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Edited by:
Christina Yong Seok Yien
Mashitah Shikh Maidin
Geetha Annavi
Wan Norhamidah Wan Ibrahim

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Department of Biology
Faculty of Science
Universiti Putra Malaysia

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Faculty of Science
Universiti Putra Malaysia
43400 UPM Serdang
Selangor Darul Ehsan
Malaysia
Tel: +603 8946 6613
http://www.science.upm.edu.my/biology

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PREFACE

From a broad economic perspective, bioeconomy refers to the set of economic activities relating to the invention, development, production and use of biological products and processes. It encompasses the production of renewable biological resources and their conversion into food, feed, chemicals, energy and healthcare wellness products via innovative and efficient technologies. Bioeconomy could make major socioeconomic contributions globally. These benefits are expected to improve health outcomes, boost the productivity of agriculture and industrial processes, and enhance environmental sustainability.

In a way the bio-economy is not a recent discovery. It has provided us with food, fuel and fibre over the centuries. In many ways we took it for granted. Today as we face into a future where the supply of fossil fuel – the driver of all economies – is finite and where the consequences of our reliance on such fuels have given rise to serious environmental problems, we are looking again to the natural world to answer our many demands – for food, fuel, fibre, new products and chemicals. Today we are more acutely aware than ever before about the need for sustainable production systems; about the importance of producing more, using less resources and polluting less. In that context we know that waste is no longer acceptable; that many by-products can also become products with a real value.

We require a revolution in our ways of thinking and doing. That revolution requires us to bring people with us on the path and to provide the knowledge and information to empower them so that they want to be a part of the revolution – a revolution that can only yield positive results. We need to harness the innovative minds of our scientists, researchers, farmers, food processors, manufacturers, entrepreneurs and many others – so that we can deliver the diverse range of goods the bioeconomy has to offer and the many innovations we have not yet thought of. With that spirit, it is very appropriate to sustainably use our bioresources. Thus, the theme chosen for this *i*-SIMBIOMAS 2014 is "Sustainable Bioresources for Bioeconomy". A greater focus on research and innovation can provide us with new products derived from biomass and new services required for realization of the bioeconomy development.

The diversity of expertise participating in this symposium makes it a remarkable scientific meeting that that can contribute in helping to meet the most pressing global challenges, such as the increasing global population, depletion of fossil fuel and natural resources, and increasing environmental pressures and climate change. By bioeconomy development we may help combat climate change, reduce waste and create new jobs.

We are proud to have been the vehicle for this symposium to run successfully.

The Organising Committee
Malaysia International Biological Symposium 2014
(*i*-SIMBIOMAS 2014)

FOREWORD

It gives me a great pleasure and privilege on behalf of the Department of Biology, Faculty of Science, Universiti Putra Malaysia (UPM) to welcome all the distinguished guests, speakers and participants of *i*-SIMBIOMAS 2014.

This scientific community meeting started in 2003 as a small-scale event. When the standard of the meeting was improved in 2009 and then upgraded to international level in 2012, it has gathered local and international participants from various institutions of higher learning and research institutes. I congratulate the organizing committee for their efforts in bringing together experts from various fields in Biology to share their findings and expertise.

Malaysia, as one of the mega-biodiversity countries has also recognised the importance of a bioeconomy. The theme chosen for this symposium: "Sustainable Bioresources for Bioeconomy", is a continuity of the previous theme "Sustainable Management of Bioresources". The fact that we face imminent limits to our present way of producing and consuming can no longer be ignored. We need to encourage an economic transition that move away from dependency on fossil resources towards sustainable production, use of biomass and bioresource management. This effort must be developed with a view to tackling societal challenges, rather than being influenced by sector based interests. A sustainable management of bioresources for bioeconomy is certainly a possible path, but requires bridge building and partnerships. A sustainable bioeconomy must take account of these challenges. This symposium is a good platform for us to share ideas and later, wherever possible, to collaborate in order to be able to address the challenges in an efficient way.

To all symposium participants, over these two days we look forward to hearing of the exciting advances you have made in your respective fields. We look forward to an active discussion of the issues presented.

Thank you.

Prof. Dr. Ahmad Ismail
Head
Department of Biology
Faculty of Science
Universiti Putra Malaysia

Plenary & Keynote

Future approach in addressing climate change

Dato' Syed Danial Syed Ariffin

Managing Director, Puncak Niaga (M) Sdn Bhd

This paper will highlight the impact of climate change, how does it affect one of our most fundamental basic needs – WATER. The occurrence of extreme weather anomalies due to climatic change, e.g. drought and flood will have a profound effect on our water source, quality and supplies and its consequences, in particular, the water rationing exercise carried out in Selangor and Kuala Lumpur in early 2014. This paper also examine the readiness of water utilities to adapt and mitigate climatic change impact in which a series of adaptation and mitigation measures, both short-term and long-term, that have been carried out by the Malaysian Government. In the search for solutions, we need to be more innovative and creative, and the answers provided should be "green, holistic and sustainable" that we should adopt in the future. Ultimately, we hope to see a future where solutions to climatic change should not disrupt the human livelihood, comfort and economic activities. In conclusion, a concerted group effort is necessary to address the climatic change impact.

Keywords: Impacts of climate change, water supplies, water resource, water quality, future approach

Development of advanced technology "Bio-logging system" and its application to conservation of wild animals and ecosystem

*Nobuyuki Miyazaki[1,2]

[1]*Atmosphere and Ocean Research Institute, The University of Tokyo, Japan,*
[2]*Marine Science and Technology Foundation, Japan*
**Corresponding author.Tel.:+81425660322; email address: miyazaki@aori.u-tokyo.ac.jp*

The Atmosphere and Ocean Research Institute, the University of Tokyo (AORI), had conducted the 10-year Multilateral Core University Program "Coastal Marine Science (2001-2010)" supported by the Japan Society for the Promotion of Science (JSPS) (Nishida, Fortes, and Miyazaki, 2011). Reviewing these activities, I set up "Bio-logging science" in Japan, which is defined as "investigation of phenomena in or around free-ranging organisms that are beyond the boundary of our visibility or experience (Boyd *et al*., 2004)". AORI established Bio-logging system (UTBLS: Bio-logging System of the University of Tokyo) using advanced data loggers and camera loggers developed with cooperation of Little Leonardo Co. Japan. This system is very useful for getting information on behavior of wild animals and their habitat use in order to make conservation of wild animals and ecosystem in the world. Thus this system can contribute to making management of the integrated coastal marine ecosystem, and to establishing the new system for "*Future Oceans*", which involves concept of human welfare. Micro-acceleration meter and salinity meter measure depth, temperature, speed, 3-dimension of acceleration and salinity in high quality. Camera logger is used for getting real scene in nature condition through animals and for confirming phenomena obtained by the data logger. So far these devices have accumulated a lot of superior knowledge for whales, dolphins, manatees, sea turtles, sea birds, fishes, etc. In this presentation, I would like to explain outline of this Bio-logging system and some scientific topics obtained by these new advanced devices with my students and colleagues.

Keywords: Advanced technology, bio-logging system, behavior and habitat use, conservation of wild animals and ecosystem, coastal management

Sustainable agriculture for future generations: The case for oil palm

*Sharifah Shahrul Rabiah Syed Alwee

Felda Global Ventures Research and Development Sdn. Bhd., Felda Biotechnology Centre, PT 23417 Lengkuk Technology, 71760 Bandar Enstek, Negeri Sembilan
**Corresponding author. email address: shahrul.a@feldaglobal.com*

Bioagriculture means farming using sustainable methods where better use of fertilizers and other agricultural inputs not only leads to improve yields but also benefits the environment and the consumer. Felda Global Ventures (FGV) launched its first sustainability report this year pledging a continuous effort towards a sustainable palm oil business end-to-end. As the world's largest plantation operator, with some 850,000 hectares of predominantly oil palm plantations across Malaysia and Indonesia, FGV is taking a lead in its commitment to agricultural sustainability. FGV is also the first complete palm oil supply chain to attain the International Sustainability and Carbon Council (ISCC). Through its subsidiary companies, FGV is embarking on various projects and programmes, via a multi-pronged technological, biological and social approach, to get the most from its production by reducing waste and increasing efficiency, while ensuring that environmental sustainability remains at the fore in its farming operations. In this paper, we will focus on our R&D efforts in supporting the sustainable agriculture mandate of FGV.

The shock of the new – Impact of molecular analyses of rheophytic and mesophytic Asian Araceae, and its role in better understanding the origins of the flora of Sunda

*Peter C. Boyce

Honorary Research Fellow, Institute of Biodiversity and Environmental Conservation (IBEC), Universiti Malaysia Sarawak, 94300 Samarahan, Sarawak, Malaysia
**Corresponding author. email address: bpeter@ibec.unimas.my*

Considerable advances using molecular analyses ("molecular systematics") has in recent years modified generic delimitation in the Araceae, established a reasonably stable internal phylogeny, and provided good estimates for divergence dating. An outcome of these analyses for Asia has been that several aroid genera hitherto considered taxonomically stable have been revealed to be polyphyletic, in some instances, notably *Piptospatha* N. E. Br., profoundly so. These taxonomic changes in turn have generated a need to reassess many of the morphological characteristics previously used to define genera, resulting in the realization that several of these key characters are homoplastic, very likely multiply-derived as advantageous in rheophytic and mesophytic habitats. On Borneo utilization of these better-understood taxa together with understanding of the canalized homoplasies that they possess has enabled a more meaningful mapping of species diversity onto geological and geographic data and through this exposed a previously obscured pattern of biomes, the existence of which impacts significantly on our understanding of the evolution of aroids, and very likely other plants, on Borneo and further afield in Sunda.

Keywords: Araceae, molecular analyses, systematics, Sunda

Invasive species is a 'cancer' for an ecological ecosystem

*Mashhor Mansor

School of Biological Sciences, Universiti Sains Malaysia 11800, Penang, Malaysia
**Corresponding author.Tel.:+604 6533518; email address: mashhor@usm.my*

Every habitat in the world is not free from colonization of invasive species. Evidently some species are more robust and resilient. These species can travel and subsequently colonized and became adapted to the ecological ecosystems. As this happens the pristine ecosystems can transform into a disturbed habitat high in invasive species. The gradual ecological changes are seldom recognized. There are several examples of noxious species. *Mimosa pigra* colonies are known to infest most part of wetland, including Mekong Delta. The seriousness of this species has brought environmental catastrophe to the Mekong Delta. River fishing industries in Cambodia and Vietnam are greatly affected by this giant mimosa. Water hyacinth (*Eichhornia crassipes*) populations have affected more than 50 countries in the world. The massive growth of water hyacinth can block and clog a river system. Therefore, basic ecological concepts of invasive species invasion and evolution should be detected and identified. Generally the adaptation of invasive species for dispersal and established should be controlled.

Keywords: Invasive species, *Mimosa pigra*, *Eichhornia crassipes,* wetland, colonization

Potential application of cloning and stem cell technologies in Malaysia

*Ramli Bin Abdullah and Wan Khadijah Wan Embong

Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
** Corresponding author.Tel.:+603 79674366; email address: ramli@um.edu.my*

Rapid advancement in reproductive technologies was shown in the last five decades. At the beginning of 21st century, cloning and stem cell research projects are actively carried out in various laboratories worldwide. As for cloning, both reproductive cloning and therapeutic cloning are the targets of research detailing on the scientific information and possible applications especially in livestock production and wildlife animals conservation. Stem cell research is mainly conducted to be applied in human regenerative medicine to treat degenerative diseases such as cardiovascular disease, Alzheimer disease and Parkinson disease. This presentation will discuss the basic technique and possible application of cloning and stem cell research in Malaysia.

Keywords: Application, protocols, reproductive cloning, stem cell, therapeutic cloning.

Towards the molecular ecology of crows and other birds

Urszula Krzeminska[1], Nur Omar Macha[1,2], Teh Ser Huy[3], Gan Han Ming[1], Song Beng Kah[1], Chris Austin[1], Sharifah Syed Hassan[2], Robyn Wilson[1] and *Sadequr Rahman[1]

[1]*School of Science, Monash University Malaysia, Bandar Sunway, Selangor*
[2]*School of Medicine, Monash University Malaysia, Bandar Sunway, Selangor*
[3]*The Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Kuala Lumpur*
**Corresponding author. email address: Sadequr.rahman@monash.edu*

Birds are an important part of the ecosystem. Malaysia is host to an abundance of birds but in addition to the native birds, Malaysia also contains introduced species. One of these species is the common house crow (*Corvus splendens*). It is amongst the most-wide-spread bird species and can have many adverse effects on native fauna and flora, including predation, competitive displacement and disease. To study population genetics of this species and to understand its colonization patterns and recent evolutionary history, a set of molecular markers has been developed. The markers include autosomal microsatellites described in the previous studies of passerine birds, mitochondrial markers, the sex-linked *CHD* gene and SNPs identified by NGS. Such a broad set of markers can be used to determine the origin of the house crow populations, gene flow among populations and the rates of changes in the nuclear genome in comparison to the mitochondrial genome. Preliminary results revealed low levels of genetic variation within crow populations in Malaysia and Singapore and confirmed population isolation based on both nuclear and mitochondrial markers. In addition, markers have also been developed to study chickens. Techniques developed for the study of crows and chickens will be used to study birds which are of high conservation concern.

Keywords: *Corvus splendens*, chickens, markers, microsatellites

Biodiversity and Conservation

Ethnobotanical survey of medicinal plants used for traditional maternal health care in Katsina State, Nigeria

*Sulaiman Sani Kankara[1,2] and Rusea Go[2].

[1]Department of Biology, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University P.M.B. 2218, Katsina, Katsina State, Nigeria
[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia 43400 Serdang, Selangor Darul Ehsan, Malaysia
**Corresponding author.Tel.:+601114255781; email address: kankarasulaiman@gmail.com*

Introduction

Medicinal plants are widely used for Pre and postnatal care in many rural areas of the world (Zumsteg and Weckerle, 2007) as such various studies (Ticktin and Dalle, 2005) have documented many medicinal plants used to treat obstetric and gynecological conditions such as birth control, complication during pregnancy and child birth and problems associated with infertility. Indigenous people of the world have used oral traditions and empirical means to compile detailed knowledge regarding the use of medicinal plants and this is being disseminated from generation to generation (Abel and Busia, 2005). Although people have been using medicinal plants to cure various ailments associated with maternal health care in Katsina State since time immemorial, the usage is never documented as information is being passed verbally from generation to generation and this poses negative impact to the Indigenous Knowledge (IK) as it will be lost as time goes on. Documenting medicinal plants used in traditional maternal health care will therefore go a long way in providing baseline data which could help in making conservation strategies and information gathered would be an invaluable source based on which pharmacological studies aimed at isolating additional compounds which could be useful in providing new drug leads would be conducted.

Materials and Methods

This study was conducted in Katsina State, Northern Nigeria (Latitude 11˚8'N and 13˚22'N; longitude 6˚52'E and 9˚20'E). Semi structured questionnaire method (Giday *et al*., 2009) was adopted to interview fifty (50) respondents from each of the randomly selected Local Government Areas (two from each of the three Senatorial Districts) to document plants used for traditional maternal health care. Information obtained was analyzed using Relative Frequency of Citation (RCF), Fidelity Level (FL) and Informant Consensus Factor (ICF). The cited plants were later collected, dried and preserved using standard herbarium techniques.

Results and Discussion

Total of one hundred and eleven (111) plant species belonging to 101 genera distributed among 50 families are used to treat various illness associated maternal health care in the study area. Family Fabaceae was the dominant family with 25 species followed by Asteraceae family with 8 species and Malvaceae family with 6 species while most of the families (31) are represented by only 1 species each. High

occurrence of family Fabaceae could be explained by the fact that most species belonging to Fabaceae family are mostly found throughout the seasons as they are adapted to withstand adverse effect of sudano sahelian regions. *Acacia nilotica*, *Guiera senegalensis*, *Gossypium barbadense*, *Artemisia annua*, *Moringa oleifera* and *Mitragyna inermis* had the highest Relative Frequency of Citation (RFC) of 0.93, 0.92, 0.89, 0.89, 0.85 and 0.85, respectively, while *A. nilotica*, *G. senegalensis*, *A. annua*, *Anisopus mannii*, and *Euphorbia convolvuloides* had the highest Fidelity Level (FL) of 100%, 100%, 95.13%, 92.49% and 85.49% respectively. From this analysis it could be seen that *A. nilotica*, *G. senegalensis* and *A. annua* had the highest combination of RFC and FL (0.93; 100%, 0.92; 100% and 0.89; 95.13 respectively). Although *G. barbadense* has RFC of 0.89, its FL is 80.52% while *M. inermis* though it has RFC of 0.85; its FL is 84.25%. *A. nilotica* was reported to be used for postpartum wound healing and it was the only plant used for that purpose as such the Informant Consensus Factor (ICF) for postpartum wound healing was 1. Most of the reported plants (68.47%) were herbs and shrubs and majority of them (84.68%) were wild species. Motlhanka and Nthoiwa (2013) however, reported that trees were mostly used for medicine in Tswapong North, Eastern Botswana. Leaves were the most frequently used plant parts (32.14). Yetein *et al*. (2013) also reported that leaves were the most frequently used plants' part for the treatment of malaria in plateau of Allada, Benin Republic. Medications were mostly prepared as decoction while most preparations were administered orally (84.64%). Oral administration is also the most common route of administration in Eastern Highlands of Papua New Guinea (Jorim *et al*., 2012).

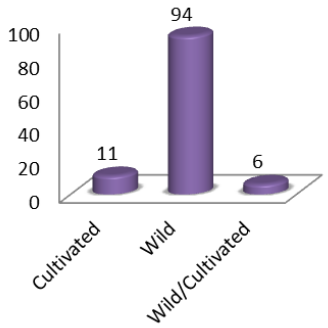

Figure 1: Domestication status of medicinal plants used for traditional maternal health care in Katsina State, Nigeria.

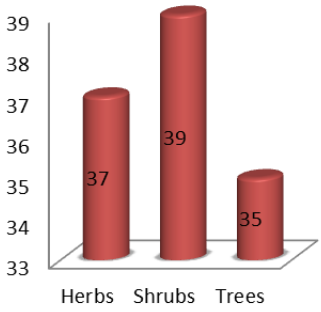

Figure 2: Habit status of medicinal plants used for traditional maternal health care in Katsina State, Nigeria.

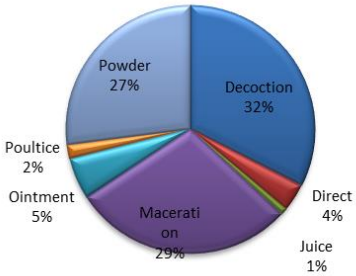

Figure 3: Mode of preparation of medicinal plants used for traditional maternal health care in Katsina State, Nigeria.

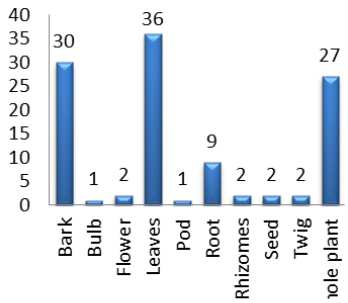

Figure 4: Plants parts used for traditional maternal health care in Katsina State, Nigeria.

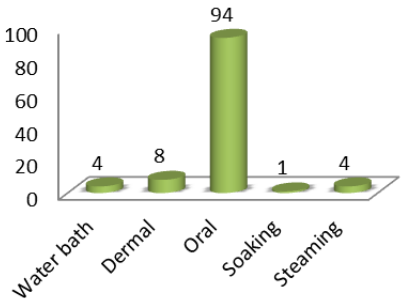

Figure 5: Routes of administration of plants used for traditional maternal health care in Katsina State, Nigeria.

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Biomass and habitat characteristics of mangrove macro algae in Miri River Estuary, Sarawak

*Abu Hena Mustafa Kamal, Mohd Masum Billah, Mohd Hanafi Idris, Johan Ismail, Hasmida Isa and Nur Raihan

Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia
**Corresponding author.Tel.:+6086 855366; email address: hena71@yahoo.com*

Introduction

Mangrove vegetations are commonly understood to be made up of a collection of woody plant species associated with different flora and fauna. Typically, they grow in anaerobic soils found in the intertidal zone of the tropical and subtropical coastlines. This coastal forest play an important role to stability and maintenance of various closely link ecosystem like seagrass, coral reef and other marine ecosystems (Karsten *et al.,* 1994). The benthic communities available in the mangrove ecosystems, benthic macro algal assemblages grow epiphytically on pneumatophores, prop roots and stems (Zuccarello *et al*., 2001). They are unique to certain mangrove habitats and an understanding of their occurrence and abundance may indicate the health of mangroves. The algal mats associated within mangrove ecosystems represent a major source of primary producers, energy source, carbon sink and storage, habitat for small estuarine invertebrates, sediment trappers and builders and nitrogen fixers.

The systemic study on benthic community structure especially on epiphytic macro algae in mangrove and their habitat characteristics in Malaysia is scarce. Few species of mangrove macro algae i.e., *Dictyota dischotama, Rhizoclonium* sp*., Gracilaria crassa, Colpomenia* sp., *Gracilaria blodgetti, Bryopis* sp*., Caloglossa lepreurii and Bostrychia radicans* are recorded from Selangor mangroves, Peninsular Malaysia (Aikanathan and Sasekumar, 1994). It is expected that the benthic epiphytic macro algal communities in the mangrove ecosystems in Malaysia may have wide number of varieties those probably have economical and ecological significance. Therefore, keeping this view in mind, this study is undertaken to investigate the baseline information on existing benthic macro algal community structure and habitat characteristics in Miri river estuary, Sarawak, Malaysia.

Materials and Methods

This study was conducted in the mangrove ecosystems at Miri river estuary (04º 24' 15.8" N and 113º 59' 20.1" E), Sarawak. The mangrove *Avicennia* sp. was the common and dominant species in this estuary and hence selected its' pneumatophores for sampling. Pneumatophores were collected randomly from inter tidal area of the estuarine river bank from 3 stations. All samples were transported to the laboratory for future process (Melville and Pulkownik, 2007). All macro algae from pneumatophores were sorted and cleaned prior to have the constant weight at 70°C for biomass. Biomass was expressed as algal weight per unit area (µg/cm^2). The physico-chemical parameters of estuarine mangrove water (pH, temperature, salinity,

turbidity, dissolve oxygen and conductivity) were detected *in situ* using multi-parameter (Model WQC-24).

Results and Discussion

Ten species of mangrove macro algae namely, *Bostrychia kelanensis*, *B. radicans, B moritziana, B. anomala, Caloglossa leuprii, C. ogasawaraensis, C. stipitata, C. adherens, Chaetomorpha linum* and *Dictyota* sp. were recorded and identified from this river estuary. The most common algae those grow in this mangrove ecosystem *were Caloglossa leuprii, C. adherens, C. ogasawaraensis, C. stipitata* and *Chaetomorpha* sp. (Table 1). The number of macro algae was found higher at the bottom (6–9 species) of pneumatophores compared to the apex (5–7 species). The species *Bostrychia* spp. were the most abundant macro algae at Station 1 for apex and followed by *Caloglossa* spp. Probably, light availability could be an important factor affecting on the abundance and distribution of these macro algae. Generally, the most of the algal species prefer the shaded area in estuarine ecosystems (Karsten *et al*., 1994). However, the species *Bostrychia* is more resistant to desiccation than *Caloglossa.* The algae, *Caloglossa* spp. were mostly common at the bottom of the pneumatophores than the apex.

Table 1: Species composition of macro algae recorded from the Miri river estuary, Sarawak.

Algae	**Station 1**		**Station 2**		**Station 3**	
	Apex	**Bottom**	**Apex**	**Bottom**	**Apex**	**Bottom**
Bostrychia kelanensis (NR)	+	+	-	+	-	-
Bostrychia radicans	-	+	-	-	-	+
Bostrychia moritziana (NR)	+	+	-	-	-	-
Bostrychia anomala (NR)	-	+	-	-	-	-
Caloglossa leuprii (NR)	+	+	+	+	+	+
Caloglossa adherens (NR)	+	+	+	+	-	+
Caloglossa ogasawaraensis (NR)	+	+	+	+	+	+
Caloglossa stipitata (NR)	+	+	+	+	+	+
Dictyota sp.	-	-	-	-	+	+
Chaetomorpha linum	+	+	+	+	+	+
Total	7	9	5	6	5	7

NR = New Record in Malaysia (Details description and taxonomic studies are ongoing)

The total biomass of macro algae was found within 47.33±68.93 to 100.84± 85.98 µg/cm^2 from pneumatophores surface. Higher total biomass of macro algae was found in Station 2 (100.84 µg/cm^2), which is comparable with the findings of other studies elsewhere (Table 2). Usually, the penumatophores that grow close to shore have had higher algal biomass than those grow in upper shore of mangroves. Besides, geographical location, nutrients uptake and size variation probably influence on the total biomass of macro algae (Saifullah and Ahmed, 2007).

Table 2: Comparison of total biomass of mangrove macro algae with other studies.

Location	**Algal biomass**	**References**
Miri, Malaysia*	47.33-100.84	Present study
Karachi, Pakistan**	6.0-9.60	Saifullah and Ahmed (2007)
Woolware Bay, Australia**	6.25-16.0	Laursen and King (2000)
Victoria, Australia**	7.18	Davey and Woelkerling (1985)
Selangor, Malaysia**	5.53	Aikanathan and Sasekumar (1994)

*DW µg/cm^2; ** DW mg/cm^2

Salinity was found higher at the bottom of the river water compared to the surface water (Table 3). The phenomenon is common in the estuarine environment as saline water is heavy and used to flow at the bottom of any tidal river during inflow and outflow. The composition and existence of macro algae was not influenced by the salinity in this study area, notwithstanding, the habitat characteristics like positioning of penumatophores and sun shine of the sampling areas could probably influence on the macro algal distribution especially *Bostrychia* sp. (Karsten *et al*., 1994).

Table 3: Recorded physico–chemical parameters of water from Miri river estuary during the study period.

Position	**Parameter**	**Station**		
		Station 1	**Station 2**	**Station 3**
Surface	Temperature (°C)	29.36±1.02[a]	29.4±1.22[a]	21.11±11.27[a]
	pH	6.34±0.14[a]	6.34±0.11[a]	8.68±3.73[a]
	Salinity (PSU)	11.1±3.95[a]	11.44±3.2[a]	16.3±11.1[a]
	Turbidity (NTU)	42.6±7.5[a]	42.7±4.7[a]	41.2±6.5[a]
	Conductivity (S/m)	1.8±0.6[a]	7.19±8.2[a]	1.8±0.3[a]
	DO (mg/l)	2.7±0.2[a]	3.14±0.7[a]	2.84±0.4[a]
	TDS (g/l)	16.8±6.1[a]	17.6±5.0[a]	16.14±3.5[a]

Bottom	Temperature (°C)	28.7±1.53[a]	28.7±1.44[a]	20.62±11.13[a]
	pH	7.11±1.00[a]	6.93±1.00[a]	11.19±6.49[a]
	Salinity (PSU)	18.5±8.8[a]	17.93±7.8[a]	22.1±10.6[a]
	Turbidity (NTU)	46.3±45.7[a]	39.8±9.3[a]	52.0±31.7[a]
	Conductivity (S/m)	3.0±1.3[a]	2.8±1.4[a]	2.9±1.2[a]
	DO (mg/l)	3.8±3.1[a]	4.14±3.0[a]	3.6±2.8[a]
	TDS (g/l)	29.9±15.5[a]	30.7±15.5[a]	29.5±14.2[a]

Means within a row at different station with same superscript letter are not significantly different (Tukey, p>0.05).

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Can birds minimize crop damages caused by insect herbivores in oil palm agro-ecosystems?

*Nor Laili Ibrahim[1], Mohamed Zakaria[1], Norman Kamarudin[3], Chong Leong Puan[2], Siti Nurhidayu Abu Bakar[1] and Badrul Azhar[2]

[1]*Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia, 43400 Serdang, Selangor*
[2]*Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, 43400 Serdang, Selangor*
[3]*Biological Research Division, Malaysian Palm Oil Board, 43000 Kajang, Selangor*
**Corresponding author.Tel.:+6012 7024929; email address: nlaili85@gmail.com*

Introduction

In Malaysia, insect herbivores are common pests in oil palm plantations. Specifically, it has been reported that 10-30% of palm leaf damage was caused by bagworms (Norman and Othman, 2006; Norman *et al*., 2010). In order to deal with the problems of bagworm infestation, oil palm stakeholders had spent tens of millions of dollars annually on pesticides (Basri, 1993). At present, one of the control techniques is through the application of chemical pesticides. In addition, the planting of beneficial plants such *as Antigonon leptopus, Tunera ulmifolia* and *Cassia cobanensis* along the roadside of the plantationscan produce nectar for various parasitoids and predators for bagworms (Ho *et al*., 2003).

In oil palm agro-ecosystem, Koh (2008) suggested that insectivorous birds provide a natural pest control which is important not only for human welfare, but also the economic through conserving the remaining natural habitats for animal and biodiversity in agro-ecosystems. It has been suggested that oil palm plantations can support the community of birds (Koh 2008; Edwards *et al*., 2010; Azhar *et al*., 2011; Jambari *et al*., 2012), many of which prey on insects (Desmier de Chenon and Susanto, 2005). To date, little is known about the use of birds as a biological control agent particularly in oil palm areas.

In this study, we investigate the association between bird (richness and abundance) and oil palm damages caused by insect herbivores such as bagworm. We also tested the prediction that bird richness and abundance; (i) in bagworm outbreak area were higher than in bagworm non-outbreak areas, and (ii) decrease with the damages levels.

Materials and Methods

Study Area

The study was conducted in an oil palm plantation estate and four oil palm smallholdings from January to April 2014. The areas were located in Selangor; Banting (2,366.49 ha), Tanjung Karang (2,063.79 ha) and Sabak Bernam (3,226 ha) which were identified as non-outbreak areas of oil palm smallholdings. All the three study areas had never been attacked by bagworms prior to our visit. These non-outbreak areas had a total area of 7,656.28 ha. The outbreak of bagworms occurred in smallholdings at Kluang, Johor (2,853.24 ha) and a plantation estate in Kemayan,

Pahang (1,500.05 ha) with total affected area of 4,353.29 ha. Both of these areas were attacked by bagworms (*Metisa plana*) prior to the survey.

Bird Sampling

Bird richness and abundance were determined within 50 meter radius at each of the sampling points. Points were spaced at 500 m apart to avoid double counting. A total of 128 point counts were used in bagworm outbreak (n=38 points) and non-outbreak (n=90 points) plantations. All bird species were detected via visually and acoustically within 10 minutes at each sampling point. Survey was made from 0700 h to 1100 h daily during clear days. The individual that flew over the point are included in these analyses. We used two field guides to identify bird species observed and their feeding guild (Jeyarajasingam and Pearson 1999; Robson 2011).

Damage Assessments

Crown and frond damage were assessed according to the method of Gilbert & Gregoire (2003) with minor modifications. We estimated the damage visually through photograph in the same point as in bird sampling within the 50 meter radius. For each point (N=128), eight palms were selected and we took only two photographs for each palm; i- crown (to show the damage on a whole palm) which is we categories the extent of damage by bagworms on the palm canopy; from middle to lower frond affected (0-50%), up to middle fronds (50-75%) or up to upper fronds (75-100%); ii- we classified the damage of frond damage as Nil (0%), Light (2-5%), Medium (10-50%) and Serious (75-100%). A total of 1,024 photos for each crown and frond damages were assessed regarding to the relative of crop damages per point count: total of percentage, X_1 (crown) or X_2 (frond)/ (eight palms).

Data Analysis

For each point counts, we calculated the species (richness and abundance) of birds between non-outbreak and outbreak areas according to their feeding guilds. We used unbalanced ANOVA due to unequal of sample size (point counts). To examine the correlation between bird richness and relative abundance with crown and frond damage, we performed Spearman's rank correlation tests. All statistical tests were performed in GenStat version 12.

Results and Discussion

Our results showed that bird richness and relative abundance in oil palm agro-ecosystem were associated with insect herbivores. We recorded a total of 3,002 birds from 49 species (13 orders; 28 families) and six feeding guilds. We found that the insectivorous species was dominant group which is 12 species (n=352 birds) and 15 species (n=1,093 birds) in insect outbreak and non-outbreak areas, respectively. There is reason to believe that oil palm agro-ecosystem is able to support a number of bird species (Koh, 2008; Azhar *et al*., 2011 and Jambari *et al*., 2012) as well as a concentration on insectivorous species.

A comparison of bagworm outbreak and non-outbreak area showed the richness and relative abundance of insectivorous birds were particularly high in non-outbreak areas (predicted mean of; richness = 4.311 species, relative abundance =

12.09 birds) than in outbreak areas (predicted mean of; richness = 3.605 species, relative abundance = 9.26 birds) (Table 1). These findings are closely related to previous studies by Koh (2008), Bael *et al*. (2008) and Perfecto *et al*. (2004) that used exclosure experiment to quantify the effects of insectivorous birds on insect herbivores (Lepidoptera larvae and arthropod) and plant damage within different agro-ecosystems. Their experiment indicate that bird exclosure consistently reduce insect herbivores and plant damage than open spaces in agro-ecosystem (assuming that bird exclosure as bagworm outbreak and open space as non-outbreak area).

A Spearman's rank correlation coefficient was significant (p = <0.05*)* with a negative relationship between bird richness and relative abundance and crown damage (overall birds; richness, r = -0.530, relative abundance, r = -0.547 and insectivore species; richness, r = -0.417, relative abundance, r = -0.427), and frond damage (overall birds; richness, r = -0.560, relative abundance, r = -0.568 and insectivore species; richness, r = -0.434, relative abundance, r = -0.432) (Table 2). These results provided striking evidence of a relationship between bird effects and crop damages. Similarly, birds responded to the arthropod outbreaks and were largely responsible for reducing arthropod abundance (Bael *et al*., 2008).

Table 1: Bird richness and abundance between bagworm outbreak area and non-outbreak areas.

Response Variable	***p***	**S.E (between predicted means)**	**Predicted mean (NO vs OB)**
Bird Richness (overall)	0.098	0.6325	10.92 ± 9.87
Relative Abundance (overall)	0.009	1.884	24.95 ± 19.92
Insectivorous Richness	0.018	0.2947	4.311 ± 3.605
Relative abundance of Insectivore	0.013	1.120	12.09 ± 9.26

Note: NO = Non-outbreak areas, OB=Outbreak areas

Table 2: The correlation of richness and abundance of birds between crown and leaves damage.

