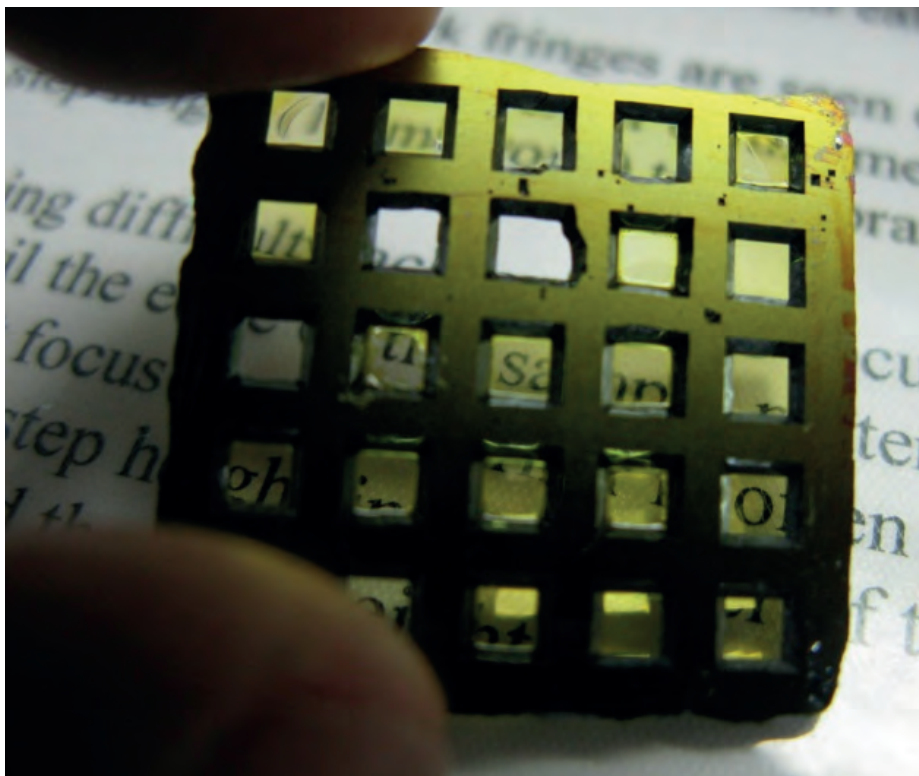


Figure 6. A photograph of microfabricated array polyimide membrane using 45% KOH with two different temperature etchings.





## CONCLUSION

We have fabricated a PI membrane on silicon substrate with silicon nitride which was used as the etching mask and stopper layer during anisotropic wet etching. The two-step process of fabrication was developed to microfabricate PI membrane on silicon using 45% KOH with different temperature processes. Based upon the results and discussion, we concluded as to the following: first-step, at boiling temperature, an anisotropic wet etching using 45% KOH had indicated a high etching rate: at the membrane edge, however, the etching of silicon was faster than at the centre. To avoid the membrane from getting thinner at the edge, in the second-step we used the same solution at a temperature of 70°C–80°C to remove the remaining silicon layer. Finally, the free standing polyimide membrane on silicon <100> substrate was fabricated successfully.

## ACKNOWLEDGEMENTS

The authors would like to thank Universiti Kebangsaan Malaysia for funding this work under the project UKM-AP-NBT-10-2009, MEMS and Nanoelectronics.

*Date of submission: May 2011*

*Date of acceptance: June 2012*

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# Advances on Bank Erosion Studies: A Review

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This paper reviews the advances made on studies related to bank erosion. Bank erosion has been an area of interest by researchers in geological, geotechnical, hydraulic, hydrology and river engineering disciplines. With anticipated global challenges from climate change impacts, bank erosion studies could support challenges faced in ensuring sustainable environmental management. The evolution in the theoretical and laboratory findings have led to the advances in bank erosion and contributed to new knowledge in the said field. This review summarises the findings of previous investigators including measurements approach and prediction of rates of bank erosion through the use of physical models and numerical approach.

**Key words:** river bank erosion; physical model; numerical model; prediction parameters; erosion simulation; high resolution techniques; measurement evaluation; researchers

Research on bank erosion has gained interest since 1959 for many reasons. Among others are their impacts to river or channel erosion, river morphology, floodplain hydraulics, and risks of flooding and lateral migration in a watershed. Increased in population have led to significant increase in the occupancy of a watershed for industrial activities and development for city centres. The consequential effects of this occupancy has resulted in damages to the river environment that include the many aspects of erosion and sedimentation apart from water pollution, a topic that has been extensively debated over years. Undoubtedly, river changes its morphology with time. The change in river morphology as agreed by landform scientists is dynamic but the change in magnitude has increased by many folds. The mechanics of erosion and sedimentation is greatly associated with the change in river morphology and a study on the impact of hydrodynamic force on river banks is of significance for a sustainable environment. Thus, as a way forward, a review on progress and advances on bank erosion studies is pertinent to establish any shortcomings with current findings.

This paper is devoted to providing a review of previous studies in bank erosion, encompassing aspects of measurements approach or technique, parameters for prediction and the modelling of erosion through physical models and numerical models.

## MEASUREMENTS OF BANK EROSION USING HIGH RESOLUTION TECHNIQUES

Sound and reliable measurement technique is important for accuracy, consistency and minimal error. Attempts have been made to review some measurement approaches or techniques proposed by some investigators in the field. Measurements have also utilized data from historical sources and observations were also gathered through botanical and sedimentological evidences. Techniques such as terrestrial photogrammetry, thermal disturbance, hydrographic resurvey and repeated photography were also exercised during the course of investigation to identify for any notable changes in planform geometry. Some investigators have also resorted to the use of sediment traps, printed section pebbles and erosion box, structures built within the study area to quantify the magnitude of erosion with respect to the factor time.

Earlier review on field measurements by Thorne (1981) on methods of measurements and monitoring of bank erosion and planform geometry changes include mapping, cross section surveys, planimetric resurvey, erosion pin, spray printing, reprofiling and side-scan sonar. The monitoring techniques have also made use of light detection and ranging (LIDAR) remote sensing technique which can be costly. This technique was first introduced in 2005 by

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Thoma *et al.* Airborne LIDAR system is a laser scanning system that sends many thousands of laser pulses to the ground every second from aircraft in a scanning pattern. The system is able to generate the terrain or topography and the morphology of the river under study. Thorne's review was however updated by Lawler in 1993 who categorises the different measurements based on timescales: long timescale (10 000 – 20 000 years), intermediate timescale (1–30 years), and short timescale (few months to a few years).

Poulenard *et al.* had used a technique known as Diffuse Reflectance Infrared Fourier Transform in 2009 which was first adopted by Pereira and Wicherson in 1999 to map riverbank elevations. The resolution versus timescale of this field measurement ranges between terrace photogram (F) and plan resurvey (D) shown in Figure 1. This method is still in the preliminary stage and a thorough investigation is required to validate its effectiveness to confirm its suitability for adoption in quantifying the magnitude of erosion.

Marked progress on technique development took place between the year 1992 and 2008. A good 18 years resulted in significant changes on the development and use of PEEP-3T system from the traditional method that uses normal erosion pins and Photo-Electronic Erosion Pin (PEEP) system introduced in 1994, an enhanced version of the erosion pins. All the methods were introduced by Lawler and Leeks in 1992, 1994 and subsequently in 2008.

The use of pins that consisted of small sized steel rods attached at certain depths of the bank to measure the changes of the bank had several disadvantages such as the disturbance of the strength of the bank and was limited to certain types of material. This method was superseded by the PEEP system (Lawler 1994) equipped with a monitoring feature for quasi-continuous monitoring of river bank erosion, deposition events and their temporal variability especially for dynamic upland headwaters. In 2008, he introduced an improved resolution system named PEET-3T that is capable of identifying detailed changes in the measurement. This is in response to the challenges in the geomorphology issues. Table 1 shows some of the available measurement techniques for bank erosion profiles.

### BANK EROSION PROFILE PREDICTION

Literature on the investigations that focus on prediction rates of erosion along river banks is very limited. Lawler in 1995 has made observations on the different processes of erosion namely fluid entrainment and mass failure and found that these features are dominant in the middle stream and lower reaches, respectively. His findings are limited to visual observations and did not include the mechanics of erosion along river banks.

A study carried out by Duan and Julien in 2005 on bank erosion rate has considered bank failure as a probabilistic phenomenon. They identified the key variables for depth-

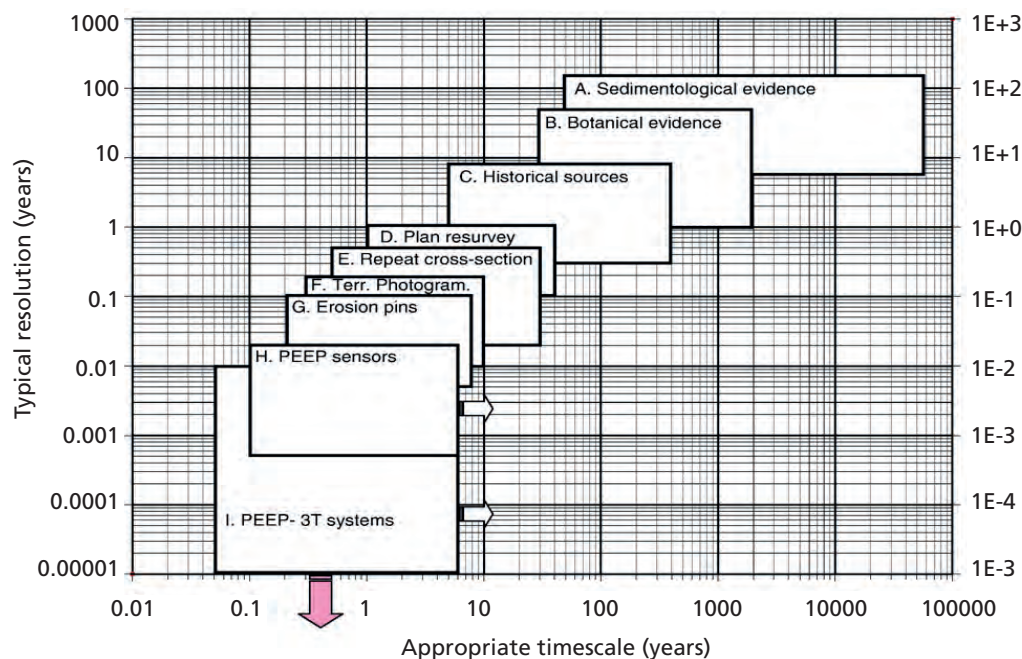


Figure 1. Relation between resolution and timescale of bank erosion field measurement techniques (Lawler 2008).

Table 1. Measurement techniques for bank erosion profiles.

Measurement Techniques	Advantages and disadvantages
Diffuse Reflectance Infrared Fourier Transform (DRIFT) (Poulenard, <i>et al.</i> 2009)	Capable of tracing the origin of suspended sediment. Small range coverage. Medium time scale. Limitation: still in the preliminary stage. Loss of fines, possible input of river material etc. may affect accuracy. Simple and cheap.
Planimetric resurvey with the use of LIDAR remote sensing (Thoma <i>et al.</i> 2005)	Measurements of volume through the use of laser scan of the area. Large area of coverage. Larger timescale (annual). Limitation: measurements can be made above waterline and measurements below waterline will not be possible due to the absorption of laser by water. Laser altimetry measurement (vertical and horizontal shifts). River gauging station measurement. Partition sediment are some of the factors that may affect accuracy. Accuracy: vertical error less than 15 cm (within manufacture specification) and no consistent error along the horizontal shift.
PEEP (Lawler & Leeks 1992; Lawler 1994) uses electronic erosion pins	Uses traditional erosion pins. Suitable for small area; study was done using four sensors (Lawler and Leeks 1992) and two sensors (Lawler 1994). Accuracy: Standard error for estimated length as low as 1.26 mm. Limitation: measured above water level. Shorter timescale: study was done at 15 minutes interval (by Lawler & Leeks 1992) or 30 minutes interval for 16 months (Lawler 1994).
PEEP-3T (Lawler 2008) uses electronic erosion pins	Improved version of PEEP with enhanced features and accuracy.

averaged bank erosion rate namely hydraulic force, concentration, particle density, particle size, toe shear stress of side slope, critical shear stress, flow depth, porosity and bank height. Two bank erosion equations were proposed that combines two elements of erosion namely basal erosion and bank failure/mass failure. Later in 2006 Julien and Torres introduced a predictor for hydraulic erosion rate of cohesive river bank. They examined the hydraulic bank erosion in terms of magnitude, duration, event peak and variability. One interesting fact about the predictor proposed by Langendoen and Alonso (2008) is the inclusion of the factor of safety for the gravitational type of failure which is subdivided into planar and cantilever failures. Their predictor for erosion rate is very similar to the one proposed by Julien and Torres. Langendoen and Alonso have also included the variables suggested by Thorne and Tovey (1981) that include failure plane angle, slice effect, effective cohesion, effective angle of internal friction, pore-water force, shear strength, weight of material, hydrostatic force, number of bank material layers in the failure block, shear, beam and tensile failure. While Langendoen and Simon (2008) have proposed an enhanced version of the predictor for factor of safety incorporating composite streambank parameter and pore pressure. Table 2 illustrates some of the bank erosion predictors by a few investigators. This illustration will be used to evaluate the reliability of the parameters used as erosion predictors.

## BANK EROSION SIMULATION — NUMERICAL AND PHYSICAL APPROACHES

Efforts have been made to simulate behaviour of bank erosion through the use of numerical and physical models. Numerical approach under the pretext of computational fluid dynamics is an approach that uses fundamental theory of fluid mechanics and hydraulics that simulates flow behaviour. The use of physical models is usually carried out under a more regulated environment with few variables kept constant.