Variables	**Data set**	**Crown Damage**	**Leave Damage**
Bird Richness	Overall	-0.530	-0.560
	Insectivorous species	-0.417	-0.434
Relative Abundance	Overall	-0.547	-0.568
	Insectivore species	-0.427	-0.432

Note: Spearman's rank correlation coefficient, p: <0.05

Conclusion

As a conclusion, this study has shown that oil palm areas with high species richness and relative abundance of birds have a potential to minimize the level of crop damages caused by insect herbivores. Hence, we recommend that palm oil stakeholders to maintain avian biodiversity.

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Difficulties in species identification of mud crabs (*Scylla* spp.) from the Philippines

*Chona Camille E. Vince Cruz and Ma. Carmen Ablan-Lagman

Biology Department, College of Science, De La Salle University, 2401 Taft Avenue, Malate, Manila

**Corresponding author.Tel.:+63 9088205189; email address: chona.vincecruz@dlsu.edu.ph*

Introduction

Scylla spp., in the Philippines, is a major export commodity with productions reaching as much as 9,000 metric tons per year (BAS, 2008). In depth studies on any one species is hampered by the sympatric coexistence of at least three species, *S. serrata, S. olivacea,* and *S. tranquebarica*, and the difficulties experienced in differentiating the three.

Molecular methods have proven to be most useful in the differentiation of the four *Scylla* species but limitations in time and resources constrain their usefulness for wild stock management in the Philippines. The morphology of the adult mud crabs provides various characteristics for use in species identification. The plasticity of these features limit their effectiveness and as an alternative, methods using morphometric characteristics of the species processed through multivariate analysis to generate species clusters can be used.

This study made use of published morphological characteristics to determine the species of *Scylla* samples acquired in 6 major fishing sites all over the Philippines. The samples were clustered into taxonomic groups using published morphometric parameters, and verified using molecular methods.

Materials and Methods

Collection of samples

Fifty samples were acquired from six coastal communities in the Philippines – Buguey, Cagayan (18.2833°N 121.8333°E), Dagupan, Pangasinan (16°02′N 120°20′E), Orani, Bataan (14.8000°N 120.5333°E), Dumaguete City (9.3167°N 123.3000°E), Roxas City (11.5833°N 122.7500°E) and Iligan City (8.2333°N 124.2500°E).

Initial species identification

Initial identification of the samples was done using the shape of the frontal lobe spine (FLS) and sizes of the carpus spines (Keenan *et al.*, 1998), the carapace color, and cheliped shape (Jirapunpipat *et al.*, 2008) (Table 1).

Morphometric clustering

Principal component analysis (PCA) was done on the seven morphometric ratios (Keenan *et al.*, 1998): Inner carpus spine (ICS)/ outer carpus spine (OCS), frontal median spine height/ frontal width, carapace frontal width/ internal carapace width, merus length/ propodus length, abdomen width/ sternum width, propodus length/internal carapace width, and inner propodus spine/ propodus length.

Table 1: Morphometric features distinguishing the four *Scylla* species.

Characters	***S. serrata***	***S. olivacea***	***S. tranquebarica***	***S. paramamosain***
FLS	Triangular	Round	Blunt	Pointed
ICS	Present	Absent	Present	Absent
OCS	Present	Present	Present	Present
Carapace color	Black	Red	Violet	Green
Cheliped shape	Distinct propodus spines; Thick curved dactyl	Blunt propodus spines; Thin elongated dactyl	Distinct propodus spines; Thin elongated dactyl	Short propodus spines; Thick curved dactyl

Molecular verification

16S rDNA and ITS-1 were amplified using DNA from the propodus muscle (Table 2). Results were viewed in a 0.7% agarose gel, stained with Sybr® Safe (Invitrogen™). PCR products of 22 samples, representing all four species, were sent out to First Base, Malaysia for sequencing. Sequences in the BLAST® database were used for verification.

Table 2: PCR conditions for the amplification of 16s rDNA and ITS-1 (Imai *et al.*, 2004).

Components:	**16S rDNA**	**ITS-1**
Buffer	10x	10x
dNTPs	2.5 mM	2.5 mM
Primers	25 pM	1.0 mM
Forward sequence	16sar-L 5'-	SP13 5'-
Reverse sequence	CGCCTGTTTATCAAAAACAT-3'	ATTTAGCTGCGGTCTTCATC-3'
Template DNA	16sbr-H	SP15 5'-
Kapa Taq polymerase	5'GGTTTGAACTCAGATCATGT-3'	CACACCGCCCGTCGCTACTA-3'
	0.5-1 uL	0.5-1 uL
	0.5 U	1 U
Conditions:		
Denaturation	94°C, 120 seconds	94°C, 90s
Denaturation, Annealing, Elongation X30	94°C,60s; 45°C,35s; 72°C,90s	94°C,30s; 56.8°C,20s;72°C,90s
Final extension	72°C, 10 minutes	72°C, 10 minutes

Results and Discussion

Common morphological mismatches

Of the 450 samples, 27.1% had an FLS that did not match the cheliped shape (Table 3). Color was also inconsistent for more than half (67.1%) of the samples. Subjective calls of these morphological markers made it difficult to differentiate *S. serrata* from *S. tranquebarica*, and *S. olivacea* from *S. paramamosain.* There is uncertainty in the identification of conjectured *S. paramamosain* samples due to the lack of reports of its presence in the Philippines (BAS, 2008).

Table 3: Common morphological mismatches in samples all over the Philippines

Mismatch Types	FLS	Cheliped shape	Incidence (%)
1	Triangular	Distinct propodus spines; Thin elongated dactyl	14
2	Blunt	Distinct propodus spines; Thick curved dactyl	8.4
3	Round	Short pointed propodus spines; Thick curved dactyl	4.7

Morphometric clustering

PCA did not result to discernible clustering of the four species. Samples with inner carpus spines were plotted separately from those without, and a slight differentiation among *S. tranquebarica* and *S. serrata* was seen but not for *S. olivacea* and *S. paramamosain* (Figure 1).

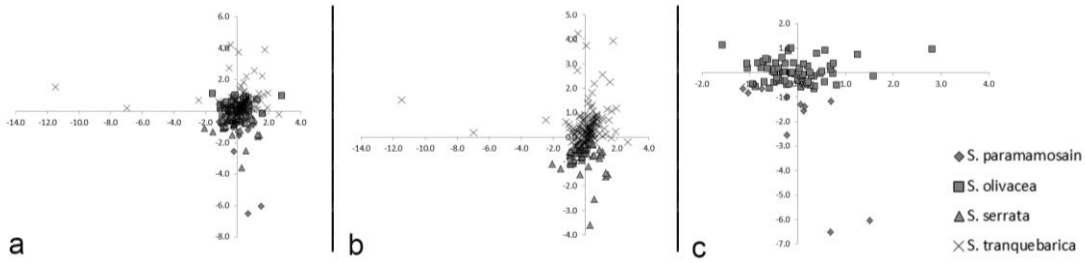

Figure 1: (a) PCA clustering of all samples (Variation: 70.09%), Clusters observed after plotting (b) *S. serrata* and *S. tranquebarica* separately from (c) *S. paramamosain* and *S. olivacea*.

Confusions in molecular identification via BLAST® Database

Thirty-six percent (36%) of sequences suggested a different species identity from those derived via morphological methods (Table 4). Natural hybridization resulting to unaccountable for morphological variation may be the cause (Imai and Takeda, 2005) or the use of differing taxonomic keys. Gel electrophoresis of ITS-1 amplicons support sequencing data except for DLSUMI 044 (Figure 2).

Table 4: Mismatches in morphological and molecular identification.

Samples	Original ID	BLAST® ID	Incongruencies
044	*S. tranquebarica*	*S. olivacea*	Presence of ICS
017	*S. paramamosain*	*S. olivacea*	Pointed FLS
032, 033, 035	*S. serrata*	*S. tranquebarica*	Triangular FLS
149, 148, 145	*S. serrata*	*S. serrata/ S. olivacea*	Two possible options

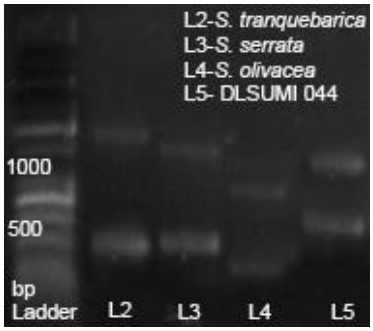

Figure 2: ITS-1 Gel.

Conclusion

Subjectivity in calling the FLS and plasticity of color make them inadequate as morphological markers for species differentiation in the genus *Scylla*. Current molecular methods were helpful in confirmation, except for instances where existing database sequences provides contradictory identities. Carpus spines, cheliped shape, and abdominal flap dimensions appear to be more consistent means for species differentiation. Alignment and construction of a phylogenetic tree from 16S rDNA sequences could help clarify molecular identities.

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Vomifoliol, a sesquiterpene isolated from *Pandanus leram*

Mohd Sharil Ahmad, Ummu Amirah Armayni, Nuruljannah Md Yusof, Hannis Fadzilah Mohsin, Sadia Sultan and *Ibtisam Abdul Wahab

Department of Pharmaceutical Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor Darul Ehsan
**Corresponding author.Tel.:+603 3258 4694; email address: ibtisam@puncakalam.uitm.edu.my*

Introduction

Screwpines (also known as pandan) are classified in the family of Pandanaceae. The *Pandanus* genus consists of about 600 to 700 plant species. The genus is given a dedicated attention, following the increased reports on the isolation and structural elucidation of their bioactive secondary metabolites (Tan *et al.,* 2008). In this study, a *Pandanus* species, scientifically named as *Pandanus leram*, was investigated. This plant is originated from Nicobar Islands, therefore, is also referred as Nicobar breadfruit. The aim of this paper is to present the ^{1}H and ^{13}C spectral evidences of a natural compound, which was isolated as colourless oil from the chloroform fraction.

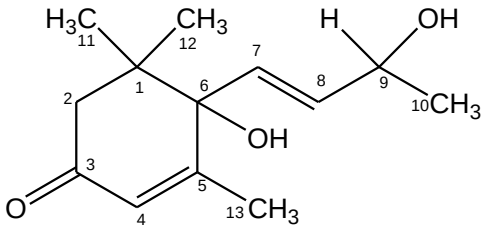
Materials and Methods

The chemicals and reagents used to carry out the experimental procedure include the following: the industrial and analytical reagent grade of hexane, ethyl acetate (EtOAc), methanol (MeOH) and chloroform ($CHCl_3$) (Fisher Scientific, UK), silica plate (Merck Kieselgel 60 F254), silica gel (Silica gel 60 0.0040 - 0.063 mm) and sea sand. The ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) data was accumulated from 500 MHz Bruker spectrometer. The extraction of the dried leaves was mentioned elsewhere (Mustafa, 2013). The purification of $CHCl_3$ fraction (60 mg), which was labelled as Fraction 09, was performed by using an open column chromatography with hexane, $CHCl_3$, EtOAc and MeOH as the mobile phases in a series of increasing solvent polarity, to yield 25 sub-fractions. A compound was later successfully isolated and subjected to NMR experiments.

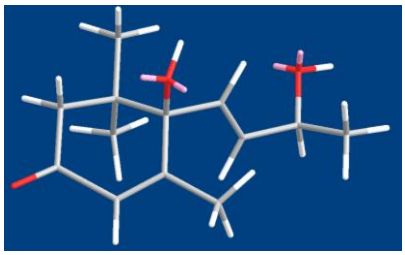
Results and Discussion

The silica thin layer chromatographic (TLC) profile of this UV-active compound was established. A brownish spot appeared, after the TLC plate was sprayed with anisaldehyde reagent, followed with a mild heating (R_f = 0.65; mobile phase = $CHCl_3$:EtOAc 10:90). The ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) data indicated that this compound belongs to the class of sesquiterpene, having 13 carbon atoms (Table 1). Four of the carbon atoms were olefinic carbons which resonated at δ_C 166.08, 125.70, 128.70 and 135.51 ppm, and corresponding to one quaternary and three methines (CH). Furthermore, three carbon signals were recognised as oxymethine, hydroxyl and carbonyl carbons that appeared at δ_C 67.32, 78.55 and 199.87 ppm, respectively. Additional signals at δ_C 18.15, 22.05, 22.41 and 23.07 ppm were attributed to methyl carbons. Finally, two signals at δ_C 49.33 and 41.03 ppm were assigned as methylene and quaternary carbon, respectively. These results were parallel with those reported in the literatures (Nair *et al.,* 2013). Therefore, it

was suggested that this compound could be identified as vomifoliol, which is similar to the one isolated from *Pandanus simplex* (Tan *et al.,* 2012). Figure 1 shows both plain and three dimensional structures of vomifoliol, generated by ChemDraw® and Chem 3D® Ultra from CambridgeSoft.



(a) Basic structure and carbon numbering.



(b) Three dimensional structures.

Figure 1 (a-b): The molecular framework of vomifoliol, a chemical isolated from *P. leram*.

Table 1: ^{1}H NMR and ^{13}C NMR data (500 MHz; CD_3OD).

C no.	δ_C (ppm)	δ_C (ppm) [a]	DEPT	δ_H (mult, *J* in Hz)	δ_H (mult, *J* in Hz) [a]
1	41.03	41.51	C	-	-
2	49.33	50.02	CH_2	2.19 (d,17); 2.55 (17)	2.24 (dd,17.1, 1.2); 2.45 (d, 17.1)
3	199.87	198.19	C=O		
4	125.70	127.31	C=CH	5.89 (t, 1.5, 1.3)	5.91 (t, 1.4, 1.2)
5	166.08	162.87	C	-	-
6	78.55	79.41	OH	-	-
7	128.70	129.36	CH	5.80 (d, 5)	5.79 (dd, 15.7, 0.54)
8	135.51	136.12	CH	5.80 (d)	5.87 (ddd, 15.7, 5.16, 1.68)
9	67.32	68.36	CH-OH	4.34 (m)	4.41 (m)
10	22.41	24.13	CH_3	1.06 (s)	1.30 (dd, 6.4, 1.68)
11	23.07	24.41	CH_3	1.03 (s)	1.01 (s)
12	22.05	23.26	CH_3	1.26 (s), 1.27 (s)	1.08 (s)
13	18.15	19.20	CH_3	1.95 (d, 1.5)	1.89 (d, 1.4)

[a] : Data as reported by Nair *et al.,* (2013).

Conclusion

It was concluded that vomifoliol was purified from the chloroform extract of *Pandanus leram.* Previously, this biomolecule was also isolated from *Pandanus simplex*. Recent literature review on vomifoliol reveals that this compound possessed the antigonococcal activity, which significantly active (63.1% inhibition) against *Neisseria gonorrhoeae*, the common gonorrhoeal pathogen (Nair *et al.,* 2013). More work on the antimicrobial properties of the *Pandanus* extract is presented (Ahmad, 2014). It is hoped that the biodiversity and chemotaxonomy of the *Pandanus* genus could be continuously investigated and learnt for future generations.

Acknowledgments

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Forest biodiversity conservation through co-management approach: Experience from Bangladesh

*Tapan Kumar Nath

School of Biosciences, The University of Nottingham Malaysia Campus, Semenyih, 43500 Selangor

**Corresponding author.Tel.:+603 87253562; email address: Tapan.Nath@nottingham.edu.my*

Introduction

Forests of Bangladesh have been under serious threats from many factors including illegal logging, deforestation, encroachment and expansion of mono plantations of exotic species. In order to arrest the forest loss and biodiversity, the government of Bangladesh had declared patches of forests as protected areas (PAs) under the provision of the Forest Act 1927, and the Bangladesh Wildlife (Preservation) Order 1973. The PA here refers to those forest protected areas designated as national parks, game reserves, wildlife sanctuaries, safari parks or eco-parks under the statute (DeCosse *et al*., 2012). Chunati Wildlife Sanctuary (CWS), one of 29 PAs, covering seven reserved forest (RF) blocks/beats of hill forests is located in the country's south-eastern region. Until the mid-1980s much of this area still comprised evergreen forests, but there has been extensive logging and encroachment since that time (DeCosse *et al*., 2012). Large tracts of forest land have been denuded and numerous species of flora and fauna lost over the past few decades. In view of restoration of lost forest and biodiversity, and enhancement of local peoples' livelihood, a co-management approach has been implemented since 2004 with the participation of local forest dependent people, forest department, non-government organizations and financial support from donor agencies including US Agency for International Development (USAID) and German Development Cooperation (GIZ). This paper presents peoples' dependency, structural composition of tree species in CWS and how co-management approach works towards forest conservation.

Materials and Methods

For vegetation survey, following stratified and systematic sampling 140 circular plots (each of 0.1 ha) were laid out in seven beats. In each plot, following data were recorded: tree species name, diameter at breast height (dbh), and number of recently felled stumps. Several group discussions were organized to collect information on co-management. Data on forest dependency were collected through household and forest trails survey. Importance value index (IVI), Shannon-Wiener diversity index, index of dominance, above-ground biomass (AGB) and basal area were estimated.

Results and Discussion

Peoples' dependency on CWS's forests

Nearly 80% respondents visited CWS more than 10 times every month for forest products collection. They use 70-80% of the collected products for household consumption and only quarter amount is sold in the markets. Surveys in 21 forest trails found that 1123 persons entered into the CWS and extracted an amount of

26,377 kg of forest products in a day whose market price is about BDT 194,989. Each collector on an average earns BDT 160 per day. Local people cultivate betel leaf on encroached forest land or lease land from other encroachers. They cultivate betel leaf in a plot (~0.04 ha) for 3-4 years and then shift to another area. In total 1594 plots were counted that covers about 64 ha of forest land.

Tree composition and structure

The vegetation survey recorded 93 tree species belonging to 36 families. The dominant species in terms of IVI are *Acacia auriculiformis* (IVI=48)*, Dipterocarpus turbinatus* (27)*, Ficus hispida* (24)*, Tectona grandis* (21)*, Shorea robusta* (13) and *Acacia mangium* (12). The average density of trees is 239 trees/ha of which 60% is composed of planted exotic species including *A. auriculiformis* (26% individuals), *A. mangium* (6%), *Eucalyptus camaldulensis* (8%)*, S. robusta* (5%)*, T. grandis* (10%) and *Gmelina arborea* (3%) and the remaining 40% stock (96 trees/ha) is composed of 86 species, most of them are indigenous. Basal area and AGB is 2.64 m^2/ha and 33.30 t/ha, respectively which is due to poor growth of trees. Diameter distribution of trees shows that nearly 90% trees are within 5-15 cm dbh class indicating that very few big trees are present in CWS and newly emerging trees are occupying the forest. Illegal cutting of trees is still occurring. The study counted 148 recently felled stumps per hectare. The Shannon-Weiner diversity index and index of dominance were 3.15 and 0.09, respectively.

Co-management structure

A four-tier governance structure was observed, formed for co-management of the CWS. The existence of the village conservation forum (VCF) at field level is little obscure. The members do not know how the VCF was formed and what their functions are. Two members, one male (leader of respective VCF) and one female form a peoples' forum (PF) in each VCF which feed the VCF's concerns and needs on to the co-management committee (CMC). A total of 14 community patrol groups (CPG) were formed whose members (20-42) in collaboration with FD guards patrol forest in their respective areas. UNDP (2013) reported that although CPG members have not completely stopped illegal logging, their worked has resulted in a considerable reduction of timber poaching. The CMC was founded with the main goal of conserving the CWS in collaboration with local communities and with government agencies acting as a supporting role (UNDP 2013). It functions with a high level of cooperation and mutual respect between its members. However, this study observed that the CMC as a body does not have very active role in making any management related decisions.

Conclusion

Since its declaration in 1986, the CWS has experienced serious threats from several sources. Thousands of local poor people depend utterly on forest products for their living. Encroachment and conversion of forest land to other land uses have been continuing throughout the CWS. Co-management has brought several positive changes in CWS management including development of a co-management governance structure, involvement of poor forest-dependent people in CWS

management, and creation of awareness among local people for conservation of CWS. Respondents commented that due to co-management vegetation coverage in the CWS has increased by more than 80% and natural regenerations are coming out. Now-a-days they often see elephants, deer, bears and other animals. However, development of a governance structure itself is not enough for effective management of natural resources unless it works properly.

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Mercury concentration in selected estuaries and coastal sediments along the Straits of Malacca

Looi Ley Juen[1], *Ahmad Zaharin Aris[1] and Fatimah Md. Yusoff[2]

[1]Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
**Corresponding author.Tel.:+603 89467455; email address: zaharin@upm.edu.my*

Introduction

Mercury is a highly noxious pollutant that could be threatens the ecosystem, bioaccumulated and biomagnified within the food chain after being released into the environment (Zahir *et al*., 2005; Maggie *et al*., 2009). Elevated mercury concentration in the human body can cause various disorders such as neurological, immunological, cardiac, motor, reproductive and genetic (Gochfeld, 2003; Cornelis *et al*., 2005; Zahir *et al*., 2005). Sediment as the great sink of pollutants in the estuaries and coastal systems could serve as an indicator of mercury pollution for the vicinity. Therefore, current study aims to profile the concentration of mercury in sediments of selected estuaries and coastal sediments along the Straits of Malacca. Besides, the concentration of mercury was compared to the permissible limit as stipulated by Canadian interim marine and freshwater sediment guideline.

Materials and Methods

Current study covers selected coastal areas and estuaries from north Kuala Kedah to South Tanjung Kupang along the Straits of Malacca. Homogenized surface sediment (0-5cm) samples were collected in triplicate and preserved in temperature less than 4°C prior transportation to the laboratory for further analyses. Air-dried sediment samples were subjected to the determination of physico-chemical characteristics (pH, electrical conductivity, salinity), and the determination of mercury concentration. Total mercury in the sediment samples was extracted using the modified method by Callasiol *et al*. (2004). The recovery of analytical procedure was checked with standard reference material for estuarine sediment 1464a and satisfactory was ranging from 97% to 108%.

Results and Discussion

The descriptive statistics for selected physico-chemical parameters sediment samples are presented in Table 1. The coefficient of variances (CV) for all measured sediment characteristics were greater than 30% (except for pH), indicating a high variation in the sediment samples between the sampling stations, due to the diverse sediment characteristics between the sampling stations.

Table 3: Descriptive analyses of physico-chemical parameters for sediment samples from the Straits of Malacca.

Matrix		Unit	Mean	Minimum	Maximum	SD	CV (%)
Sediment	pH	-	6.24	2.85	7.97	1.63	26.14
	EC	mS/cm	18.53	8.92	46.37	6.4	34.51
	Sal	ppt	11.04	4.97	30.13	4.24	38.4
	THg	µg/kg	61.43	16.55	114.02	23.25	37.85

SD = standard deviation, CV = coefficient of variation

The highest mercury concentration (114.02±1.54 µg/kg) was recorded in station 11 from Kuala Juru whereas the lowest mercury concentration (16.55±0.61 µg/kg) was identified at station 24 (Figure 1).

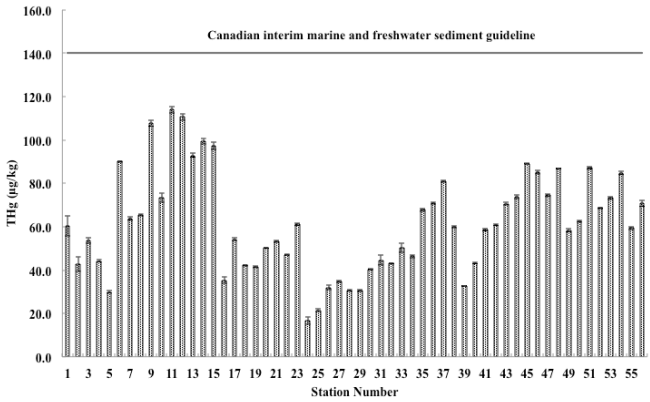

Figure 1: Total mercury concentrations for selected estuaries and coastal sediments from the Straits of Malacca as compared to Canadian interim marine and freshwater guideline.

According to Sadiq (1992), 50.0 µg/kg of THg concentration could be used to represent the background mercury concentration in uncontaminated sediment. Practically, 66% of estuaries and coastal sediment samples collected from the Straits of Malacca had a THg concentration that exceeded the background mercury level (Figure 2).

Only 34% of all the sampling stations had <50 µg/kg of THg in their sediment samples. However, the THg concentrations in the sediment samples were all below the permissible limit when compared to Canadian interim marine and freshwater sediment guideline (140 µg/kg) (Gaudet *et al*., 1995).

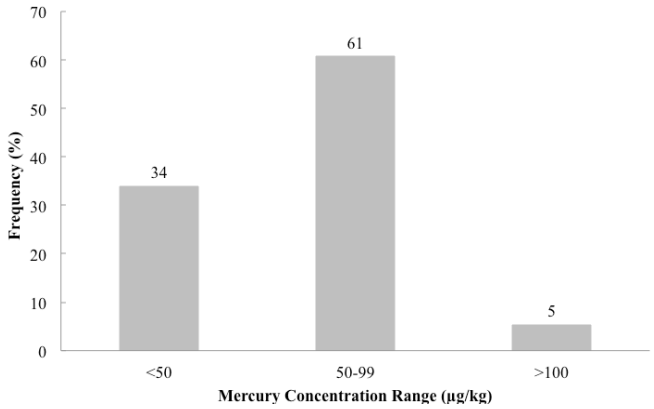

Figure 2: Frequencies of mercury concentrations in selected estuary and coastal sediments from the Straits of Malacca.

Conclusion

THg concentrations in current study were all below 140 µg/kg (Canadian interim marine and freshwater sediment guideline). The profiling of THg concentration will provide updated information and good insights for future mercury pollution and monitoring studies along the Straits of Malacca.

Acknowledgements

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Effect of yeast extract and coconut water on protocorm proliferation and growth development of *Dimorphorchis rossii*

*Jualang Azlan Gansau[1,2], Roslina Jawan[1] and Sharon J. Spiridrin[2]

[1]*Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
[2]*Unit for Orchid Studies, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah. Malaysia*
*Corresponding author.Tel.:088 320000 (ext. 5729); email address: *azlanajg@ums.edu.my*

Introduction

Orchidaceae is one of the largest plant families and comprises about more than 800 genera and 20 000-30000 species (Bektas *et al.,* 2013). *Dimorphorchis rossii* is among the orchid species that belong to the *tribe* Vandeae and *sub tribe* Aeridinae (Beaman and Wood, 2001). The ongoing forest clearance and fires from Bukit Hempuen to Telupid area (Chan *et al.,* 1994; Wood *et al.,* 2011) has contributed to the declined number of this exquisite orchid. Thus, plant tissue culture technique had been applied throughout this present studies to conserve this orchid by means of culturing the protocorms of *Dimorphorchis rossii* in basal media supplemented with complex additives. Growth and development of the protocorms were observed to determine the most effective concentration of the complex additives and basal media used.

Materials and Methods

Proliferation and shoot development of protocorm

Three months old protocorms of *D. rossii* (1.0-2.0mm in size) were cultured on plastic Petri dish containing 25ml of media of full nutrients strength Murashige and Skoog (MS) (Murashige and Skoog, 1962) supplemented with complex additives such as coconut water (10-20%, v/v) and yeast extract. All treatments fortified with 2% (w/v) sucrose. The cultures were grown at 25±2^{o}C and the illumination provided for 16 hours by cool white fluorescent tubes (Philips, Malaysia).

Data analysis

All experiments were performed in a Completely Randomized Design (CRD) and repeated in 5 replicates. Cultures were observed using Dino Lite Digital Microscope. Parameters observed in this studies are percentage of protocorm proliferation, mean number of new protocorms, percentage of protocorm formed leaf, mean number of new leaf, percentage of protocorm formed root, mean number of new root, length of leaf/root (mm) and percentage of necrosis. Analysis of variants (ANOVA) was performed on the data and mean values were compared by using Duncan Multiple Range Test (DMRT) at $p<0.05$.

Results and Discussion

In this present studies, 0.2% yeast extract recorded the highest percentage of protocorm proliferation (41.67±0.51%) compare to other treatments (Table 1). Yeast extract normally enhances growth in media containing relatively low concentration of nitrogen or where vitamins are lacking and have been shown to have some unusual properties which may relate to its amino acid content (George *et al.,* 2008). According to Tokuhara and Mii (2003), yeast extract used at low concentration (0.1-1.0 g/l) enhance cell proliferation and protocorm-like bodies (PLBs) formation of *Phalaenopsis and Doritaenopsis* orchid. For the protocorm development, 10% coconut water (78.33±0.42%) recorded the highest percent of protocorm forming leaf and root (66.67±0.48%) and the lowest percentage of necrosis or protocorm dead (6.67±0.63%). However, the highest mean number of new leaf was found in 15% coconut water (6.28±3.90). Coconut water can be beneficial to some orchid plant where it can induce proliferation and development of protocorm into complete seedling but the reasons for the beneficial effects are not clear (Arditti, 2008). Besides that, coconut water also has been found to be beneficial for inducing growth of both callus and suspension culture and for induction of morphogenesis (George, 2008). This was supported by findings done by Pinaki and Shyamal (2004), Murdad *et al.* (2006) and Piri *et al.* (2013) where incorporation of coconut water into basal media resulted in high percentage of proliferation and enhances shoot regeneration from protocorm of *Vanda teres, Phalaenopsis gigantean* and *Acampe papillosa* respectively.

Table 1: Effect of complex additives added in MS medium on protocorm proliferation observed after 130 days of culture.

Complex additives		**Percentage of protocorm proliferation (%±SD)**	**Mean number of new protocorm (±SD)**
Control		33.33±0.49^{b}	2.67±1.60^{c}
Yeast extract (YE)	0.1	12.50±0.34^{d}	3.00±2.08^{a}
	0.2	41.67±0.51^{a}	1.94±2.35^{e}
	0.3	31.25±0.48^{b}	1.50±1.02^{f}
Coconut water (CW)	10	11.67±0.32^{d}	2.80±2.25^{b}
	15	16.67±0.39^{c}	2.33±1.38^{d}
	20	8.33±0.29^{e}	1.67±1.44^{f}

Significant at P<0.01 level. Means in a column followed by a same letter(s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.

Table 2: Effect of complex additives added in MS medium on shoot development observed after 130 days of culture.

Complex additives		Percentage number of protocorm with leaf (%±SD)	Mean number of leaf (±SD)	Length of leaf (mm±SD)	Percentage number of protocorm with root (%±SD)	Mean number of root (±SD)	Length of root (mm±SD)	Percentage of necrosis (%±SD)
Control		41.67±0.51^{f}	4.83±3.82^{c}	1.09±0.59^{c}	16.67±0.39^{d}	2.33±1.51^{a}	0.68±1.69^{d}	16.67±0.39^{c}
CW (v/v)	10	78.33±0.42^{a}	5.12±3.57^{b}	3.43±2.46^{a}	66.67±0.48^{a}	2.27±1.31^{a}	2.82±2.93^{b}	6.67±0.63^{a}
	15	66.67±0.49^{c}	6.28±3.90^{a}	2.48±2.02^{b}	58.33±0.52^{b}	2.22±1.50^{a}	3.71±3.76^{a}	16.67±0.39^{c}
	20	33.33±0.49^{g}	3.50±3.33^{e}	0.94±0.49^{d}	25.00±0.45^{c}	1.50±1.08^{c}	1.12±2.25^{c}	66.67±0.49^{e}
YE (w/v)	0.1	75.00±0.45^{b}	2.81±1.76^{f}	1.27±0.96^{c}	25.00±0.45^{c}	0.88±0.60^{d}	0.24±0.47^{e}	12.50±0.34^{b}
	0.2	50.00±0.52^{e}	4.11±2.87^{d}	0.89±0.74^{e}	25.00±0.45^{c}	1.83±1.24^{b}	0.26±0.48^{e}	16.67±0.40^{c}
	0.3	56.25±0.51^{d}	4.33±2.78^{d}	0.89±0.56^{e}	25.00±0.45^{c}	0.75±0.45^{e}	0.18±0.32^{f}	37.50±0.50^{d}

Note: CW-coconut water. YE-yeast extract. Significant at P<0.01 level. Means in a column followed by a same letter(s) are not significantly (P<0.05) different according to Duncan's Multiple Range Test.

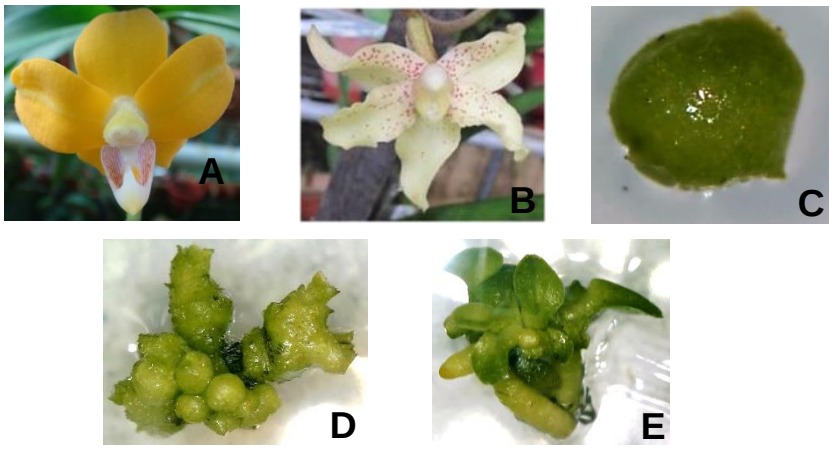

Figure 1: Different stages of seed germination and development of *Dimorphorchis rossii.* (A-B) Flower of *Dimorphorchis rossii* ; (C) Protocorm ; (D) Proliferation of protocorms; (E) Growth and development of protocorm (Bar=1mm).

Conclusion

From this present study, it may conclude that the requirement of nutrients for protocorm proliferation and development of *D. rossii,* had been determine where MS

basal medium was the best basal media and the effect was enhanced by addition of coconut water or yeast extract. This protocol can be used for ex vitro conservation of *D. rossii,* threatened and valuable orchid species in Sabah.