### Bank Erosion Simulation Using Numerical Approach

Applications of numerical approach used to simulate and predict bank erosion phenomena have gained popularity among researchers. Chen and Duan (2006) felt that the approach could be cost effective. They classified the approach into one-dimensional, two-dimensional and three dimensional. Devauchelle and co-researchers had in 2008 introduced the one-dimensional erosion law but this was only for laminar flow on cohesionless soil.

Several other studies undertaken by few researchers between the year 2001 and 2010 include Mosselman (1998), Langendoen *et al.* (2001), Duan *et al.* (2002), Dulal *et al.* (2010), Duan and Julien (2005), Wang *et al.* (2008), Jia and



Table 2. Bank erosion predictors by a few investigators.

Investigators	Bank erosion predictors	Predictors
Duan & Julien (2005)	Bank erosion with a combination of two elements as follows: Basal erosion (Duan 2001)	Depth-average bank erosion rate due to hydraulic force.
	$\bar{\xi} = \sin \bar{\beta} \sqrt{\frac{C_L}{3\rho_s}} \left( 1 - \frac{C}{C_*} \cos \bar{\beta} \right) \left( 1 - \frac{\tau_{bc}}{\tau_{b0}} \right)^{\frac{3}{2}} \sqrt{\tau_{b0}}$ $q_{br}^b = \frac{\bar{\xi}(1-p)h_b}{\sin \bar{\beta}}$ <p><math>\bar{\xi}</math> = Depth-averaged bank erosion rate <math>\bar{\beta}</math> = Average bank slope <math>C</math> = Concentration <math>C_L</math> = Lift force coefficient <math>C_*</math> = Concentration <math>\tau_{bc}</math> = Critical shear stress for basal erosion <math>\tau_{b0}</math> = Shear stress at the toe of the bank slope <math>\rho_s</math> = Density sediment particle <math>q_{br}^f</math> = Net volume of sediment contribution <math>p</math> = Porosity <math>h_b</math> = Flow depth at near-bank</p> <p>Bank failure / mass failure</p> $q_{br}^f = \bar{\xi} \Delta h_{bank} (1-p)$ <p><math>q_{br}^f</math> = Sediment material eroded per unit channel width from bank failure <math>\Delta h_{bank}</math> = bank height above the water surface <math>p</math> = Porosity.</p>	Concentration suspended sediment. Particle density. Particle size. Shear stress at toe of bank slope. Critical shear stress. Flow depth. Porosity. Bank height.
Julien & Torres (2006)	This study used common model of hydraulic <i>erosion rate</i> of cohesive river bank $E = k(\tau - \tau_c)$ <p><math>E</math> = Lateral erosion rate <math>k</math> = Erodibility coefficient <math>\tau</math> = Applied shear stress by flow <math>\tau_c</math> = Critical shear stress for entrainment.</p>	Hydraulic bank erosion in terms of magnitude, duration, event peak and variability.





Table 2 (cont.). Bank erosion predictors by a few investigators

Investigators	Bank erosion predictors	Predictors
Langendoen & Alonso (2008); Langendoen & Simon (2008)	<p><i>Hydraulic erosion</i></p> <p>Lateral erosion rate equal to cohesive bed-material:</p> $E = M \left( \frac{\tau_{sb}}{\tau_c} - 1 \right)$ <p><math>E</math> = Lateral erosion rate  <math>M</math> = Erosion rate  <math>\tau_{sb}</math> = Shear stress exerted on bank material  <math>\tau_c</math> = Critical shear stress</p> <p>Gravitational failure is subdivided into planar failure and cantilever failure with the respective factor of safety.</p> <p>Planar failure:</p> $FS = \frac{\sec \beta \sum_j (L_j c'_j + N_j \tan \phi'_j - U_j \tan \phi_j^b)}{\tan \beta \sum_j W_j - F_w}$ <p><math>FS</math> = Factor of safety  <math>\beta</math> = Failure plane angle  <math>j</math> = Slice index  <math>L_j</math> = Length of the slice base  <math>c'_j</math> = Effective cohesion  <math>N_j</math> = Normal force on the base of the slice  <math>\phi'_j</math> = Effective angle of internal friction  <math>U_j</math> = Pore-water force on the base of the slice  <math>\phi_j^b</math> = Angle indicating the increase in shear strength for an increase in matric suction (negative <math>U_j</math>)  <math>W_j</math> = Weight of the slice  <math>F_w</math> = Horizontal component of the hydrostatic force exerted by the surface water on the streambank.</p> <p>Cantilever failure:</p> $FS_s = \frac{\sum_j (L_j c'_j - U_j \tan \phi_j^b)}{\sum_j W_j}$ <p><math>FS_s</math> = Factor of safety for shear failure  <math>j</math> = Index of bank-material layers in the failure block.</p>	<p>Hydraulic erosion predictor for shear stress and erosion rate</p> <p>Gravitational predictors as reported by Thorne and Tovey (1981) included failure plane angle, slice effect, effective cohesion, effective angle of internal friction, pore-water force, shear strength, weight of material, hydrostatic force, number of bank material layers in the failure block, shear, beam and tensile failure.</p>

co-researchers (2009) and Jia *et al.* (2010). Summary of their study description and their significant findings using numerical approach is given in Table 3.

### Bank Erosion Simulation Using Physical Models

The construction of physical models were used to validate the simulations derived through numerical modelling. Table 4 shows a summary of the proposed laboratory models for bank erosions for different types of bank materials.

Laboratory erosion model of non-cohesive material was initiated by Friedkin in 1945. Many studies in the later years were based on Friedkin (1945) and this is evident in the Ruther and Olsen study in 2007. Laboratory experiment similar to Friedkin (1945) was used to validate the three-dimensional computational fluid dynamics to model free-forming meander evolution. Besides Friedkin (1945), model experiment was upgraded by Duan and Julien (2005), in terms of meander formed and relationship of meander formation to water discharge, sediment load, bank composition and valley slope to validate the numerical simulation of the inception of channel meandering.

Chen *et al.* (1985) had developed a model to determine the adequacy of a channelization scheme for Salt River near Sky Harbor International Airport, Phoenix, Arizona. This model included the two types of bank and bed erosion. Jang and Shimizu (2005), developed laboratory model to validate their numerical simulation relating to wide, shallow channels with erodible banks.

On the other hand, Nagata *et al.* (2000) also developed a non-cohesive laboratory model. This model was used widely to validate the numerical experiment. Chen and Duan (2006) used this laboratory model to verify the applicability of their numerical model to simulate the widening processes of a sine-generated channel. Jia *et al.* (2010) used laboratory model similar to that of Nagata *et al.* (2000) to verify the ability of their three-dimensional modelling on bank erosion and morphological changes. In terms of cohesive laboratory model, only Papanicolaou (2001) has made a study on this. The model was used to determine the erosive strength of the cohesive soil.

Dulal *et al.* (2010) had conducted a study on stratified bank material. The experiment was used to validate a two-dimensional bank erosion model by adding slump block phenomena on two layers of bank material in laboratory modelling. This experiment was an in depth study on slump block phenomenon with zero effect on the variation of structure and composition of stratified soil. They have also developed a laboratory scale model of stratified river bank with cohesive soil in the upper layer and non-cohesive soil in the lower layer. The focus of their study

was on the evolution of bank erosion that complemented the study on slump block phenomena. Another important study by Chu-Agor and co-researchers (2008) focussed on the influence of seepage in their lysimeter experiment on stratified channel. Their experiment used three types of material which is silty loam in the upper layer, loamy sand in the middle and clay loam in the bottom layer. They have not considered actual river flow, but emphasis was on the seepage flow through stratified bank. Table 5 illustrates the numerical models of bank erosion documented by ASCE in 1998.

### CONCLUSION

Bank erosion prediction is important in the fields of hydraulics, erosion and sedimentation as this is highly associated with lateral migration issues in rivers and the adjacent land that leads to change in river morphology. Several measurement techniques were proposed to measure the change in river morphology using high resolution equipment or systems. The use of these equipments or systems could be very costly and would induce higher cost in generating the morphology of rivers.

Review on the use of parameters facilitated the selection of the most significant parameters as predictors to rates of bank erosion. Nevertheless, further studies are required to identify other influential factors that determine the rates of bank erosion. Selection of the appropriate predictors is pertinent and formed the basis to any modelling studies. The series of methods reviewed were confined to measuring the change in bank erosion profile and river morphology with respect to time while the mechanics of erosion needed to be fully understood. As in many numerical models, the use of finite element in modelling structural behaviour formed the basis of the modelling machine. While many would agree that the stiffness of any structural member is more or less fixed but the variability in the properties of soil under the actions of hydrodynamic force would be an uphill task.

The review has also highlighted the different variables that might have great influence on the rates of erosion thus, it was also important to evaluate their degree of significance.

Another point of interest is on the influence of seepage to the strength and structure of the river bank. The study which was conducted by Chu-Agor and co-researchers (2008) can be an element of importance in the forthcoming research.

In conclusion this review facilitated the identification of suitable measurement technique, common predictors and elements of importance which could be incorporated in the intended study.

Table 3. Studies on bank erosion using numerical approach by selected investigators

Investigators	Description of study	Significant findings
Mosselman (1998)	Proposed two dimensional depth-average model of river morphology. Has included bank erosion mechanism and provision to account for the associated planform changes and input of bank erosion product.	Poor agreement between numerical model and observation due to formulation of bank erosion mechanism/calibration bank erodibility and the absence of a bank accretion mechanism in model.
Langendoen <i>et al.</i> (2001)	Proposed CONCEPTS (Conservation Channel Evolution and Pollutant Transport System) is a process-based dynamic computer model that simulates open-channel hydraulics, sediment transport, and channel morphology and included bank material stratigraphy.	Predict undercutting of streambanks but under-predicts the retreat of top outside bank at bend apex approximately two meters (because critical shear stress to initiate lateral erosion at toe bank was too small).
Duan <i>et al.</i> (2002)	Proposed EnCCHE2D is a 3D flow field meandering channel. Model considers effect of secondary flow and transversal component of gravitational force. The model simulates bed load transport rate and quantifies advanced and retreat of bank from proposed bank erosion equation. Sediment transport rate is based on the sediment concentration and velocity of flow.	Enhanced CCHE2D with EnCCHE2D (capable of predicting quasi three dimensional flow field and shear stress distribution on the bed).
Dulal <i>et al.</i> (2010)	A 2D morphological model for bed and bank to reproduce evolution process of free meandering that considers effect of slump block. The model features moving boundary fitted co-ordinate system that simulates naturally shaped river system. The model consists of a depth-average flow equation and the advection phase of momentum equation [Cubic interpolated pseudo-particle (CIP) numerical technique]. The sediment continuity equation for bedload sediment transport considers the effect of secondary flow.	Simulation and experiment observation worked well with meandering process with constant width maintained along the meandering length. Slump block works effectively for damping bank erosion. Meandering was found to be affected by the discharge fluctuation. Channel migration was found independently and separately in inner and outer bank. Channel width almost constant keeps its width constant while making expansion and contraction due to discharge fluctuation.
Duan & Julien (2005)	A 2D study model to simulate formation process of meandering channels. Separate bank erosion model (Duan 2001) and advance and retreat bank lines model (near-bank mass conservation equation).	Good agreement between numerical simulation and experiment. Deposited eroded bank material at toe of bank does not guarantee retreat of bank line. Suspended sediment is less significant to bank erosion while bedload is significant to migration. Bank material from caving bank supplements sediment deposit on point bar when bed and bank material are the same.

Table 3 (cont.). Studies on bank erosion using numerical approach by selected investigators.

Investigators	Description of study	Significant Findings
Wang <i>et al.</i> (2008)	A 2D model that simulates longitudinal and lateral channel deformation in braided reach of the Lower Yellow River. A composite model consisting of depth average 2D flow, sediment transport sub-model, and a bank-erosion sub-model.	The model incorporates a new technique that simulates the degradation and aggradation process allowing the two sub-models to be closely combined.
Jia <i>et al.</i> (2009)	A 2D model CCHE2D to simulate river channel change and bank erosion process using a depth averaged model for ChoShui River. Bank erosion model based on general hydrodynamic and sediment transport model (CCHE2D). Considered basal erosion and mass failure based on Osman & Thorne (1988 a,b) and Hanson & Simon (2001).	Enhanced CCHE2D that simulates the secondary flow effects on suspended sediment and bed load sediment transport. Considers surface erosion and mass failure mechanism. Counter moving boundary (bank) problem and dynamically vary the mesh by using mesh stretching technique. Simulated sediment transport and bank erosion was in good agreement.
Jia <i>et al.</i> (2010)	A 3D model that simulates morphological change in alluvial channel due to bank erosion in the Shishou bend of the middle Yangtze river. Takes into consideration double layer and other factors affecting rate of bank erosion (longitudinal length failed bank, thickness of each layer, erosion-resisting effect of cohesive material from top layer). Has also considered cantilever failure proposed by Thorne & Tovey (1981).	Proposed equivalent channel-forming discharge can improved calculation efficiency for feasibility study (reduced 73% completion time). Model is capable to predict complicated riverbank composition.

Table 4. Laboratory model of bank erosion.

Investigators	Laboratory model	Bank material
Friedkin 1945	–	Non-cohesive
Chen <i>et al.</i> 1985	Morphology of river (prototype river)	Non-cohesive
Nagata <i>et al.</i> 2000	–	Non-cohesive
Papanicolaou 2001	Erosional strength of the soil	Cohesive
Jang & Shimizu 2005	Braided channel (validate)	Non-cohesive
Duan & Julien 2005 (Upgrade from Friedkin 1945)	Inception of channel meandering (validate)	Non-cohesive
Chen & Duan 2006 (same as Nagata <i>et al.</i> 2000)	Widening processes of a sine-generated channel (validate)	Non-cohesive
Ruther & Olsen 2007 (same as Friedkin <i>et al.</i> 1945)	Free-forming meander evolution (validate)	Non-cohesive
Chu-Agor <i>et al.</i> 2008	Effect seepage flow	Stratified (layer)
Jia <i>et al.</i> 2010 (same as Nagata <i>et al.</i> 2000)	Bank erosion and morphology change (validate)	Non-cohesive
Dulal <i>et al.</i> 2010	Slump block phenomenon (validate)	Stratified (layer)



Table 5. Numerical models of bank erosion (ASCE 1998).

Model	Cohesive	Bank material		Heterogeneous
		Non-cohesive	Layer	
Darby-Thorne	Yes	No	No	No
CCHEBank	No	Yes	No	No
Kovacs-Parker	No	Yes	No	No
Wiele	No	Yes	No	No
RIPA	Yes	No	No	No
Simon <i>et al.</i>	Yes	No	No	No
Pizzuto	No	Yes	No	No
STREAM2	Yes	No	No	No
GSTARS	–	–	–	–
Fluvial-12	–	–	–	–
Alonso-Combs	Yes	No	No	No
WIDTH	Yes	No	No	No
Mosselman <i>et al.</i> 1998	–	–	–	–
CONCEPTS (Langendoen <i>et al.</i> 2001)	–	–	Yes	No
Habibullah <i>et al.</i> 2001	Yes	No	No	No
CCHE2D (Duan <i>et al.</i> 2002)	No	Yes	No	No
Jang & Shimizu 2005	No	Yes	No	No
Duan & Julien 2005	Yes	No	No	No
Chen & Duan 2006	No	Yes	No	No
Wang <i>et al.</i> 2008	Yes	No	No	No
Devauchelle <i>et al.</i> 2008	No	Yes	No	No
CONCEPTS (Langendoen <i>et al.</i> 2009)	–	–	Yes	No
CCHE2D (Jia <i>et al.</i> 2009)	No	Yes	No	No
Dulal <i>et al.</i> 2010	No	No	Yes	No
Jia <i>et al.</i> 2010	No	No	Yes	No

## ACKNOWLEDGEMENTS

The authors would like to thank the Fluvial & River Engineering Dynamics (FRiEnD) Institute for Infrastructure Engineering and Sustainable Management, Faculty of Civil Engineering, Universiti Teknologi MARA and the Research Management Institute of Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia for the facilities and provision of grants for this study.

*Date of submission: May 2011*

*Date of acceptance: July 2012*

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# Computational Studies on the Process of Peptide Bond Formation

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Finding a proper transition structure for the peptide bond formation process can lead to a better understanding of the role of the ribosome in catalyzing this reaction. A potential energy surface scan was performed on the ester bond dissociation of the P-site aminoacyl-tRNA and the peptide bond formation of P-site and A-site amino acids. The full fragment of initiator tRNA<sub>i</sub><sup>met</sup> attached to both cognate (met) and non-cognate (ala) amino acids as the P-site substrate and the methionine as the A-site amino acid was used in this study. Due to the large size of tRNA, ONIOM calculations were used to reduce the computational cost. This study illustrated that the rate of peptide bond formation was reduced for misacylated tRNA without the presence of ribosomal bases. This demonstrated that there were indeed specific structural interactions involving the amino acid side chain within the tRNA<sub>i</sub><sup>met</sup>.

**Key words:** peptide bond formation; tRNA; ribosome; potential energy surface; ONIOM calculations; non-cognate aminoacyl-tRNAs

The process of protein synthesis starts with the complex of initiator tRNA<sub>i</sub><sup>met</sup> and smaller ribosome binding to the mRNA strand and finding the start codon (i.e. AUG) by moving along the strand downstream (5' to 3'). After the interaction of the tRNA<sub>i</sub><sup>met</sup> anticodon to the mRNA codon, the larger ribosomal subunit binds to the complex and places the initiator tRNA<sub>i</sub><sup>met</sup> in its P-site (Basavappa & Sigler 1991). The elongation cycle starts when the second amino acid binds to the ribosomal A-site and forms a peptide bond with the methionine in the PTC (peptidyl transferase center). Due to the induced fit mechanism (Bochevarov & Sherrill 2005), the elongation stage occurs in an accelerated forward rate for the cognate codon-anticodon interaction. On the other hand, the near/non-cognate codon-anticodon interaction results in a slow forward reaction rate compared to the reverse one during the accommodation of the aminoacyl-tRNA (aa-tRNA) in the A-site. This effect is known as the kinetic proofreading which is caused by the lack of induced fit mechanism. The kinetic proofreading process is only limited to the codon-anticodon interaction and not the amino acid side chain. In addition, it is not known whether the ribosome specifies the amino acid side chain in the A-site. Thus, it remains unclear where the misacylated tRNA (in case of occasional mistakes during the tRNA aminoacylation process (Echenique & Alonso 2007) goes wrong (Dale *et al.* 2009). Concerning this matter and using the assumption of which

the ribosome's specificity for the amino acid side chain is not limited to the initial selection (Dale *et al.* 2009), we performed computational calculations on the process of peptide bond formation using the full fragment of initiator tRNA<sub>i</sub><sup>met</sup> attached to cognate (methionine) and non-cognate (alanine) amino acids as the P-site substrates and methionine as the A-site amino acid. This study enables us to compare the process of peptide bond formation between cognate and non-cognate aminoacyl-tRNAs. Since the transition structure of the peptide bond formation can not easily be observed (due to the fast rate of peptide bond formation i.e. 20/sec), we calculated the potential energy surface (PES) for this reaction to estimate the transition state (TS) for both cognate and non-cognate amino acids attached to the tRNA<sub>i</sub><sup>met</sup>. Due to the large size of the molecule, we used the ONIOM (Our Own N-layered Integrated molecular Orbital and molecular Mechanics) calculations to have accuracy while saving the time. The calculations are performed in the absence of ribosomal bases and the results are compared with the ones carried out empirically with presence of the ribosome. This comparison will help one to understand the importance of ribosomal bases in catalyzing the process.

## Computational Methods

The full fragment of tRNA<sub>i</sub><sup>met</sup> is used as the P-site tRNA for formation of the first peptide bond. The X-ray crystal

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structure of this tRNA is obtained from Protein Data Bank (www.rcsb.org) (ID code: 1YFG) (Effraim *et al.* 2009). The calculations are carried out using Gaussian 03 program and the molecular visualizations are performed with Gauss View (Gaussian 03 *et al.* 2003). Unlike other computational studies on the same process (Gindulyte *et al.* 2006; Monajemi *et al.* 2009), we used the full fragment of the tRNA molecule. The model excludes the surrounding PTC of the ribosome due to the large size of the molecule and limited computational resources. Since the A-site tRNA is not directly involved in this reaction, we used the amino acid itself to save the computational cost (including both tRNAs together in the calculation results in nearly 5000 atoms which makes it impossible to compute with the available computer hardware). Due to the large size of this molecule (near 2500 atoms), the ONIOM(QM/MM) method is used to have high accuracy with reasonable time. ONIOM is a hybrid method that overcomes the size limitation in computational analysis on large molecules (Schmeing & Ramakrishnan 2009). This method enables one to divide the system into two (high and low) or three (high, medium and low) different layers. In this study, we used a two-layered ONIOM method, QM for the region where the chemical reaction (peptide bond formation) was occurring, and MM for the rest of the tRNA which was important enough to consider given its interaction with the high region. The energy expression for ONIOM(QM/MM) method was:

$$E^{\text{ONIOM}} = E^{\text{Model,High}} + E^{\text{Real,Low}} - E^{\text{Model,Low}} \quad (1)$$

where,  $E^{\text{Model,High}}$  is the energy of the QM region with a high level of calculation,  $E^{\text{Real,Low}}$  is the energy of the MM region with a low level of calculation and  $E^{\text{Model,Low}}$  is the energy of the QM region with the low level of calculation (similar to the one used in  $E^{\text{Real,Low}}$ ).

### S-Value Test

This study involves bond breaking/forming process which requires more concentration in choosing a proper layer for the QM and MM regions in order to have a continuous PES from the reactants to the product (for more information please refer to reference (Schmeing & Ramakrishnan 2009). For the continuity of the PES, the MM contribution to the ONIOM energy must be continuous and for the latter to occur, the S-value (substituent value) must be the same for the reactants and the product and the MM terms must cancel each other in Equation 1. In other words, the Equation 2 must be zero (Schmeing & Ramakrishnan 2009).

$$\Delta S^{\text{MM}} = S^{\text{MM}}(\text{TS, reactant connectivity}) - S^{\text{MM}}(\text{TS, product connectivity}) \quad (2)$$

A zero  $\Delta S$  is a result of a continuous MM energy contribution in reactants and product which ends up with

a continuous potential energy throughout the reaction. To verify a more appropriate partitioning in our system, we have used this method for three different partitioning (Figure 1). The most appropriate partitioning is the one with  $\Delta S$  closer to zero (Table 1). According to this partitioning model, the MM region contains 2361 atoms where for the cognate met-tRNA<sub>i</sub><sup>met</sup>-met with 2431 atoms in the real system, 70 atoms are in QM region and for the non-cognate ala-tRNA<sub>i</sub><sup>met</sup>-met with 2424 atoms in the real system, 63 atoms are in QM region.

### Convenience of Using the DFT Method over MP2 for the Model System

Looking at the bond breaking/forming requires both accuracy and speed in the calculations. Methods such as single and double excitation coupled cluster (CCSD) or configuration interaction (CI) are too expensive to be used in our ONIOM calculations. Furthermore, Configuration Interaction method fails in explanation of the chemical reactions because of lack the size consistency. Thus, we have carried out some comparison calculations which can determine an efficient method to study the process of bond breaking (Schweisguth & Moore 1997). Although the MP2 method is the simplest and least expensive single reference correlated method which is regarded as accurate in the literature (Stortchevoi 2006), the results of our previous studies on this process indicates the aptness of DFT method for bond breaking/forming studies. This is due to the fact that the MPn methods in general do not give a good description for the bond breaking/forming processes and fall apart when the system is not at equilibrium (Schweisguth & Moore 1997; Trobro & Aqvist 2005). Thus we used the DFT method using the hybrid Becke three-parameter functional with the correlation functional of Lee, Yang, and Parr (B3LYP) for the model system while the real system is handled with a molecular mechanics method i.e. UFF. To consider accurate representation of bonding between atoms, we used a split valance basis set with one polarization function i.e. 6-31G(d).

## RESULTS

### PES of the Peptide Bond Formation for the Full Fragment of tRNA

The PES of the peptide bond formation was calculated for the cognate met-tRNA<sub>i</sub><sup>met</sup>-met and the non-cognate ala-tRNA<sub>i</sub><sup>met</sup>-met as a function of peptide and ester bond variation from 1.2 Å to 4 Å (the natural peptide bond length is around 1.34 Å) with the step size of 0.2 Å (Figure 2). The process of peptide bond formation for the cognate structure occurred by attacking a lone pair from a nucleophile (i.e. the amino group of the methionine) to the carboxyl group with an electrophile leaving group attached to it (i.e. hydroxyl of the ribose sugar). The cleavage of the C-O (ester) bond



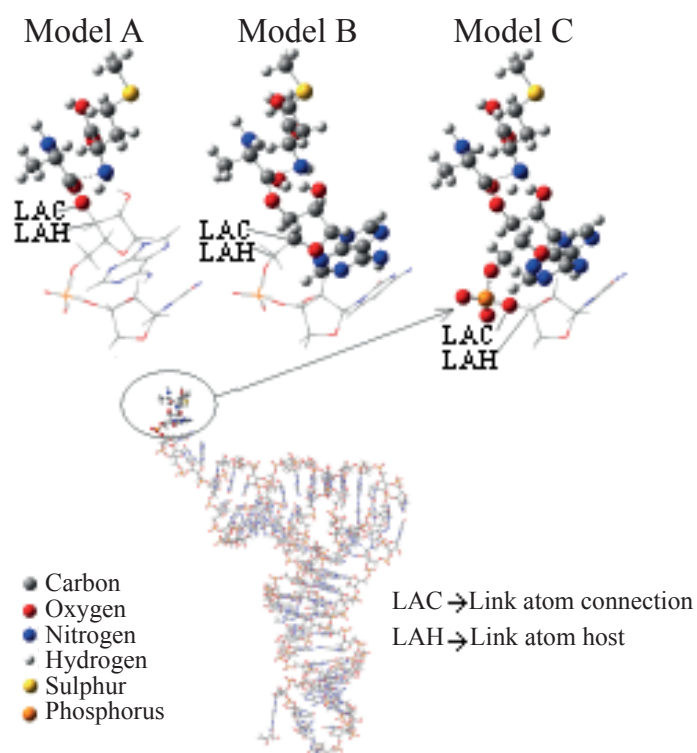


Figure1. Three different partitioning models for the aa-tRNA molecule where the peptide formation occurs.