Acknowledgments

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Waste marine barnacles as solid catalyst for methyl ester preparation using catfish (*Pangasius*) fat as feedstock

Intan Shafinaz Abd Manaf[1] and *Gaanty Pragas Maniam[1,2]

[1]*Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia*
[2]*Central Laboratory, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia*
**Corresponding author.Tel.:+60 16 4110236; email address: gaanty@hotmail.com*

Introduction

The growing awareness concerning the environmental issues on energy supply and usage have recently been the topic of interest in research. Among the various alternative energy discovered, biodiesel is one of the promising blended fuel to substitute petroleum derived diesel which offers friendly and sustainable environment. Boey *et al.(* 2011), Boey *et al.* (2009), Nakatani *et al*. (2009) and Obadiah *et al.* (2012) revealed that many type of heterogeneous catalyst from waste, such as waste egg, crab, and oyster shells; bone and ash. Barnacle is successful creatures with abundant and diverse populations. Scientists have identified about 1,445 living species, of which 900 are barnacle. Their abundance can create serious and expensive fouling problems on ship bottoms, buoys and pilings. Astachov *et al.*, 2011 stated that, in less than two years, 10 tons of barnacle can become attached to a tanker. In the present work, the transesterification catfish fat using barnacle as a catalyst was attempted.

Materials and Methods

Materials

The raw material used in this work is catfish fat was collected from restaurant in Gambang, Malaysia. The barnacle shell was obtained from Tanjung Lumpur beach. The chemicals were purchased from Sigma-Aldrich company (Switzerland) include methyl heptadecanoate as an internal standard GC grades (> 99.1%). Methanol (anhydrous, ≥ 99.8%), hexane (anhydrous, ≥ 99.8%) was purchased from Hamburg (Germany).

Preparation and characterizations shells as catalysts and oil from catfish

The shell was cleaned using water to remove dirt and fibrous matters. Then the shells were dried in an oven at 105 °C. The shell was then ground in a pestle and mortar to obtain the gross powder and further fine ground with a dry-mill blender and sieved through 75 µm mesh before being subjected to heat treatment in furnace at 900 °C. Catfish fat was obtained from local restaurant in Gambang, Malaysia. The CaO was identified by X-ray diffraction (Rigaku), FT-IR (PerkinElmer Spectrum 100), FE-SEM with electron dispersive X-ray (EDX) (JSM-7800F). The catalyst was examined by thermogravimetric analysis (TGA).

Transesterification

The conversion of used catfish oil to biodiesel was performed in a 50 ml 2-neck round bottom flask equipped with a reflux condenser and magnetic stirrer. Biodiesel was isolated by centrifuged at 4000 rpm for 5 min, to further separate the layers (methyl ester, glycerol and catalyst, and then excessive amount of methanol) was evaporated before the chromatographic analysis. The concentration of methyl ester (ME) in the sample was quantified using GC-FID (Agilent 7890A) by following the European procedure EN 14214.

Results and Discussion

X-Ray diffraction and electron dispersive X-ray (EDX) result show that, upon thermal activation, the shell transformed into CaO, the active ingredient that catalyzes the reaction. In addition, the result showed that the methyl esters content of barnacle was achieved at 93.6 ± 0.03 respectively with the 3 h reaction duration at 65 ºC. Optimization of reaction parameters revealed that MeOH:oil, 12:1; catalyst, 4 wt.% as optimal reaction conditions of both catalyst. Figure 1 below shows the methyl ester conversion with different type of optimizations.

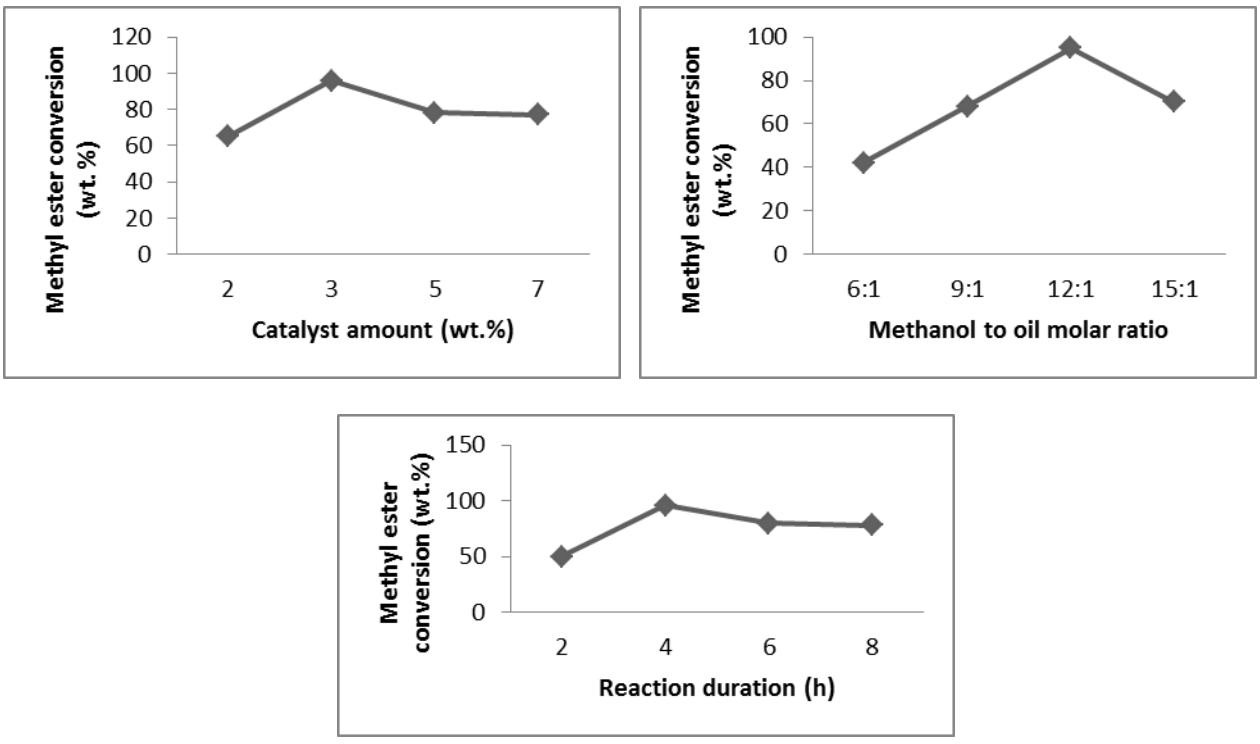

Figure 1: Effect of catalyst amount, methanol to oil molar ratio and reaction duration for methyl ester conversion.

Conclusion

Methyl esters (ME) content of barnacle was at 93.6 ± 0.3 with the 3 h reaction duration at 65 ºC. Optimization of reaction parameters revealed that MeOH: oil, 12:1; catalyst, 4 wt. % as optimal reaction conditions.

Acknowledgements

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Orchids in the summit region of Gunung Jerai, Kedah

Nur Adilah Auyob[1], Syaizwan Zahmir Zulkifli[1], Farah Alia Nordin[2], Vhenosha A/P Annathurai[1] and *Rusea Go[1]

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan.
[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang.
**Corresponding author.Tel.:+603 89466634; email address: rusea@upm.edu.my*

Introduction

Species diversity and distribution survey of Orchidaceae was conducted at the summit region in Gunung Jerai, Kedah. Orchidaceae are the most advance family among angiosperms which can adapts to vary type of habitats (Dressler, 1981). However, they can mostly be found in highlands due to the high humidity and low temperature climate (Shahril, 1999; Rusea *et al*., 2011).

Gunung Jerai also known as Kedah Peak is one of an isolated mountain in Malaysia. The peak is at 1,214 meters above sea level and it sweeps down on all sides into flat lowlands, currently paddy fields and rubber estates. Geologically, Gunung Jerai is formed by granite as the core, and quartzite covering most of the upper part of the mountain. Quartzite will metamorphose into sandstones. As the result, hill heath forest is produced in the summit region which is at 700 meters and above. This forest is characterized by its small stature, low and open canopy, small and often gnarled trees (Ali and Mohamed, 2006).

The uniqueness of its formation has attracted many botanists and collectors that collected the plants including orchids since 1845 until now (Anis, 2002). However, there was no intensive study on the diversity of orchids in Gunung Jerai, Kedah even though it has been botanized over 169 years. Based on the literatures, all plants including orchids species had been collected, which was published as a checklist of plants found in this area.

Materials and Methods

The study was carried out by collecting the samples in five trails which were located at least 700 meters above sea level. The samples were collected randomly within 10 m^2 along the trail. The samples were preserved by using standard herbarium technique after Bridson and Foreman (1992) and some non-flowering samples were kept in the UPM's glasshouse as living collection (Rusea *et al.*, 2011). Meanwhile, Shannon-Wiener Index (H') was used to calculate the diversity index, which focused on the species richness and species abundance (Heip *et al.*, 1998). The value of H' is ranging 0 to 5, which the greater the value, the higher the diversity.

Results and Discussion

A total of 73 species that represent 42 genera have been collected. All the species were identified based on their morphological characteristics, which are vegetative and floral structures by referring to orchid flora publications from this region and herbarium specimens. Based on individual and population count, the most common

species found in this study is *Apostasia nuda, Arundina graminifolia, Corybas geminigibbus, Podochilus microphyllus, Podochilus muricatus, Podochilus tenuis*, and *Spathoglottis plicata*. There are 15 new records to Kedah, 4 endemic species, and 1 new record to Malaysia (Table 1).

Table 1: List of new records and endemic species.

Taxa	**Status**	**Distribution (Turner,1995)**
Anoectochilus geniculatus Ridl.	New record to Kedah	Pg
Bryobium hyacinthoides (Blume) Y.P.Ng & P.J.Cribb	New record to Kedah	Kl; Tg;Pk;Sl;Sp
Bulbophyllum apodum Hook.*f.*	New record to Kedah	South of Peninsular
Bulbophyllum brevipes Ridl.	New record to Kedah	Gunung Bujang, Ml; Pk; Fraser's Hill, Ph
Bulbophyllum linearifolium King & Pantl.	Endemic	Kd; Pk; Ph; Sl
Bulbophyllum sigaldiae Guillaumin	New record to Malaysia	Thailand
Calanthe lyroglossa Rchb.*f*.	New record to Kedah	Pk; Ph
Chrysoglossum ornatum Blume	New record to Kedah	Fraser's Hill, Ph
Coelogyne swaniana Rolfe	New record to Kedah	Taiping Hill, Pk
Corybas geminigibbus J.J.Sm.	Endemic	Gunung Jerai
Crepidium rheedei Blume	New record to Kedah	Kl; Pk
Dendrobium hughii Rchb.*f.*	Endemic	Kd; Ph; Sl; Jh
Eria pilifera Ridl.	New record to Kedah	Pk; Ph
Lecanorchis malaccensis Ridl.	New record to Kedah	Ph; Jh
Malleola sylvestris (Ridl.) Garay	New record to Kedah	Kl; Pk
Paphiopedilum callosum var. *sublaeve* (Rchb.*f*.) P.J.Cribb	Endemic	Gunung Jerai
Plocoglottis lowii Rchb.*f*	New record to Kedah	Ph
Trichoglottis lanceolaria Blume	New record to Kedah	Fraser's Hill, Ph; Pulau Tioman & Gunung Panti, Jh
Trichoglottis winkleri J.J.Sm.	New record to Kedah	Kota Gelenggi, Ph
Zeuxine strateumatica (L.) Schltr.	New record to Kedah	Ml

Jh=Johor; Kd=Kedah; Kl=Kelantan; Ml=Malacca; Pg=Penang; Ph=Pahang; Pk=Perak; Sl=Selangor; Sp=Singapore; Tg=Terengganu

Based to the Shannon-Wiener Index calculation, H' value is 3.79. This value indicates that the species at the summit region is quite diverse and abundant. The present of all five subfamilies of Orchidaceae collected within a small area further support the value is moderately in a good range for species diversity.

Conclusion

Although the same trails had been visited for several times, but chances of discovering new records was always evident with two to three species identified as new records in every visit. Therefore, it is obvious that Gunung Jerai harbors a very diverse orchid species and need to be studied further comprehensively in order to reveal the true status of diversity that could be utilized as data to support conservation plan.

Acknowledgements

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Inoculation of blue green algae in paddy soil using artificial plots

Sohana Shabnam, Elena Wong Ai Meng, Hishamuddin Omar, Shahrizim Zulkifly and *Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43200 UPM serdang, Selangor Darul Ehsan, Malaysia

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Introduction

Blue-green algae are well known to fix nitrogen in submerged paddy fields and increase soil's natural fertility. The inoculation of algae in soil known as algalization has been reported to have beneficial effect on rice grain yieldsin rice producing countries in Asia (Roger and Kulasooriya, 1980). In this study, experiments were carried out to propagate beneficial filamentous blue-green algae isolated from paddy fields and to inoculate these into the test plots to observe their adaptation and the changes the inoculation brings to the plants.

Materials and Methods

Sampling and culturing of Blue-green algae

Three Samples of blue-green algae were collected in Sangai Manik and were cultured in Fogg's media (Rangaswami and Bagyaraj, 2005). Cultures were monitored for the growth of the blue-green algae to know the dominating species. Throughout the mass culture process, the pH, temperature and light intensity were being monitored.

Preparation of artificial plots and algalization

Six artificial plots (64 cm x 64 cm x 28 cm) were made using cement bricks and plastics at the base. Among these three were used as control. Seedlings of MR219 seeds from BERNAS (MARDI, 2001) were transplanted in the artificial plots. Fertilizers were applied according to the schedule given by KADA (2002). After fifteen days of transplanting algae inoculation was carried out by spreading the algae at the rate of 1500 ml per plot.

Results and Discussion

Cultures were monitored under the microscope to record any changes in the growth of algae and were identified. The results showed that, in Fogg's meida the green algae such as *Crucigenia* sp*., Scenedesmus* sp**.,** and *Actinastrum* sp*. w*ere the predominant species for the initial few days then blue green algae *Oscillatoria* sp*.* became dominant after 20 days of culturing. At the end of culture period (about 45 days) *Nostoc* sp*.* replaced *Oscillatoria* sp*.* as the leading species (Figure 1).

The change in pH of the media and algae species is given in the following Table 1.

Table 1: Changes in pH and the dominating algal species in the culture media.

Culture Age (Days)	**pH**	***Species in the inoculated plots**	**Algal density (cell.ml^{-1})**
1-10	7.5	*Crucigenia* sp.	1.37 x 10^5
		Actinastrum sp.	1.12 x 10^5
		Scenedesmus sp.	0.62 X 10^5
11-20	7.68	*Scenedesmus* sp.	1.75 x 105
		Actinastrum sp.	1.00 x 105
		Ankistrodesmus sp.	0.87 x 105
21-30	7.95	*Scenedesmus* sp.	1.21 x 105
		Nostoc sp.	1.00 x 105
		Oscillatoria sp.	0.68 x 105
		Actinastrum sp.	0.62 x 105
		Spyrogyra sp.	0.37 x 105
31-40	8.20	*Nostoc* sp.	2.24 x 105
		Oscillatoria sp.	1.78 x 105
		Scenedesmus sp.	1.00 x 105
		Anabaena sp.	0.25 x 105
		Spyrogyra sp.	0.12 x 105

*Graham and Wilcox, 2000.

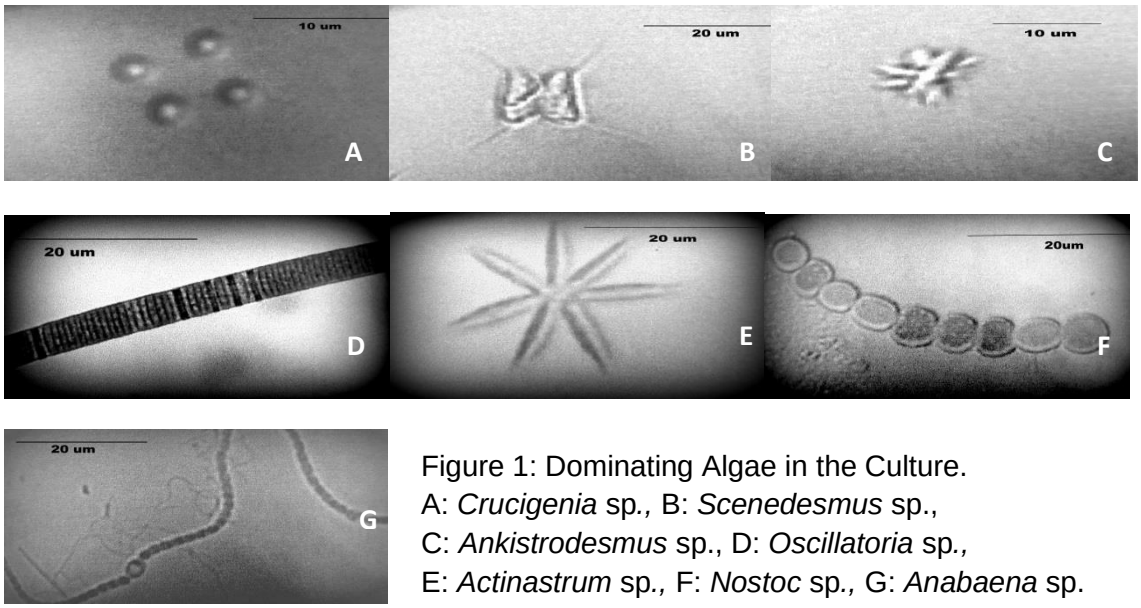

Figure 1: Dominating Algae in the Culture.
A: *Crucigenia* sp., B: *Scenedesmus* sp.,
C: *Ankistrodesmus* sp., D: *Oscillatoria* sp.,
E: *Actinastrum* sp., F: *Nostoc* sp., G: *Anabaena* sp.

The Value of pH in the culture media increased towards alkaline conditions. Roger and Kulasooriya (1980) stated that the optimum pH for growth of blue green algae range from 7.5-10.0 and the lower limit is about 6.7 to 7.0. Thus, the increase in pH and the decrease in the growth of green algae by day 20 seemed to favor the growth of blue green algae. Nevertheless it appears that blue green algae extract dissolved inorganic Carbon at high pH values more than other algae do. The increased in pH may be due to the dissolved inorganic Carbon and the photosynthetic activity of the algal biomass.

In field, before algalization the pH of all plots did not differ much from each other. After inoculation pH of the inoculated plot increased to a maximum value of 7.32 (Figure 2) and then decreased to the original value of the soil. On the other hand, the pH value was neutral to near neutral in the un-inoculated plots where no algae grew naturally.

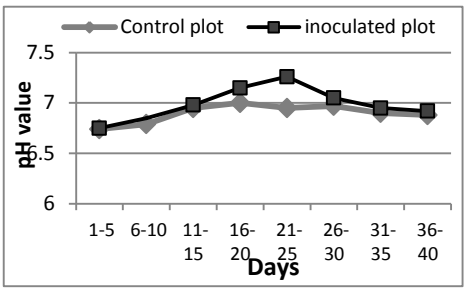

Figure 2: pH values of the plots.

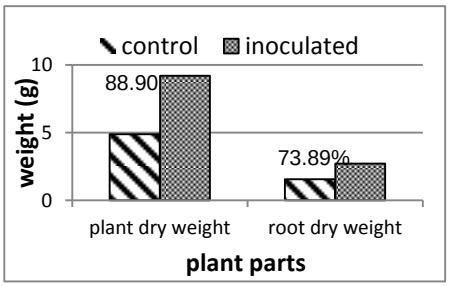

Figure 3: The weight of plant roots of 40 days old taken from the plots.

Plant samples of 40 days old plants were taken from both plots. The plants and roots taken from the inoculated plot were much bigger in size, compared the plants taken from the control plots. The percentage increase over control for dry weight of plants and roots were 88.90 and 73.89 respectively (Figure 3). The

production of growth substancesand vitamins by algae might be responsible in part for the greater plant yield (Rangaswami and Bagyaraj, 2005).

Conclusion

The experiment showed the blue green algae *Oscillatoria* sp. and *Nostoc* sp. could be grown successfully in Fogg's media to inoculate in outdoor plots. As the experiment was continued for 40 days, the algalization effect on crop yield could not be analyzed in this study, though its effect on growth of plant parts was found to be positive.

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Vanilla of Peninsular Malaysia

Mohd Akmal Mohd Raffi[1], *Rusea Go[2], Nur Ashikin Psyquay Abdullah[1],Thohirah Lee Abdullah[3] and Ghizan Saleh[3]

[1]*Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia 43400 Serdang*
[2]*Department of Biology, Faculty Science, Universiti Putra Malaysia 43400 Serdang*
[3]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia 43400 Serdang*
**Corresponding author.Tel.:+6012 6788810; email address: rusea@upm.edu.my*

Introduction

Natural vanilla is extracted from at least three *Vanilla* species from Subfamily Vanilloideae of the Orchidaceae family, among which *Vanilla planifolia* is the most known species. It is native to Mexico and supplies 95% of the world commercial natural vanilla. This species does not occur naturally in Malaysia but there are seven species of vanilla, *V. abundiflora, V. aphylla, V. borneensis, V. havilandii, V. griffithii, V. montana and V. kinabaluensis* that are indigenous to Malaysia. However, they are not cultivated and no documentation on the economic significance has been studied and published. Currently, *V. planifolia* is considered as threatened with habitat fragmentation and destruction at its origin and susceptibility to environmental disruptions. To date, there are 110 vanilla species recognized globally and the potential of each species still remains a mystery, as the genus is not comprehensively studied. Currently, all natural species including the widely cultivated *V. planifolia* are considered as threatened as habitat fragmentation and destruction at its natural habitat, and susceptibility to environmental disruptions has caused massive reduction of population in the wild.

Materials and Methods

Field observation and samples collection

Field observations and random samplings were carried out at selected locations from December 2011 to October 2014. Most of the samples were photographed in their natural habitat and important characteristics and features were documented. Cuttings of appropriate sizes were collected depending on the length and availability of samples found. The locality of each accession was documented and the coordinates were taken. The living samples were collected for *ex-situ* conservation as germplasm and planted in a glasshouse (5A), Ladang 2, Universiti Putra Malaysia.

Leaf morphological variations analysis

The leaf characteristics of each accession such as length, width, shapes, texture, and petiole length were examined. The scores were analysed using multivariate analysis in Multivariate Statistical Package (MVSP) software to analyze the similarity matrix between the species using Gower's General Coefficient index. A dendrogram was developed by using Unweighted Pair Group Method with Arithmethic Mean (UPGMA). Dichotomous keys were further developed based on descriptions by

Holttum (1953), Seidenfaden and Wood (1992) and Soto Arenas and Cribb (2010) and the current collected samples.

Results and Discussion

Recent diversity studies on Peninsular Malaysia's vanilla species revealed that the distributions of the genus are best described as constantly sparse, widespread and in many habitats (Rabinowitz, 1981). Wild vanillas have been recorded in lower montane forest, heath hill forest, hill dipterocarp forest, lowland dipterocarp forest, peat swamp forest and coastal hill forest. The species located at the northern Peninsular Malaysia could withstand the distinct drought season and in contrast another species was recorded to grow vigorously in the wettest area in the country. Germplasm containing several species, which include *V. griffithii*, *V. borneensis* and *V. aphylla* with a new prospect species is established. Among all of the local *Vanilla* species, *V. griffithii* is seen as the most common in Malaysia. Its capability to tolerate with wide range of environmental conditions and high probability to reproduce sexually are the main factors making them to be a superior local vanilla.

The absences of the reproductive structures have complicated the taxonomic revision, which served as the fundamental process for the utilization study of the local species. However, the native species were observed to have differences in vegetative morphological characteristics especially on the leaf part. Morphological analysis from leaf characteristics showed variations indicating species identification is possible using leaf morphology as descriptors. In general, *Vanilla* from Peninsular Malaysia shared common feature of membranous leaf but varied in apex, base and overall shapes. The leaf shapes were either elliptic to narrowly elliptic with variations in acuminate type of apex or symmetrically or asymmetrically rounded base (Figure 1). The length, width and petiole length were observed to be significantly influenced the classification the examined samples into species level. This preliminary study on leaf variations showed potential new records of species to Peninsular Malaysia, *V. abundiflora* and *V. havilandii* agreeable to descriptions from Soto Arenas and Cribb (2010).

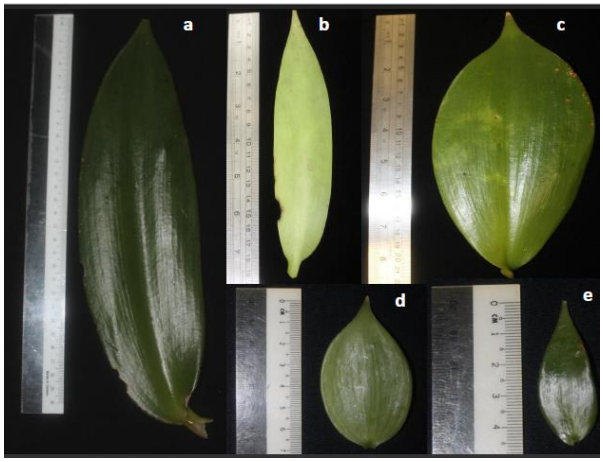

Figure 1: Leaf variations among *Vanilla* species from Peninsular Malaysia (a) *V. albundiflora* (b) *V. havilandii* (c) *V. sp. novo*. (d) *V. griffithii* (e) *V. aphylla*.

Conclusion

Peninsular Malaysia is blessed with variety of *Vanilla* species and in many habitats. The qualitative and quantitative characteristics of the leaves can be optimized as the species delimitating factor or key taxonomic character.

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In vitro propagation of an epiphytic orchid *Dimorphorchis lowii* through shoot tip culture

*Jualang Azlan Gansau and Juddy E. Jainol

Faculty of Science and Natural Resources, Universiti Malaysia Sabah
88999 Kota Kinabalu, Sabah
**Corresponding author.Tel.:+608 8320111; email address: azlanajg@ums.edu.my*

Introduction

Orchid *Dimorphorchis lowii* is one of the species that has been gaining much interest over the years because of its unique dimorphic flowers, which means it has two different flowers in one spike. It has the potential to be exported as an ornamental flower because of its one of its kind, beautiful and fragrant flowers. It is also one of the rarest epiphytic orchids found principally in the altitude of 600-1800m on Mount kinabalu, Mount Tombuyukon and Mount Trus Madi in Sabah (Crib *et al*., 2008). Because of the increasing popularity, endangered (included in CITES-Appendix II), very rare, thus difficult to locate, it is important to establish a technique for multiplication to produce a large number of plantlets and subsequently conserving this species. Shoot tip culture is found to be an efficient method for the production of large number of plantlets in a short period of time. Pant *et al.* (2011) reported that commercial orchids are produced by tissue culture and this technique is used routinely in many countries for large-scale production of orchid seedlings. To date, there has been no documented report available on *in vitro* propagation of this species. Therefore this paper would be the first to describe the protocol for *in vitro* propagation of *D. lowii* using shoot tip explant.

Materials and Methods

Protocorms developed from seed culture were transferred to ½ strength MS medium (Murashige and Skoog (1962) containing 2% (w/v) sucrose and 0.2% (w/v) yeast extract. Multiple shoots from these cultures were cut and separated individually and used as explants. The explants were inoculated on KC medium (Knudson C, 1946) with different concentrations of plant growth regulators, BAP (0, 0.5, 1.0, 2.0, 3.0, 4.0 mg/l), and Kn (0, 0.5, 1.0, 2.0, 3.0, 4.0 mg/l). Data collection and sub-culturing were done at four-week intervals for the duration of 180 days of culture. In the rooting development, individual shoots (3-6mm in length) each with two to three expanded leaves were detached from the shoot and were cultured in KC medium containing different combination of NAA, IAA, and IBA (0, 1.0 mg/l IAA + 0.5 mg/l NAA, 2.0 mg/l IAA + 0.5 mg/l NAA, 1.0 mg/l IAA + 0.5 mg/l IBA and 2.0 mg/l IAA + 0.5 mg/l IBA). Data collection and sub-culturing were done at four-week intervals for the duration of 120 days of culture. All data were subjected to analysis of variance (ANOVA) and means were compared by the Duncan's multiple range test at $P < 0.05$ using the SPSS ver. 20 (SPSS Inc., USA). Plantlets were removed from the culture flasks and were washed thoroughly and treated with Ancom Thiram 80 (fungicide). The rooted plantlets were then planted in the plastic pots containing coco peat and sphagnum moss (2:1) and covered with clear plastic bags for 30 days and maintained under

humidity.

Results and Discussion

The shoot proliferation and multiplication of *D. lowii* under influence of different concentrations of BAP and Kn was investigated (Table 1). Among the treatments tested, the highest number of shoots per explant (5.05 shoots) with mean length of 9.13 mm was observed in the medium supplemented with 2.0 mg/l Kn while the PGR-free medium gave the lowest number of shoots (Figures 1A & B). Panwar *et al.*, (2012) reported that further multiplication was achieved by subculturing shoot clumps of *Eulophia* orchid on medium containing 4.64 µM Kn after 4 weeks of culture. Ng *et al.* (2011) also reported that kinetin at 4.0 µM gave the highest number of secondary PLBs of *Paphiopedilum* orchid, which then further grew into complete plantlets when cultured in PGR-free medium. High rooting response was achieved on KC supplemented with 1.0 mg/l IAA with 0.5 mg/l IBA (75.83%) with a mean number of 1.98 roots per shoot and mean length of 13.02 mm (Figure 1D). The most commonly used auxins in orchid tissue culture media are the naturally occurring IAA and the three synthetics NAA, IBA and 2,4-D (Arditti and Ernst, 1993). These auxins are said to intensify the number of adventitious roots by increasing the level of endogenous contents of enzymes. They are considered to have an increased effect on cell division, elongation and differentiation (Pant and Shrestha, 2011). In the present study, a lower concentration of IAA (1.0 mg/l) as opposed to a higher concentration (2.0 mg/l) is suitable for root formation when in combination with 0.5 mg/l IBA rather than NAA. This is in agreement with the result obtained by Liu *et al.* (2002) where he described that IBA is physiologically a more active auxin than NAA in promoting root initiation as it acts as a precursor for endogenous IAA. The *in vitro* well-developed rooted plantlets of *D. lowii* were successfully hardened on potting mixture containing coco peat and sphagnum moss (2:1). This simple and efficient procedure for regenerating a large number of plantlets from shoot tip cultures could be used for large-scale propagation and *ex situ* conservation of this endangered species.

Table 1, results represent mean ± SD of ten replicated experiments after 180 days of culture. The different letters within a column indicates a significant difference at $p<0.05$ level. Table 2, results represent mean ± SD of ten replicated experiments after 120 days of culture. The different letters within a column indicates a significant difference at $p<0.05$ level.

Table 1: The effect of different concentrations of BAP and Kn on shoot proliferation of *D. lowii.*

Treatment (mg/l) BAP	Kn	**Percentage of survival**	**Percentage of explant forming shoots**	**Mean number of shoots per explant**	**Mean Length of shoot (mm)**
0.0	0.0	50.00 ± 23.57^{def}	37.50 ± 31.73^{bcde}	1.67 ± 1.00^{bc}	6.60 ± 5.28^{abc}
0.5	0.0	85.00 ± 17.48^{ab}	54.17 ± 33.85^{abc}	1.85 ± 1.12^{bc}	4.38 ± 2.31^{bc}
1.0	0.0	77.78 ± 26.35^{abc}	65.74 ± 19.29^{ab}	2.12 ± 0.67^{bc}	4.49 ± 1.59^{bc}
2.0	0.0	75.00 ± 26.73^{abcd}	51.04 ± 37.65^{abc}	2.38 ± 1.44^{bc}	4.26 ± 2.02^{bc}
3.0	0.0	70.00 ± 22.97^{bcd}	40.00 ± 35.09^{bcd}	1.93 ± 1.14^{bc}	4.69 ± 3.41^{abc}
4.0	0.0	86.11 ± 18.16^{ab}	50.93 ± 38.96^{abc}	2.08 ± 1.39^{bc}	7.20 ± 5.81^{abc}
0.0	0.5	44.44 ± 30.05^{ef}	26.85 ± 18.05^{cde}	3.66 ± 3.01^{ab}	3.82 ± 3.45^{c}
0.0	1.0	52.78 ± 19.54^{cdef}	51.85 ± 43.66^{abc}	2.98 ± 2.03^{bc}	8.04 ± 7.09^{ab}
0.0	2.0	83.33 ± 25.00^{ab}	80.56 ± 22.44^{a}	5.05 ± 2.81^{a}	9.13 ± 3.31^{a}
0.0	3.0	67.50 ± 31.29^{bcde}	32.50 ± 30.03^{bcde}	1.80 ± 1.44^{bc}	5.04 ± 4.53^{abc}
0.0	4.0	37.50 ± 35.84^{f}	23.33 ± 18.62^{cde}	1.67 ± 1.03^{bc}	5.08 ± 3.85^{abc}

Table 2: The effect of IAA, IBA and NAA on root formation from regenerated shoots of *D. lowii.*

Type of Auxin (mg/l)	**Percentage of survival**	**Percentage of shoots forming roots**	**Mean number of roots per shoot**	**Mean Length of root (mm)**
Control	82.50 ± 16.87^{ab}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
1.0 IAA+ 0.5 NAA	85.71 ± 19.67^{ab}	70.24 ± 22.49^{a}	1.58 ± 0.89^{a}	10.29 ± 6.58^{a}
2.0 IAA+0.5 NAA	77.50 ± 21.89^{b}	64.17 ± 32.41^{a}	1.58 ± 1.00^{a}	6.57 ± 3.79^{a}
1.0 IAA+ 0.5 IBA	97.50 ± 7.91^{a}	75.83 ± 29.78^{a}	1.93 ± 0.96^{a}	13.02 ± 11.23^{a}
2.0 IAA+ 0.5 IBA	91.67 ± 12.50^{ab}	58.33 ± 23.19^{a}	1.19 ± 0.60^{a}	10.35 ± 6.16^{a}

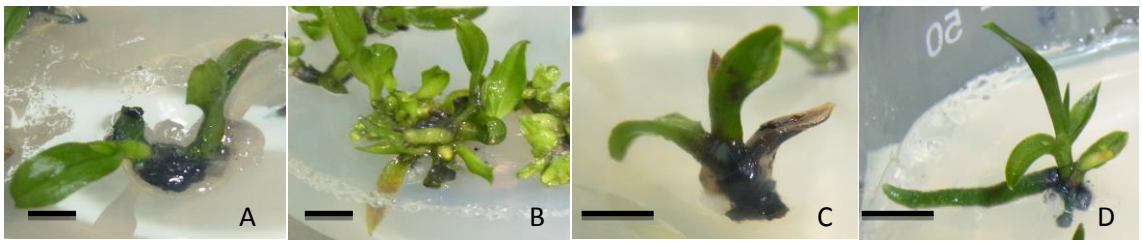

Figure 1: The proliferation of multiple shoots on (A) PGR-free medium and (B) medium containing 2.0 mg/l Kn. Root formation was absent in (C) PGR-free medium but was observed in (D) medium containing 1.0 mg/l IAA + 0.5 mg/l IBA (Bar is 5 mm).