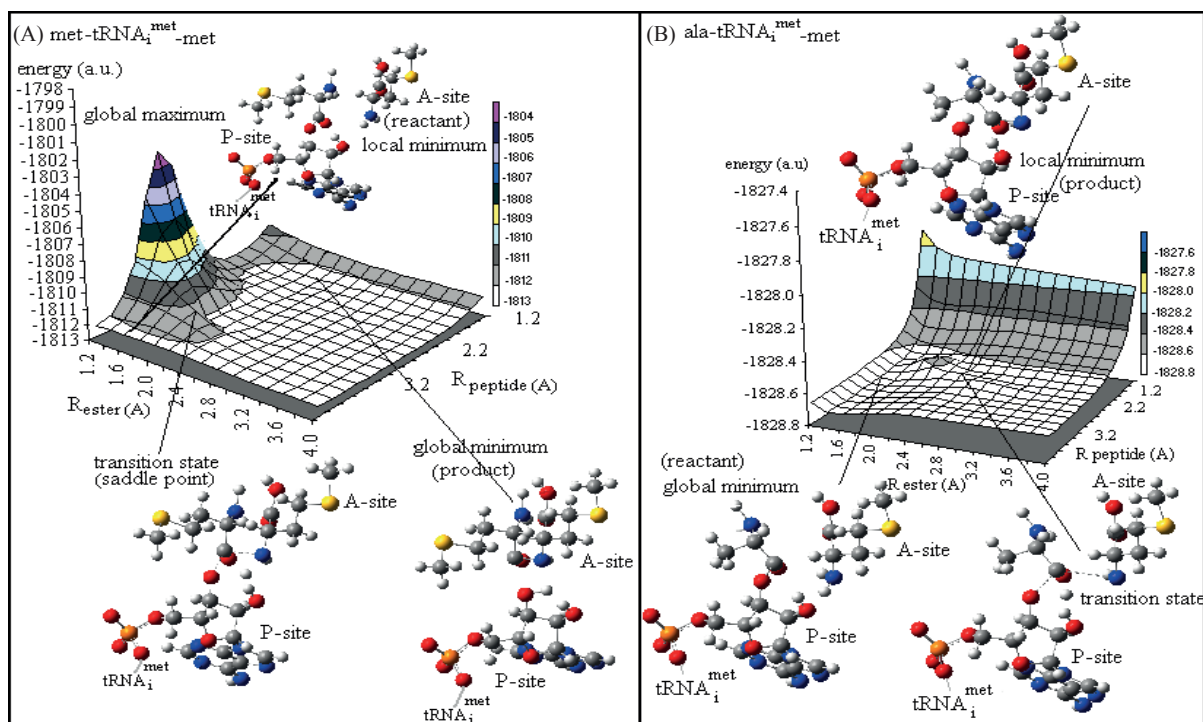


Figure 2. The PES for (A)  $\text{met-tRNA}_i^{\text{met}} - \text{met}$ , (B)  $\text{ala-tRNA}_i^{\text{met}} - \text{met}$ .

and the formation of the C-N (peptide) bond occurred simultaneously and formed TS. As it is shown in figure 2-A, there was a path from reactant to the equilibrium of the peptide bond at 3.4 Å and an ester bond distance of 2 Å. This point was the TS which led the reactants to the product. The system exhibited a large maximum as the scan approached small R-ester and intermediate R-peptide. This was understood as overcrowding would occur at these small bond distances. The PES showed a relative stability at higher R-peptide and lower R-ester compared to the other parts of this surface. However, the system was less likely to undergo chemical reaction as the R-ester increased and the R-peptide decreased. This indicated the relative stability of the product (after the formation of the peptide bond). Additional to this, a minimum was observed at R peptide of 1.6 Å and R ester of 2.2 Å. The minimum was rather flat and it was rather difficult to pinpoint the actual minimum of this potential well.

However, the situation was quite different when the non-cognate amino acid (i.e. alanine) was attached to this tRNA (Figure 2-B). This surface was almost similar to the previous one in a sense that decreasing the peptide bond from 2 Å to 1.2 Å in a constant ester bond decreased the stability of the molecule. However, this instability was less severe in the cognate structure. Conversely, the global maximum which was observed in both surfaces was less intense in the non-cognate structure. Furthermore, the location of global maximum at the ester bond of 2 Å and the peptide bond of 2.4 Å resulted in late TS i.e. at the ester bond of 2.6 Å and the peptide bond of 2.4 Å. The late TS might result in a low rate of ester bond

dissociation and peptide bond formation for the non-cognate structure.

### PES of the Peptide Bond Formation for the Short Fragment of tRNA

To have a better understanding of the chemical reaction, we decreased the size of the molecule to the level that only one nucleotide (adenine) was attached to alanine. This study enabled us to optimize the whole structure and get more accurate energies using the DFT/B3LYP method. Furthermore, comparing this study with the ones carried out with presence of the tRNA body could help us to understand the effect of the tRNA body on the amino acid side chain as well as the reaction itself.

Since we were comparing the PES for this reaction with the ones carried out using the full tRNA incorporation, we used rigid scan for the peptide and ester bond variation to avoid the optimized structure at each point. The PES for this process (Figure 3) explained the ester bond dissociation between alanine and adenosine<sup>ala</sup> during the nucleophilic attack from the amino group of methionine to the hydroxyl group of alanine-adenosine<sup>ala</sup> as well as peptide bond formation between the carboxyl group of alanine and the amino group of methionine.

As it was observed, global minimum was where the peptide bond was 1.4 Å and the ester bond was 1.6 Å (product). The pass which was shown in a red line was a possible reaction path along the reaction co-ordinate towards the product. The highest energy on this pass was the

Table 1. The *S*-value test for three partitioning models proposed in Figure 1.

Three different partitioning models	$\Delta S^{MM}$
Partitioning model A	0.0004497
Partitioning model B	0.0006148
Partitioning model C	0.0000993

Table 2. Comparison between TS co-ordinates of three PESs in this study and other relevant studies.

Study	Molecule	Transition state co-ordinate	
		Peptide (Å)	Ester (Å)
Long fragment (this study)	met-tRNA <sub>i</sub> <sup>met</sup> -met	3.4	2.0
	ala-tRNA <sub>i</sub> <sup>met</sup> -met	2.4	2.6
Short fragment (this study)	ala-adenosine-met	3.6	1.4
Short fragment in ribosomal bases (Gindulyte <i>et al.</i> 2006)	ala-ribose-ala-ribose	1.57	1.9
Short fragment in ribosomal bases (Wallin and Åqvist 2009)	ala-ribose-ala-ribose	6-member	1.55

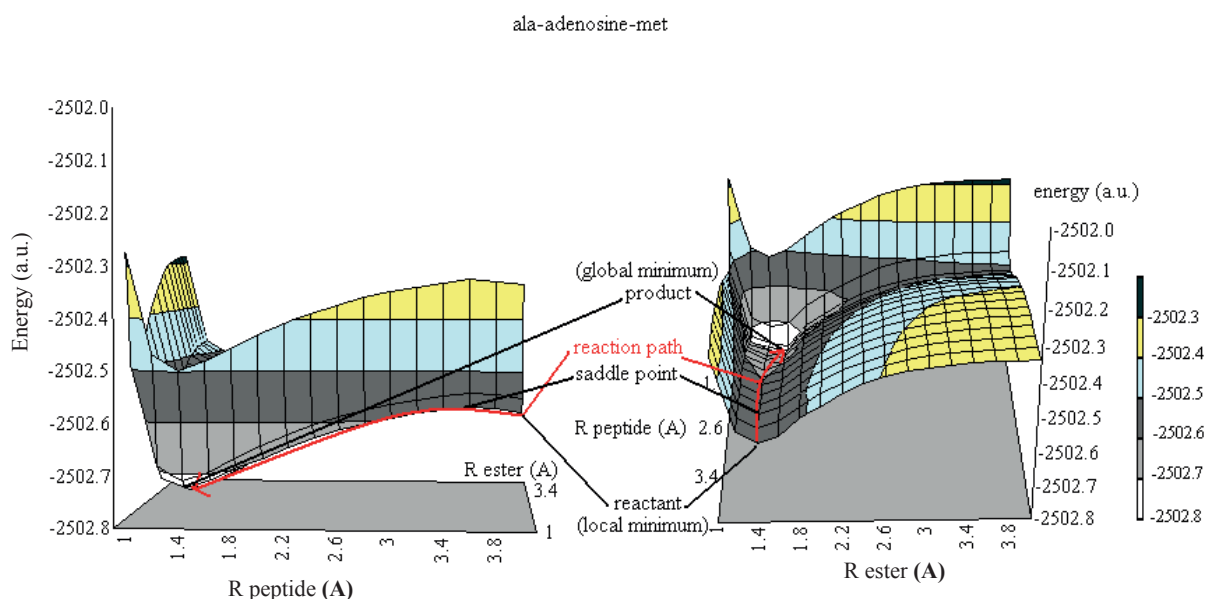


Figure 3. PES for the short fragment of non-cognate ala-tRNA<sub>i</sub><sup>met</sup>-met i.e. ala-adenosine-met.

saddle point where the structure was at its TS (i.e. the ester bond of 1.4Å and the peptide bond of 3.6Å). The early TS and the high steep of the path towards the global minimum indicated the tendency of the reactant towards forming the product. This was in a good agreement with the TS co-ordinate for met-tRNA<sub>i</sub><sup>met</sup>-met reaction. Table 2 shows the comparison of the TS co-ordinates on the PES for the three structures of cognate and non-cognate aminoacyl-tRNAs and ala-adenosine-met. The similarity of the transition state co-ordinates in other studies using the short fragment of the tRNA molecule (i.e. the ribose of the adenosine with the absence of purine base) which indicated the effect of the tRNA<sub>i</sub><sup>met</sup> molecule on the reaction (Table 2). Although the transition structures in some of those studies are not 4-membered (6-membered due to the presence of 2'-OH of the ribose as a proton transfer in the reaction) (Wallin & Åqvist 2009), there are some similarities between the forming peptide bond length and dissociating ester bond length in the transition structures. Those studies mainly focus on the rate in which the reactions occur in different conditions and the role of the tRNA molecule in the reaction was not taken into consideration.

## DISCUSSION

There is a significant difference between the TS co-ordinates of ala-tRNA<sub>i</sub><sup>met</sup> and the other two molecules: the late TS indicates the refusal of this molecule to react with methionine and form the peptide bond. The peptide bond formation for the cognate amino acid (i.e. methionine)

attached to the tRNA<sub>i</sub><sup>met</sup> shows the tendency for forward reaction while the non-cognate structure tends to remain in its initial condition. This occurs when the non-cognate amino acid is in the P-site and the next amino acid is about to be attached to it (meaning that the non-cognate amino acid is already attached to the nascent polypeptide chain). Therefore, this process cannot be corrected and the only way which can prevent the creation of improper protein is the premature termination of the peptide bond formation and dissociation of the ribosome to the environment.

However, since this study is on the initiator tRNA<sub>i</sub><sup>met</sup>, there is a possibility that this behaviour is only limited to this tRNA due to its structural differences with the other elongator tRNAs (Vreven *et al.* 2006). Therefore, further studies on the process of peptide bond formation for cognate and non-cognate elongator aa-tRNAs will be carried out in the future in order to investigate the structural behaviour of the elongator tRNAs during the peptide bond formation process.

## ACKNOWLEDGEMENTS

I would like to acknowledge the financial support provided by the Institute of Research Management and Consultancy of the University of Malaya.

*Date of submission: May 2011*

*Date of acceptance: June 2012*

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# Challenges and Importance of Traceability of *Halal* Raw Materials in Processed Meat Products: A Review

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Research and development trends will continue to design innovative composite foods in which muscle proteins are combined with non-conventional animal products, non-meat proteins and functional food additives, many of which have lost their original inherent properties and characteristics. Composite food are products with meat, non-meat proteins, fats, carbohydrates and functional ingredients such as pre-emulsion, probiotics, enzymes, bioactives, peptides, hormones, emulsifiers, gelatin, animal fats/oils, alcohol and visceral tissues. Traceability of *halal* meat raw materials should start at the point of animal breeding, production to the stage of *halal* slaughter, processing operations and final point of consumption. Traceability of food additives used in the food industry remains a major hurdle for the Muslim community seeking *halal* food. The processes and technological advancements made in raw material processing, ingredient extractions, modifications, purification and resynthesized into many food ingredients make the question of traceability and solving of the materials and processes that are *halal* a monumental task. Food is only *halal* if the entire food chain from farm to table, is processed, handled and stored in accordance with the *syariah* and/or *halal* standards or guidelines, such as in the *Jabatan Kemajuan Islam Malaysia (JAKIM): General guidelines, Malaysia Standards MS 1500:2009* and *Codex Alimentarius (Food Labeling)*. Here lies the challenge and importance of traceability to verify the 'wholesomeness' of the sources of *halal* raw materials and final meat-based food products.

**Key words:** *halal*; food ingredients; food packaging; composite food; processing operations; standards; guidelines

Muslims in Malaysia are concerned about the raw materials, ingredients and additives utilized in the production of *halal* food products. Traceability of *halal* meat raw materials should start at the point of animal breeding, production to the stage of *halal* slaughter and processing operations. All sources of food are *halal* except for the following sources including the products and derivatives which are considered *haram* such as land and aquatic animals; plants; mushroom and micro-organism; natural minerals and chemicals; drinks; sauces and condiments; and genetically modified foods. All land animals are lawful as food, except dogs; pigs; the animals that are not slaughtered according the *syariah* law; animals with long-pointed teeth, e.g.: tiger; predator birds like eagles; animals which are forbidden to be killed like bees and ants; and animal enjoined by Islam to be killed like snake. All aquatic animals are *halal*, except poisonous, intoxicating or those hazardous to health. Puffer fish is *haram* except when the poison has been effectively removed. Natural minerals and chemicals are all *halal* except those which are poisonous, intoxicating or hazardous to health. All additives and preservatives must comply with

Food Regulations 1985. Foods and drinks containing and/or by-products of genetically modified organisms (GMOs) or ingredient made by use of genetic material of animals that are non-*halal* by *syariah* law are not *halal* (JAKIM 2011).

Handling of raw material mechanically using pumps, pneumatic conveyors, conveyor belts, forklifts and cranes should ensure that the machinery part in contact with foods are not *haram* or contaminate. These include lubricants from pig fat, animal grease and body parts. Food ingredients and additives should be derived from *halal* animals slaughtered according to *syariah* law. Food ingredients and additives can also be derived from intermediate products such as fats and oils, frying oil, shortening, butter, margarine, ghee (animal source), *vanaspati* (vegetable ghee) and blended oil. All sources must be *halal*. Rennet (Rennin), one of the food additives added to agglutinate milk and aids the formation of typical cheese flavour and texture, is derived from either stomach of young animals, or microbes e.g.: *Escherichia coli*.

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Protein hydrolysates are considered *halal* such as hydrolyzed vegetable proteins which are added for flavour, water binder, thickener, conditioning agent, gelatin which is partial hydrolysis of collagen from bones and skins of cattle and sheep. However toxic or carcinogenic components such as 3-monochloropropane-1,2-diol derived from fish sauce which is hazardous to human is classified as *haram*. Traceability of food additives used in the food industry remains a major hurdle for the Muslim community seeking *halal* food. The process and technological advancements made in raw material processing, ingredient extractions, modifications, purification and resynthesized into thousands of food ingredients make the question of traceability and solving of materials and processes that are *halal*, a monumental task. Food is only *halal* if the entire food chain from farm to table, is processed, handled and stored in accordance with the *syariah* and/or *halal* standards or guidelines, such as in Malaysia — the *Jabatan Kemajuan Islam Malaysia (JAKIM): General Guidelines, Malaysia Standards MS 1500: 2009* and *Codex Alimentarius (Food Labeling)*.

In Thailand and Malaysia, further processing has increased steadily, with about 20% of the processed birds going for further processing (Babji 1993). This figure is expected to increase in Asia especially in China and Indonesia. Price intervention and classifying poultry as a controlled food item by the government has resulted in low profit margin for whole birds. Producers are motivated to increase the value added products which give high profit margins. Locally manufactured products are developed specifically for local markets. The range of value added chicken products do not compete with imported products like Tyson's or Orca Meyer's.

The expected shift from red meat to white meat, and the growth of fast food chains would place the poultry products in the forefront. The focus is on down stream processing emphasizing on quality, to meet health, nutritional and quality standard requirements such as Hazard Analysis and Critical Control Points, Quality Assurance Programme and *halal* Assurance System (*JAKIM*) of importing and exporting countries.

Fat provide succulence, texture and flavour, all of which are altered if fat is removed. Reducing salt in processed meat may be detrimental to emulsion stability, cook-yield, flavour, and juiciness. Moving upstream, meat scientists are working with medical scientists to improve the health fullness of ruminants. Modification of the fatty acid content of meat products through changes in animal feeds and product changes are aimed at reducing the amounts of fatty acids which can potentially elevate human blood cholesterol.

Unconventional protein resources such as mechanically deboned meat, connective tissues, skin and surimi-like

materials are carefully formulated to yield a wide range of meat based food products, many imported or locally produced. One needs to ponder the question of origin of raw materials and address the issues related to *halal* status of such food items.

Modern blending and marinating technology, pre-emulsion, gelation, and coating techniques produced what consumers demand: quality meat products which are nutritious, healthy and wholesome (*halal*). Functional ingredients are currently in demand in the processed meat industry simply because they are able to replace fatty tissues and yet retain the quality characteristics associated with good meat products. Thickeners, binders, fillers, fat replacers and fat extenders are becoming more useful in producing healthy food. In fat replacement systems, the majority of fat is replaced by water. Water replacement alone makes products too soft (Yackel & Cox 1992).

Food additives, such as phosphates, carrageenan and isolated soy proteins are technologically blended to produce quality meat products. Certain texturising agents can be used to improve the eating texture of fat reduced processed meats without necessarily mimicking the texture of fat. Cereal starches, rusks, brans and potato starches are also replacing part of the meat protein and fat, due to the demand for increase in fiber and carbohydrate in their diets. Processing variables such as time and temperature for meat protein and carbohydrates are different, thus requiring research and development to achieve quality products.

The concept of restructured meat products has attracted increasing attention in both research and commercial areas (Smith 1989). New processing technologies are involved in the production of meat products with low fat and new ingredients with functional properties aimed at producing healthy products. Of significant importance is the emergence of gelatinized connective tissue protein from low grade beef as raw material in processed meat (Osborn & Mandigo 1995). The broiler- and fish-based industries have blended in sophisticated processing technologies, coupled with the latest in composite, restructured and formulated recipes to produce a wide variety of convenient meat products. In addition, large local processing plants have utilized efficient processing operations to minimize animal wastes and by-products, improving their functional and nutritional properties and producing economical good quality value-added meat products (Babji 1993; Babji & Ong 1993; Babji & Seri Chempaka 1995; Babji 1996). Currently, the private sectors are well informed on the availability of modern processing technologies combined with appropriate ingredients blended with the meat to yield meat products. One of the important protein functionalities, in processed meats is thermally induced gelation of muscle proteins. Thermal gelation changes of the muscle proteins account for many textural and functional properties including gel strength, gel elasticity, water holding

capacity, and encapsulation of other food constituents of the processed meat products (Fukazawa *et al.* 1961; Asghar *et al.* 1985; Autio *et al.* 1989; Autio & Mietsch 1990). Coating technology added bite and crispiness of texture powdering, battering and breading enhance the appearance and taste characteristics associated with desired colour and flavour (Cunningham & Suderman 1981). Some researchers reported better quality chicken products using the flour pre-dust-batter-flour or batter-breading methods (Nakai & Chen 1986). The technological implementations of plant proteins and other food ingredients have resulted in a wide range of modern processed meat products currently available in the Asian region. Isolated soy protein in further processed poultry marination secures optimum penetration into the meat membrane. Tumbling, massaging and curing, improved flavour, juiciness, and yield for both coated and uncoated meat products.

Blending of flavour ingredients and aromatic components has enabled manufacturers to enhance the blend raw tastes of meat and poultry products by marinating technologies and self basting, giving new possibilities to a wide range of flavours into meat products. Frozen batter and breaded bone in products or coated flash fried and frozen meat used to be of poor quality due to poor batter adhesion and high oil absorption during frying. Today, hot air ovens and hot air fryers eliminate these negative side effects. These new technologies control humidity and air temperature, producing quality and uniform processed meat and poultry products (Hoogenkemp 1992). The co-extrusion technology using Rheon machineries, has resulted in a vast range of meat products with pastry and dough mix such as the *pow* with meat fillings and the *dim sum* with all sorts of fillings currently available in the market.

A new processing technology with specialized ingredient was developed by the Protein Technologies International U.S.A., an emulsion preparation using isolated soy protein, animal fat and ice water emulsified in a vacuum chilled bowl chopper to produce a pre-emulsion. This was then combined with high quality surimi to yield excellent composite product in terms of sensory acceptability as well as meeting the health needs of consumers.

Processing operations are also important in the production of quality products. Chopping sequence for comminuted meat products need to be properly followed to achieve desirable, and quality end products. Product development must be complemented by industry needs to produce *halal* value-added products that meet the demand of Muslim consumers. In Asia, traditional meat and poultry products of high fat, cholesterol, salt and chemical additives will have to give way to 'lite and lean' types of products. While this change is significant in developed nations, Asia is still fully utilizing its meat resources to the fullest. Fatty tissues are fully utilized in products like *Lap Cheong*,

Taiwanese sausages, *Naem*, *Chorizo* and *Longanisa*, being still popular largely amongst the low income communities. It makes little sense for product development technologists in Asia to research into detailed aspects of meat quality. The priorities for research should be re-orientated with emphasis on quality control and health monitoring and *halal* assurance rather than repeating basic meat science and technology knowledge such as tenderness, juiciness and water holding capacity of meat.

In more developed countries, a major research area has emphasized on the wide variability related to eating quality, and how to reduce variations. Asian poultry scientists and technologists should not get too carried away with known facts that do not matter much to the vast majority of Asian population. One has to ask what the Muslim and non-Muslim consumers really want and how to captivate these two potentially growing market? Do we produce more meat with growth-promoting hormone products, enzymes and food ingredients and fail to meet demands for *halal* meat products? This is what research planners in Asia need to address; is it worth pursuing new methods of improving efficiency that involved feed additives, hormone application, and genetic manipulation, resulting in issues related to *halal* food production? Research trends will continue to design innovative food in which muscle proteins are combined with non-meat proteins. The composite type products with meat, non-meat protein, fat, carbohydrate and functional additives will be more visible than a major challenge in food ingredients and raw material traceability.

### ISSUES IN HALAL FOOD PACKAGING AND PROCESSING

Asian market display of meat products in supermarkets showed no less innovation in packaging capability. Packaging companies from the West have applied information on trends, distribution patterns and advanced packaging technology to solve problems and added value to Asian poultry products. These include not only optimum packaging materials, but also equipment, technical support and graphic design. However, efforts in packaging technology have neglected the *halal* aspects of food packaging globally. Malaysia, in its effort to ensure *halal* food are wrapped/packed in proper materials have included *Halal Standards MS1500:2009 Guidelines on Packaging (Malaysian Standard 2009)*. *Halal* packaging materials must not contain raw materials considered *najis* such as sausage casings from pig intestine or unslaughtered animal packaging materials must not be contaminated with *najis* and food transported should be physically separated from non-*halal* food and *najis*. Packaging should also be carried out in a clean and hygienic manner in clean sanitary environment. Packaging manufacturers must comply with the *halal* requirement if claims are made on the *halal* status

be it from any form of packaging materials. Here again, the question of source/origin of films/packaging materials become issues that need clear definitions as to its intent and usage. Waxes and coatings may be from animal fats, while metal cans and drums can be contaminated with lard or *haram* animal fats.

Concerns by Muslims on *halal* meat and meat products need to be considered in the production of *halal* food from farm to table food chain. These include raw meat imports, local slaughter, processing operations/equipment, packaging, storage, transportation, food ingredients, additive adulterants, biotechnology and genetically modified animals, food safety and food quality aspects of *thoyyibah*. Animal products are particularly addressed in issues related to *halal* food production as many products from the animal industry are available in the market. Gelatin, enzymes, emulsifier, blood, mono/diglycerides, alcohol, lard, tallow, viscera and hormones are directly or indirectly added or included intentionally or unintentionally to produce ingredients, additives and food for the consumers. Thus the importance of traceability for all these raw and processed materials is of most importance for the Muslim consumers.

*Date of submission: May 2011*

*Date of acceptance: July 2012*

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## Acknowledgement of Referees

The Academy of Sciences Malaysia and the Editorial Board of the *ASM Science Journal* (*ASM Sc. J.*) wish to express sincere appreciation and gratitude to the following respected referees, for their valuable and profound technical evaluation of manuscripts for  
*ASM Sc. J.* vol. 6, nos. 1 & 2, 2012:

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# Impact of Climate Change on Water Resources: Global and Malaysian Scenarios

A.S. Mohd Armi<sup>1</sup>, A.S. Afiza<sup>2</sup> and A. Mohd Ramzi<sup>3</sup>

Climate is the word which often used to describe the long-term mean state of the atmosphere and this includes the corresponding temperature, humidity and wind strength. Climate can also refer to the oceanic state, the cryosphere (snow and sea-ice), the biosphere and sometimes even the lithosphere (Earth's crust). Meteorologists and atmospheric scientists profess that climate is the condition that you expect; weather is the condition that you get. The dynamical systems are therefore full of complicated processes which induce chaotic variations and changes due to the external factors enforcing of the systems. For the Earth's climate, we usually think of trends in global average quantities (especially surface temperature) as indicators of climate change. When the trend leads to a change larger than the natural variability, most certainly a statistically significant change has occurred.

The current issue of climate change is closely related to water resource systems, especially in terms of water usage. A lot of discussions have been made on this matter followed up with a special Intergovernmental Panel on Climate Change (IPCC) publication dedicated to water and climate change dating back to the 19th IPCC Session held in Geneva in April 2002, where the Secretariat of the World Climate Programme—Water and the International Steering Committee of the Dialogue on Water and Climate requested that the IPCC prepare a special report on water and climate. The dialogue concluded that climate change and water resource were very important issues which needed to be discussed and effective actions should be taken to curb any problems.

Climate variability already makes a large impact on water supply and protection. Millions of people are affected every year by droughts and floods. Future climate change is likely to make things worse. Many people within the water sector are aware that climate change would affect water resource management and system.

## Assessment of Global Scenario

In global-scale assessments, basins are defined as being water stressed if they have either a per capita water availability of below 1000 m<sup>3</sup> per year (based on long-term average runoff) or a ratio of withdrawals to long-term average annual runoff of above 0.4. A water volume of 1000 m<sup>3</sup> per capita per year is typically more than what would be required for domestic, industrial and agricultural water usage. Water usage, particularly for irrigation, generally increases with temperature and decreases with precipitation; there is no evidence however of climate-related trends for water usage in the past. This could be due, in part, to the fact that water usage had been mainly governed by non-climatic factors resulting in the poor quality of water-usage data in general and of time-series data in particular.

Previous studies by Prof Pavel Kabat (University of Wageningen, Netherlands) and Paul Reiter (International Water Association) had outlined the challenges of global changes with increases of population, environmental pressures and increasing demands of water for domestic water usage in the agricultural sector. Climate change contributes to global change by rising sea levels, increasing floods, storms and droughts with differing impact for different regions. The authors observed that as we were not able to cope with the current variability, we need to gear up to be prepared for future changes. 'Stationary is dead' quoted a recent scientific article, when leading hydrologists seem to have changed position to attribute critical importance to climate change and that we needed to think of the possibilities of step changes and how to cope with them. State leaders are very concerned about the impact of climate change. The problem is that the current management regimes are not robust enough. Some countries have started to prepare tools on how to mainstream climate factors into their programme and are planning to cash in

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on their big investments to cope with it. Globally, one of the problems is that we do not have enough data. There are many gaps in our observations and monitoring which need investment and a concerted international effort, for instance in satellite technology imaging. We also have a lack of understanding and ability to link climate change scenarios and hydrological models to have a hydrological impact at city, rural or utility levels when the forecasts are surrounded by uncertainty and socio-economic factors. We have to find a recipe of response and create a portfolio of measurement to address the impact of climate change so that it would not be only about building storage, other structural measurements or awareness.