Conclusion

A protocol was established for *in vitro* propagation of *D.lowii* using shoot tips as the starting material. Shoot multiplication was best seen in KC medium added with 2.0 mg/l Kinetin. The best rooting performance was observed in shoot tips that were cultured in KC medium added with 1.0 mg/l IAA with 0.5 mg/l IBA. It is hoped that this successful protocol for micropropagation using shoot tip culture can be applied in a commercial scale with the aim to mass-produce and introduce this species in their natural habitat.

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Orchid survey in heath forest of Terengganu

Siti Fatimah Md Isa[1], *Rusea Go[1], Jamilah Mohd. Salim[2] and Christina Yong Seok Yien[1]

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor
[2]Department of Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu
**Corresponding author:Tel.:+6012 678 8810; email address: go_rusea@yahoo.com*

Introduction

Heath forest is a type of tropical moist forest occurs on acidic sandy soil that are often lacking in nutrient. In the coastal area of Terengganu, the area of heath forest is known as Beach Ridges Interspersed with Swales (BRIS) soil having sand texture and the presence of spodic horizon. The soil usually poses a great challenge in low inherent soil status, poor nutrient and water holding capacities, excessive drainage, high surface temperatures and a very high moisture stress. Thus it is well known that the biodiversity in this forest area is poor when compared to other lowland forest. This has been motivated us to survey the diversity of orchid species in this drought area. We are expecting to document more epiphytic orchid rather than terrestrial orchid. This would be the first study specifically on orchid species in heath forest of Terengganu.

Materials and Methods

The survey was carried out in two BRIS areas of Terengganu; Dungun and Setiu from 2013 to 2014. All macro morphology characters such as vegetative and floral structures were observed and recorded in the collection book for later identification. Where necessary, the sterile plants were collected as living collections and will transplanted in the greenhouse of Universiti Putra Malaysia (UPM) for ex-situ conservation and identification once they flowered. Non-sterile and other sterile plants were collected as herbarium collection and processed following the standard herbarium specimen preparation techniques as outlined by Bridson and Forman (1989). Specimens were identified using the characters and the identification keys described in the revised version of Seidenfaden and Wood (1992), Holttum (1957) and Comber (1990, 2001). All the herbarium specimens were kept in the Herbarium of the Biology Department at the Faculty of Science, UPM.

Results and Discussion

We managed to document 23 species of orchid survive in the study areas. 18 species were identified as epiphyte and 5 species as terrestrial (Table 1).

Table 1: List of Orchid Species found in the Heath Forest of Terengganu.

Bil.	Subfamily	Genus	Species	Habit
1	Epidendroideae	Arachnis	*Arachnis flos-aeris* (L.) Rchb.f.	Epiphyte
2			*Arachnis hookeriana* (Rchb.f.) Rchb.f	Epiphyte
3		Bromheadia	*Bromheadia finlaysoniana* (Lindl.) Miq.	Terrestrial
4		Bulbophyllum	*Bulbophyllum clandestinum* Lindl.	Epiphyte
5			*Bulbophyllum corolliferum* J.J.Sm	Epiphyte
6			*Bulbophyllum purpurascens* Teijsm. & Binn.	Epiphyte
7			*Bulbophyllum vaginatum* (Lindl) Rchb.f.	Epiphyte
8		Callostylis	*Callostylis pulchella* (Lindl) S.C.Chen & Z.H.Tsi	Epiphyte
9		Coelogyne	*Coelogyne foerstermannii* Reichb.f.	Epiphyte
10		Crepedium	*Crepidium carrii* (Seidenf. & J.J.Wood) M.A.Clem. & D.L.Jones	Terrestrial
11		Cymbidium	*Cymbidium finlaysonianum* Lindl.	Epiphyte
12			*Cymbidium rectum* Ridl.	Epiphyte
13		Dendrobium	*Dendrobium acerosum* Lindl.	Epiphyte
14			*Dendrobium aloifolium* (Bl.) Reichb.f.	Epiphyte
15			*Dendrobium crumenatum* Sw.	Epiphyte
16			*Dendrobium leonis* (Lindl.) Reichb.f.	Epiphyte
17		Phalaenopsis	*Phalaenopsis pulcherrima* (Lindl.) J.J.Sm	Terrestrial
18		Plocoglottis	*Plocoglottis lowii* Rchb.f.	Terrestrial
19		Polystachya	*Polystachya concreta* (Jacq.) Garay & H.R.Sweet	Epiphyte
20		Taenophyllum	*Taenophyllum pusillum* (Willd.) Seidenf. & Ormerod	Epiphyte
21			*Thrixspermum calceolus* (Lindl.) Rchb.f.	Epiphyte
22			*Thrixspermum trichoglottis* (Hook.f.) Kuntze	Epiphyte
23	Orchidoideae	Zeuxine	*Zeuxine affinis* (Lindl.) Benth. Ex Hook.f.	Terrestrial

Conclusion

The result obtained support our theory where we found more epiphytic orchid that anchor on the tree trunk rather than terrestrial orchid that grow on the ground given that the poor condition of the sandy soil texture is not favourable for survival of terrestrial orchid. This result can be added in current databases of information on plant diversity in heath forest of Terengganu and also can be used as the basic data resources for further assessment on orchid species in the area.

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Species diversity and utilization of medicinal plants in Khok Dong Keng Public Forest, Na Dun District, Maha Sarakham Province, Thailand

Tawatchai Tanee[1,2], *Runglawan Sudmoon[2,3], Arunrat Chaveerach[2,3], Penkhae Thamsenanupap[1] and Sombat Appamaraka[4]

[1]*Faculty of Environment and Resource Studies, Mahasarakham University, Mahasarakham 44150, Thailand*
[2]*Genetics and Environmental Toxicology (GET) Research Group, Khon Kaen University, Khon Kaen 40002, Thailand*
[3]*Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[4]*Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44150, Thailand*
**Corresponding author.Tel.:+66 86 8568735; email address: rsudmoon@yahoo.com*

Introduction

Maha Sarakham Province covers only 0.78% natural forests comparing to its whole area. Because of this very small area of forest, this province is one of the driest areas in Thailand. Khok Dong Keng Public Forest, Na Dun District is one of the most important forests of the province. The forest and its resources have been intensively utilized without concerning of conservation of this limited area of the forest of this province. This research aimed to explore medicinal plants in the Khok Dong Keng Public Forest concerning their species diversity, abundance and utilization. Traditional healers and villagers who live around the forest were interviewed to get information of the usages of medicinal plants for curing disease. This information will be useful for public circulating as well as for setting up the management system for the community and the forest.

Materials and Methods

Species diversity of medicinal plants was observed in the eight 20×20 m^2 plots in Kok Dong Keng public forest. The Shannon's diversity index (Shannon & Weaver, 1949) was taken into account. Medicinal properties were investigated by interviewing the forest ranger who is expert in medicinal plants. The medicinal plants utilization was addressed by the questionnaires with two traditional healers and 330 villagers who live around the forest area. Finally data from all the informants were evaluated based on informant consensus factor (ICF) and fidelity level (FL). The ICF is presented for a particular treatment. It was calculated from $ICF = [n_{ur} - n_t] / [n_{ur} - 1]$; where n_{ur} is a number of informants using plants for a treatment, and n_t is a number of species used for that treatment (Gazzaneo *et al*., 2005). Whereas, the FL is presented for each plant species showing the specificity of the plant to disease. It was calculated from $FL(\%) = [N_p/N] \times 100$; where N_p is a number of informants that claim a use of the plant species for a particular treatment, and N is a number of informants that use the plant for any treatments (Ugulu, 2012).

Results and Discussion

The plot sampling (Figure 1), with a guide from the forest ranger showed 80 species of the medicinal plants (Figure 2). These included trees, shrubs and herbs. They showed species diversity index (*H'*) of 3.74. Apart from the forest, the two traditional healers have used 71 medicinal plant species, of which 37 species were found in the forest. Whereas, the data from 330 questionnaires revealed that the villagers have used 106 species, 38 common species were observed in the forest, for curing 46 diseases. Concerning these data, the informant consensus factor (ICF) ranges between 0 and 1. The highest ICF includes using *Chromolaena odorata* (L.) R.M.King & H.Rob. for hemostatic and *Phyllanthus emblica* L. for antiscurvy, for

Figure 1: Location of Kok Dong Keng public forest (red line) near Phrathat Nadun (star) the famous place housing Bhudha's relics and the eight sampling plots (yellow pins).

Figure 2: Examples of medicinal plants in Kok Dong Keng public forest.

examples. The higher the IFC the more people use the same plant for the specific treatment. Furthermore, specificity of the plant to the treatment was determined by fidelity level (FL) which ranges from 25 to 100%. The species with 100% FL are *C. odorata*, *Cissus quadrangularis* L., and *Solanum incanum* L., for examples, which have been used for hemostatic, antihemorrhoid, and expectorant, respectively. Some of these usages have been supported by scientific researches, such as aqueous extracts of *P. emblica* was effective protecting agent against the clastogenicity (Ghosh *et al*., 1992). Recently, Pandith *et al*. (2013) evaluated that *C. odorata* extract affected hemostatic and wound healing by promoting fibroblast cell migration and proliferation. Also accelerating wound healing enzymes, a vasoconstrictor, and a platelet aggregator were increased, while, antiplatelet aggregator was decrease during the treatment with the extract.

Conclusion

There are many useful medicinal plants which can be foods, spices, or vegetables. Ideally we can eat these plants as protective medicines. Unfortunately only people at age of higher than 60 know and use the plants. Therefore, the medicinal plant utilization data should be collected and publicized.

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The influence of elicitor Cu^{2+} and Fe^{2+} to asiaticoside level of pennywort (*Centella asiatica* L. urban) callus

*Evika Sandi Savitri, Feni Dwi Khoriroh and Luluk Lugiati Sholikhah

Department of Biology, Faculty Science and Technology,
Maulana Malik Ibrahim Islamic State University Malang, Indonesia
**Corresponding author.Tel.:+62 817531500; email address: evikasandi@yahoo.com*

Introduction

Pennywort (*Centella asiatica* L. Urban is a medicinal plant used in traditional herbal medicine industry in Indonesia. *Centella asiatica* contains various levels of secondary metabolites belongs to triterpenoid glycosides include asiaticoside, medekacocide, asiatic acid, acid medekacocide (Prabowo, 2002: Kimura, 2008).

Asiaticoside can be used for drug treatment of Alzheimer's disease because these compounds have been shown have potential to protect cells against cell death of β-amyloid. Asiaticoside also has antidepressant activity and increase the production of granulocytes to repair wounds and burns(Kimura, 2008).

Production of bioactive compounds through tissue culture can be enhanced by elicitation. The metal used is metal Cu^{2+} and Fe^{2+}. According to Marscher (1995), metal ions Cu^{2+} is an essential micronutrient for all living creatures as well as a cofactor of many enzymes and plays an important role in electron transport, redox reactions in various metabolic pathways.

Redox reactions and metal ion homeostasis is closely related and can cause oxidative stress. Metal ions Fe^{2+} is one of the most important nutrients for plants because Fe is required in the synthesis of chlorophyll, plays an important role in energy transfer. Fe is part of several enzymes and proteins and function in plant respiration and metabolism are also involved in nitrogen fixation. Fe is an element of enzyme activator (Cheu *et al*., 2010). Fe^{2+} play a role in activating the enzymes in the pathways of secondary metabolite from terpenoids groups, such as the enzyme DXP synthase.

Materials and Methods

This study was an experimental study using completely randomized design.The treatment used is various concentration of metal ions Fe^{2+} on callus subculture medium namely 0, 90, 100, and 110 mM. with 4 replications to obtain 16 trials. Cu^{2+} ion concentration treatments used consisted of 4 levels: Cu0 = control (0 mM), Cu1=30 mM , Cu2 = 35 mM, Cu3 =40 mM with 4 replications to obtain 16 trials

Results and Discussion

Giving Cu^{2+} with different concentrations showed significant differences. Treatment with the administration of 40 mM Cu^{2+} has the highest levels of compounds asiatikosida 4.1595 g / 100g. The lowest levels asiaticoside compounds show by the control (without Cu^{2+}) which was 3.6235 g / 100g (Figure 1).

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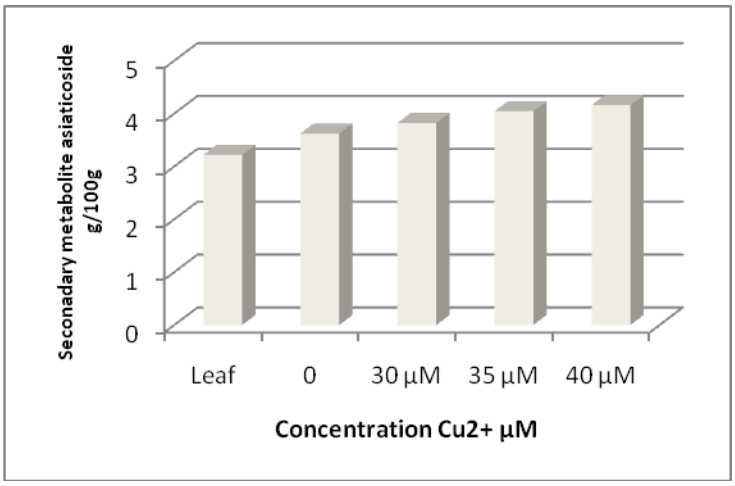

Figure 1: Secondary metabolite asiaticoside in the leaf and callus *Centella asiatica* in the treatment Cu^{2+}.

The content of the fresh leaves *Centella asiatica* has asiatikosida compound levels lower than that of the untreated callus, where the levels of the compound in the callus is 3.623 g / 100g, while the fresh leaves of 3.218 g / 100g.

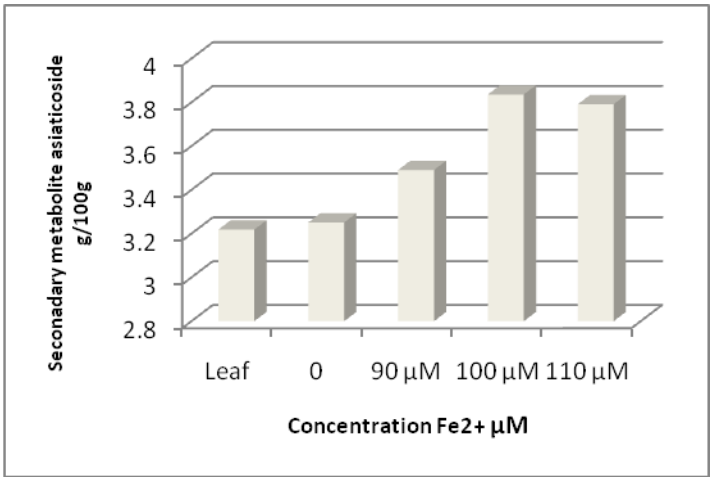

Figure 2: Secondary metabolite asiaticoside in the leaf and callus *Centella asiatica* in the treatment Fe^{2+}.

The asiaticoside elicited increased after using metal ions Fe^{2+} (Figure 2). Media which were supplemented with 100 mM Fe^{2+} was the best medium to produce the highest content of asiaticoside 3.836 (g / 100g). Treatment Cu^{2+} showed higher levels than using Fe^{2+}. The presence of elicitor will affect the biosynthesis of secondary metabolites in plant cells because it activates genes that encode enzymes in the biosynthetic pathways of secondary metabolites (Jing Wu, 2009).

Conclusion

Elicitor Cu^{2+} with 40μM concentration resulted in the high levels production of secondary metabolites asiaticoside 4.1595 g / 100g. Elicitor Fe^{2+} with a concentration of 100 mM asiaticoside produce secondary metabolite 3.836 g / 100g. Elicitor Cu2 + produces higher levels asiaticoside compared with Fe^{2+}

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The effect of endogeic earthworm, *Pontoscolex corethrurus* (Müller, 1857) on banana infected by blood disease – a histological study

Suk-Kuan Teng[1], [*]Nor Azwady Abd. Aziz[1], Muskhazli Mustafa[1], Rozeita Laboh[2], Intan Safinar Ismail[3,4] and Sujithra Devi A.[1]

[1]*Department of Biology, Science Faculty, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*
[2]*Horticulture Research Centre, Malaysian Agriculture Research and Development Institute, MARDI Headquarters, 43000 Serdang, Selangor, Malaysia.*
[3]*Department of Chemistry, Science Faculty, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*
[4]*Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*
**Corresponding author.Tel.:+603 89466632; email address: azwady@upm.edu.my*

Introduction

The occurrence of blood disease of banana in Malaysia is another challenge to the industry besides Moko and Fusarium wilt (Kogeethavani *et al*., 2013; Teng *et al*., 2014). Therefore, the search for effective approach to halt the spread of the disease is desired in view of the severity and damages reported in Indonesia (Supriadi, 2005). Endogeic earthworms are soil-burrowing earthworms that have close interaction with soil and plant root systems. Previous studies have documented the role of endogeic earthworms in improving plant health (Lafont *et al*., 2007; Loranger-Merciris *et al*., 2012), thus increasing their resistance towards pathogens and diseases. The present study was conducted to determine the effect of endogeic earthworm, *P. corethrurus* on the histology of stem and root of the blood disease infected banana.

Materials and Methods

Twenty eight banana plantlets (8 weeks' old Berangan variety) were purchased from a nursery in Banting, Selangor and maintained in the glasshouse for a week. The plantlets were divided into four treatments (Control, A; Blood disease bacterium, B; *P. corethrurus* and BDB, C and *P. corethrurus*, D) with seven replicates each. They were arranged in completely randomized design (CRD).

Five adult *P. corethrurus* were introduced into the soils of treatment A and C. After two weeks, 5 ml of BDB inoculum (10^8 cell/ml) were inoculated into each plantlet in treatment B and C through drenching method (Kusumoto *et al*., 2004). Root sections of the plantlets were injured with a sterilized needle (0.5 x 16mm). Five millilitres (5ml) of the BDB inoculum was carefully drenched onto the soil. Five millilitres of sterilized distilled water (SDW) was inoculated onto plantlets in treatment D using the same procedure and served as the control. The plantlets were observed for disease symptoms for 3 weeks. All the plantlets were harvested after 18 days after inoculation (DAI). The root and stem of each plantlet were sampled and preserved in formalin acetic acid (FAA) solution.

In the laboratory, the cross sections of stem and root were subjected to the standard procedures for preparations of plant histological microscopic slides. The samples were subjected to a series of dehydration using ethanol at different concentrations, embedded into wax blocks, sectioned into 8 µm thin layers and stained with Safranin O and Fast Green stains. The nuclei, chromosomes and lignified cell walls appeared red when stained with Safranin O whereas cytoplasm and cellulosic cell walls appeared green when stained with Fast Green (Ruzin, 1999). The slides were then viewed under compound microscope and images were photographed for documentation.

Results and Discussion

The severity of blood disease was slightly reduced in the presence of *P. corethrurus* upon harvest at 18 DAI. Although the plantlets in treatment C showed symptoms of BDB infection, however it was less severe compared to plantlets in treatment B (second plantlet from the left in Figure 1).

Figure 1: Banana plantlets of different treatments upon harvest at 18 DAI

Histological observation on the stem and root tissues of plants in treatment A and D were similar, indicating the presence of *P. corethrurus* did not cause harmful effect towards the plants. The stem section of plant in treatment B collapsed, leaving a hollow region at the centre (Figure 2b). This may be due to the BDB infection that blocked the vascular tissues. This eventually caused the damage and death of stem tissues. A number of red pigments were observed at the centre of the stem section of plant in treatment C (Figure 2c). This was most probably due to the accumulation of dead lignified tissues. Besides that, both stem sections of treatment B and C have less nuclei and appeared red compared to those in treatment A and D. These indicated to the destruction of tissues due to the presence of BDB. However, the stem tissue damage observed in treatment C was slightly less compared to its counterpart in treatment B.

The histological structure of root tissues in treatment A and D had a typical monocotyledon structure as described by Mateille (1994). Meanwhile, the metaxylems of root tissue in treatment B appeared blocked and darkened (Figure 3b). This might be due to the accumulation of the pathogen (BDB) or organic compounds produced by the host in response to infection. Thus, prevented the movement of water and minerals from the root and caused the plants to wilt. However, the metaxylems of root tissue in treatment C were large without blockage

(Figure 3c). This may be due to the presence of *P. corethrurus* that lessen the effect of BDB infection. Therefore, the disease symptoms were slightly less severe.

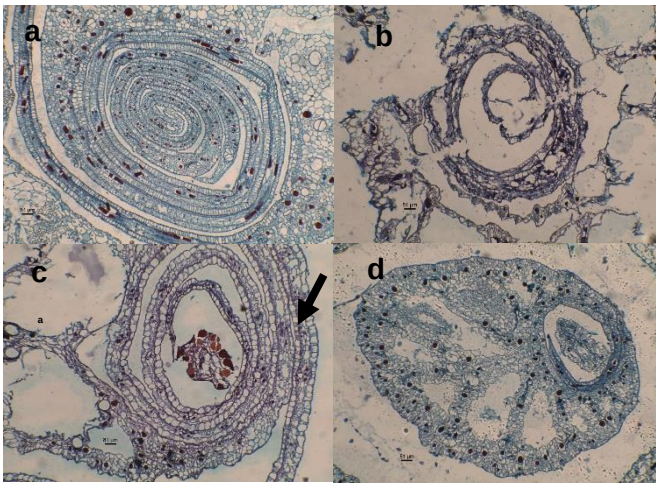

Figure 2: Stem cross section (a) Treatment A (Control); (b) Treatment B (BDB); (c) Treatment C (PC + BDB) and (d) Treatment D (PC)

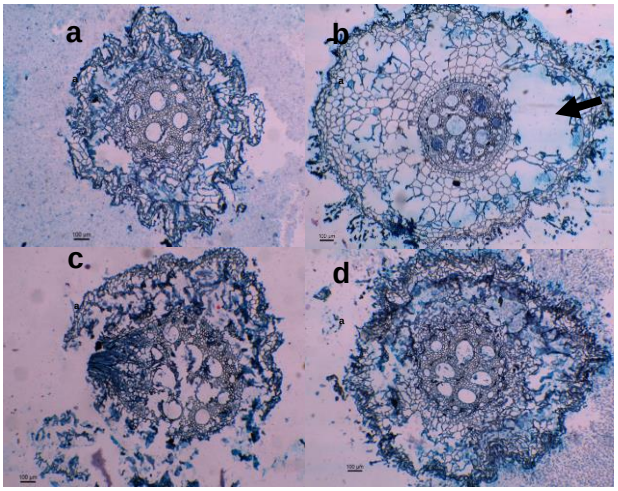

Figure 3: Root cross section (a) Treatment A (Control); (b) Treatment B (BDB); (c) Treatment C (PC + BDB) and (d) Treatment D (PC)

Conclusion

Although banana exhibited BDB infection in the presence of *P. corethrurus*, the severity of the disease was slightly less. *P. corethrurus* could be a potential soil bioremediation agent in improving plant health and reduce the effect of BDB infection.

Acknowledgements

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Species composition of Lianas in Berembun Virgin Jungle Reserve, Negeri Sembilan, Malaysia

*Mohd-Ridzuwan, B. E., Wan Juliana, W. A. and Latiff, A.

School of Environmental and Natural Resources Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
**Corresponding author.Tel.:+6016 4129868; email address: kgpalas@gmail.com / mohdridzuwan@forestry.gov.my*

Introduction

Virgin Jungle Reserve (VJR) is one of the twelve forest classification of Permanent Reserved Forests under National Forestry Act with an area of 23,002 hectares that represent various forest types in Peninsular Malaysia. One of this is Berembun Virgin Jungle Reserve located at Berembun Forest, Negeri Sembilan, Malaysia and about 200 km south east of Kuala Lumpur, the capital city of Malaysia. This reserve is bordering to the traditional Malay villagers in the west and extends to the Banjaran Titiwangsa in the east. The objectives of VJRs are to serve as permanent nature reserves and natural arboreta, as a control of production forest areas that are being exploited and silviculturally treated; and undisturbed natural forest areas for general ecological and botanical studies of fundamental importance (Wyatt-Smith, 1950).

The Berembun VJR is a lowland dipterocarp forest with hot and wet around the year, a very fertile soil, easily accessible place and an important place in socio-economic activities. The temperature is around 27^{o}C to 30^{o}C. The rainfall ranges from 1,018 mm to 2,243 mm with an average of about 1,871 mm per annum. An increased of urbanisation in the nearby area and potentially causes negative impact on natural resources for meeting human needs such as food, fodder, fuel and timber.

Since 1970s, large tract of forest lands had been loss due to the conversion into agricultural fields, buildings, roads, industrial zones and urbanisation. This leads to the loss of forest biodiversity, forest fragmentation, change in forest structure, vegetation and species composition. Many changes had occurred on forest vegetations, plant communities, natural resources and the environment. Unfortunately, there is no detail record and documentation of such changes on forest biodiversity and vegetation in Berembun Forest. As such it is important to have an account of plant communities to be protected and preserved from extinction through conservation programme. This paper describes the species composition of lianas of plant communities in Berembun VJR. It also focuses on the importance value index of the species in the study area.

Materials and Methods

Four plots of 50 m x 50 m (0.25 ha) were established, and each plot was divided into 10 m x 10 m subplots (0.01 ha) laid out downward perpendicular to the altitudinal gradient. The protocol used to measure the diameters of lianas at breast height (dbh) followed Gerwing *et al*. (2006) and Schnitzer *et al*. (2008). All lianas $\geq$1 cm dbh were tagged in all four 50 m x 50 m plots and in 10 m x 10 m subplots, enumerated and voucher specimens were recorded and documented. BiodiversityR software (R Development Core Team 2005; Kind and Core, 2005), R sads package (Prado and

Miranda 2013) and PAST statistics software package (Hammer *et al*. 2001) all were used to calculate the species composition and the importance value index.

Results And Discussion

The study recorded 23 families, 45 genera, 68 species and 246 individuals in 1 ha plot at Compartment 32, Berembun Forest Reserve. In comparison, secondary lowland forest at Pasoh, recorded a total of 1,628 individuals represented by 31 families, 65 genera and 167 species *in 50 hectares plot* (Nurfazliza *et al*., 2012). The most abundant species in this study was *Omphalia bracteata* (27 stems). The second most abundant was *Fibraurea tinctoria* (13), followed by *Friesodielsia glauca* (12), *Mitrellia kentii* (12), *Spatholobos maingayi* (12), *S. ferrugineus* (11), *Tetracera fagifolia* (9), *Byttneria maingayi* (7), *Friesodielsia affinis* (7), *Millettia sericea* (7), *Parameria polyneura* (7), and *Pyramidanthe prismatica* (22). Other recorded species had less than 7 individuals each.

Importance value index (IVi) showed clear variation between species. The highest IVi value was that of *Omphalea bracteata* (36%) in Plot 1 followed by *Spatholobus ferrugineus* (26%) in Plot 2, *S. maingayi* (23.7%) in Plot 3 and *Willughbeia tenuiflora* (35.3%) in Plot 4.

Conclusion

The primary forests of Berembun VJR harbours a substantial amount of species composition of lianas that contribute a significant contribution to the forest biodiversity in Peninsular Malaysia. The findings will further enhance our knowledge on climbers as an important component of forest dynamics. Microhabitats of lianas such as along rivers should be maintained to ensure a continued ecological stability and must be free from anthrophogenic disturbances.

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Batch process for bio-hydrogen production on small scale bioreactor from palm oil mill effluent (POME)

*A. Fakhrul'l-razi, N. K. Nik Norfadilah and Razif M. Harun

Department of Chemical and Environmental Engineering, Universiti Putra Malaysia 43400 Serdang

**Corresponding author.Tel.:+603 8946304; email address: fakhrul@upm.edu.my*

In this study, treatment of palm oil mill effluent (POME) was carried out under anaerobic fermentation process to produce bio-hydrogen by micro flora. Experiment was investigated in 500mL bioreactor under mesophilic operation at and different pH values. Raw POME was collected from cooling pond which is final discharge of effluent from the mill and POME sludge was collected from the anaerobic pond of a POME treatment plant at Labu palm oil mill. The source of inoculum used was POME sludge as hydrogen producing bacteria. A batch reactor was set up producing hydrogen at an optimum parameter of pH 5.5 and 10% POME sludge (w/v) with a maximum hydrogen production yield of 5988.96 mL H2/ L-med. The maximum hydrogen percentage in the biogas was 36% obtained at pH 5.5. Throughout the study, there no methane gas was observed in the evolved gas mixture.

Keywords: Bio-hydrogen, POME sludge, raw POME, microflora

Identification of bioactive compounds produced by bacteria-based biopesticides, *Bacillus thuringiensis* ATCC 10792 produced in shrimp pond sludge

Noormasshela Ulul Azmi, Nurul Shaziera Abdul Ghafar, Cheong Jee Yin, Safiya Yakubu, Azleen Ahmad Adli, Nor Azwady Abd. Aziz and *Muskhazli Mustafa

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

**Corresponding author.Tel.:+603 89466631; email address: muskhazli@upm.edu.my*

Biological controls of destructive pest in forest and agriculture sector using bacteria were proven to minimize the problems caused by the usage of chemical insecticides. However, their application is limited due to high production cost through fermentation. In this study, shrimp pond sludge and wastewater were used as an alternate culture medium for bacteria-based biopesticides production. It has been determined by previous part of this study that *Bacillus thuringiensis* ATCC 10792 in hydrolysed shrimp pond has showed the highest potential to be used as bacteria-based biopestecides based on their high growth rate ($7.44x10^6$ ± 5.16 CFU/ml) and sporulation ($5.90x10^6$ ± 7.88 CFU/ml). Bioassay of entomotoxicity also showed high mortality rate, disruption on the target insect physically and affect the life cycle of the target insect. Hence, proteomic analysis using SDS-Page and MALDITOF-MS were conducted to identify the bioactive compound that contribute to the toxicity of the bacteria-based biopesticides produced. Separation and identification of the bioactive components produced by the bacteria during the fermentation were done and two types of protein were produced by the bacteria. Superoxide dismustase and spore coat protein were identified to be produced during the fermentation and respectively play an important roles to the toxicity of the bacteria. This study showed the potential of *Bt* ATCC10792 to be used as biopesticides.

Keywords: Toxin, SDS-Page, MALDITOF-MS, protein

Biodiversity and succession of freshwater algae in Hutan Simpan Ayer Hitam Forest Reserve, Puchong Selangor

*Shahrizim Zulkifly, Intan Farhanna Uyob and Noraishah Sahar

Department of Biology, Faculty Science, Universiti Putra Malaysia, 43400 Serdang
**Corresponding author.Tel.:+603 89466645; email address: shahrizim@upm.edu.my*

The succession and dominant of freshwater algae were studied in the north lake of Ayer Hitam Forest Reserve. Sampling was performed for thirteen weeks, from the end of the November 2013 to the end of the February 2014. Water sampling for biological analysis was done during the thirteen weeks. The structural community was determined by population density, class of algae and diversity indices. The community of freshwater algae consisted of 15 species from 5 different phyla which are Ochrophyta, Dinophyta, Charophyta, Cyanophyta and Chlorophyta. The Dinophyta had the highest total density in the thirteen weeks and two species from three species of Dinophyta were the most conspicuous species in the succession which are *Gonyaulax apiculata* and *Gymnodinium palustre*. The dominance of Dinophyta was during week 2, 3, 6, 7, 10 and 11. Dinobryon sertularia from phylum Ochrophyta had the highest density and being dominant during week 1, 4 and 5. For the Cyanophyta, *Anabaena subcylindrica* had the highest density as compared to the other species of Cyanophyta. *Anabaena subcylindrica* was being dominant during week 8, 9 and 13. For diatom *Navicula* sp. (Ochrophyta), the highest density was obtained during week 12. The Shannon-Weaver index had the highest during week 12 and the lowest during week 4. The highest Evenness index occurred during week 3 and the lowest during week 4. The pattern of succession and dominant of freshwater algae in a north lake of AHFR was associated with the physico-chemical parameter especially nutrient status, pH, temperature and light intensity.

Keywords: Algae, freshwater, succession, dominant, north lake of Ayer Hitam Forest Reserve, physico-chemical parameter

Anatomy of selected *Lygodium* species in Chini Lake, Pahang

*Nurnida Mohd Kamal, Noraini Talip, Mohamad Ruzi Abdul Rahman and Nor Fairuz Ramli

School of Environmental Science and Natural Resourses, Faculty Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi
**Corresponding author.Tel.:+6012 6045620; email address: nurnidakamal@yahoo.com*

A study on the frond of two species of *Lygodium* namely *L. flexuosum* and *L. microphyllum* was undertaken. The objective of this study is to verify the anatomical characteristics between these 2 species. Sample was collected from Chini Lake, Pahang and method used in this study was sectioning using sliding microtome. The samples were observed after going through the clearing, colouring, dehydration and mounting process. Result showed that three cross section which were lamina, midrib and margin can be used to differentiate between *L. flexuosum* and *L. microphyllum*. Whereas, for the stipe cross section, epidermal peel and clearing of these 2 species showed no different in their anatomical characterization. As for conclusion, results for leaves anatomical characteristics of *Lygodium flexuosum* and *L. microphyllum* can be very useful as additional data for species differentiation and also in identification of species.

Keywords: Anatomy, frond and *Lygodium*

Bioeconomic study a marine phytoplankton chaetoceros calcitran by using commercial and formulated fertilizer

Khairunnisa Abdul Rahman, Abd. Malek Abdul Rahim, Shahrizim Zulkifly, Hishamudin Omar and *Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor.

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Studies were carried out on two different aspect of marine diatom, *Chaetoceros calcitran*. First is the measure of cell growth base on number of cells and dry weight in different culture media such as LCP, LCP + clewat 32, Japanese Media and Conway Media as reference. The second study is looking at the effect of growth in different tank such as glass tank, conical fiberglass tang and rectangular fiberglass tank. All the culture of *Chaetoceros calcitran*, was carried out in outdoor tank, salinity of 23.25 % and ambient air temperature of 27-36 °C. Throughout the study, the weather is sunny. Results shows that *Chaetoceros calcitran* cultured in Conway media has the highest cell at 1.843 x 106 cells (maximum cell number) and 0.870 g/L (maximum dry weight). The Cultivation cost for Conway media and LCP were RM 41.227 and RM 74.833 respectively. The results from second showed growth are in the glass tank which is 1.687 x 106 cell/ml (maximum cell growth) and 0.783 g/L (maximum dry weight). Cultivation in a glass tank also showed that the lowest production cost at RM 45.191 /g dry weight compared to the fiber conical tank and rectangular fiber glass tank.

Keywords: Conway medium, *Chaetoceros calcitran*, marine phytoplankton, diatom, LCP

Bioeconomic study of *Chlorella vulgaris* in outdoor tank culture by using different types of fertilizer

Tunku Ainna Tunku Azmir Shah, Loo Bee Gan, Shahrizim Zulkifly, Hishamudin Omar, and *Ahmad Ismail.

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor.

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Four different culture mediums that were Bold Basal, commercial fertilizer, modified fertilizer and modified Bold Basal were used to cultivate *Chlorella* sp. in outdoor tanks. The modified fertilizer was formulated according to the NPK ratio in Bold Basal medium (control). Clewat 32 (0.01 g/L) was used in modified Bold Basal to replace the micronutrient in Bold Basal medium. The growth of *C. vulgaris* in outdoor culture was determined daily by cell count and dry weight every two days. The result of this study shows that, culture in modified fertilizer has no effect in improving the total production in outdoor culture. Meanwhile, culture in Bold Basal medium has relatively higher productivity (1.14 g/m2/day) at its optimum if compared to modified Bold Basal medium at 1.08 g/m2/day. Thus, the production cost per gram in 100 L outdoor tank culture was less expensive for Bold Basal medium which is about RM 21.13/g but moderately higher in modified fertilizer (RM 26.75/g). Bioeconomic studies revealed the weakness particularly with respect to the biological component. Possible weaknesses in the biological component are low productivity, unsuitable strain of *Chlorella* sp., weather, culture technique and scale of production.

Keywords: Bold basal medium, *Chlorella vulgaris,* cost, bioeconomic, outdoor

Comparison of tree composition between logged-over and virgin forest at Ulu Muda Forest Reserve in Kedah, Malaysia

*M. Mohd Nizar, I. Faridah-Hanum, K.R. Hakeem

Faculty of Forestry, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
**Corresponding author. email address: mohdnizarmardan@gmail.com*

Introduction

Most of rainforest today presented in the country are classified into 16 forest types based on climatic, edaphic, and biotic factors. According to Symington (1943), definition of this three factors were the important fact to describe the differentiation types of forest. The forest of Peninsular Malaysia was divided into 3 groups comprising of littoral, lowland and mountain or hill forests by Faxworthy (1922), were each further subdivided into subgroup. According to Symington (1943), delimit of the forest based on the altitudinal zoned which is Lowland Dipterocarp Forest, Hill Dipterocarp Forest, Upper Hill Dipterocarp Forest, Mountain Oak Forest and Mountain Forest. The Flora of Peninsular Malaysia will take at least 20 years to complete where about 400-500 species a year (Saw and Chung, 2005). Generally, the tropical forests are known have a higher species diversity. Followed by other studies before this, for example Faridah Hanum *et al*. (2001a and 2001b), Rusea *et al*. (2001) state biological diversity research has been carried out to assess the species richness, diversity, and the similarity of various forest ecosystems. Hill Dipterocarp Forest was classified by Whitmore (1984) when the forest in the ranges from 300m to 800m above sea level (a.s.l). Usually, these forests commonly produce for productive and protective functions. That means, hill forests types filled by specific species which is by several unique timber tree and commonly different from lowland forest such as floristic composition of the dominants in the upper layer and main tree storey (Abdul Rahman *et al.,* 2002).

Material and Methods

Study Site

This study was conducted in Ulu Muda Forest Reserve (UMFR), Kedah. This study will be covered four hectare which is three hectare from compartment 28A with an area of 108.98 ha and one hectare from compartment 35A as a control plots. The soil types of UMFR are classified as Baling, Tai Tak, Serdang, Bungor and Kuala Brang Series (DOA, 1994). The Baling and Tai Tak series are yellowish brown finely textured, well drained and moderately deep soil. The climate changes at this study area are highly rainfall and temperature which is uniformly high throughout the year. The mean temperature in nearest station (Hospital Baling) is in February until July (average 26.9 °C), the minimum occurs during September until December (average 25.9 °C). The average annual rainfall for this area about 2153.9 mm per year and distribution of rainfall occurs with a major peak in October and November, and minor peak starting January until end of February. Mean annual rainfall is 1615.44 mm (2003-2013). Average elevation for this area was 465 m a.s.l

Field surveys and data collection

For this study, total area were be covered are two hectare. One hectare was held covered in compartment 28A and compartment 35A (VJR) as a control plots. For each plot, 0.1 ha size of main plot will be established. The plot size were 50m x 20m and placed along a transect line. Total plots for this study is 20 plots which is 10 main plots in compartment 28A and another 10 plots in compartment 35A (VJR). Distance between two plots along the transect line were 50m. The degree for baseline is 83°, and for the transect line on the left side is 353° and for the right side is 173°. Each plot were be surveyed by enumerated, identified the species, and measured the dbh and height of tree. Among the data recorded were the diameter of breast height (dbh) ≥ 1cm was measured at 1.3 above the ground, height of tree, and name of species.

Statistical analysis

Data were transferred into the Excel spreadsheet and the data will be analyzed using. Quantitative analysis will conduct on the data collected from the 2 ha plots. Species composition and diversity will be calculated including Important Value Index (IVI) and Family Value Index (FVI), and Species Richness which is to describe the species composition at that study area. For this study, by using SDR 4 (Species Diversity and Richness) some equation will be used to calculate the species diversity such as Shannon-Wiener index of diversity, Simpson's index for diversity, and Alpha Fisher's index for diversity (Diversity indices). Then, species Richness also were being applied used Margalef index of species richness, second order Chao of species richness, and second order for Jackknife of species richness. Lastly, species Evenness (Evenness indices) were applied such as Simpson's measure of evenness, and the evenness index (E).

Result and Discussion

DBH and basal area

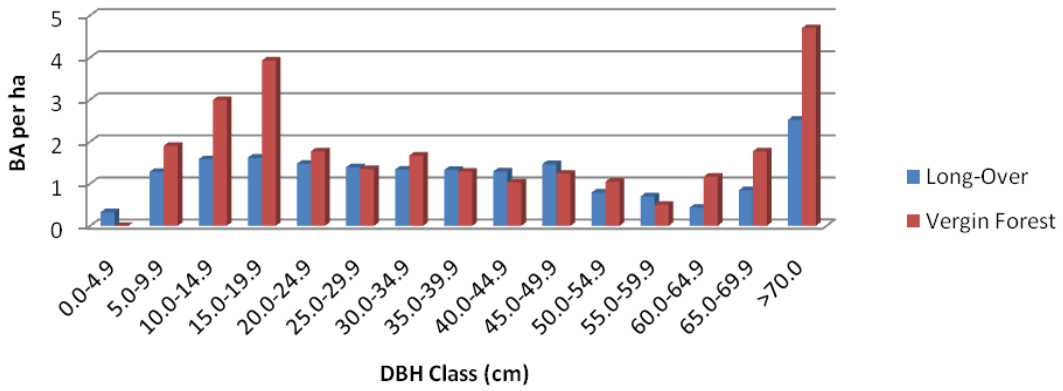

Figure1.

Figure 1 shows the distribution of basal area by dbh class for two different areas. Firstly, for log-over area, total basal area per ha for 1110 stems was 21.17 m^2ha^{-1} and for VJR area, total basal area per ha was 26.35 m^2ha^{-1} (976 stems/ha). Family from Euphorbiaceae with (860 individuals) or 287 stems/ha for log-over area and 213 stems/ha for VJR area has a large basal area. Based from the graph, basal area for

VJR area was highest than log-over area. That was because, more solid tree with highest dbh already cut-off and only small tree with small or mid-size dbh just left in log-over area. For example, trees with dbh >70 cm in VJR area is highly than log-over, but we can see in dbh class 45 until 49.9 cm, log-over area is more highly than VJR area.

Height and density of trees

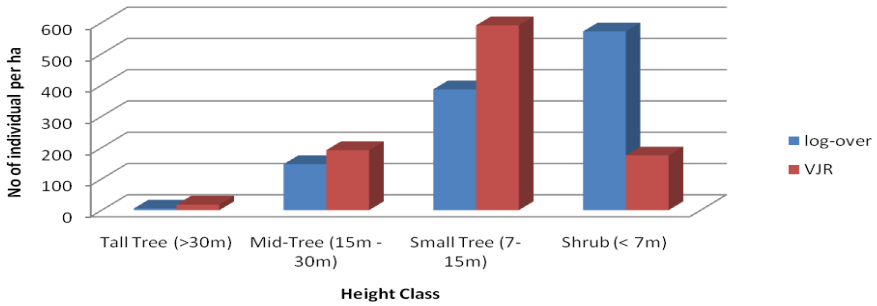

Figure 2.

Figure 2 shows the distribution of no of individuals per ha by height class. From this figure, we can see height class for small tree, mid-size tree, and tall tree are dominant by VJR area than log-over area. This is because, no logging process have been done at VJR area and after logging be done, we can see at this figure shows the structure of forest in log-over are totally different. More shrubs can be found after logging because of the open gap and more sunlight direct to species that need more light (light-demand species). From here, effect of log-over with conventional method can be destroyed small tree and changes the structure of species composition.

Table 1: Summaries of mean tree density per ha based on log-over and VJR area in Ulu Muda Forest Reserve, Kedah.

Area	Mean Tree Density / ha	Abundance Family
Log-over	1110 stems/ ha	Euphorbiaceae (287 stems/ha)
VJR	976 stems / ha	Euphorbiaceae (213 stems/ha)

According to Table 1, the abundant species come from family Euphorbiaceae for both area which is for log-over area contribute 287 stems/ha and VJR contribute 213 stems/ha. Based from mean tree density per ha shows log-over area about 1110 stems/ha and VJR about 976 stems/ha. From the result, we can assume that after logging activities have been done, more species from family Euphorbiaceae can be found and this is also shows effect from conventional logging increase number of species which need more light and easily growth in open gap.

Diversity, Evenness and Richness

Based on Table 2, it shows the comparison between log-over area and VJR area. From the result, we can see log-over area already disturb and in process to recovery. Shannon-winner shows VJR area is more highest than log-over area. This is shows that the situation of log-over is already disturb and low of diversity. Also, simpsons for

evenness shows VJR is less than log-over area. That means, VJR area is more diverse and highest number of species than log-over area which is slow to recovery. So, effect conventional logging also is a main causes log-over area right now need more time to recovery to re-structure the composition and species diversity same like VJR area.

Table 2: Description of tree species diversity, evenness, and richness in log-over and VJR area, UMFR, Kedah.

Plot	Shannon-Winner	Simpsons, E	Simpsons, D	Alpha-Fisher	Jack 2	Chao 2	Margalef
Log-over	4.50	0.37	0.88	56.94	216.27	230.63	24.31
VJR	4.62	0.33	0.89	64.83	188.10	226.60	26.00

Conclusion

As a conclusion for this study, log-over area need more time to recovery for the species composition, structure and diversity. Using conventional logging method in log process also can make highest damage to small trees and also can changes the situation of forest. Using our system right now, Forest Department need more clear about this problem and need to control activities log in our forest. If this problem didn't take action as soon as possible, more damage can be done and for sure our ecosystem in forest also disturb. In addition to contributing to our understanding of species richness and diversity, the result I presented also creates an opportunity to address new research questions. Conservation biology seeks to develop tools and methods that increase investment and success in conservation planning. The consensus has been that much more research is needed to provide directly relevant and applicable evidence for a better understanding of the hill dipterocarp ecosystem. Monitoring, assessment, research, and planning should be in continues interplay, because UMFR need more time to recovery especially in this log-over area. Thus, an improvement management to ensure the preservation and protection is necessary for sustainability of environment and development. Study about hill dipterocarp still need more research and need more advance to improve our management system in future. Unsupervised logging method must be stop because the effect is too high in our biodiversity and can be control by implement in our law.

Acknowledgements

This study has been partly supported by the research grant "Tree species composition, diversity, structure and physical environment in Ulu Muda Forest Reserve, Kedah 8-years after conventional logging methods" (Research Grant No: 9199757).

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Different salinity effects on the mass cultivation of spirulina (*Arthrospira platensis*) using urea as nitrogen source in Oman and Malaysia

Hafidh Al Mahrougi[1], Mohamed Amar Naqqiuddin[2], Jackson Achankunju[1], Hishamuddin Omar[2] and *Ahmad Ismail[2]

[1]College of Agriculture and Marine Science, Sultan Qaboos University.
[2]Biology Department, Faculty of Science, Universiti Putra Malaysia 43400, Serdang, Selangor.
**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Mother earth is facing multitude of problems such as desertification, diminishing cultivation land and malnutrition. One way to overcome these problems is through cultivation of Spirulina (*A. platensis*). This paper focused on the different effects of salinity and comparative climate patterns to the mass production of *A. platensis* in Oman and in Malaysia. With extremely contrasting environment, *A. platensis* has unique ability to grow in both tropical (Malaysia) and arid (Oman) outdoor conditions. Mass cultivation has been carried out at different salinity 5, 15, 25 and 35 ppt for 10 days with triplicates in both countries. The total volume of culture in each tank and land photobioreactor was maintained at 100 L and 50L respectively. In Oman, the highest optical density (ABS) was 1.691 ± 0.099 at 5 ppt significantly different and higher than 25 and 35 ppt ($p < 0.05$). Though, the highest biomass (g L^{-1}) achieved with 35ppt, 0.848 ± 0.039 was not significantly different from other salinity concentration ($p > 0.05$). While in Malaysia, the highest optical density (ABS) recorded with Spirulina dry weight was collected from Spirulina culture treatment with 5 ppt, 0.974 ± 0.052 was not significantly different from other salinity treatments ($p > 0.05$) and the dry weight at 0.575 ± 0.032 g L^{-1} was significantly different to 25 and 35 ppt ($p < 0.05$). Highest average mean ± SE of pH in Oman and Malaysia were recorded with salinity treatment of 15 ppt, 10.60 ± 0.058 and 10.20 ± 0.037.

Keywords: Spirulina, *Arthrospira platensis*, salinity, photobioreactor, climate pattern

Effect of herbal supplements on antimicrobial activity of kombucha tea

Kalia Zhumabekova[1], *Bibigul Zhumabekova[2], Lyudmila Mamonova[3], Zauresh Rymzhanova[2] and Natalia Tarassovskaya[2]

[1]*Line Brew Bottlers» Brewery, Almaty, Kazakhstan*
[2]*Pavlodar State Pedagogical Institute, Pavlodar, Kazakhstan*
[3]*Kazakh Academy of Nutrition, Almaty, Kazakhstan*
**Corresponding author.Tel.:+77012252144; email address: bibigul_kz@bk.ru*

Introduction

Kombucha tea is a health beverage made by incubating the Kombucha "mushroom" in tea and sugar. Kombucha is not a mushroom but rather a poorly defined consortium of yeasts and bacteria. Studies have shown that drinking Kombucha has increased resistance against cancer, capable of preventing cardiovascular and digestive system diseases. It stimulates the immune system and reducing of inflammation.

Each microorganism of symbiotic Kombucha association produces metabolites with antimicrobial activity against many pathogenic and opportunistic microorganisms. The aim of this work was to study the effect of various herbs infusions on the synthesis of Kombucha tea's metabolites having antimicrobial activity.

Materials and Methods

The object of the study were natural mixed Kombucha culture widespread and used by the population of Kazakhstan, association of yeast and acetic acid bacteria from kombucha and the Kombucha drink.

Cultivation of pure microorganisms' cultures from natural populations was performed in stationary conditions in fermentors with a working volume of 700 ml on a liquid aqueous medium containing 6% sugar and 0.1 % tea leaves at a temperature of 28^0C. After 3 days of cultivation up to 3.5 pH value of culture liquid, samples taken from the fermenter were plated on selective media: wort agar, plain agar, agar hydrolyzate of milk. There were the following microorganisms: *Hanseniaspora apiculata, Saccharomyces sp., Torulopsis sp., Acetobacterioum xylinum, Acetobacterium aceti*, isolated and identified from natural kombucha [1-2]. Selected association of the microorganisms further grew in semi-continuous conditions of cultivation (by daily out pouring half of the culture liquid and up toping the same amount of solution) [3-4]. Culturing of microorganisms was carried out in a liquid containing sugar (from 4 % to 10 %) and 0.1 % of the extract of the tea leaf.

We used seven opportunistic bacterial cultures (*Enterobacter aerogenes, Escherichia coli, Salmonella enteritidis, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilus*) as the test culture. The test cultures were cultivated on relevant elective medium [5]. pH of solution was measured by an electronic pH meter. The cells number of cultured microorganisms was determined by counting grown colonies after plating on agar medium. Antimicrobial activity of the Kombucha culture liquid was determined by its diffusion in agar medium contaminated by respective test culture, followed by measuring zones of their growth

delay [6].

To study the effect of herbal additives on antibiotic activity of beverage we used aqueous extracts of medicinal herbs (sage *Salvia officinalis* L., common marigold *Calendula officinalis* L., thyme *Thymus vulgaris* L., peppermint *Mentha × piperita* L., oregano *Origanum vulgare* L., lemon balm *Melissa officinalis* L., St. John's wort *Hypericum perforatum* L.). The obtained data were processed by mathematical methods of variation statistics on a personal computer using the program «Excel» [7-8].

Results and Discussion

Results of studies of the antimicrobial properties of the Kombucha product with aqueous herbal extracts showed that, in both control and experimental cases the inhibition of the test cultures was observed (Figure 1). However, in the presence of some herbs this effect was greatly amplified. In the control variant the highest antibacterial activity of the Kombucha product was noted with respect to the *Enterobacter aerogenes* and *Staphylococcus aureus*.

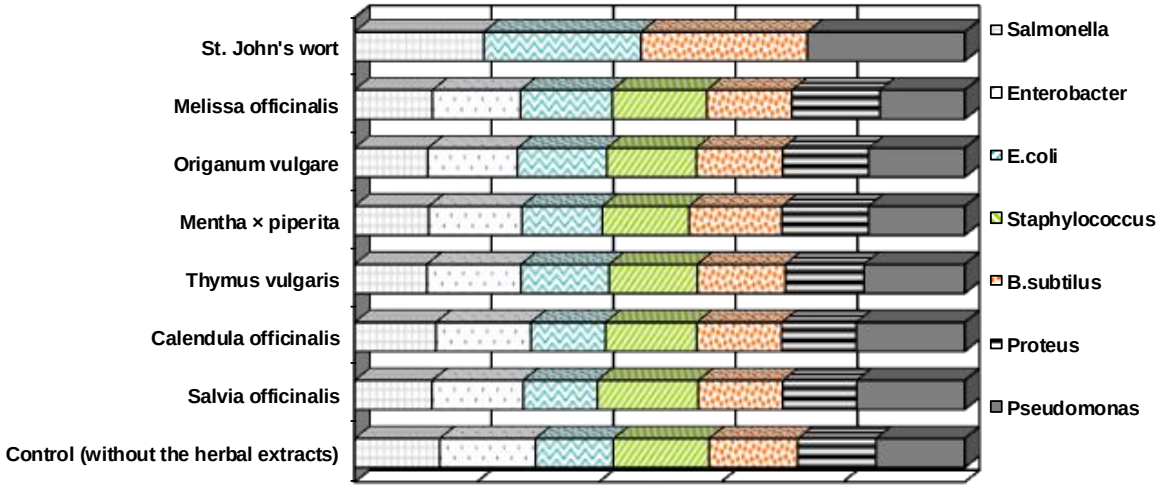

Figure 1: Variation of antimicrobial activity of 'kombucha tea" in the medicinal plants extracts presence (inhibition zone of the test cultures growth, in mm)

Adding the sage extract to the culture medium stimulates antagonism to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The presence of calendula extract caused a significant reduction in the *Pseudomonas aeruginosa* growth zone as compared to the control variant.

Thyme extract enhances the antimicrobial activity against *Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilus*.

The presence of the extract of mint and oregano in the culture medium increases antagonism to all test cultures, except *Salmonella enteritidis*. It is interesting to note that none of the added plant does not contribute to the antibacterial activity with respect to this culture compared with the control.

Adding the lemon balm extract in the culture medium significantly inhibits the growth of *Escherichia coli* and *Proteus mirabilis*.

The therapeutic effect of many medicinal plant species currently used in medical practice, but also in food industry due to the presence of various biologically active substances, which determine a particular physiological effect in the human organism. Physiologically active substances of plants have diverse composition and belong to different classes of chemical compounds. It is proved that the optimal effect on the human body provides the whole complex of these substances, and not the individual components [9].

The changes of antibacterial activity of the "tea fungus" association in our studies can be explained by its enrichment by complex of biologically active components in herbs. For example, the sage leaves contain complex substances such as essential oils, alkaloids, flavonoids, phytoncides, *Salvini* vegetable antibiotic that has antimicrobial and fungistatic properties. *Salvini* antibiotic delays reproduction of *Staphylococcus aureus*.

Calendula flowers contain essential oils, flavonoids, saponins and alkaloids. Calendula also has antimicrobial and anti-inflammatory activity, reduces inflammation of the gastrointestinal tract, of liver, of biliary tract and respiratory tract. These properties enhance the antibacterial activity of the kombucha beverage based on calendula flowers.

Thyme herb contains essential oils, flavonoids and tannins. It has antiseptic, antibacterial and anti-inflammatory action. This is probably also enhances the antibacterial activity of the kombucha beverage obtained with the addition of thyme extract.

Mint and oregano increase the activity of the drink with respect to all the studied test cultures due to the presence in them of flavonoids, essential oils, terpenoids, phenol carbonic acids and other biologically active components.

Melissa does not contain substances with expressed antimicrobial activity, but it has substances that may contribute to increased metabolism of the "tea fungus" association itself, which in turn promotes the synthesis of substances causing antibacterial activity. It's known that Kombucha from lemon balm tea exibited antimicrobial activity against prokaryotic microorganisms independently of their cell wall structure (both Gram-positive and Gram-negative bacteria), while there was no observed activity against eukaryots (yeasts and moulds) [10].

It is noted that the presence *Hypericum* extract in the culture medium, unlike other herbs significantly reduces antimicrobial activity against all taken to study the test cultures. The flowers of St. John's wort contains a sufficient number of substances with antimicrobial activity, which perhaps inhibits the development of "tea fungus", slows down its metabolism. It explains the decrease of antibacterial activity of Kombucha beverage based on the *Hypericum* extract.

Conclusion

Thus, all studied extracts of medicinal herbs, except of *Hypericum* or enhance antibacterial activity of a "tea fungus" drink, or leave it unchanged, depending on the test culture and the used plant species. An increase of antimicrobial activity of Kombucha drink under the influence of herbal extracts can be used in the future for developing new drinks variants with increased biological activity for use in the treatment and prevention of various diseases.

Acknowledgments

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Effects of $NaNO_3$ and KNO_3 in kosaric fertilizer on the growth of *Arthrospira platensis*

Norsalwani Muhamad Nor, Halisa Bajis, Hishamudin Omar and *Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor.

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

This study was carried out to determine whether the replacement of $NaNO_3$ to KNO_3 in Kosaric media can give better growth to *Arthrospira platensis*. The growth of *A. platensis* in different culture media was which are Kosaric media and Kosaric media with the modifications of $NaN0_3$ to KNO_3 at concentration of 0.75, 1.25, and 1.75g/L in indoor condition. The pH value for Kosaric media shows the highest reading which is 9.63, followed by both modified Kosaric media with the addition of 1.25g/L KNO_3 and 1.75g/L KNO_3 which have the same reading of 9.58, and 9.56 for modified Kosaric media with the addition of 0.75g/L KNO_3. Cell dry weight is 2.13mg/L for modified Kosaric media, I.78mg/L for modified Kosaric media with the addition of l .25g/L KNO_3, 1.61 mg/L. for modified Kosaric media with the addition of 0.75g/L KNO_3 and 1.18mg/L for Kosaric media. While for chlorophyll a content, Kosaric media shows the highest value which is 2.79mg/L, followed by modified Kosaric media with the addition of 1.75b/L KNO_3 (1.65mg/L), modified Kosaric media with the addition of 1.25g/L KNO_3 (1.29mg/L), and modified Kosaric media with the addition of 0.75g/L KNO_3 (1.09mg/L). Kosaric media is still the best culture media for culturing of *A. platensis*. Except for cell dry weight, the modified Kosaric media with the addition of 1.75g/L KNO_3 shows the highest reading. The alternative commercial fertilizer that are cheaper than Kosaric media are hoped to replace Kosaric media in culturing *A. platensis*.

Keywords: Kosaric medium, *Arthrospira platensis*, $NaNO_3$, KNO_3.

Enhancing barn owl population in rice fields through installation of tree nest boxes

*Badrulhadza Amzah[1], Mohd Khusairy Khadzir[2], Mohd Fitri Masarudin[3] and Hafizi Yahya[3]

[1]*Strategic Planning & Innovation Management Division, MARDI Headquarters, 43400, Serdang*
[2]*Mechanization & Automation Research Center, MARDI Seberang Perai, 13200, Kepala Batas*
[3]*Rice & Industrial Crop Research Center, MARDI Seberang Perai, 13200, Kepala Batas*
**Corresponding author.Tel.:+603 89437678; email address: bhadza@mardi.gov.my*

Introduction

Rice field rat, *Rattus argentiventer* Robinson & Kloss is one of the major pest of rice. One of the control measure for this pest is by using barn owl or *Tyto alba* Scopoli. Barn owl is an effective biological control agent for rice field rat. Population of barn owl can be enhanced by erecting artificial nest boxes in rice field areas as places for the birds to take shelter and to breed (Badrulhadza, 2012). At present, most nest boxes found in rice fields were made from fiber glass and erected on iron poles. However, the use of iron poles is costly and can easily be stolen due to their high value. In MADA for example, many of these pole nest boxes were damaged and out of order because of these incidence. Current status of barn owl 'iron pole nest boxes' in MADA areas is shown in Figure 1. Depleting of this type of nest box will eventually lead to reduction of barn owl population in rice fields. To overcome this situation, installation of artificial nest boxes made from cheaper materials on trees seems to be ideal and practical. The use of plywood is also much cheaper than fiber glass.

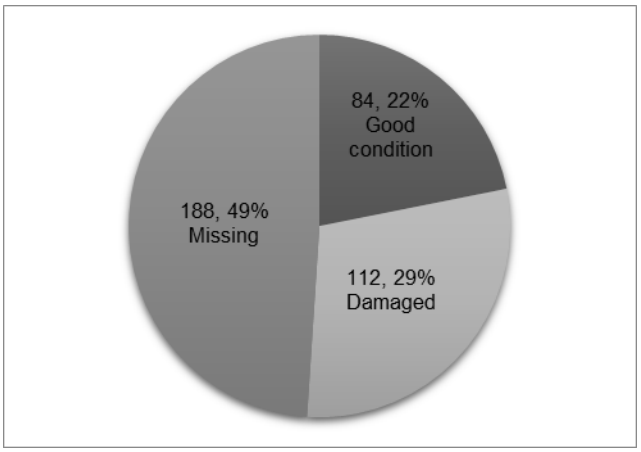

Figure 1: Current status of barn owl 'iron pole nest boxes' in MADA areas (Update April 2013) (Badrulhadza and Khusairy, 2013)

Materials and Methods

This study was carried out in MARDI Seberang Perai Research Station. The study consisted of two parts which were data recording before installation (Main season 2012/2013) and after installation (Off season 2013) of tree nest boxes. Rice damage percentage and rat population status in MARDI Seberang Perai were recorded in both seasons. Rice damage percentage assessment was done during ripening stage by randomly selecting several rice plots within the station. A trap barrier system (TBS) also was set up to monitor the rat population. Number of rat caught using TBS was used as indicator of their population in the field.

In the meantime, twenty barn owl nest boxes were made from plywood (Dimension: 67.5 cm length x 42.5 cm height x 37.5 cm width) (Figure 2) and painted with white paint to increase the shelf life.

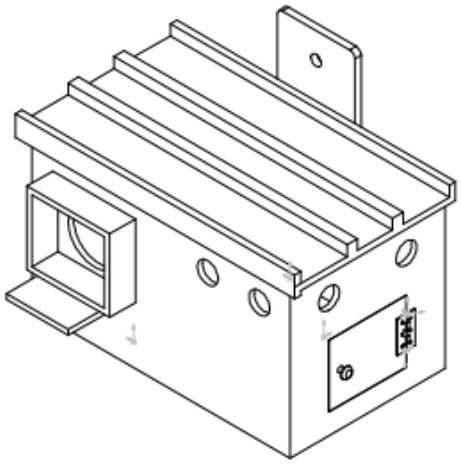

Figure 2: The design of the tree nest box

These boxes then were attached to the randomly selected coconut trees within the rice field area in MARDI Seberang Perai at 15-20 feet height during Off season 2013. It was expected that the barn owls will occupy the nest boxes naturally. These boxes will provide shelter and breeding ground for barn owls so that their population can increase greatly as these birds cannot make their own nest. Weekly observations were done to monitor the boxes for any sign of barn owl's activities (Number of adults, eggs, owlets, dead rats, rat pellets, rat skulls, owl's feathers).

Results and Discussion

Table 1 shows that average crop damage by rat attack was 25.28% and 19 individual rats were caught through trap barrier system (TBS) in one-month period before the installment of the tree nest boxes (Main season 2012/2013). Table 1 also shows that after installment of 20 unit boxes (Off season 2013), the average crop damage was reduce to 5.94% and only 4 individual rats were caught by TBS in three-month period of sampling.

Table 1: Crop damage percentage & rat population status before & after installation of barn owl 'tree nest boxes' in MARDI Seberang Perai

	Before	**After**
Average crop damage attack by rats	25.28%	5.94%
Number of rat caught in trap barrier system	19	4

This proved that the barn owl population in MARDI Seberang Perai was increased successfully. Several adult owls were observed occupying and actively roosting in the nest boxes. Predation activity also can be sensed by observing the undigested bones and fur of the rats in pellet forms found inside the nest box.

The cost per unit of this plywood nest box is far cheaper than wooden and fiber glass type. Materials and labor cost per unit for plywood nest box is about RM161.40 while fiber glass box is RM497 (without iron pole cost) and wooden board box is RM691 (without iron pole cost). Installment of tree nest boxes without the need of using iron poles also greatly reduce the cost. This environmental friendly 'automated rice field rat control' technology can easily be made by the farmers themselves and can simply be applied in their fields.

Conclusion

Tree nest boxes are still new in Malaysia and have never been implemented in our rice field areas. It has high potential usage as this tree nest box is a low cost technology, environmental friendly and emphasizes on the role of natural pest control. Installation of high number of tree nest boxes in rice fields will increase population of barn owls naturally. This will eventually reduce the rice field rat attack on the crop and at the same time increase the yield.

Acknowledgements

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Fatty acid profile of *Chlorella vulgaris* commercial cultured in different concentration of fertilizer and salinity

Norhafizah Osman, Mah Szu Han, Mohammad Amal Noor Azmai, Hishamudin Omar and *Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Chlorella vulgaris could be consumed as a complete supplementary food in the diet. *Chlorella* sp. is high in fatty content, especially Essential Fatty Acid (EFA). *Chlorella vulgaris* was cultured in different culture media, and then FAME were extracted from the sample following Ostrowski and Divakaran method to the study the fatty acid profile. The result showed the polyunsaturated fatty acid reached to the highest percentage on the seventh day of the cultivation. The cell growth as well as the dry weight reached the peak dung the same day. By looking at the PUFA content in different medium, the modified Bold Basal medium has the highest PUFA content among all. The result also showed that the raw of omega-3: omega-6 is about 2:10. This showed that *Chlorella vulgaris* could be taken as a balance food.

Keywords: *Chlorella vulgaris*, Essential Fatty Acid (EFA), bold basal medium, omega-3, omega-6

Fusarium species isolated from post-harvest fruit rot of tomato

*Nur Ain Izzati Mohd Zainudin and Nur Baiti Abd Murad

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

**Corresponding author.Tel.:+603 89466642; email address: ainizzati@upm.edu.my*

Introduction

Post-harvest fruit rot of tomato is one of the most important diseases, caused by filamentous fungi including *Fusarium* species. The harvested tomatoes are very sensitive and liable for contaminations of *Fusarium* species due to its juicy epicarp, which facilitate the fungal hyphae to penetrate severely into the fruit (Abu Bakar *et al.,* 2013). Infection may occur at any time during postharvest handling, from harvest to consumption. The chronology of the infection mechanism begin when fungal hypae is penetrating the tissue beneath the fruit surface, then, causing internal black rot and the process is continuous until the fruit collapses and the fungus will be able to infect the nearby fruits (Bartz *et al.,* 2013).

Recently, Abu Bakar *et al.* (2013) reported, in Malaysia, many *Fusarium* species were isolated from various vegetables and fruits but were not well documented with comprehensive information on its diversity on tomato. They found five *Fusarium* species - were identified morphologically as the causal agents of fruit rot of tomato in Malaysia, which is *F. semitectum, F. oxysporum, F. equiseti, F. subglutinans* and *F. solani*.

Realizing the information of *Fusarium* species associated with fruit rot of tomato is limited in tropical area, including Malaysia, therefore in this study, we identify and characterize some *Fusarium* strains based on translation elongation factor 1-α (*tef1-α*) gene sequence.

Materials and Methods

Thirty-four strains of *Fusarium* species were isolated from infected samples of tomato obtained in Selangor, Malaysia used for *tef1-α* gene (partial) sequencing. DNA extraction was done using the Ultra Clean® microbial DNA isolation kit (MO-BIO, Carlsbad, CA, USA) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) amplification was performed in a TProfessional Standard Thermocycler (Biometra Company) using PCR master mix (Promega, Madison, WI, USA) following the manufacturer's recommendations. The *tef1-α* gene was amplified using primer pair of EF1 (5'-ATGGGTAAGGAGGACAAGAC-3') and EF2 (5'-GGAAGTACCAGTGATCATGTT-3') (Geiser *et al.,* 2004).