We all know that climate change is more than a warming trend. Increasing temperatures would lead to changes in many aspects of the weather and indirectly touch water resources management factors such as wind patterns, the amount and types of precipitation, including the types and frequency of severe weather events that might be expected to occur currently or in the future.

### Malaysian Experience

Malaysia has always been vulnerable to extreme climatic events such as typhoons, floods and drought. We expect climate change to exacerbate these vulnerabilities. Our projections show that by 2050, Malaysia would be

hotter with a temperature rise of up to 1.5°C. We also expect more rainfall extremes—intense rainfall in the wet period and a lack of rainfall in the dry period. This could lead to a higher high flow which meant that there could be more severe floods and lower low flows or longer droughts. The sea level is also expected to rise anywhere from 15 to 95 centimeters over a hundred-year period, threatening the increasing urban population in our coastal areas. We still need more in-depth assessments to be able to answer with confidence in terms of the exact impact on irrigated and rainfed agriculture, public water supply, hydropower generation, erosion and sedimentation of river basins and other areas. However, preliminary findings indicate that Malaysia could be a net carbon sink rather than an emitter. This meant that most of the climate changes that we are currently experiencing were likely due to emission from the more developed economies, but that is not to say that we are blameless. Land use changes such as deforestation, followed by inconsiderate highland agricultural activities and rapid urbanization with scant regard for sound stormwater management practices, have had already made an impact on Malaysia's hydrological regime. These can be observed by the increase of storm runoff that lead to severe floods, the reduced river baseflows compounded with indiscriminate river pollution that lead to public water restrictions during droughts, or the erosion, sedimentation, and landslides experienced last December in Cameron Highlands, the

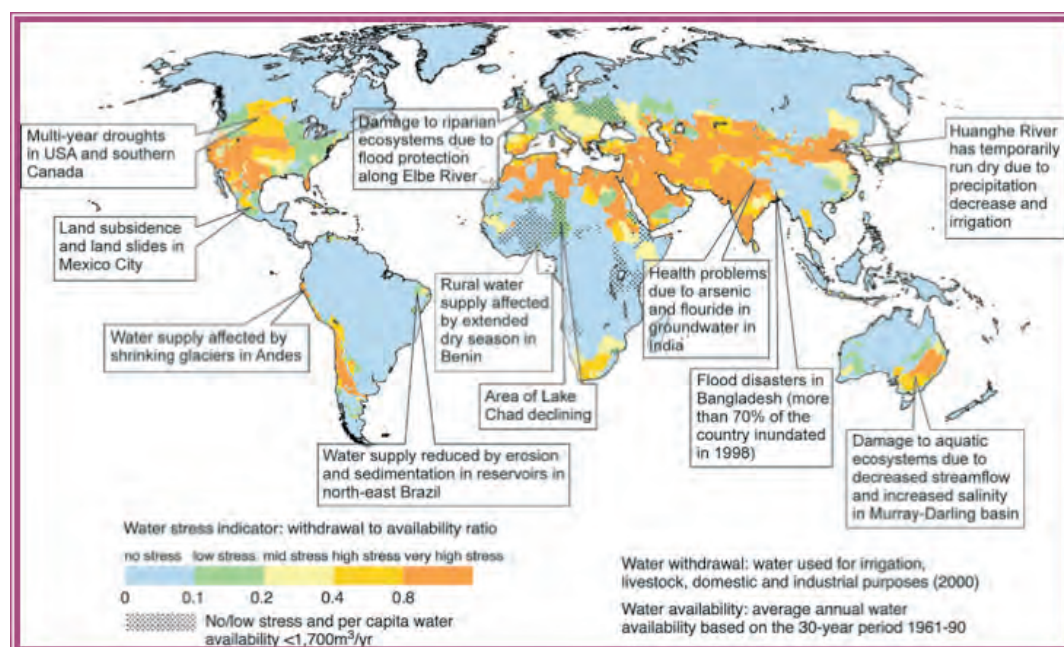


Figure 1. The current vulnerable freshwater resources and their management; in the background, is a water stress map based on WaterGAP.

Table 1. A comparison of sectoral integrated impact for four climatic scenarios (percentage change from 2060 base results).

Climatic scenario	Impact sector				Integrated impact
	Land area	Food demand	Agricultural water productivity index	Water Resources	Consumer—producer surplus
UKMO <sup>1</sup>	– 5	– 3	– 45	– 13	– 23
GISS <sup>2</sup>	– 5	– 1	– 13	+ 18	– 6
GFDL <sup>3</sup>	– 5	– 1	– 36	– 78	– 52
'Low-end'	– 5	0	+ 10	+ 14	– 10

<sup>1</sup> United Kingdom Meteorological Office model (Wilson & Mitchell 1987)

<sup>2</sup> Goddard Institute for space studies model (Hansen *et al.* 1983)

<sup>3</sup> Geophysical Fluid Dynamics Laboratory model (Manabe & Wetherals 1987)

Klang Valley and Johor. Figure 1 shows the map for current vulnerable of freshwater resources, their management and a comparison of sectoral with integrated impact for four climatic scenarios.

is real. They also need to accept that actions to adapt with our vulnerabilities should be immediately put in place. We can do this by integrating the various sector policies and securing the participation of all stakeholders in Malaysia and other countries.

## CONCLUSION

Over the last century, the earth's climate has changed. It is a serious global, long-term problem which involves complex interactions. A lot of evidence suggests that most of the observed factors contributing to the crisis over the last 50 years can be attributed to human activities. Since the early 1970s, from the first Earth Summit in Stockholm, Malaysia has considered climate change in its planning initiatives. The evidence of climatic changes has become clearer as the scientific analyses have improved. Thus, we are constantly assessing our priorities and strategies to prevent the problem from worsening. To ensure, that our water resources will always be secure and ready for use. We need to create awareness in the public and the policy makers so that they will acknowledge that the climate change issue

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# Electrons Making Waves in the Sky: The Ionosphere and Us\*

When Nobel Laureate Sir J. J. Thomson was awarded the 1906 Nobel Prize in Physics for the discovery of the electron and for his theoretical and experimental investigations on the conduction of electricity by gases, little did he realise that a Kampung Baru, Kuala Lumpur born boy will continue his work on the study of electrons. Sir Thomson experimented with cathode rays and demonstrated that all materials are made up of sub-atomic particles that he called corpuscles, later to be termed electrons. On the other hand, the Kampung Baru urbanite studied the interaction of electrons with radio waves in the sky.

The earth is enveloped in an atmosphere that comprises gaseous molecules and charged particles. The heavier molecules would be hugging the earth, while the lighter ones would be found higher until the edge of near space. This upper atmospheric region contains mainly electrons that is dislodged from the neutral molecules by the intense radiation energy from the sun. The heavier positively charged ions would settle at a lower height compared to the lighter electrons that form an ocean of negatively charged cloud.

The chemistry of the ionosphere determines the production of electrons whereby a quasi stable state is reached as the electrons would not be produced ad infinitum since they would constantly recombine with the less abundant positively charged ions. However, there is always a net number of electrons available. The solar energy loses its momentum as it penetrates deeper into the atmosphere with different wavelengths absorbed at different heights. This more or less forms stratified ionospheric regions. Only energy in the visible, infrared, and ultraviolet spectrum reaches the surface of the earth. If not for the presence of the thick atmosphere, life on earth cannot exist as it would be radiated by the solar gamma and X-rays.

This electronically charged rarefied medium in the presence of the earth's magnetic field is a form of plasma. Electromagnetic waves in the radio spectrum is greatly affected by it. Radiowaves that normally propagate in a straight line at the speed of light in vacuum would be impeded and slow down when traversing this ionised

plasma. The direction of the waves can also be bent and if conditions are right will return back to earth. The reflection properties of the ionosphere makes it a useful natural mirror for radiowaves of low enough frequencies to travel beyond line-of-sight. This is what makes it possible for shortwave radio broadcasts around the world.

The electrons are also not uniformly distributed. Pockets of high electron density forms plasma bubbles. This could cause interruptions to radiowaves in the microwave spectrum. Hence it would affect satellite signals and cause "flickering" or scintillation of the amplitude of the signal. The accuracy of GPS is also greatly affected by changes in electron densities.

The variability of the ionosphere makes it an interesting medium to study and also to find applications. Prof Ahmad Faizal Mohd Zain (Universiti Malaysia Pahang) rejuvenated the study of the ionosphere in the equatorial region of Malaysia. He set up the first Wireless and Radio Science Centre (WARAS) in Batu Pahat, Johor, that operated the first digital ionosonde in Malaysia to probe the ionosphere. The Malaysian ionospheric critical and gyro-frequencies was also determined. An important observation that was reported is the presence of a higher ionospheric region at around 700 km to 900 km. He used GPS receivers to measure the number of electrons and mapped this over Malaysia. Another aspect of ionospheric studies is to measure the earth's magnetic field which was carried at WARAS.

In a recent Inaugural Lecture of the Academy of Sciences Malaysia, a short history of ionospheric research in Malaysia and the work carried out to better characterise the equatorial ionosphere over Malaysia was reported by Prof Ahmad Faizal. Apart from its applications in radio communications, its usefulness as a good diagnostic and prediction tool for earthquake detection and solar weather was also expounded. Information on how the ionosphere could affect submarine and satellite communications was also revealed during the lecture. A monograph of the lecture is scheduled to be published in the near future.

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## Announcements

# Proceedings: Impact of Climate Change on Water Resources and Their Consequences to Major Economic Sectors

Climate change is a global phenomena and Asia Pacific, where Malaysia is located, is expected to be the worst affected. Global warming as a result of climate change is manifested in changes in hydro-meteorological events, such as floods and droughts, in which extreme events increase in intensity and occurrences. This will impact on water resources management and will affect the function and operation of existing water systems and water infrastructure, including water supply, hydropower, structural flood defenses, navigation, and drainage and irrigation systems. Water underpins all socio-economic activities and will be of major concern because of the chain-reaction and the impact on food and national security. While too much and too little water causes distress to mostly vulnerable groups, the impact on Malaysia's economic development such as in tourism, health, industries and agriculture can be devastating, as has been analysed in the 2011 Thailand flood.

Rising sea levels, floods, prolonged droughts and water-borne diseases are among threats that we must face if we do not have correct adaptive capacities to mitigate the impacts. Adaptation measures require the use of "usable knowledge" to develop resilience and no-regrets measures. These include retrofitting existing infrastructures, increase water storage, and improve water use efficiencies, engineering hardy new plant varieties, having an efficient public health system and an effective integrated and holistic planning protocol in development projects not only to ensure climate change impacts are minimal but also to ensure sustainable development.

Therefore, there is the need to mainstream policies on climate change as major inputs into national development plans. We must adopt optimal combinations of measures in mitigation and adaptation, hand-in-hand, to finding the most suitable and cost-effective approach to combat climate change impacts.

A proceedings entitled the *National Conference on the Impact of Climate Change on Water Resource and Their Consequences to Major Economic Sectors* was published by the Academy of Sciences Malaysia (ASM); it arose from a Conference organised by ASM in collaboration with the Ministry of Natural Resources and Environment Malaysia (NRE), National Hydraulic Research Institute of Malaysia, and the Ministry of Science, Technology and Innovation Malaysia to present an *ASM Study Report on the Status*

*of Climate Change Impact on Water-related Issues* and to receive feedback from all stakeholders. The objectives of the Conference were:

- To create awareness among stakeholders on the key issues in water and climate change
- To obtain an overview of the impact of climate change at sectoral and national levels; and
- To identify issues, gaps and challenges of climate change at sectoral and national levels.

The 118-page publication includes articles on climate changes related to: the economics in South East Asia; water resources and supply; national policy; agriculture—impact and adaptation strategies under water-limited environment; adaptation programme; impact on health and ecosystems; sustainable land-use planning and the like. The paper presenters hailed from the Asian Development Bank; Fellows of the Academy of Sciences Malaysia; NRE; Malaysian Agricultural Research and Development Institute; Department of Irrigation and Drainage Malaysia; Ministry of Energy, Green Technology and Water, Malaysia; Institute of Medical Research; Forest Research Institute Malaysia; and the Federal Department of Town and Country Planning, Peninsular Malaysia.

Other stakeholders at the national level were also invited to share their expertise and present papers detailing the issues and adaptive strategies in their respective sectors. Arising from this National Conference, a series of stakeholder consultative workshops will be held subsequently to identify and streamline the required strategic actions to address the water-related issues arising from the potential impact of climate change in Malaysia.



(The Proceedings, including slide presentations in CDs (pdf) are available for sale at RM60/USD20.) Enquiries can be directed to: [publication@akademisains.gov.my](mailto:publication@akademisains.gov.my)





## Announcements

### MAHATHIR SCIENCE AWARD FOUNDATION (904190-H)

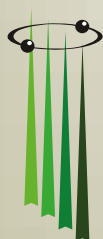
He is known as the “Father of Hybrid Rice” and has won many awards for his innovative breakthrough. In 2011, he was named as the winner for Mahathir Science Award in recognition of his courage to think independently, out of norm, in his rice breeding work. Prof Yuan LongPing, the Director General of China Hybrid Rice Research and Development Centre, through his innovation has resulted in the development of hybrid rice, a staple food of the tropics and has consequently revolutionised global rice production and has led to improved sustainability.