The digested DNA was electrophoresed on 1% agarose gels. The gels were stained with ethidium bromide and the DNA fragments were visualized under ultraviolet (UV) light. The images were photographed using DOC PRINT system (Vilber Lourmat, USA). The DNA fragment with size 700bp was purified for *tef1-α* sequence analysis (Nitschke *et al.,* 2009). Target DNA fragment was excised from

the gel and purified using QIAquick® gel extraction kit (QIAGEN, USA) following the manufacturer's recommendation.

The purified PCR products were sent for DNA sequencing. The *tef1-α* gene region was sequenced using ABI3730XL sequencer (MyTACG Bioscience Company, MY). The *tef1-α* gene sequences of the strains were compared to the sequences in GenBank by using the Standard Nucleotide BLAST network services for similarities present in National Centre for Biotechnology Information (NCBI) database accessed through MEGA software analysis version 6.06 (Tamura *et al.,* 2013). The species of the *Fusarium* strains were recorded.

Results and Discussion

All 34 strains used in *tef1-α* gene sequencing showed a clear single DNA fragment was amplified corresponding to the expected molecular fragment size of the *tef1-α* region, 700 bp. These PCR amplification results were predicting that all the tested samples were belonged to the *Fusarium* species. The identification results of all strains as determined by partial DNA sequence of their *tef1-α* region showed the strains were identified as *F. solani* (17 strains), *F. oxysporum* (7 strains) including *F. oxysporum* f. sp. *lycopersici* (6 strains), *F. verticillioides* (2 strains) and a single strain of *F. proliferatum* and *F. equiseti* respectively (Table 1).

Table 1: *Fusarium* strains isolated from post-harvest fruit rot of tomato in Selangor characterized based on *tef1-α* gene sequence.

Species	Strain no.	Location	Closest species match and GeneBank accession number	Similarity (%)
F. solani	B616T, B627T, B664T, B1350T	Serdang	*F. solani* strain FRC S1746 (DQ247263)	99
	B780T	Sungai Buloh		
	B684T, B698T, B702T, B1356T	Kajang		
	B777T	Sungai Buloh		
	B752T	Bandar Sunway		
	B722T	Semenyih	*F. solani* strain NRRL 52798 (JF740866)	99
	B1351T, B1352T, B1353T, B1355T	Ampang	*F. solani* strain FRC S623 (DQ247324)	99
	B1354T	Kajang	*F. solani* strain NRRL 52699 (JF740782.1)	99
F. oxysporum	B635T, B622T, B695T, B688T, B696T	Kajang	*F. oxysporum* NRRL 52691 (JF740776.1)	99
	B725T	Puchong	*F. oxysporum* strain 11-227 (KF728241.1)	98
	B1358T	Selayang	*F. oxysporum* strain NRRL 25099 (JF740726.1)	99
F. oxysporum f. sp. *lycopersici*	B633T, B645T	Serdang	*F. oxysporum* f. sp. *lycopersici* strain FOLR2 (DQ837692.1)	99
	B711T, B713T	Kajang		
	B717T, B718T	Semenyih		
F. equiseti	B1349T	Serdang	*F. equiseti* strain Q12002 (KF208617)	99
F. proliferatum	B1357T	Serdang	*F. proliferatum* strain NRRL 43545 (EF452971)	99
F. verticillioides	B1359T, B1360T	Selayang	*F. verticillioides* strain NRRL 25102 (JF740729)	99

The *tef1-α* sequence of all *Fusarium* strains were blasted with standard nucleotide sequence database of GeneBank showed that the similarity percentages of the strains ranged at 98 to 99%.

In this study, six strains were identified as *F. oxysporum* f. sp. *lycopersici*. Based on morphological characteristics, the *Fusarium* strains cannot identified into forma speciales level, however, by sequencing the *tef1-α* gene, some of the strains can be classify into forma speciales. *tef1-α* gene is translates an important part of the protein translation machinery, where it is a good single locus identification tool in *Fusarium* and shows high sequence polymorphism among closely related species compared to the other intron-rich portions of protein coding genes. Moreover, non-orthologous copies of the gene have not been detected in the genus (Nitschke *et al.,* 2009).

Conclusion

Five *Fusarium* species were discovered from post-harvest fruit rot of tomato samples, namely, *F. oxysporum* (including *F. oxysporum* f. sp. *lycopersici*), *F. solani, F. equiseti, F. proliferatum* and *F. verticillioides* based on *tef1-α* gene sequence analysis.

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Genetic relations by banding patterns and the highest efficiency sequences combination of *rpo*B and *psb*A-*trn*H spacer for barcoding Solanaceae

*Arunrat Chaveerach[1,2], Tawatchai Tanee[2,3], Arisa Sanubol[1,2], Nantiya Kwanda[2], Kittiya S. Suwannakud[2,4], Lamyai Neeratanaphan[2,5] and Runglawan Sudmoon[1,2]

[1]*Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Genetics and Environmental Toxicology (GET) Research Group, Khon Kaen University, Khon Kaen 40002, Thailand*
[3]*Faculty of Environment and Resource Studies, Mahasarakham University, Mahasarakham 44150, Thailand*
[4]*Faculty of Public Health, Chalermkarnchana College, Sisaket 33000, Thailand*
[5]*Department of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
**Corresponding author.Tel.:+66 82 3095690; email address: raccha@kku.ac.th*

Introduction

Solanaceae is a family of important species, which has been used in Thailand and worldwide for edible, medicinal, and ornamental plants. The most important species of the family for foods is *S. tuberosum* which is recorded as the fourth most important crop in the world, after wheat, rice, and maize (Somani, 2009; Grover *et al*., 2012). Many species, especially the *Solanum* and *Capsicum* species, are used as vegetables and food flavoring. Other examples of useful species are: *Nicotiana tabacum* which is important for cigarette production; *Brunfelsia*, *Cestrum*, and *Petunia* which are commonly used as ornamentation. These wide uses have led to the cultivation leading to genetic variations in the species and in the genus. Therefore, genetic relations of the economic family Solanaceae should be evaluated using DNA fingerprinting and some partial specific regions called barcode.

Materials and Methods

Plants of family Solanaceae were explored and collected in many provinces throughout Thailand. Two individuals of each species were undergone DNA extraction, DNA fingerprinting using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers, and DNA barcoding using *rpo*B gene, *rpo*C gene and *psb*A-*trn*H spacer region (http://www.kew.org/barcoding/update.html; 28 January 2009). The fingerprints were documented as diallelic characters (present = 1, absent = 0) and used to construct a dendrogram using the NTSYSpc 2.1 software (Rohlf 2000). DNA barcoding sequences were aligned to get genetic diversity.

Results and Discussion

The constructed dendrogram (Figure 1) shows polyphyletic groups of the genera *Capsicum* and *Solanum* accordingly to their high number of species. With a high number of characters which widely distributed in the genomes, the polyphyly interpretation is possible, though these do not follow the evolutionary concept that a same group, species, genus, family, or others, must be monophyly (Simpson 2006).

The genetic similarity (S) of intraspecific are 0.85-0.92 (for *Capsicum* species), interspecific species are 0.75-0.94, and intergeneric species are 0.70 to 0.86. According to Weier *et al*. (1982), the S of 0.85-1.00 can be recognized as a part of the same species, while a criterion of 0.65 usually defines the genus level. However, the ultimate interpretation is dependent upon the researchers' knowledge of the species examined. Therefore, we suggest that they are the same species, same genera as earlier classified because their morphological characteristic variations are not enough for new species/genera designation. In this matter, the polyphyly and low genetic similarity within a group can be caused from most of the species have been economic and cultivated plants leading to have high variation with crop production and hybridization. The unchanged morphological characteristics may be related to changing rate of genes to phenotypes.

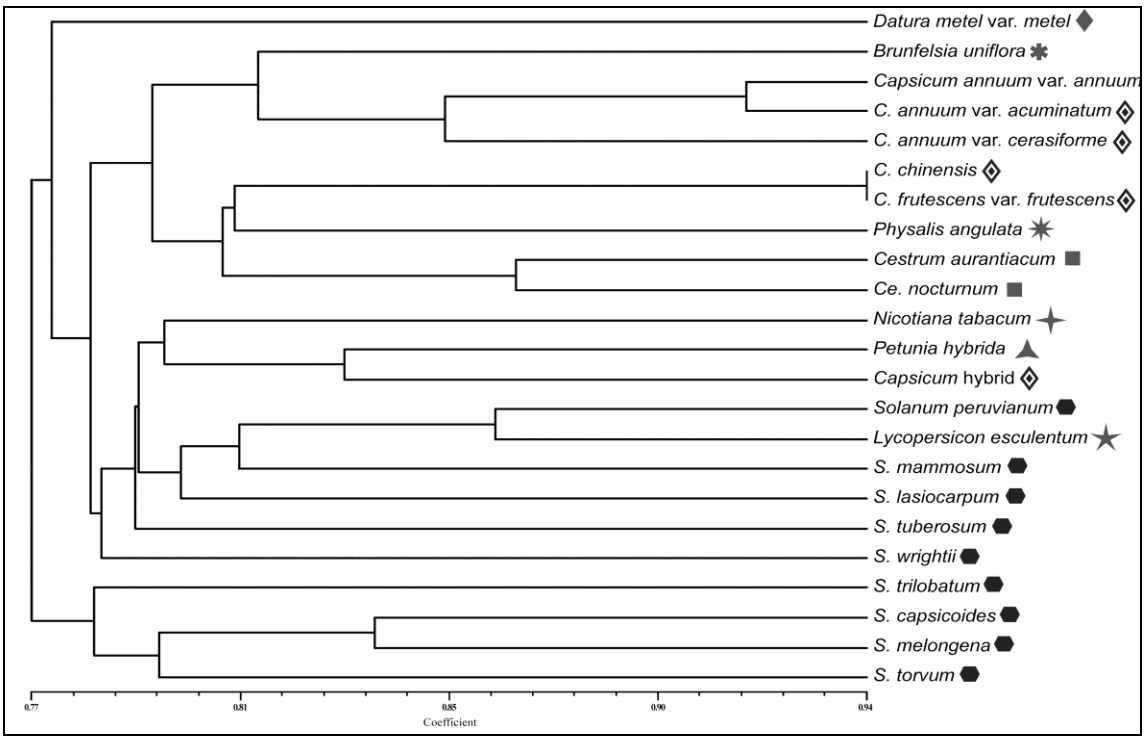

Figure 1: Dendrogram constructed from combination of RAPD and ISSR bands showing polyphyletic groups of the genera *Solanaceae* (⬣) and *Capsicum* (⟐).

The high efficient barcoding regions *rbc*L, *rpo*B, and *psb*A-*trn*H spacer (CBOL Plant Working Group, 2009; Hollingsworth, 2011; Kwanda *et al.,* 2013) were tested for Solanaceae species. The sequence analyses indicated the genetic distance (D) values of intraspecific and interspecific species. The values are mostly efficient enough to use a single region except for some species/genera pairs which revealed no sequence divergence. There is more than one region preferable for barcoding of this plant group. Moreover, when sequence combinations were tested, the highest genetic distances are 0.299 to 0.765 from the combinations of *rpo*B + *psb*A-*trn*H spacer. However, the genetic distances of intraspecific species, *Capsicum* varieties 0.688-0.697 and interspecific species, *Capsicum* species 0.383-0.717 are not in accordance with the concept of barcoding that low variation within a species, whereas high variation among species. These may be caused from they are

cultivated species and, additionally, are polyphyletic group. GenBank accession numbers for these barcoding sequences are JX856284-325, JX856337-46, KC551923-25.

Conclusion

The polyphyly and low genetic similarity within the Solanaceae species, as shown by the RAPD and ISSR markers can be caused from most of the species have been economic and cultivated plants leading to have high variation with crop production and hybridization. The unchanged morphological characteristics may be related to changing rate of genes to phenotypes. With the Solanaceae plant model, the authors propose the combination of the two regions, *rpo*B + *psb*A-*trn*H spacer, and a single region, the *psb*A-*trn*H spacer, to be used for barcoding in the other flowering plant groups.

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Gonad development of hard clam (*Meretri xlyrata*)

*Hadi Hamli, Mohd Hanafi Idris, Abu Hena Mustafa Kamal and Amy Halimah Rajaee

Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, P. O. Box 396, 97008 Bintulu, Sarawak, Malaysia

**Corresponding author.Tel.: +6086 8555373/5382; email address: hadihamli@gmail.com*

Hard clam are mollusc belongs to bivalvia class that dominating the benthic area from deep sea to lowland area particularly tropical area. This invertebrate is suspension feeder that feeds upon plankton, detritus and dissolved organic matter as the source of metabolic energy to keep it grow. Those foods source are abundance at the open sea which make hard clam proper for mariculture practice. Hard clam production through mariculture gradually increase each year even still behind oyster and cockle production. Knowledge regarding gonad stage development is important for hard clam culture in order to determine proper condition for spawning. Development of bivalve gonad is categorized into resting, early development, late development, mature, spawning and spawned. Gonad development and gender of *Meretrix lyrata* can be identified accurately through histological study.

Keywords: *Meretrix lyrata,* gametogenesis, histological

How to rapidly accelerate biodiversity inventory in places where most of the species are unknown?

*Kong-Wah Sing[1,2], Khairunnisa Syaripuddin[1,2] and John-James Wilson [1,2]

[1]*Museum of Zoology, Institute of Biological Sciences, Faculty of Science, University of Malaya 50603 Kuala Lumpur*
[2]*Institute of Biological Sciences, Faculty of Science, University of Malaya 50603 Kuala Lumpur*
**Corresponding author.Tel.:+603 79674112; email address: singkongwah@um.edu.my*

Introduction

Biodiversity inventory is the first step for most ecological research, providing data points for monitoring changes in biodiversity over time and space. For example, conservation planning usually requires knowledge of where certain species are found or of locations high in biodiversity (e.g. Mohamed and Zakaria Ismail, 2005). Yet, for all but the most well-known groups (e.g. butterflies, birds) the taxonomic investment required for inventories can be prohibitive to their successful and timely completion (Smith *et al.*, 2005). The difficulty in recording species identities accurately and efficiently is magnified by the fact that most species have never been recorded before (are undescribed), particularly in regions of the world where biodiversity inventory might be most important - tropical sites high in biodiversity, but at risk from rapid biodiversity loss.

DNA barcodes are a short, standardized region of DNA that are unique to a species, and could provide a solution to the taxonomic impediment to biodiversity inventory (Floyd *et al.*, 2009). DNA barcodes can be generated from freshly collected specimens quickly and cheaply in a basic molecular laboratory. A bioblitz is a short, intense period of specimen sampling in an attempt to record all the living species (or all the species of a large group e.g. arthropods, insects) within a designated area (Laforest *et al.*, 2013). DNA barcodes can be integrated into bioblitz in a two-step process. In step one, DNA barcodes are analysed to determine the number of species units sampled by the inventory, know as molecular operational taxonomic units (MOTU). In step two, the DNA barcodes are matched against a reference library of DNA barcodes of known species origin to assign a Linnaean name (species or higher taxon; Wilson *et al.*, 2011) to the MOTU. When it is not possible to assign a Linnaean species name to a MOTU, the MOTU can still be treated as a species for the purpose of biodiversity inventory. This may reflect a gap in the DNA barcode reference library for a described species, or may be the first record of a new species and therefore, constitutes the first step on the pathway to a formal species name (see Maddison *et al.*, 2012). We propose that bioblitz, coupled with DNA barcoding, can provide a rapid and accurate biodiversity inventory for a tropical site and tested the feasibility of this approach through a DNA barcoding bioblitz conducted on the campus of University Kebangsaan Malaysia on 10 December 2012.

Materials and Methods

The bioblitz was conducted at a 500m^2 site, close to the Genetics Laboratory between 12 noon and 12 midnight. We employed four different specimen collection methods – (a) Manual collection by three persons with sweep nets and forceps, during daylight hours; (b) Ten pitfall traps, set at 12 noon, collected at 12 midnight; (c) One malaise trap, set at 12 noon, collected at 12 midnight; (d) Manual collection with forceps from one light trap, during darkness hours. All arthropod specimens were rapidly sorted into individual 1.5ml microcentrifuge tubes.

On return to the lab a single leg was removed from each specimen and placed into a fresh tube. As this was a preliminary study we selected only a few specimens for DNA barcoding choosing specimens from amongst the different collection methods and choosing morphologically distinct specimens. DNA was extracted from the samples using Xytogen Animal Extraction Kit (Xytogen, Australia) following the manufacturer's guidelines. Next, the DNA barcode (COI-5' mtDNA; Floyd *et al.*, 2009) was PCR amplified. The reaction tube contained 12.5µl EconoTaq® PLUS GREEN PCR 2x Master Mix (Lucigen Corporation, USA), 11 µl ddH_2O, 0.25 µl Forward Primer (10 pmol/µl), 0.25 µl Reverse Primer (10 pmol/µl), and 0.5µl DNA template. Primers and thermocycling protocol (COI Fast) followed Wilson (2012) for a first (LepF1/LepR1) and second (MLepF1/LepR1) pass of PCR. The PCR products were visualized on an agarose gel. In the case of no bands, the PCR was repeated for that DNA template. The PCR products were sent for sequencing with the LepR1 primer by a local company. The DNA barcodes were received from the sequencing company through email, edited, aligned and uploaded to BOLD (http://www.boldsystems.org; Ratnasingham and Hebert, 2007; following Wilson, 2012). The DNA barcodes are available in BOLD public dataset: DS-UKMBB.

The DNA barcodes were downloaded from BOLD and sorted into MOTU using the Automated Barcode Gap Discovery (ABGD) web interface (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html; Puillandre *et al.*, 2012). This method has been showed to perform well with sympatric datasets, is fast and user-friendly and does not have any special computational requirements since it is implemented in a web interface (Paz and Crawford, 2012). The DNA barcodes were submitted to the BOLD identification engine "Full Database" and species assignments were made using a >99% sequence similarity threshold. When there was no match within 99%, higher taxon assignments were made following Wilson *et al.* (2011).

Results and Discussion

The whole process from collection to a completed inventory took three working days and cost approximately 7.25 USD (RM23.50) per DNA barcode obtained (Table 1). The ABGD analysis sorted the DNA barcodes into 62 MOTU. Of these 62 MOTU, 18 could be assigned a Linnaean species name, 21 could be assigned a genus name, 37 a family name and 58 an order name. The details of the assignments are available in an excel file attached to the BOLD dataset. We found that a small number of people can collect hundreds, if not thousands, of specimens over this time period using the four collection methods. While the work is intensive, it requires no specialized equipment. Instructions for home construction of malaise traps and

sweep nets can be found through online searches. These collection methods are easily standardised, scalable and repeatable across many sites. Likewise, the DNA barcoding lab methods we optimized for this study provide an economical and time efficient system for evaluating biodiversity (Table 1). More high-tech approaches have been developed (e.g. Zhou *et al.*, 2012), but these are currently beyond the reach of most agencies conducting biodiversity inventory in Malaysia. Our approach however requires only the most basic molecular laboratory equipment. Of the MOTU lacking a Linnaean species name we see this as their first step towards a formal name. They are currently "dark taxa" except they are not completely dark, being searchable (through BOLD) and interoperable between future biodiversity inventories (Maddison *et al.*, 2012).

Table 1: Approximate cost and time taken for completion of DNA barcoding bioblitz.

Step	**Equipment**	**Consumables**	**Approximate cost of consumables per specimen (USD)**	**Time taken (h)**
Specimen collection	Sweep nets, forceps, plastic cups, small shovel, malaise trap	1.5ml microcentrifuge tubes	0.03	12
Specimen sorting	Forceps	96-well plate	0.03	2
DNA extraction	Pipette, PCR machine	Xytogen kit, Pipette tips	0.71	0.5
PCR	Pipette, PCR machine	PCR Master mix, Primers, ddH_20, 0.2ml PCR tube, Pipette tips	0.31 (+0.31)	5 (+5)
PCR check	Gel electrophoresis rig, transilluminator	Agarose, TAE buffer, DNA ladder (0.5µl), GelRed DNA stain	0.31 (+0.31)	1.5 (+1.5)
DNA sequencing			5.86	48
Sequence editing, alignment, upload	Personal Computer (internet connection)			1
Analysis of DNA barcodes	Personal Computer (internet connection)			1
TOTAL			**7.25 (+0.62)**	**77.5**

Conclusion

Biodiversity inventories are vital for monitoring changes in biodiversity over time and space, essential for conservation planning. Yet, most species have never been recorded before, especially in tropical regions which are high in biodiversity but at risk from rapid biodiversity loss. Bioblitz coupled with DNA barcoding that required

only basic molecular laboratory equipment, can provide a cost effective, rapid and accurate biodiversity inventory for a tropical site. For MOTU lacking a Linnaean species name we see this as their first step towards a formal name. They are currently “dark taxa”, except they are not completely dark, being searchable through the Barcode of Life Datasystems and interoperable between future biodiversity inventories.

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Identification and quantification of marine siderophores using high-performance liquid chromatography with electrospray ionization mass spectrometry

Khairul N. Mohamed[1] and Martha Gledhill[2]

[1]*Universiti Putra Malaysia, Faculty of Environmental Studies, Department of Environmental Science, 43400 Serdang, Selangor, Malaysia*
[2]*University of Southampton, School of Ocean and Earth Science, National Oceanography Centre Southampton, Southampton SO14 3ZH, United Kingdom*
**Corresponding author. email address: k_nizam@upm.edu.my*

Siderophores are low-molecular-weight organic compounds (500 - 1500 Da) produced by heterotrophic bacteria and highly selective for Fe(III). These complexation of iron by siderophores has the potential to affect both the solubility and bioavailability of iron (III) in seawater. We have determined the ferrioxamine siderophores in the seawater samples from high latitude North Atlantic Ocean The identification and quantification of this siderophore type chelate was done by using recently developed high-performance liquid chromatography-mass spectrometry methods. Five different siderophore type chelates were detected and the compounds comprised of two groups; ferrioxamines and amphibactins group. In the dissolved phase, three types of hydroxamate siderophore have been identified; Ferioxamine B (FOB), Ferrioxamine G (FOG) and ferrioxamine E (FOE)**.** Concentration of dissolved FOB, FOG and FOE are extremely low between 0–135 x 10^{-18} M. Our present data indicated the presence of low concentrations of dissolved siderophores in the high latitude of the Atlantic Ocean and suggest that siderophore distributions are both spatially and temporally variable.

Molecular data supports morphological-based species identification: A case study in gaharu producing tree, *Aquilaria*

Lee Shiou Yih, Faridah Hanum Ibrahim, Mohd Nazre Saleh, *Rozi Mohamed

Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia
43400 UPM Serdang, Selangor, Malaysia
**Corresponding author.Tel.:+603 89467183; email address: rozimohd@upm.edu.my*

Introduction

Aquilaria (Thymelaeaceae), endemic to the Indomalesia region, is an endangered gaharu-producing tropical tree. Due to the high price of gaharu, *Aquilaria* attracts a great number of local planters, who cultivate them in large scales. Demands for seedlings drive planters to bring in foreign species to diversify their planting materials. Traditionally, identification of *Aquilaria* species and related genera relies on the morphological characteristics. However, without their reproductive parts, it is quite easy to misidentify them. As descriptions of *Aquilaria* saplings are still lacking, we opt to conduct sapling identification based on their vegetative characteristics. To provide supporting evidence to the morphological-based identification, we analyzed the *trn*L-*trn*F intergenic spacer in the chloroplast genome from the species understudy. This region is regarded as a DNA barcode and is often used in conducting phylogenetic study from the intra-species to the inter-family levels (Tsai *et al.*, 2006). We found that the *trn*L-*trn*F intergenic spacer is useful for identification of several *Aquilaria* species and it complements findings based on morphological observations.

Materials and Methods

One-year-old local *Aquilaria* species planted in polybags and grown in the shade house were used in this study. The species and its origin were: 1) *A. malaccensis* from Lentang Seed Centre, Karak, Pahang, 2) *A. hirta* from Ladang Merchang, Terengganu, and 3) *Aquilaria* sp. 1 from Semenggoh Forest, Sarawak. Voucher specimens are kept at the Faculty of Forestry's herbarium.

Fresh leaves were pulverized in liquid nitrogen and total genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen, Germany). The *trn*L-*trn*F region was amplified using the forward and reverse primers, 5'GGTTCAAGTCCCTC TATCCC3' (Taberlet *et al*., 1991) and 5'TCTGCTCTACCAGCTGTGCT3' (Eurlings and Gravendeel, 2005), respectively. PCR was conducted using the 2X Mytaq RedMix (Bioline, UK), primers at 0.5 mM each, and 50 ng DNA templates. PCR program was: 1min at 94°C; 35 cycles for 30 sec at 94°C, 40 sec at 55°C and 40 sec at 72°C, and a final 5 min extension at 72°C. PCR products were gel electrophoresed, purified and sequenced (1st Base Laboratories, Singapore).

Sequences were aligned using ClustalW. Pair-wise nucleotide sequence divergences were calculated using the Kimura 2-parameter (K2P) model (Kimura, 1980), and the neighbor-joining (NJ) analysis using MEGA 6 with a total of 1000 replicates (Tamura *et al.*, 2013).

Results and Discussion

Observations revealed that *A. hirta* sapling can be recognized through the densely hairy twigs, young shoots and axillary buds, undersurface of leaf, margin, petiole and midrib besides the strongly raised midrib, and usually unbranched habit. *A. malaccensis*, through the many branches and white spots along main axis, entire leaf margin, slightly hairy leaf undersurface and midrib, and glabrous petiole, and *Aquilaria* sp.1 from the lower branches that are nearly perpendicular to the stem and wavy leaf margin.

The length of the *trn*L-*trn*F region for eleven different *Aquilaria* species varied from 356-451 bp with 20 variable sites. Nucleotide composition is strong A+T bias (average 66.4% for all codon) where a percentage of T (41.6%) is higher than A (24.8%). Based on K2P (Table 1), the interspecific distances between *A. malaccensis*, *A. beccarania* and *Aquilaria* sp.1 are zero. Although they display close genetic distance, we find that it is possible to differentiate the three species based on their vegetative characteristics as reported above. The maximum interspecific distance within *Aquilaria* species (0.0199) was found in *A. yunnanensis*, displaying a further genetic distance with other *Aquilaria* species in this study. Despite being easily confused with *A. sinensis* and *A. malaccensis* from their vegetative description (Flora of China Editorial Committee, 1999), differences in genetic distance makes it possible to tell *A. yunnanensis* apart from other *Aquilaria* species.

Table 1: K2P intraspecific distance (bold on diagonal) and interspecific distance (below diagonal) for *trn*L-*trn*F sequence of selected *Aquilaria* species.

	1	2	3	4	5	6	7	8	9	10	11
1	**0.0000**										
2	0.0028	**0.0000**									
3	0.0000	0.0028	**0.0000**								
4	0.0000	0.0028	0.0000	**0.0000**							
5	0.0085	0.0056	0.0085	0.0085	**n/c**						
6	0.0056	0.0028	0.0056	0.0056	0.0085	**n/c**					
7	0.0085	0.0056	0.0085	0.0085	0.0113	0.0085	**n/c**				
8	0.0085	0.0056	0.0085	0.0085	0.0000	0.0085	0.0113	**n/c**			
9	0.0114	0.0085	0.0114	0.0114	0.0113	0.0114	0.0142	0.0113	**0.0113**		
10	0.0085	0.0056	0.0085	0.0085	0.0000	0.0085	0.0113	0.0000	0.0113	**n/c**	
11	0.0170	0.0142	0.0170	0.0170	0.0199	0.0170	0.0199	0.0199	0.0171	0.0199	**n/c**

Note: Aquilaria species represent in the table, 1 *A. malaccensis*, 2 *A. hirta*, 3 *Aquilaria* sp. 1, 4 *A. beccarania*, 5 *A. citricarpa*, 6 *A. crassna*, 7. *A. khasiana*, 8 *A. parvifolia*, 9 *A. sinensis*, 10 *A. urdantensis*, 11. *A. yunnanensis*

The phylogenetic tree (Figure 1) revealed that *Aquilaria* and *Gyrinops* are closely related with support level between 42 – 65%, and are grouped together apart from *Gonystylus*. This explains why *Gyrinops* is often confused with *Aquilaria* based on morphological characteristics. However, using sequence analysis, it is clear that *Aquilaria* species are in different clusters than *Gyrinops*.

Conclusion

In this study, three local *Aquilaria* saplings were described in the absence of reproductive parts. We also analyzed their relationship with related genera found in the GenBank at DNA level. We demonstrated that sequence analysis can be a suitable approach to support species identification up to species level in *Aquilaria*, hence providing solution to growers to validate their planting materials. However,

additional regions should be sequenced and tested before a more conclusive identification scheme could be in place.

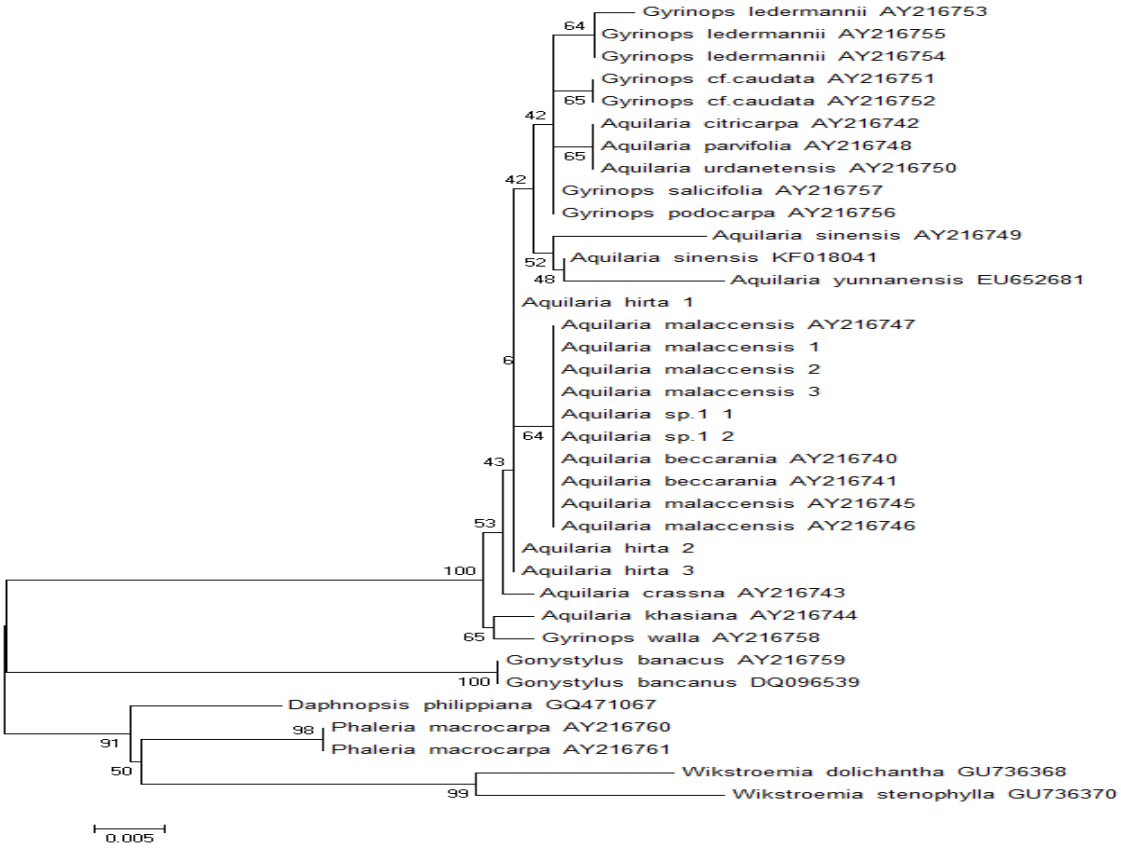

Figure 1: Neighbor-joining analysis of *trn*L-*trn*F for *Aquilaria* and its related genera.

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Morphological characterization of *Setosphaeria rostrata* and *Cochliobolus* species isolated from rice in Selangor

Nor Azizah Kusai[1], Mohd Termizi Yusuf[2], Shahrizim Zulkifly[1], Azmi Abd Razak[3] and *Nur Ain Izzati Mohd Zainudin[1]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Department of Microbiology, Faculty of Biotechnology and Biomolecule Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[3]*Malaysian Agricultural Research and Development Institute, Seberang Perai Research Station, 13200 Kepala Batas, Pulau Pinang*
**Corresponding author.Tel.:+603 89466642; email address: ainizzati@upm.edu.my*

Introduction

Rice (*Oryza sativa)* found in all over continents plays an important role to human that serve as food. The most common diseases of rice are narrow leaf spots (*Cercospora oryzae*), brown spots (*Cochliobolus miyabeanus*), blast (*Pyricularia oryzae*) and leaf smut (*Enthyloma oryzae*) (Groth *et al.*, 1990; Ou, 1985).The typical symptoms of leaf spot of rice are varied from size and color of the spots(Chin & Muhd Amin, 1986). The sizes were range from minute spot up to large which caused necrotic lesion on leaves. All these diseased greatly give impact to the reduction of yield of rice.

Setosphaeria sp. and *Cochliobolus* sp. were classified in the order Pleosporales that cause disease associated with grasses (Manamgoda *et al.*, 2012). Several studies showed that *Setosphaeria* as potential biological control agent to control weed in rice field (Tosiah *et al.,* 2009; Tsukamoto *et. al.*, 1997). *S. rostrata* were reported associated with rice seed (Cardona, 2007), banana and human (Wu & Turgeon, 2013). In addition, *C. eragrostidis* and *C. geniculatus* have a large host range caused diseased of leaf spot and blights (Manamgoda *et al.,* 2011).

The objective of this study was to identify the fungi isolated from diseased-rice leaves samples based on their morphological characteristics.

Materials and Methods

Diseased-rice leaf samples showing leaf spots symptom were obtained from five locations in Selangor – Pasir Panjang, Sawah Sempadan, Sekinchan, Sungai Besar and Sungai Burung. All samples were cultured in complete media with xylose (CMX) added with streptomycin.

Single-spore isolation was carried out on water agar (WA) and transferred to new CMX to obtain the pure cultures. CMX and WA with rice straw (modification of (Mothlagh & Kavian, 2008) were used for species identification based on their morphological characteristics following Sivanesan (1986).

Results and Discussion

Thirty-nine isolates were obtained from diseased-rice leaves from all sampling areas. Based on the morphological characteristics, 36 isolates were identified as *S. rostrata*.