This year, Mahathir Science Award Foundation is calling the nomination for 2013 Mahathir Science Award to scientists, researchers and institutions who share the same aspiration as Prof Yuan LongPing and other Mahathir Science Award past winners. The Foundation is pleased to receive nominations from scientists, researchers and institutions that has made internationally recognized scientific breakthrough in pioneering tropical research which has brought greater positive impact on the well-being of society. **Submit the nomination now and visit our website for further information.**



**2011**

Tropical Agriculture



#### Prize

One Award will be conferred each year covering any of the four categories.

#### Categories

Tropical Medicine  
Tropical Agriculture  
Tropical Architecture and Engineering  
Tropical Natural Resources

**The prize for the Award is RM100,000.00. A gold medal and a certificate will also be presented.**

#### Selection Criteria

Scientific Breakthrough  
Impact of the breakthrough  
Solving problems of the tropics

A selection exercise is carried out through a stringent vetting process by an evaluation committee comprising Fellows of the Academy of Sciences Malaysia, an international panel of technical experts and Nobel Prize winners.

**Submission:  
Before  
31 March 2013**

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## Announcements

# TRSM

TOP RESEARCH SCIENTISTS MALAYSIA

an Academy of Sciences Malaysia initiative

### Vision

To build a culture of excellence in scientific research

### Mission

To develop a knowledge hub of top notch Malaysian Research Scientists

The Top Research Scientists Malaysia (TRSM) project is an ASM initiative in line with its focus on fostering a culture of excellence in science, technology and innovation (STI). The project aims to identify and recognize the leading research scientists in Malaysia. The output of this project is a TRSM profile that would provide a centralized point of reference on leading scientists in Malaysia along with information on their research areas. The convention of the best minds in the country would create a hub for knowledge dissemination that can spur the growth of STI in Malaysia.

Malaysian research scientists contribute significantly to the economic growth of the nation via generation and dissemination of knowledge, wealth creation and the productive pursuit of scientific excellence for the benefit of humanity. In relation to this, the TRSM project intends to acknowledge their contributions and showcase the accomplishments of Malaysian research scientists as role models of excellence, mentors to the next generation and leaders to forge ahead with the STI agenda of the nation.

The accumulation of expertise through this initiative would simplify the process of identifying areas of need and interest to the country which subsequently allows the selection of the best candidate to pursue those areas. In addition, the TRSM list would serve as a gateway for the international scientific community, in particular academia, industries and international STI organizations that wish to seek top-notch STI expertise in Malaysia. The TRSM project would also be a good reference guide to facilitate nomination of outstanding Malaysian candidates for various national and international STI awards.

## Objectives

- To develop a list of leading research scientists
- To recognize and showcase top Malaysian research scientists as drivers of the national STI agenda
- To identify Malaysian research scientists with pioneer mindset to move the country forward in an innovation-led economy
- To build a critical mass of leading research scientists

## Eligibility

- The recognition is given to Malaysian research scientists working in Malaysia whose outstanding achievements in STI have been nationally and internationally recognised
- At the time of application, the candidate should be actively involved in research in the last 5 years with at least 10 years cumulative contribution towards the progress of STI

## Application

- Online application is open throughout the year at [www.mytopscientists.org](http://www.mytopscientists.org)





## Selection Criteria

### Privileges

- Facilitation by ASM for attachment to world-renowned research laboratories and centres of excellence by leveraging on ASM's international and national linkages
- International Fund Facilitation Programme (IFFP) to obtain international R&D grants
- Profiled in leading media
- Featured in STI databases as 'Top Research Scientists Malaysia' for promotion and positioning
- Featured in a book on 'Top Research Scientists Malaysia' to be published by ASM
- Preference for research funding

The Top Research Scientists Malaysia (TRSM) will be selected through an objective and standardised scoring mechanism based on the following criteria:

- (i) Knowledge Generation
- (ii) Knowledge Dissemination
- (iii) Impact of Research Output



For further information, kindly contact:

**The TRSM Secretariat**

Academy of Sciences Malaysia

902-4, Jalan Tun Ismail

50480 Kuala Lumpur

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# Young Scientists Network—Academy of Sciences Malaysia

What seemed miles away and uncertain finally materialised. A network, platform, foundation for the nation's outstanding young scientists. Young Scientists Network—Academy of Sciences Malaysia (YSN-ASM) was the given name. The proposal was presented by Prof Mohd Basyaruddin Abdul Rahman in June 2010 during the Lindau announcement/press conference. The first initiative took place in December 2011 when four young scientists (Prof Basya, Dr Abhimanyu Veerakumarasivam, Dr Azwani Sofia Ahmad Khair and Dr Normi Mohd Yahaya) under the tutelage of an experienced senior scientist (Prof Abdul Halim Shaari) convened in a meeting room in Universiti Putra Malaysia to lay the foundation of YSN-ASM. More 'bricks' were laid the following year with a more significant involvement of other excellent young scientists across the country who responded to the Academy's call to solidify the establishment of YSN-ASM.

The effort was timely as other nations across the globe have announced the establishment of such chapters in their own respective countries, i.e. Die Junge Akademie in Germany, De Jonge Akademie in The Netherlands, the Royal Society of Edinburgh Young Academy of Scotland, the National Academy of Young Scientists in Pakistan, the Young Academy of Sweden, the South African Young Academy of Science, Young Academy of Japan, Thai Young Academy and Philippine Academy of Young Scientists. All

such establishments are the result of or spin-offs from the worldwide organisation for young scientists known as the Global Young Academy (GYA) under the InterAcademy Panel. Malaysia's YSN-ASM will be the latest addition to this growing list.

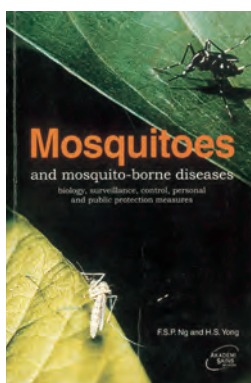
In line with GYA, YSN-ASM will serve as a strong platform where excellent young, local scientists with diverse background and expertise can interact, co-ordinate, organize and meet both national and international challenges. It will also be a platform for talented young scientists across disciplines to collaborate and create opportunities for career development with any young scientists in the country or abroad. It will serve as the centre where the gap between young and senior scientists is established and consolidated where mentorship, policy consultation by senior scientists with fellow young scientists is highly encouraged for the sustainability of the scientific community. Last but not least, YSN-ASM will be a source of role models for future scientists in training at high schools and universities which is important for expanding the nation's scientific capacity in the near future.

With the impending official launch of YSN-ASM due in December 2012, it would signify the first out of many scientific excellence and contributions by YSN-ASM for the research community and the general public in Malaysia.

**[Enquiries (e-mail): [akma@akademisains.gov.my](mailto:akma@akademisains.gov.my)]**







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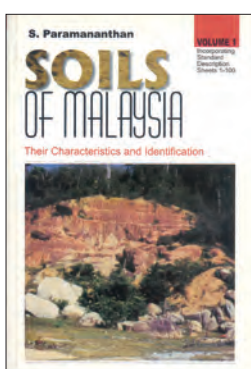
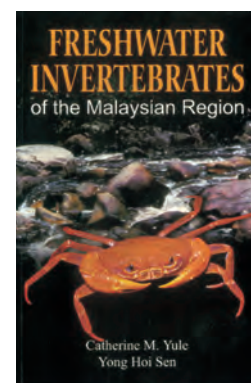
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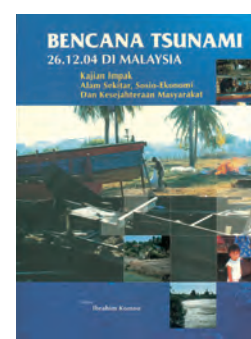
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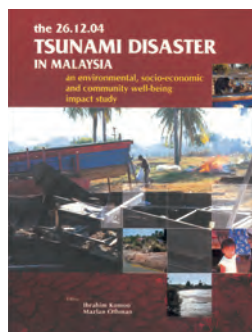
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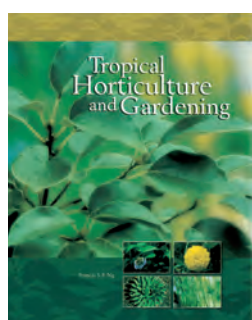
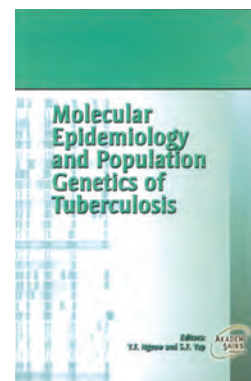
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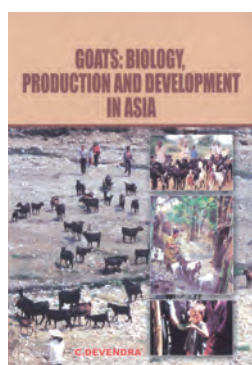
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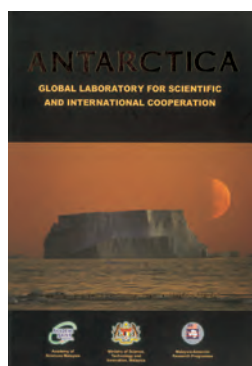
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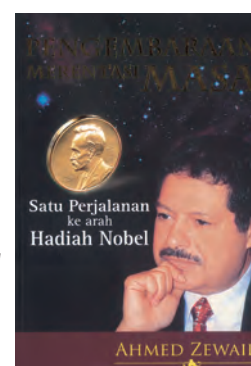
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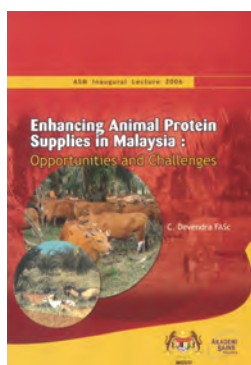
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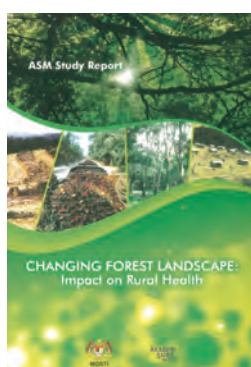
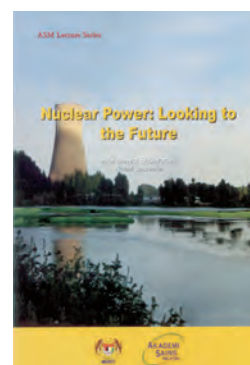
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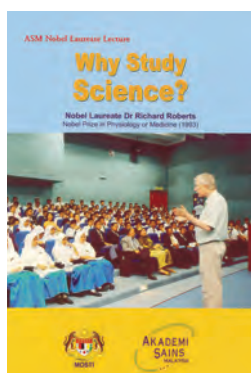
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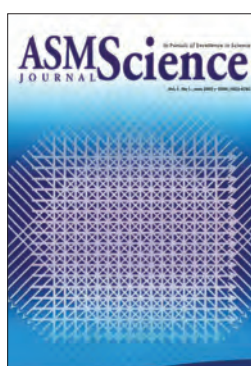
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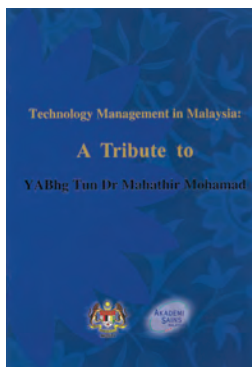
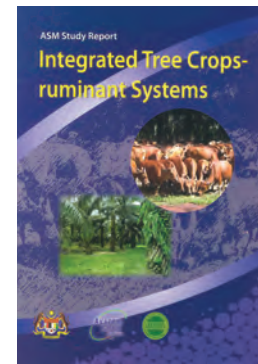
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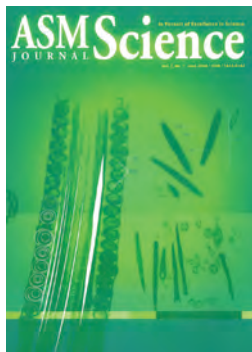


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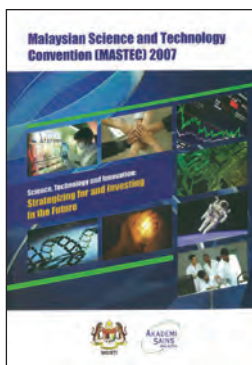
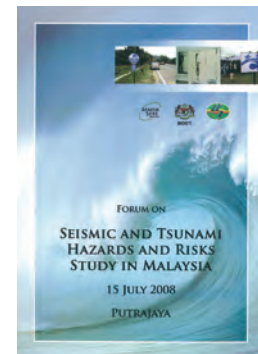


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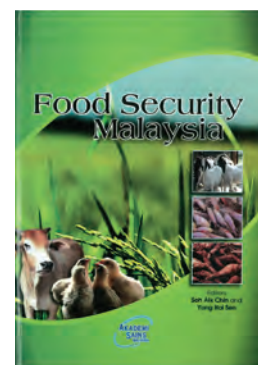
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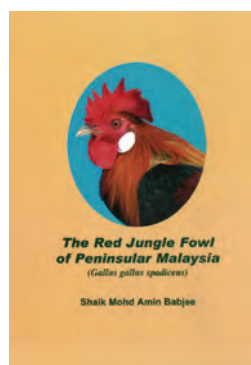
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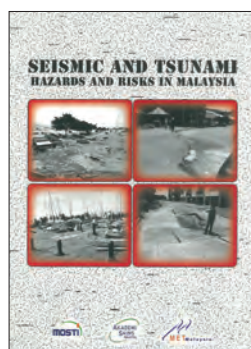
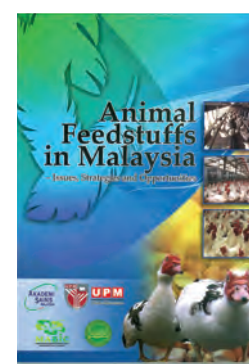
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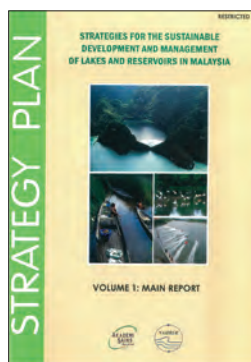
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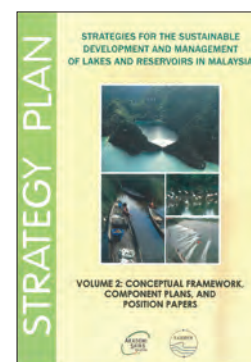