The remaining isolates were identified as *C. geniculatus* (2 isolates) and *C. eragrostidis* (1 isolate). All isolates belong to order Pleosporales(Berbee, 1996).

Based on observation, the pigmentation of *S. rostrata* were initially light brown, become dark with age and have sparse mycelium on CMX. *S. rostrata* have cylindrical, ellipsoidal with straight to slightly curve conidia with 5 to 15 pseudoseptate. The conidia has truncated end with basal end darker than other septa. The size of conidia was range between 155.7 - 249.4 µm x 9.5 – 17.7 µm.

For *Cochliobolus* species, *C. geniculatus* and *C. eragrostidis* have small conidia with usually 3-septate. Both *Cochliobolus* species have swollen intermediate cell.*C. eragrostidis* have dark brown pigmentation, cottony mycelium with concentric ring. Conidia of *C. eragrostidis* were usually ellipsoidal with middle septum are darker than other septate. Size of conidia measures was between 17.39 – 20.38 µm x 7.78 – 12.08 µm.This characteristic can be differentiate with *C. geniculatus* which have fusiform with geniculate shape of conidia with three to four septate. The size of conidia was measured around 20.56 – 26.20 µm x 7.60 - 10.50 µm. The pigmentation of *C. geniculatus* was dark black pigmentation with white to grey dense mycelium on CMX.

Conclusion

Three species of fungi were successfully isolated from diseased-rice leaves showing leaf spots symptoms were *S. rostrata*, *C. geniculatus* and *C. eragrostidis*. This is the new record of*S. rostrata* isolated from rice leaves.

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Novel biomass crops in Malaysia, potential and challenges, napier grass as an example

Nor Azira Ishaq, *Ibraheem Alshareef and Soh A. C.

Crops for the Future Research Centre (CFFRC), University of Nottingham Malaysia Campus, Jalan Broga, Selangor, Malaysia

**Corresponding author.Tel: +603 89248799; email address: Ibraheem.alshareef@cffresearch.org*

Introduction

Fossil fuel use is a major contributor to the global warming (NBC, 2007); hence with the interest in renewable and clean energy sources to reduce carbon dioxide and greenhouse gas emissions responsible for it. World energy demand is expected to grow by 50% by 2030 (NBC, 2007) and, renewable energy sources are expected to play a significant role in meeting this demand.

If bioenergy is expected to meet the future energy demand, it is essential to diversify the biomass sources and lead biomass crop diversification. Crop diversification is not only essential for the economic sustainability but also sustainability of the whole cropping system, from the ecological and environmental perspectives. Continuous monoculture cropping system has been attributed to lead to soil fertility reduction, spread of pest and disease and increase chemical pesticides and less efficient resource use.

Oil palm has been contributing significantly to the agricultural economy in Malaysia. In 2012, the industry generated RM 85 billion in export earnings representing 5.6% of Malaysia's total earning (Arshad *et al*., 2013). Most biomass in Malaysia is derived from agricultural wastes, over 85% of which comes from the monoculture of oil palm (Shuit *et al*., 2009). However, this overdependence on oil palm leads to a vulnerable agriculture economy and raises some issues about the negative impacts of monoculture on the environment.

Around the world, a number of candidate energy crops are being tested. Some of these crops e.g maize, sugarcane, and soybean have been well established in the bioenergy sector and some are still in the early stage of biomass research e.g. Napier grass. Because several studies have indicated the desirable properties of Napier in terms of the biomass production, this paper will explore the potential of Napier to complement oil palm in Malaysia.

Malaysia and oil palm plantation

The most important agricultural commodity crop in Malaysia is oil palm. There is currently 6.6 million hectares of land for agricultural use in Malaysia, of which 5 million hectares (75 percent of total) is used for oil palm plantations (Azam-ali *et al*., 2013). In Malaysia, oil palm is the most productive source for biomass. However, Plantations of oil palm support much fewer species than do forests and often also fewer than other tree crops. Habitat fragmentation and pollution, including greenhouse gas emissions are also attributed to be the negative impacts of oil palm plantation. At least 1.0 million ha of forest was estimated to have been replaced by oil palm between 1990 and 2005 (Koh and Wilcove, 2007).

Potential of Napier as a biomass crop

According to Zewdu (2005), different accessions of Napier grass yielded different contents of dry matter. Rengsirikul *et al*. (2013) studied a range of cultivars: Dwarf, Muaklek, Bana, Taiwan, A148, Common, Wruk wona, Tifton and Kampheng San for biofuel production in central Thailand. High biomass yields were recorded for taller cultivars compared to shorter cultivars. Tifton and Wruk wona produced 58 t/ha and 52 t/ha respectively indicating their good potential for ethanol production.

According to Tessema *et al*. (2010), the frequency of defoliation had a significant effect on the dry matter yield of this crop with reduced frequency increasing the dry matter yield. A long re-growth period with a tendency towards maturity would further enhance the dry matter yield suggesting that long harvest interval is needed for high herbage yield.

This crop had been comprehensively studied for cellulosic feedstock production (Sanderson and Adler, 2008). Forage crops have commonly been chosen as potential biofuel crops. This could be because farmers are already familiar with the harvesting, management and cultivation of the crops. Besides, the crops offer broad options (forage or biofuel or both) in terms of management and land utilisation (Sanderson and Adler, 2008).

Napier grass has also demonstrated its potential as a cellulosic biofuel crop in the southern United States and other parts of the world. Ohimain *et al*. (2014) found that the gross calorific value of the elephant grass bagasse ranged from 15.76–17.07MJ/kg, which is comparable to values recorded for other better known crop biomass.

Table1: Estimated land use and space in Malaysian oil palm plantations (adapted from Azam-Ali *et al*., 2013).

Current Use	**Area (ha)**
Land under oil palm	5,000,000
Land not suited to cultivation of oil palm	353,400
Space around oil palm before canopy closure	380,000
Space under oil palm canopy (10% of total)	500,000
Space within pylon corridors (5% of total)	250,000
Potential available space	1,130,000

Potential of Napier grass to complement oil palm

There a lot of space within and around the oil palm plantation can be used for crop planting including Napier grass. Azam-Ali *et al*. (2013) estimated the land use and space in Malaysian oil palm plantations (Table1). Napier grass easily spreads and colonises wastelands and grow along marshes and ditch banks, in forest clearings and margins. Napier grass can grow even on poorly drained clay soils through the gamut of soil types to excessively drained sandy oils. While light shade can ensure its survival, Napier grass cannot survive under a closed canopy tree.

Conclusion

This paper has raised the issue of Napier grass potential as a complementary to oil palm in Malaysia. Whilst we focused on Malaysia, the issue is relevant to oil palm

and Napier elsewhere. Research on Napier as one of the crops for the future focuses on improving the plants themselves, through plant breeding and selection, and the challenge for the future is how to take practical steps to prove all the findings introduced in this paper. Whilst this paper showed that there is a high potential for Napier to complement oil palm, introducing Napier as an additional crop to oil palm is a big challenge.

Napier grass is one of the potentially good crops for second generation biomass sources. All of the biotic and abiotic requirements for cultivation of Napier grass can be fulfilled by Malaysia's environment. However, based on research by Rengsirikul *et al.* (2010), it is derived that the best season to harvest the crop would be during the rainy season. Besides that, soil moisture to grow plant also plays an important role in yielding a high dry matter yield. Once adequate moisture obtained, the optimum temperature for harvesting this crop would be in the range between 35°C - 40°C. This crop would grow well in Malaysia because almost all of the factors can be fulfilled.

Another important factor would be optimising the genetic resources of the crop. By manipulating the vast genetic variety of the crop, a stronger and better accession of the crop could be generated that can produce a higher yield of dry matter.

The possibility of Napier grass planted in East Asia to be infected with diseases is low, because the vectors responsible in spreading the disease is rare in Malaysia.

Although Napier grass has vast potential of its biomass, planting Napier grass on a large scale might be challenging and harder to be easily carried out, as it require human expertise to introduce the best varieties to grow in Malaysia with optimum yield. There is also need for a policy and interests within the government sector to include Napier grass in their Biofuel Program.

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Small mammals distribution peculiarities within Kazakhstan Irtysh river biotopes

*Bibigul Zhumabekova, Zharkyn Kabdolov, Yerbol Issakaev, Nurlan Sarbassov and Gulzhan Orazbayeva

Pavlodar State Pedagogical Institute, Pavlodar, Kazakhstan
**Corresponding author.Tel: +77012252144; email address: bibigul_kz@bk.ru*

Introduction

The Irtysh River is a river in Siberia and Kazakhstan and is the chief tributary of the Ob River. The Ob-Irtysh system forms a major drainage basin in Asia, encompassing most of Western Siberia and the Altai Mountains. The territory of the Kazakhstan part of an Irtysh river basin is almost the center of the Eurasian continent, than is caused the originality of its ecological conditions and existence of various ecosystems. The small rodents and insectivorous are component of many ecosystems and convenient indicator for an assessment of natural and anthropogenous biotopes.

The purpose of our work was the comparative analysis of ecological groups and biodiversity of small mammals in various biotopes within the Kazakhstan Irtysh biotopes: the closed biotopes (forest), the open biotopes (steppe) and the human settlements.

Materials and Methods

Standard methods of population survey for small mammals were used in this study [1]. Gero's traps were set in various biotopes: natural (forest and steppe zones) and anthropogenically changed (human settlements zone). The research territories are differing according to physical and geographical characteristics and the degree of economic development. The field material was collected within 2011-2013. There were 1500 trap-nights in total. We captured 349 small animals of 25 species. The species identification of animals were carried out with using of diagnostic morphological and craniometrical features [2].

Small mammals communities in habitats were analyzed by using of the following indicators: Margalef's diversity index, Evenness index**,** Berger–Parker index [3-5].

Results and Discussion

During the period of work were captured 25 species of small mammals (Table 1). Most numerous are following micromammals: *Apodemus uralensis, Mus musculus, Allocricetulus eversmanni, Apodemus agrarius, Microtus arvalis, Myodes rutilus, Meriones tamariscinus, Sorex tundrensis.* Depending to ecologically associated with humans all captured species were divided into three groups: synanthropes, hemisynanthropes and exoanthrops [6].

The majority of the found species of small animals within Kazakhstan Irtysh river biotopes belong to exoanthrops. They are *Sicista subtilis, Salpingotus crassicauda, Crocidura suaveolens, Allocricetulus eversmanni, Dipus sagitta, Phodopus sungorus, Microtus arvalis, Microtus economus, Microtus gregalis,*

Stylodipus telum, Allactaga elater, Pygeretemus pumilio, Sorex tundrensis, Ochotona pusilla, Lagurus lagurus, Hemiechinus auritus, Allactaga major, Allocricetulus eversmanni pseudocurtatus, Phodopus roborovskii and *Meriones tamariscinus*. Exoanthrops avoid human neighbourhood. They need rather untouched, natural biotopes for life [7].

Synanthropes are contrast to exoanthrops. Synanthrope animals are those species which regularly live in the territory of human settlements or in human constructions (residential buildings, shops, foodstuff storage places, etc.), where they form constants or periodically arising independent or semi-dependent populations [8]. We classified one species – *Mus musculus* as synanthrope. Facultative synanthropes (or hemisynanthropes) are presented by four species: *Myodes rutilus, Apodemus uralensis, Apodemus agrarius, Cricetulus migratorius*. They may exhibit varying degrees of sinanthropy depending on the season and part of the area.

Among small mammals of the Kazakhstan Irtysh biotopes prevail inhabitants of open biotopes. They are presented by species: *Sicista subtilis, Salpingotus crassicauda, Allocricetulus eversmanni, Cricetulus migratorius, Dipus sagitta, Phodopus sungorus, Microtus arvalis, Microtus gregalis, Stylodipus telum, Allactaga elater, Pygeretemus pumilio, Ochotona pusilla, Lagurus lagurus, Allactaga major, Allocricetulus eversmanni pseudocurtatus, Crocidura suaveolens, Microtus economus, Sorex tundrensis, Myodes rutilus, Apodemus agrarius, Phodopus roborovskii* and *Meriones tamariscinus*.

Crocidura suaveolens, Microtus economus, Sorex tundrensis, Myodes rutilus, Apodemus uralensis, Apodemus agrarius and *Hemiechinus auritus* were found in the forest (closed) biotopes. The inhabitant of human settlements is the sinanthropes *Mus musculus*. Human presence and his activities significantly reduces area suitable for wildlife habitat.

Table 1: Species composition and number of captured small mammals within Kazakhstan Irtysh River biotopes (2011-2013).

No	Species of micromammals	Quantity, samples
1	*Apodemus uralensis*	38
2	*Mus musculus*	31
3	*Allocricetulus eversmanni*	29
4	*Apodemus agrarius*	27
5	*Microtus arvalis*	25
6	*Myodes rutilus*	25
7	*Meriones tamariscinus*	22
8	*Sorex tundrensis*	22
9	*Sicista subtilis*	17
10	*Microtus economus*	15
11	*Salpingotus crassicauda*	14
12	*Dipus sagitta*	14
13	*Microtus gregalis*	14
14	*Cricetulus migratorius*	13
15	*Crocidura suaveolens*	12
16	*Allactaga elater*	12
17	*Phodopus sungorus*	6
18	*Stylodipus telum*	4
19	*Pygeretemus pumilio*	3
20	*Ochotona pusilla*	1
21	*Lagurus lagurus*	1
22	*Allocricetulus eversmanni pseudocurtatus*	1
23	*Phodopus roborovskii*	1
24	*Hemiechinus auritus*	1
25	*Allactaga major*	1
	In total	349

Comparing the habitat distribution of ecological groups of small mammals in natural and anthropogenically modified habitats of Kazakhstan Irtysh biotopes, it can be noted that the steppe and forest zone are represented by facultative sinanthropes and exoanthrops in different proportions. Exoanthrops dominate in the steppe, there are 14 % facultative sinanthropes. In the forest zone, hemisynanthropes and exoanthrops are presented practically equally (43% and 57%).

There are *Crocidura suaveolens, Microtus economus, Sorex tundrensis, Myodes rutilus, Apodemus agrarius* in both a forest zone and the steppe. *Apodemus uralensis* dominates in the forest zone but *Allocricetulus eversmanni* dominates in the steppe.

Measuring biological diversity of small mammals in natural and anthropogenically modified habitats shows the high Margalef's diversity index and the high Evenness index with a low Berger–Parker index of domination in small mammals community within forest and steppe compared with rates in the human settlements area (Figure 1).

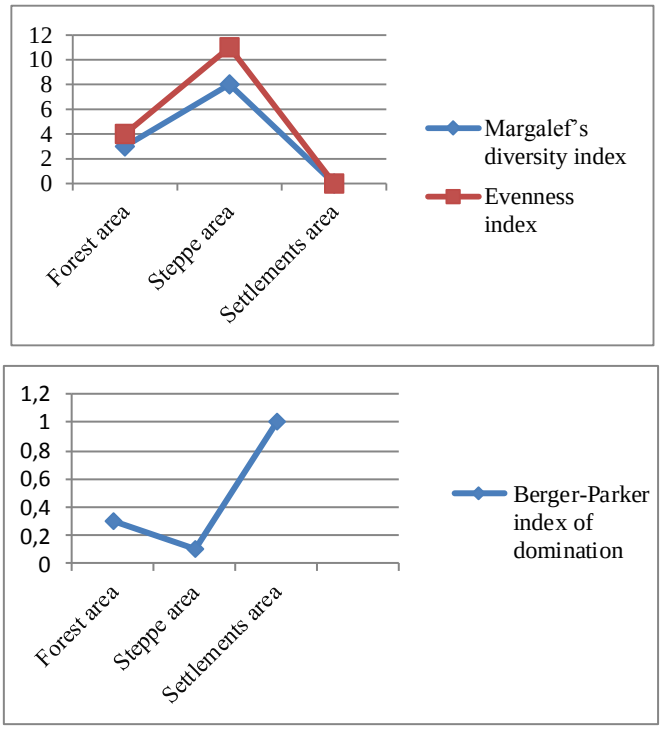

Figure1: Ecological index in small mammals community within the natural and anthropogenically changed biotopes (Berger–Parker index for *Apodemus uralensis* in a forest, for *Allocricetulus eversmanni* in a steppe and for *Mus musculus* in the human settlements area).

High indicators of the Evenness index and species diversity in a forest zone and a zone of steppes show the balance and the maturity of small mammals communities on these areas.

Conclusion

The fauna of small mammals within Kazakhstan Irtysh river biotopes is represented by 22 species of rodents and 3 species of the insectivorous. The majority of the found species are inhabitants of open biotopes and belong to ecological group of exoanthrops. The small mammal community of steppe is characterized by the high Margalef's diversity index and the high Evenness index with a low Berger–Parker index of domination (*Allocricetulus eversmanni*). Exoanthrops prevail in the steppe (86%), here 14% of micromammals are hemisynanthropes. Diversity of landscape elements creates optimal conditions for living of most plastic rodent species in the steppe biotope. Hemisynanthropes and exoanthrops in a forest zone are represented practically equally (43% and 57%). A species diversity of small mammals in the forest zone is slightly poorer than in the steppe zone, but the high Evenness index and the low Berger–Parker index of domination (*Apodemus uralensis*) in a forest zone show a balance and maturity of small mammal communities in this area. In a zone of human settlements a small mammals species diversity is very poor and is represented by dominant - a house mouse.

The comparative analysis of the small mammals distribution within Kazakhstan Irtysh biotopes reveals some patterns and trends that reflect the characteristics of animal ecology, as well as indicates differences in environmental conditions of different habitats, formed under the influence of anthropogenic impact.

Acknowledgments

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The cell structure and growth rate of three species of marine microalgae (*Chlorella vulgaris*, *Tetraselmis chuii* and *Isochrysis* sp.) before and after cryopreservation

Muna A.Omar Abdul Kader, Aizatul Adzwa Mohd Basri, Shahrizim Zulkifly, *Hishamudin Omar and Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor.

**Corresponding author.Tel.:+603 89466617; email address: hishamom@upm.edu.my*

Three species of marine phytoplankton *Chlorella vulgaris*, *Tetraselmis chuii* and *Isochrysis* sp. were cultured using Conway media in 20 °C with the light intensity of 90 µmol m-2s-1 and a photoperiod of 12:12 h L/D cycle. Cells were harvested in stages at logarithmic and stationary phases. Equilibration time for cyroprotectant exposure was 10 to 20 minutes before the samples was placed in the programmable freezer for a controlled rate of cooling at -1 °C /min. The temperature was reduced uniformly from 200C until it reaches -40 °C where the samples were maintained at this temperature for 30 minutes before direct immersion into liquid nitrogen at -196 °C. Samples were stored in liquid nitrogen for one week. Percentage of viable cells was relatively higher in the stationary phase for the three species that is 95.13 % for *Chlorella vulgaris*, 79.09 % for *Isochrysis* sp. and 69.36 % for *Tetraselmis chuii*. The growth rate of viable cells for *Chlorella vulgaris* a both logarithmic and stationary phases and *Tetraselmis chuii* at logarithmic phase showed insignificant differences ($p > 0.05$) before and after cryopreservation. *Tetraselmis chuii* of stationary phase and *Isochrysis* sp. of both phases gave significant differences ($p < 0.05$) for growth rate before and after cryopreservation due to photo-oxidation. The cell structure after cryopreservation showed disorganization and abnormality in the ultra-structure but this did not seem to affect the growth rate of the viable cells.

Keywords: *Chlorella vulgaris*, *Tetraselmis chuii*, *Isochrysis* sp., conway media

The effectiveness of commercial fertilizer N:P:K (15, 15, 15) and (12:12:17) with magnesium and iron to increase the growth of *Arthrospira platensis*

Mohamed Amar Naqqiuddin, Chang Kuai Peng, Hishamudin Omar and *Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia
43400 Serdang, Selangor.
**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Introduction

Arthrospira platensis (Spirulina) is unlike majority algae as it has unique ability to thrive high alkaline water bodies (Belay, 1993). Due to this prior advantage, *A. platensis* has been highly suggested to be mono mass cultured into commercial scale. Fertilizer as the nutrient sources plays significant role towards improving yield of *A. platensis.* However in Malaysia, weather patterns are seen as huge drawback causing troubles to the adaptation and acclimatization process of microalgae cultivation. Advanced fertilizer formulas were tested and created from different cheap nutrient sources to break through current high market prices. Light has been one of the fundamental factors contributing the growth of *A. platensis.* At optimum level of lights, the population of cells expands following the persistence of lights. In Malaysia, it is too unsafe to implement open channel cultivation due to different causes such extreme sunlight exposures; contamination risks; temperature disarray, thus it was highly recommended to culture *A. platensis* in closed photobioreactor (Torzillo, 1997). Also at a certain level, overly released ammonia could be harmful to the dominant growing algae (Boussiba and Gibson, 1991). Zarrouk and Kosaric media are usually used as growth medium for *A. platensis* (Zarrouk, 1966; Tompkins *et al*., 1995). Nitrogen sources play significant task affecting productivity of *A. platensis* biomass as well as the protein and chlorophyll contents. Urea or ammonium were also used as nitrogen sources for growth enhancement and cost reduction (Soong, 1980). Besides, majority commercial fertilizer contains some other useful microelements supplemented to enhanced even higher yield of *A. platensis*. Thus, this study was hold to determine the effectiveness of media consists of commercial fertilizer N:P:K (12:12:17), magnesium and iron in growth of indoor and outdoor culture alternatively for *A. platensis* cultivation.

Materials and Methods

Microalgae and Medium Preparations

A. platensis was obtained from Plant Physiology Laboratory, Biology Department, Faculty of Science, University Putra Malaysia. Commercial fertilizer N:P:K (15:15:15) and N:P:K (12:12:17) produced by MARDI were used in this experiment with control of Kosaric media (Tompkins *et al*.,1995) as *A. platensis* growth fertilizer medium. 0.05 g/L Fe and 0.1 g/L Mg were added to each commercial fertilizer medium treatments. Supplementary A5 micronutrients were added following Tompkins *et al*. (1995). Each fertilizer treatments of *A. platensis* culture (25L) in aquarium tanks had 2 sets of triplicates (1: indoor condition; 2: outdoor condition). The growth was compared to Kosaric medium as control which differs in N, P, K ratio and

micronutrient supplement (Mg). For outdoor condition culture, acclimatization period of 3 days were arranged for sufficient adaptation into new environment. Cultivation was experimented for a period of 4 days.

Analysis and Statistical Procedures

For dry weight measurements, 50ml samples were filtered through pre-dried and pre-weight GF/C Whattman filter papers (Borowitzka *et al*., 1991). Dry weight was calculated in mg/L after the filter paper was dried in oven at 105°C for 24 hours. Optical density was measured to determine the growth rate of *A. platensis* by using spectrophotometer. Optical density was estimated to count cell density by using wavelength at 620nm for the whole set of triplicates of the culture. pH measured with WTW pH meter (PH 330) and light intensity with Licor 182 (LI-250) were collected every alternate day for 2 weeks of cultivation cycle. One-way ANOVA was used to determine the differences among treatments at 95% confidence level.

Results and Discussion

The mean of indoor light intensity was 14.09±2.59 $\mu molm^{-2}s^{-2}$, while in outdoor condition; the light intensity was 24.21±13.39 $\mu molm^{-2}s^{-2}$. Air temperature was ranged between 29.5-30.7°C in indoor condition; 29.0-31.9°C in outdoor condition. Indoor cultured condition has remarkably more increment in pH compared to outdoor condition cultures. Growth fertilizer of both sets of (12:12:17+Fe+Mg and Kosaric) shown significantly higher optical density (OD) readings compared to fertilizer set of 15:15:15+Fe+Mg for cultures of indoor condition, whereas Kosaric medium set cultured *A. platensis* has shown significantly highest OD reading compared to the commercial fertilizers (Figure1). Highest dry mass (mg/L) collected from 12:12:17+Fe+Mg, commercial fertilizer: 73 mg/L for indoor grown culture condition. However, dry mass collected from all sets in outdoor condition were significantly lower compared to indoor condition.

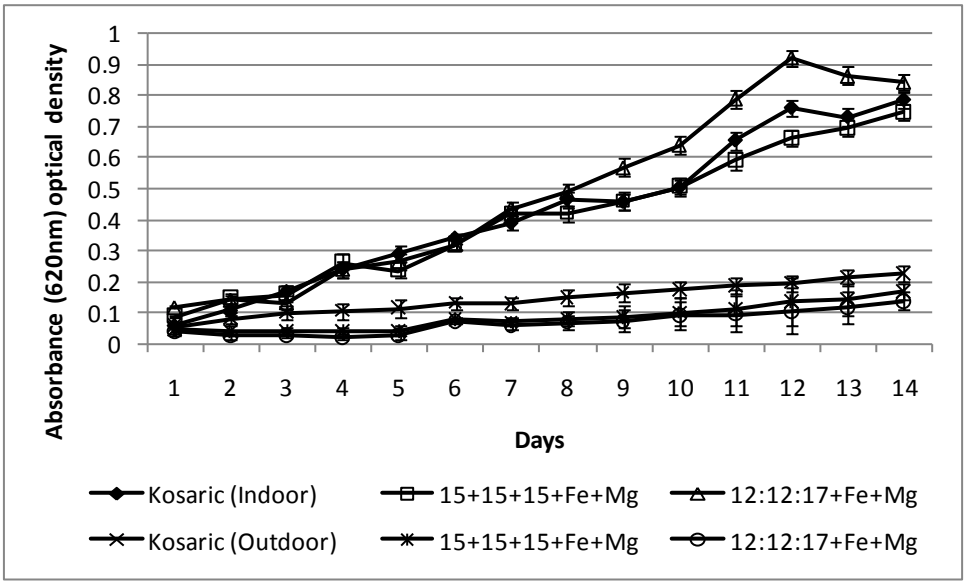

Figure 3: Average optical density of grown Arthrospira platensis with 2 sets of indoor and outdoor conditions using fertilizer mediums: Kosaric; commercial fertilizer, 15+15+15+Fe+Mg and 12:12:17+Fe+Mg. (Values presented as mean±SE; n=3).

Every year, tropical countries are facing problems of high cloud covers that have been shading sun rays. As light were not received well, the growth rate of *A. platensis* were inhibited. Indoor cultures were placed under constant light sources, Philips TLD fluorescent lights that helped improving growth of *A. platensis.* Different in nutrient sources used in *A. platensis* cultivation showed varies yield of *A. platensis* (Rangel-Yagui *et al*., 2004). As expected, Fe and Mg are essential in enhancing growth rate of indoor cultures; however there was no significant growth improvements shown by Fe and Mg supplemented commercial fertilizer compared to normal Kosaric medium in outdoor condition cultured *A. platensis*. Moreover, the acclimatization period may have to be extended up to more than 1 week to give more time for adaptation process. Inability to adapt in new changed environment especially to outdoor condition (inconstant parameters) could probably effects the growth rate and yield of *A. platensis*.

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The extraction and characterization of chitosan from *Cunninghamella elegans*

*Nur Syahmina Rasudin[1], Kalaivani Nadarajah[2] and Shamarina Shohaimi[1]

[1]Department of Biology, Faculty Science, Universiti Putra Malaysia, 43400 Serdang
[2]School of Environmental and Natural Resource Sciences, Faculty Science and Technology,The National University of Malaysia, 43600, Bangi
**Corresponding author.Tel.:+6012 4531515; email address: syamina_85@yahoo.com*

Introduction

Chitosan is a naturally occurring biopolymers that obtained from crab and shrimp shells (Kumar, 2000). Chitosan is a biodegradable polysaccharide derived from deacytelation of chitin (poly-N-acetyl-D-glucosamine). Chitosan is a biopolymers that has a wide variety of applications such as in pharmaceuticals products, food industry, waste water treatment and medical industry (Synoweici and Khateeb, 2003). Chitosan also could be extracted from fungi where it was found primarily as the main component of the fungal cell wall, especially in Zygomycetes (Arcidiacono and Kaplan 1992). The objectives of this study were to extract chitosan from *Cunninghamella elegans* and to determine the degree of deacytelation, molecular weight and colour of the chitosan extracted from fungi *Cunninghamella elegans*.

Materials and Methods

Chitosan production and extraction

Cunninghamella elegans was cultured in Potato Dextrose Agar (PDA) for 5 days. The spores from PDA was used to generate spore suspensions. The fungi were grown in complex medium YPG (Yeast Peptone Glucose). The spores (inoculum size 1x107) were inoculated aseptically into autoclaved growth medium (110oC for 10 minutes). Fermentation was carried out in 5L fermenter with 1L medium maintained at 30°C with aeration for 72 hours. Chitosan was extracted from the mycelium using the White *et al*. (1979) methodology with alkayl and acid treatment.

Chitosan Characterization

The degree of deacetylation of the chitosan samples was determined by the calculation of the glucosamine percentage by using First Derivative Ultraviolet Specthrophotometry (FDUVS) (Muzarelli & Rochetti, 1985). Forty milligrams (mg) of chitosan was diluted in 10ml acetic acid (0.1M) and read spectrophotometrically at 190 to 240 nm. Molecular weight of chitosan was determined using Ubbelohde tube at 26°C through viscometric analysis. Seventy five miligrams (mg) chitosan was diluted in 30ml acetic acid to produce 0.25% chitosan dilution. Curves for ηsp/concentration versus concentration (whereby ηsp – specific viscosity) were plotted and extrapolated in order to obtain the intrinsic viscosity, [η] ([η]= [ηsp/c]c→0) (Dawn *et al.*, 2004). The average molecular weight was calculated based on the Mark-Houwink equation: $[\eta] = KM^a$, where K and a are coefficients related to the Ubbelohde tube and the molecular weight of sample. Colour of chitosan was measured using a Minolta spectrophotometer (Model CR300 Japan). The chitosan

sample was put in a transparent plastic bag (4×4 cm). Color Hunter readings indicate L for lightness, (+a) for redness and (b+) for yellowness.

Results and Discussion

Cunninghamella elegans produced about 0.11 to 0.47 grams of mycelia biomass in the duration of 72 hours of culturing (Figure 1). Chitosan was extracted at the highest amount of 60th or 72nd hours culturing which was in the late exponential phase of growth and the extractability of Chitosan increased with the increment of biomass (Dawn, 2004). The quality of the chitosan obtained in this study was measured through the degree of deacetylation, molecular weight, and lightness values. The degree of deacetylation obtained from *Cunninghamella elegans* chitosan was 77.08%. The degree of deactylation of chitosan depends on several factors such as incubation temperature, incubation period and acid concentration (Dawn, 2004). The molecular weight of fungal chitosan observed was 4.318 x 10^3 Da. Molecular weight of chitosan produced might be affected by the type of medium growth due to the presence of elements such as ferum and manganese ions or certain substrates that may slow down cause the activities of chitin synthase (Jaworska and Konieczna 2001) Lightness value, L for chitosan extracted from *Cunninghamella elegans* was 79.86 indicates that chitosan have higher lightness value.

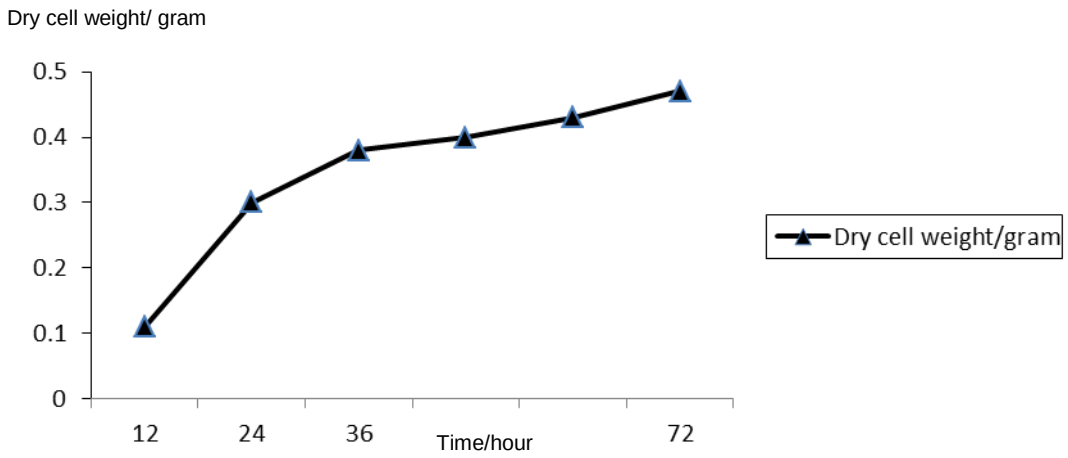

Figure 1: Profile of mycelia production from *Cunninghamella elegans* grown on YPG.

Conclusion

The results obtained from this study showed that chitosan extracted from *Cunninghamella elegans* had high degree of deacytelation, average molecular weight and higher lightness colour of chitosan and indicates that *Cunninghamella elegans* has a potential to be as a source of chitosans and for use in the commercial production of chitosans in the future.

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Tree species richness and importance value of a regenerated hill dipterocarp forest, after supervised logging

Seyed Mousa Sadeghi[1,2], *Faridah-Hanum Ibrahim[1], Wan Razali Wan Mohd[1], Kamziah Abd Kudus[1] and Khalid Rehman Hakeem[1]

[1]Faculty of forestry, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
[2]Natural Resources and Agricultural Research Center of Bushehr Province, Varzesh Street, Bushehr 7515643373,I.R.Iran
**Corresponding author.Tel: +60 133 277172; email address: smbooraki@gmail.com*

Introduction

Hill dipterocarp forest (HDF) of Ulu Muda Forest Reserve (UMFR) is dominated by dipterocarp trees (Symington *et al*., 2004). The species richness of HDF is high (Latiff and Faridah-Hanum 2005; Saiful *et al*., 2008). The number of species, genera and families of logged-over HDF in UMFR declined immediately after conventional logging which was 24.1%, 16.2% and 12.2%, respectively (Saiful, 2002). The importance of evaluation of past changes in species composition of logged-over HDF is related to: (1) At least two-third of our planet's terrestrial biodiversity is in the tropical forest ecosystems. (2) obligate forest species (Those species which found in the native forests) are basically more vulnerable than partly dependent species (those species which grow at forest edge) on forest habitat to ultimate extinction from forest loss (Gardner *et al*., 2009). Hence, the research on species composition of logged HDF is necessary to help decision makers in the forestry sector.