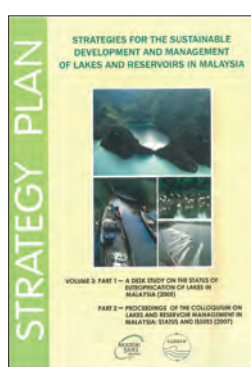
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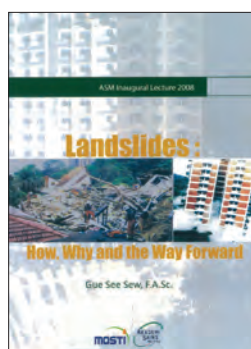
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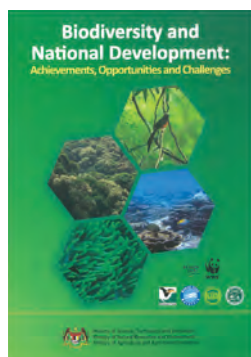
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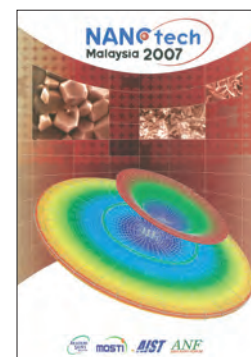
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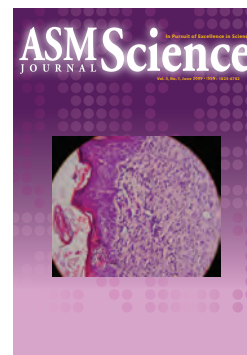
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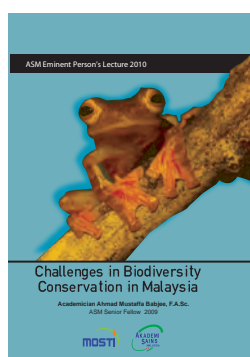
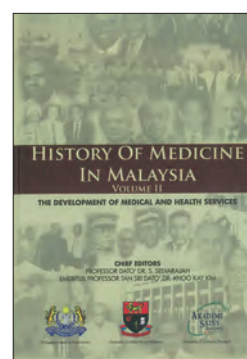
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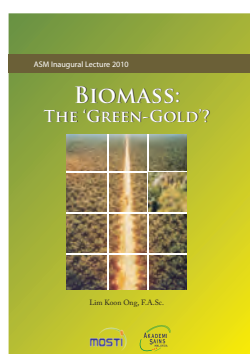
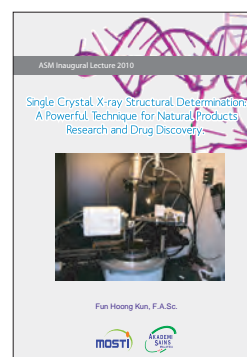
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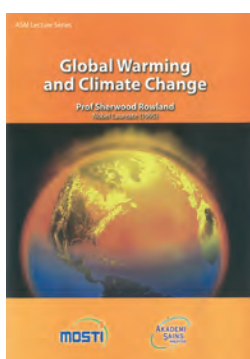
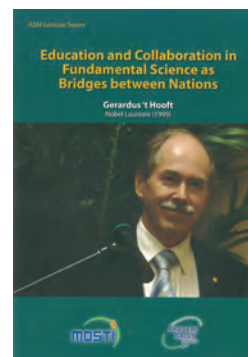
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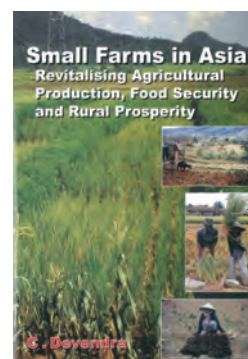
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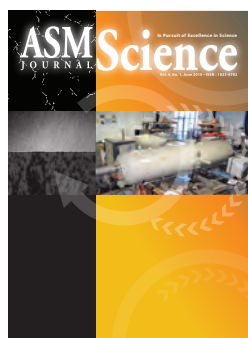
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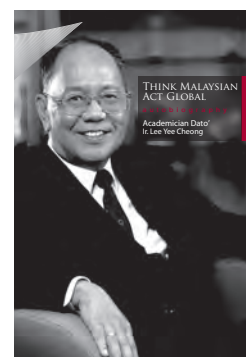
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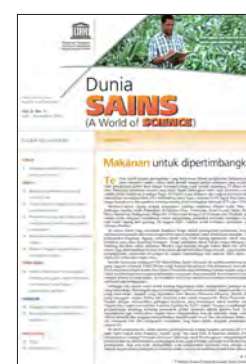


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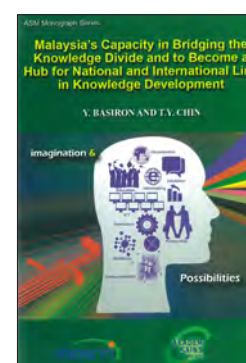
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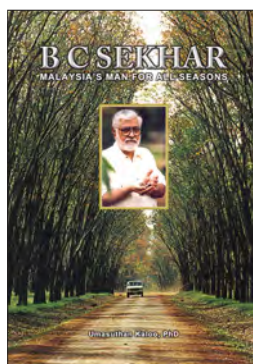
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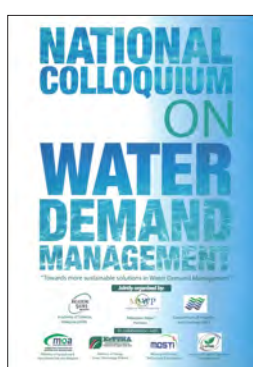
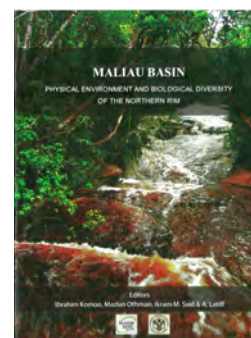
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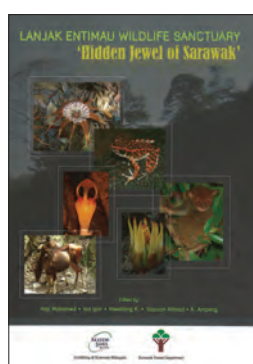
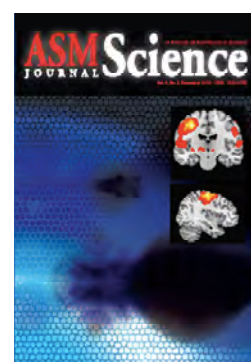
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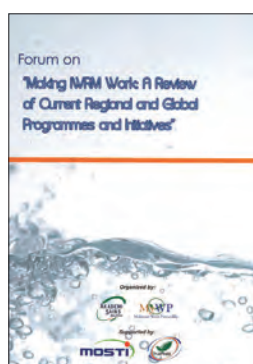
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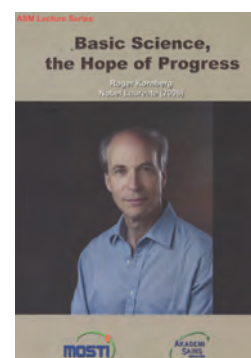
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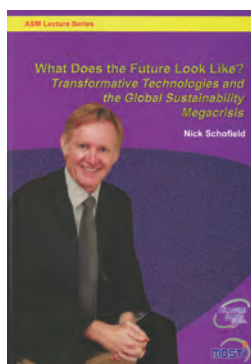
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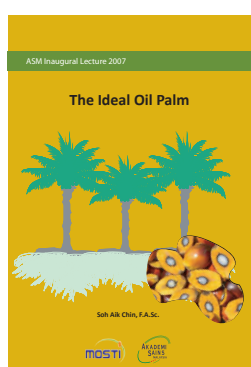
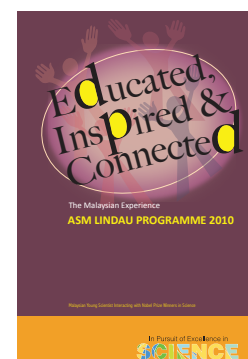
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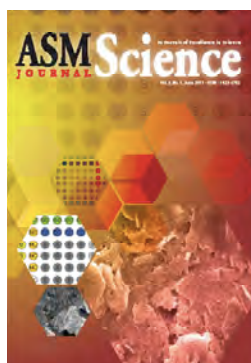
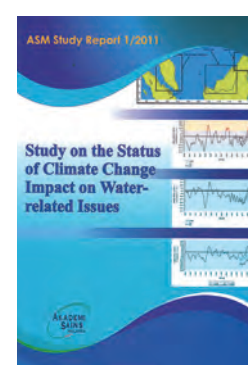
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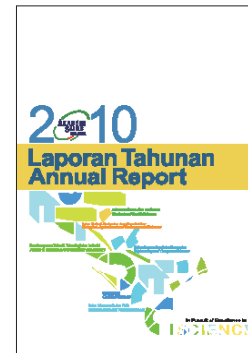
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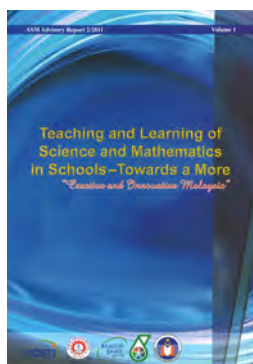
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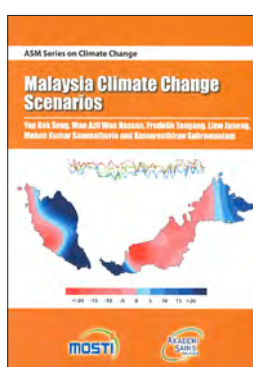
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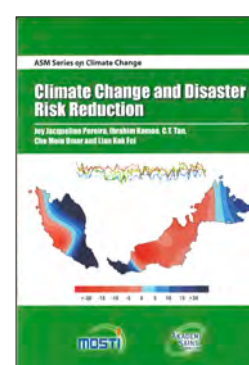
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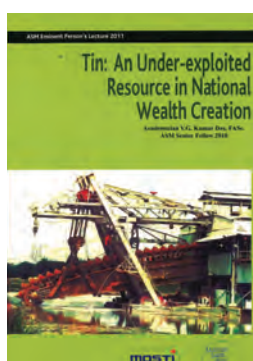
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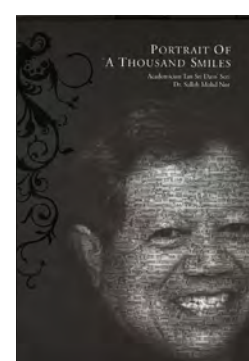
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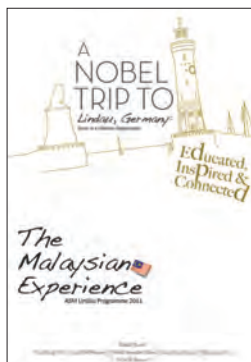
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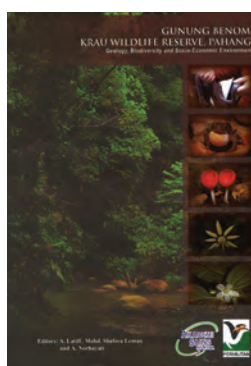
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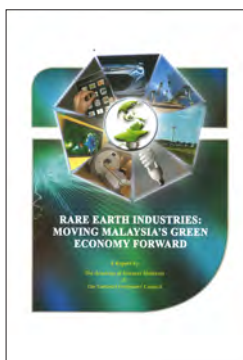
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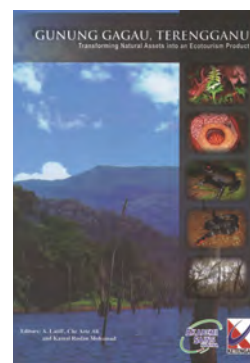
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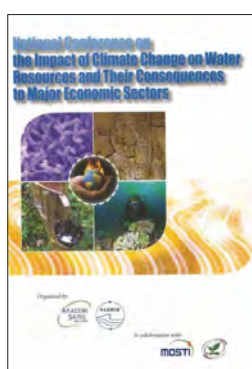
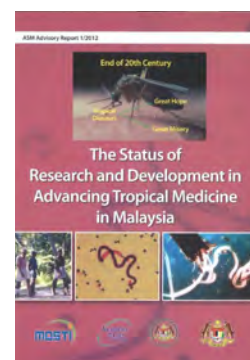
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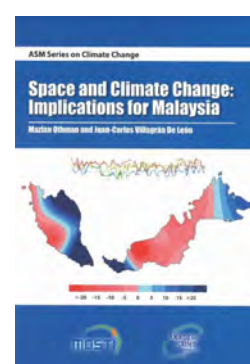
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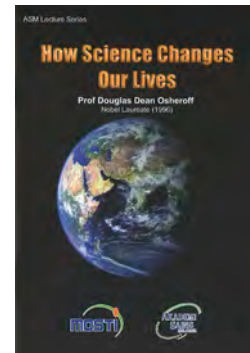
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