Effects of supervised logging on HDF in UMFR after intermediate period are not studied yet. Here, we investigate the tree species richness and importance value of HDF 12 years after logging. Also, we compare our data with available published data (from primary and logged-over forest) to find the changes of species composition.

Materials and Methods

The research was conducted at HDF, UMFR in Kedah, Malaysia (Compartment 25 A). The study site is hilly land with altitude ranges from 419 to 555 m. The mean value of rainfall and temperature is 2869 mm and 26-29◦C, respectively. The forest was subjected to supervised logging operations from 2000 to 2001.

Tree inventory was undertaken in the 20 plots (50 m × 20 m) with systematic sampling layout. All individuals were enumerated, measured and identified. In each sampling plot the diameter at breast height (DBH) of trees ≥ 1 cm was measured. The sample of un-identified individual was collected, labeled, oven-dried and conserved at Herbarium of Faculty of forestry, University Putra Malaysia for additional identifications.

According to Curtis and McIntosh (1951) method the importance value index (IVI) of species was calculated. The summation index of relative frequency (RF), relative density (RD) and relative dominance (RDo) was considered as (IVI) as follows:

RF= (Frequency of species/Total frequency of all species) × 100
RD= (Number of individual of species/Total number of individual of all species) × 100
RDo = (Basal area of a species/Total basal area of all species) × 100
The basal area (BA) estimating was done by following equation:
BA (m^2) = [π × $(DBH)^2$]/40000
According to Mori *et al*. (1983) method the family importance values (FIV) of forest community were computed. The summation of relative diversity (RDi), relative density (RDe) and relative dominance (RDo) was determined as FIV:

RDi = (Number of species in family/Total number of species) × 100
RDe = (Number of individual in family/Total number of trees) × 100
RDo = (Total basal area of family/Total basal area) × 100

Results and Discussion

Out of 2096 individuals in two ha of sampling area, 63 families, 173 genera and 380 species and one variety was found. Five most diverse families contributed 37.6% of the total species richness. Euphorbiaceae contributed the highest value (49 species) followed by Fabaceae (27 species), Lauraceae (25 species), Annonaceae (22 species) and Rubiaceae (20 species). In term of FIV, of 63 families five top families contributed 42.4% of FIV. Euphorbiaceae provided highest level (51.09) followed by Dipterocarpaceae (28.10), Annonaceae (18.20), Lauraceae (15.87) and Fabaceae (13.80). The minimum value of FIV was related to Rutaceae (0.14). At species level five top species contributed (9.9%) of IVI. Results showed that the study site was dominated by Dipterocarpaceae, due to 40% of five top species was from dipterocarp trees. *Shorea macroptera* Dyer provided highest level of IVI (9.26).
In term of species richness, the supervised logged-area at UMFR was dominated by dipterocarp trees but some pioneer species such as *Macaranga* spp., *Croton* spp. from Euphorbiaceae, *Neolamarckia cadamba* (Roxb.) Bosser and *Neonauclea pallida* (Reinw. ex Havil.) Bakh.f. ssp. *malaccensis* (Gand.) Ridsdale from Rubiaceae were presented in the site. In contrast, conventionally logged forest (UMFR) was dominated by pioneer species: *Macaranga gigantea* (Rchb.f. & Zoll.) Mull.Arg., *Macaranga hosei* King ex Hook.f., *Mallotus griffithianus* (Mardan *et al*., 2013) because out of five top species in term of IVI, three of them (60%) were pioneer species. Same results were reported in a supervised logged forest at Deramakot Forest Reserve, Sabah Malaysia and conventionally logged forest at Tangkulap Forest Reserve in Sabah, Malaysia (Imai *et al*., 2013). In another logged-over HDF at Tekai Tembeling Forest Reserve as a comparison, Dipterocarp trees dominated the area (Kamziah *et al*., 2011). As a comparison in the primary forest the dipterocarp trees were dominant (Saiful, 2002; Imai *et al*., 2013). Hence, the pattern of supervised logged-over forest was similar to primary forest but it was not so regarding conventionally logged forest. In the primary HDF at UMFR at family level, the highest level of FIV was contribution of Dipterocarpaceae. However, in our study area, the biggest amount of FIV was related to Euphorbiaceae. Because although, big trees were from dipterocarp groups, their frequency was low hence, at the family level the FIV of dipterocarp group was lower than non-dipterocarp tress. So, at the family level the regenerated forest needs more time to resemble the original level of

FIV of primary HDF. However, at the species level in both primary and supervised logged-over HDF dipterocarp trees were dominant in the HDF at UMFR. Same results was carried out in primary and supervised logged-over forest at Deramakot Forest Reserve Sabah, Malaysia (Imai *et al*., 2013).

Conclusion

In comparison with conventionally logged forest we concluded that supervised method could reduce the environmental damage to forest ecosystem since, pattern of mixed dipterocarp forest was identified in supervised logged site 12 years after logging.

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Tributyltin-resistant bacteria from contaminated surface sediment

Abdussamad Abubakar[1], Muskhazli Mustafa[2], Wan Lutfi Wan Johari[3], *Ferdaus Mohamat-Yusuff[4], Syaizwan Zahmir Zulkifli[2] and Ahmad Ismail[2]

[1,3]Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Environmental Forensics Research Centre ENFORCE, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
**Corresponding author.Tel.:+603 89468023; email address: ferdausmy@upm.edu.my*

Resistance to tributyltin (TBT) was examined in pure bacteria cultures isolated from TBT-polluted sediments. We defined a TBT-resistant bacterium as one which grows in a TBT concentration above the reported concentration at the sampling site (Kong Kong Laut), which was less than 1000µg/l. More than 15 pure colonies of bacteria were isolated which are mostly Gram negative and more than 80% percent of these isolates are possible TBT-degrading bacteria due to their ability to resist TBT concentration of up to 1000µg/l. All TBT-resistant bacteria are potential TBT degrading bacteria but may not degrade the TBT. However, TBT degrading bacteria must be TBT resistant bacteria. These TBT-resistant bacteria were also examined for their biodegradability and they shows capability of degrading TBT, suggesting that these microorganism can utilize the carbon source in the pollutant. This study has successfully shown that these bacteria isolated from Kong Kong Laut are potential TBT-degrading bacteria and this also paves a major pathway for sustainable remediation solution.

Keyword: Tributyltin, resistance, biodegradation, sediment, bacteria

Ecology

Estimation of GHG emission from different land use changes associated with oil palm plantation

*Nurul Izzati Mat Akhir[1], Faradiella Mohd Kusin[2] and Ferdaus Mohamat-Yusuff [1]

[1]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang*
[2]*Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
**Corresponding author.Tel.:+013 3371402, email address: nurulizzatimatakhir@gmail.com*

The release of nitrous oxide (N_2O) from agricultural activities contributes to the increase of greenhouse gases in the atmosphere. In this study, the amount of nitrogen fertilizer used in an oil palm plantation of different stages (immature and mature) was estimated. Data of fertilizing scheme at the oil palm plantation for oil palms varying in age (planted between 1986 and 2009) was used. Estimation of nitrous oxide emission and the resulting CO_2-equivalent emission were calculated for each category of the oil palm. The amount of N-fertilizer applied were between 194-260 kg N/ha. The resulting N_2O emissions were between 2.75-3.13 kg N_2O-N/ha, which corresponds to CO_2-equivalent of between 261.99-346.94 kg CO_2-eq/ha. Despite increase of N_2O emission from immature stage until maturely-developed up to 20 years, there is no clear relationship between N_2O emission per ha and the age of oil palm. Generally, the N_2O emissions found in this study are still low compared to the default value for synthetic nitrogen fertilizer-induced emissions for tropical regions.

Keywords: CO_2-equivalent, global warming, nitrous oxide, N-fertilizer, oil palm plantation

Utilization of Java Medaka (*Oryzias javanicus*) in ecotoxicological studies

*Shahrizad Yusof and Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia

**Corresponding author.Tel.:+603 89466636; email address: izad@upm.edu.my*

An increasing number of chemicals due to human activities enters our environment every day. This study promotes Java medaka (*Oryzias javanicus*) as test organism for the impacts of these chemicals. Java medaka is a small tropical fish native to Malaysia, Indonesia, Singapore, Thailand and Vietnam, commonly found in abundance in estuaries. The fish have been successfully cultured in the laboratory in ambient temperature and controlled photoperiod of 14 hours light and 10 hours dark. Some ecological aspects of the fish in their natural habitat were studied in order to understand their response to chemicals. The fish occur abundantly all year round in the west and south coasts of Peninsular Malaysia. They migrate tidally within a large salinity range (1.2 – 29.0 ppt), making them suitable to represent the coastal environment. The sensitivity of different life stages of Java medaka to environmental pollutants was tested. All life stages of the fish have been utilized and they have shown particular sensitivity. The embryos were sensitive to low concentrations (0.01 – 0.05 ppm) of heavy metals (Cd, Hg, Pb, Cu and Zn) in terms of developmental impairments. In exposure to glyphosate-based herbicide embryonic death was found to be the most prominent response while swimming disorder was observed in the juveniles. Teratogenecity is another developmental endpoints in embryos exposed to the pollutants tested. The responses shown by all the life stages of the fish indicated that this fish species can be a useful to investigate short term and long term effect of pollutants in the future.

Keywords: Java medaka, test organism, pollution, coastal areas, environment

Ag and As concentrations in mangrove snails, *Nerita lineata*

*Cheng Wan Hee[1], Yap Chee Kong[1], Mohamad Pauzi Zakaria[2], Ahmad Zaharin Aris[2] and Tan Soon Guan[3]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia.*
[2]*Environmental Forensics Laboratory, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.*
[3]*Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
**Corresponding author. email address: wheec@hotmail.com*

Introduction

Trace metals are naturally present in the environment and could exhibit in high levels which will cause harmful effects on the marine coastal ecosystem (Fritsch *et al*., 2011). In order to assess the pollution levels of the environment, sediments and marine gastropods were commonly used as biomonitors of trace metals. The objective of this study is to determine the background concentrations and the ability of *Nerita lineata* to reflect the trace metal levels of the environment.

Materials and Methods

Sampling of snail and sediment samples was conducted in April 2011 from 10 sites of the western and southern part of Peninsular Malaysia. The snails were dissected into tissue parts (shells, opercula and soft tissues) and sediments were dried separately at 60 °C until constant dry weights are achieved. The samples were then digested with 7 ml of HNO_3 65% + 1 ml H_2O_2 30% for the snails and 9 ml of HCl + 3 ml of HNO_3 65% for sediments in a microwave digester. Analysis was done by using the Perkin Elmer SCIEX ELAN DRC-e ICP-MS for Ag and As.

Results and Discussion

Tables 1 and 2 show the concentrations of Ag and As in the snails and sediment collected from 10 sites of the western and southern Peninsular Malaysia. The average concentrations (µg/g dry weight) of Ag and As in the shells of the snails were 0.013 and 0.041, respectively. While the average concentrations (µg/g dry weight) of Ag and As in the opercula of the snails were 0.007 and 1.572, respectively. As for soft tissues, the average concentrations (µg/g dry weight) of Ag and As were 0.039 and 5.182, respectively. The average concentrations (µg/g dry weight) of Ag and As in sediments were 0.674 and 34.823. The pattern of trace metal accumulation of all the metals were in the decreasing order of soft tissues > opercula ≥ shell. It has been reported in previous studies that essential metals were recorded higher in the soft tissues compared to the shells of gastropods (Cravo, 2004; Yap *et al*., 2010). Metals, in this study, such as As are basic essential elements required for the normal functions of organisms while non-essential metals such as Ag exhibit toxicity risks (Perkins and Gadd, 1993; Liden *et al*., 2011). Pearson's correlation coefficient showed that there were no relationship between the snails (shells, opercula and soft

Table 1: Heavy metals levels (Ag and As) in the shells, opercula and soft tissues of the *N. lineata* collected from 10 sites of Peninsular Malaysia.

	Shells		Opercula		Soft Tissues	
Site	**Ag**	**As**	**Ag**	**As**	**Ag**	**As**
Kpg. Pasir Puteh	0.03 ± 0.0006[b]	0.033 ± 0.002[a,b]	0.006 ± 0.002[c]	0.028 ± 0.002[a]	0.085 ± 0.004[c]	4.301 ± 0.265[a]
Sg. Ayam	BDL	0.041 ± 0.005[a,b,c]	0.029 ± 0.001[d]	0.146 ± 0.025[a]	0.022 ± 0.002[a,b]	5.089 ± 0.068[a]
Jetty to Pulau Ketam	BDL	0.056 ± 0.016[b,c]	0.002 ± 0.001[a,b]	0.351 ± 0.164[a]	0.047 ± 0.007[b]	4.742 ± 0.0434[a]
Sg Janggut	BDL	0.008 ± 0.001[a]	0.003 ± 0.0002[a,b]	0.054 ± 0.037[a]	0.027 ± 0.003[a,b]	3.713 ± 0.181[a]
Kukup	BDL	0.022 ± 0.004[a,b]	< 0.001[a]	0.039 ± 0.004[a]	0.024 ± 0.001[a,b]	6.831 ± 0.147[b]
Lukut	BDL	0.088 ± 0.019[d]	BDL	0.038 ± 0.013[a]	0.089 ± 0.012[c]	9.02 ± 0.084[c]
Kpg. Sg. Melayu	BDL	0.021 ± 0.004[a,b]	BDL	0.007 ± 0.004[a]	0.019 ± 0.002[a,b]	5.063 ± 0.237[a]
Tg. Langsat	BDL	0.02 ± 0.001[a,b]	BDL	0.792 ± 0.4156[b]	0.028 ± 0.010[a,b]	3.493 ± 0.836[a]
Sepang	BDL	0.048 ± 0.006[a,b,c]	0.002 ± 0.0002[a,b]	0.061 ± 0.004[a]	0.009 ± 0.001[a]	4.758 ± 0.264[a]
Tg. Piai	0.001 ± 0.0005[a]	0.072 ± 0.007[c,d]	0.004 ± 0.0008[b]	0.057 ± 0.006[a]	0.04 ± 0.009[b]	4.814 ± 0.433[a]

Table 2: Heavy metals levels (Ag, As, Be and Cr) in the sediments collected from 9 sites of Peninsular Malaysia.

Sites	**Ag**			**As**		
Kpg. Pasir Puteh	0.385	±	0.003[a]	25.580	±	0.207[a,b]
Sg. Ayam	0.338	±	0.072[a]	21.809	±	1.857[a]
Jetty to Pulau Ketam	0.236	±	0.090[a]	41.981	±	11.250[b]
Sg. Janggut	0.794	±	0.091[a]	33.176	±	0.905[a,b]
Kukup	0.133	±	0.012[a]	38.948	±	0.809[a,b]
Lukut	0.341	±	0.079[a]	37.285	±	0.801[a,b]
Kpg. Sg. Melayu	1.686	±	0.355[b]	59.488	±	0.786[c]
Tg. Langsat	0.469	±	0.145[a]	24.331	±	0.135[a,b]
Sepang	1.684	±	0.243[b]	30.813	±	1.174[a,b]

tissues) and sediments (P>0.05) which could possibly indicated that i) regulation of metals in the tissues of the snails (Rainbow *et al*., 2002), ii) uptake of trace metals are influenced by physico-chemical and environmental factors, such as biological and physiological character of the organisms, spatial distribution of metals in the sediments and variations occurred by seasons (Rainbow *et al*., 1990; Mourier *et al*., 2011).

Conclusion

This study provides the background data of trace metals (Ag, As, Be and Cr) levels in the snail *N. lineata* collected from 10 sites of Peninsular Malaysia. However, the current results showed that the snails is not a good biomonitor for the trace metals.

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Lethal concentration 50 (LC50) of tributyltin chloride (TBTCl) on nauplii of brine shrimp (*Artemia salina*)

Najla Mohamed Abu Shaala, *Syaizwan Zahmir Zulkifli, Ahmad Ismail and Mohammed Noor Amal Azmai

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Corresponding author.Tel.:+ 603 29466627; email address: syaizwan@upm.edu.my

Introduction

Fouling is an undesirable phenomenon when takes place on ship hulls, fish net cages, or oil rig supports. It is a major problem for the shipping industry because friction between the hull and seawater is increased, which in combination with the increased weight of the fouling organisms can lead to a considerable increase in fuel consumption (Panagoula *et al*., 2002). Fouling is also implicated in the spread of invasive species across the world's oceans, which according to (Bartley and Minchin, 1996) are regarded as a threat to aquatic fauna, causing demise or the native species to vanish by competitive exclusion, introduction of diseases, or predation. Brine Shrimp *Artemia* species are not considered as sensitive as other screening instruments or organisms (Nunes *et al*., 2006) they have some important advantages including constant commercial availability all year round, cost efficiency, ease of culture, short life-cycle, no feeding required during the assay and great offspring production (Vanhaecke *et al*., 1981).

Materials and Methods

Hatching procedure followed the one described in ARC-test, standardised short- term toxicity test with *Artemia* nauplii (Persoone and Vanhaecke, 1981). The hatching medium used was artificial seawater of normal seawater salinity (35 $g.l^{-1}$). For test approximately 0.5 g cysts of *A. salina* was incubated in 500 ml seawater in a cylindroconical tube at a temperature of 25 ± 1 °C and with lateral illumination (1000 lux) for 24hr. All the cysts were kept in continuous suspension by aeration provided by a small air tube extending to the bottom of the hatching device. After 24 the test was carried out in small petri dishes. Ten nauplii were transferred with a Pasteur pipet into each dish. After that, the dishes were filled with 10 ml of the respective concentrations of the toxicant, and incubated at a temperature of 25 ±1 °C for 24 hr. Then, the petri dish was placed on the stage of the dissection microscope and estimate mortality of 10 larvae were transferred and recorded. The nauplii were considered dead if no movement of the appendages was observed within 10 sec. After that, the percentage mortality was calculated from the total number of dead larvae for each concentration.

Results and Discussion

The respective 24-hour mortality values of *A. salina* nauplii for TBTCl are shown in Figure 1, which presents the relationship between mortality rates and increasing concentration of TBTCl respectively. LC_{50} values (X) as determined by the Probit (Y)

in this case of TBTCl concentrations were 400 ng.l^{-1}, 420 ng.l^{-1}, 450 ng.l^{-1}, 455 ng.l^{-1}, 460 ng.l^{-1}, 465 ng.l^{-1}, 470 ng.l^{-1}, 475 ng.l^{-1}, 480 ng.l^{-1}, 485 ng.l^{-1}, 490 ng.l^{-1}, 500 ng.l^{-1}, respectively. Upper and minimum limits of LC_{50} values of TBTCl were 420 ng.l^{-1} and 490ng.l^{-1}. LC_{50} values for TBTCl determined in the present study is 470 ng.l^{-1}. These results indicate that in this system TBTCl is toxic.

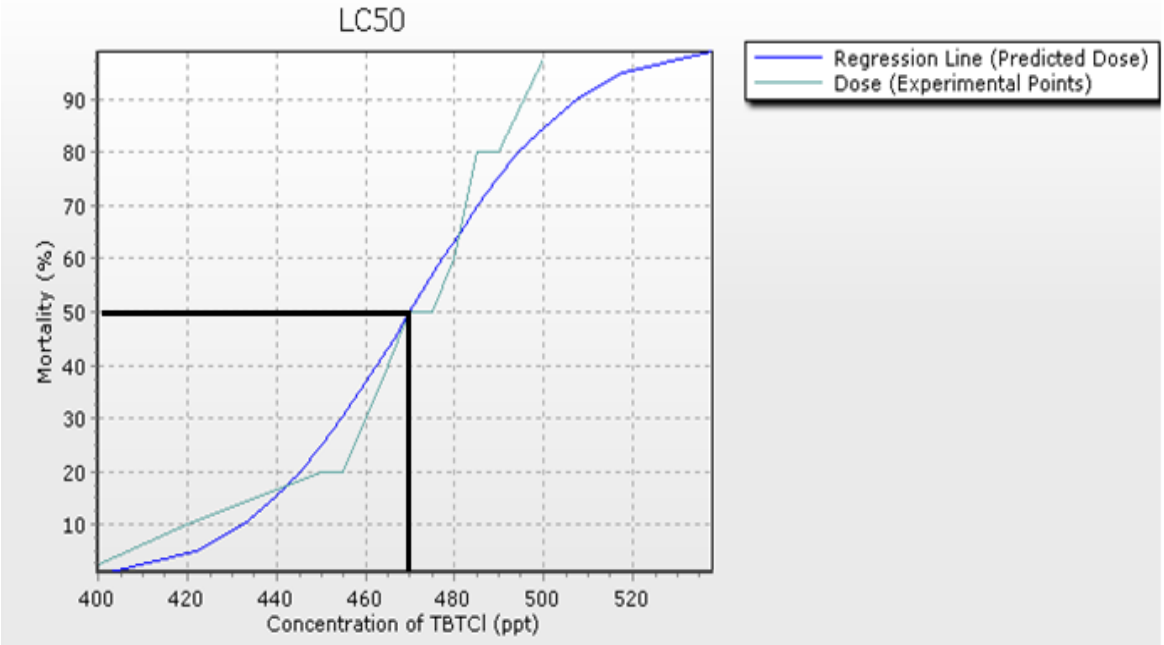

Figure 1: Relationship between mortality rates and increasing concentrations of TBTCl.

In the mid-1960s, the tributyltin based antifouling paints were widely used because they were proved more effective. Some years later, it was found that TBTCl had harmful effects to non-targeted aquatic organisms. Measurements of TBTCl concentration in U.S. water since 1991 indicate that there has been no risk of acute toxicity to aquatic organisms since 1994 and risk of chronic toxicity is considered as low as at 1996 levels (Cardwell *et al*., 1999). Based on these findings there is a strong concern to use TBTCl. Therefore, must be find alternatives as less hazardous than TBTCl. Also, any alternatives to TBTCl must be thoroughly evaluated before concluding that it is less hazardous. The results of the present study may add some information towards this direction, clarifying the higher magnitude of TBTCl toxicity. Furthermore, this study will add-up information on the potential of nauplii of *A. salina* for bioassays of biocides.

Conclusion

LC50 values for TBTCl determined in the present study is 470 ng.l^{-1}. These results indicate that in this system TBTCl is toxic; however, TBTCl is proven environmentally toxic substances. As it was earlier stated, acute toxicity alone does not give enough information about the environmental impact of using such an antifouling agent.

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Litter production in relation to some climatic variables in a mangrove ecosystem of Sarawak, Malaysia

*Mohammed Muzammel Hoque[1], Abu Hena Mustafa Kamal[1], Mohd Hanafi Idris[1], Osumanu Haruna Ahmed[2] and Md. Masum Billah[1]

[1]*Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia*
[2]*Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia*
**Corresponding author.Tel.:+6016 8767480; email address: hoque_doe@yahoo.com*

Introduction

Mangroves are the world's most productive ecosystems (Nagarajan *et al*., 2008), which are frequently inundated by tidal action. During this action numerous materials and nutrients are exchanged to the nearby ecosystems (Ye *et al*., 2011). Generally, litter production provides energy via increasing soil organic matter and is the indicator of forest fertility. This litter production usually varies from place to place and species to species. Apart from this, the variability of litter production is also influenced by climatic variables. Unexpectedly, few studies of litter production in tropical forests emphasize on climate variables (Custodio-Filho *et al*., 1996). Although Sarawak is endowed with numerous pristine estuarine mangroves, studies on the ecological roles especially on primary production are less, which leads to incomplete understanding of mangrove functions in this region. Therefore, this study was carried out to investigate the species and temporal patterns of litterfall production of Kuala Sibuti mangrove, Sarawak, Malaysia in relation to few climatic variables. Findings of this study would help to understand the productivity and roles of an undisturbed mangrove of Sarawak, which, will be useful for sustainable conservation and management of this forest in future.

Materials and Methods

The study area, Kuala Sibuti mangrove (3°59´25.76´´N and 113°43´51.6´´E) is located at the edge of South China Sea, Sarawak, Malaysia. The forest is dominated by *Rhizophora apiculata* and class-4 (Watson 1928). From the river bank to inner side, the whole forest is almost flat with very little increase in elevation at the edge of the forest. Three sampling plots, each 100 m x 100 m (river estuary, middle and last part of the forest) were selected considering the structure, density and topography of the forest to represent the whole forest. Twelve (12) square litter traps of 1 m x 1 m mouths (4 in each sample plot) were fixed randomly under the forest canopy. The trapped litterfalls were collected at monthly interval from January to December 2013. The litterfalls of individual trap were sorted into leaves, twigs, stipule, flower, propagules and others, and air dried for 24 hours. The air dried litterfall components were then oven dried at 80°C until get the constant weight and weighted. Monthly climate data of the study area (Jan-Dec 2013) was collected from the nearest meteorological station. Seasonal variation (Intermediate: Jan-Apr; Dry: May-Aug; Wet: Sep-Dec) of total and species wise different components of litterfall production

were compared by two way analysis of variance (ANOVA) followed by DMRT using SAS 9.2 version. Pearson's correlation coefficient analysis was performed to determine the influence of climatic variables (temperature, wind speed and rainfall) on total as well as various components of yearly litterfall production.

Results and Discussion

Except for few months, the overall total litterfall production of Kuala Sibuti mangrove was not fluctuated throughout the year. Usually, the mangrove *R. apiculata* showed significant seasonality in litterfall production in many mangrove forests of the world (Bunyavejchewin and Nuyim, 2001). However, in contrast, it was not observed for the litterfall production (total and different components) for both the *R. apiculata* and *Xylocarpus granatum* species in Kuala Sibuti mangrove forest (Table 1). This could probably be due to geographical position of the study area like tropical climate, where climatic variations are very negligible. A total of 1640.82 g/m^2 dry weights of litterfall consisting of leaves, stipules, flowers, propagules, twigs and others was produced annually (Table 1). Leaves were the highest contributory components (57.21%) followed by propagule (11.89%), flower (10.85%), twigs (8.56%) and stipules (8.45). Out of the total litterfall production, *R. apiculata* alone contributed 92.94% followed by *X. granatum* (4.01%) and others (3.05%). Flowering of *R. apiculata* was found profound in dry season especially in June-August, and following the sequence of flowering, fruiting of *R. apiculata* was observed highest in wet season. The total litterfall production of Kuala Sibuti mangrove was highest compared to other mangroves in Malaysia (Table 2). The higher production of litterfall in Kuala Sibuti mangrove could probably be due to pristine condition of the forest, tree maturity and/or frequent inundation by adequate fresh water flow (Lugo and Snedaker, 1974).

Table 1: Seasonal (g/m^2/season±SE) and annual total litterfall production (g/m^2/yr) of various components of dominant and co-dominant species of Kuala Sibuti mangrove.

		Litter Components					
Species	**Season**	**Leaf**	**Stipule**	**Flower**	**Propagule**	**Twigs**	**Total**
R. apiculta	Inter	342.39±0.76^{a}	51.57±0.56^{a}	37.17±0.49^{b}	39.03±0.55^{b}	61.53±0.71^{a}	531.69±0.98^{a}
	Dry	302.63±0.73^{ab}	37.17±0.58^{a}	86.80±0.64^{a}	30.94±0.61^{b}	42.92±0.48^{a}	500.46±1.11^{a}
	Wet	248.90±0.66^{b}	49.90±0.67^{a}	53.80±0.65^{ab}	112.70±0.62^{a}	27.61±0.40^{a}	492.90±0.62^{a}
Total (yearly)		893.92±0.29	138.63±0.20	177.77±0.23	182.66±0.28	132.06±0.20	1525.05±0.31
X. granatum	Inter	16.47±0.25^{a}	0	0.20±0.08^{a}	9.24±0.43^{a}	2.00±0.16^{b}	27.91±0.50^{a}
	Dry	16.30±0.31^{a}	0	0	3.22±0.29^{a}	5.43±0.09^{a}	24.95±0.29^{a}
	Wet	11.95±0.32^{a}	0	0	0	0.96±0.07^{b}	12.91±0.33^{a}
Total (yearly)		44.73±0.10	0	0.20±0.02	12.45±0.12	8.39±0.06	65.77±0.14
Other species							50.00
Grand total							1640.82

Means within a column for each species with different superscript letters are significantly different (n= 48, Duncan Multiple Range Test) (P<0.05)

Except rainfall (101.3-691.2 mm), temperature (27.95-28.95°C) and wind velocity (1.8-2.52 kph) of the study area were not fluctuated annually. The total and major

components of litterfall production of both species were not significantly influenced by the climate variables (Table 3).

The insignificant influence of climate variables on overall litter productivity indicates that the higher productivity of this forest may be due to the other influential variables like, tree stand density, maturity, pristine in nature, vegetation heredity and nutrients availability in this mangrove ecosystem.

Table 2: Comparison of litterfall production estimates of *R. apiculata* from Malaysian mangroves.

Species	Location	Latitude	Dry wt of litterfall ($g/m^2/yr$)	Source
R. apiculata	Sungai Merbok, Malaysia	5°40´N	1007.40	Ong *et al*., 1980
Rhizophora sp.	Kuala Selangor, Malaysia	3°15´N	1576.80	Sasekumer and Loi, 1983
R. apiculata (Planted)	Matang Mangrove, Malaysia	4°50´N	697.15-1138.8	Gong *et al*., 1984
R. apiculata	Matang Mangrove, Malaysia		762.85	Gong *et al*., 1984
R. mucronata + *R. apiculata*	Siar Beach, Lundu, Sarawak	1°45´N	572.00	Saberi, 1989
R. apiculata+*X. granatum*+ Other species	Kuala Sibuti Mangrove, Sarawak	3°59´N	1640.82	This study

Table 3: Pearson correlation coefficient and significance level for total and various components of litters with climatic variables of Kuala Sibuti mangrove, Sarawak.

Species	Components	Mean temp (°C)	Rainfall (mm)	Wind speed (Kph)
R. apiculata	Leaf	0.548^{ns}	-0.017^{ns}	-0.179^{ns}
	Stipule	-0.049^{ns}	0.345^{ns}	-0.345^{ns}
	Flower	0.303^{ns}	-0.622*	0.354^{ns}
	Propagule	-0.38^{ns}	0.317^{ns}	0.144^{ns}
	Twigs	0.127^{ns}	-0.372^{ns}	-0.43^{ns}
	Total Litter	0.204^{ns}	-0.063^{ns}	-0.078^{ns}
X. granatum	Leaf	0.213^{ns}	-0.282^{ns}	-0.158^{ns}
	Propagule	0.487^{ns}	0.118^{ns}	-0.228^{ns}
	Twigs	0.668*	-0.624*	0.243^{ns}
	Total Litter	0.538^{ns}	-0.231^{ns}	-0.167^{ns}

** Significant at P<0.01; ns; not significant at P>0.05; *significant at P<0.05; for litterfall components (leaf, stipule, flower, propagule, twigs) of each species, n=144; for climatic variables, n=12.

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Determination of trophodynamic structure in the mangrove area of Tanjung Kupang, Malaysia: in sight of stable isotope analysis

Munirah Hanapiah[1], *Syaizwan Zahmir Zulkifli[1], Aqilah Mukhtar[1], Ferdaus Mohamat-Yusuff[2,3] Amirul Azuan Md Joni[1,3] and Ahmad Ismail[1]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
**Coressponding author.Tel.:+603 89466627; email address: syaizwan@upm.edu.my*

The Malaysian coastal water area comprises of various ecosystems including the mangrove ecosystem. Nowadays, many areas have been reclaimed and developed due to high demand from community and industries. Deteriorating environmental health in a particular area will indirectly affect the stability of the existing food web. Current study aimed to investigate the existing trophodynamic structure in the mangrove area of Tanjung Kupang using stable isotope analysis. A total of 40 samples was collected from the area. All the samples had undergone stable isotope preparation method and was analyzed by the used of CF-IRMS to obtain dual isotope signatures ($\delta^{13}C$ and $\delta^{15}N$). Animal species had $\delta^{13}C$ values ranging from ~-27.02 to -14.83 ± 0.54‰ meanwhile for plants part range from ~-30.17 to -19.89 ± 1.04‰ meanwhile, $\delta^{15}N$ value reflects ~4.09 to 15.89 ± 0.56‰ and 3.00 to 5.42 ± 0.22‰ for animal and plants, respectively. Calculation of trophic level estimation (TLE) and Discriminant Analysis (DA) could be used to strengthen the trophic positioning of a species. In coming years, further in depth investigations should be conducted to create a comprehensive trophodynamic structure of food web in the area. This preliminary study is important in planning strategies for better management and conservation of mangrove ecosystem important bioresources who rely on the area.

Keywords: Trophodynamic structure, stable isotope analysis, food web, mangrove ecosystem, trophic level

Distribution of heavy metals (Cd, Cu, Fe, Ni, Pb, and Zn) in the different parts of *Asystasia gangetica* in Kg. Ayer Hitam, Sg. Kembung, and Chukai, Peninsular Malaysia

Chew, W. Y.[1], *Yap, C. K.[1], Umi Kalsom, Y.[1] and Zakaria, M. P. [2]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia*
[2]*Centre of Excellence for Environmental Forensics, Faculty of Environmental Studies*
**Corresponding Author. email address: yapckong@hotmail.com*

Introduction

Heavy metals pollution in Malaysia is mostly contributed from the urban activities and urban wastes. As development in Malaysia increase rapidly, heavy metals pollution is raising health concerns to the public. Hence, in this study, we are looking for an alternative monitoring method of which it could be cost saving and at the same time give us better heavy metals pollutions data in the surrounding area. Therefore, *Asystasia gangetica* was selected as biomonitor of heavy metals pollutions. *Asystasia gangetica* (L.) Anders. (Subspecies micrantha) is a native herb from India. It was brought in to Malaya during British colonial period as a cover crop in rubber plantations (Kiew and Vollesen, 1997). However, this species has become invasive weed in Malaysia. Nowadays, this plant is clearly visible along the roadside of urban and rural areas. This plant was selected for heavy metals pollutions study as a biomonitor in Peninsular Malaysia for its population abundance, for it is easily recognised and for the advantage it holds as sessile organisms. The objective of this study is to determine the heavy metal concentrations in different parts of the plant in different sites with various heavy metals pollution input.

Material and Methods

The sampling of the target species was collected during 26 June until 23 July, year 2011, in Kg. Ayer Hitam (Selangor), Sg. Kembung (Selangor), and Chukai (Terengganu). The samples was brought back to lab and separated into different parts, namely Pericarp, Seed, Flower Stalk, Leaf, Stem, and Root. All samples were washed thoroughly prior to the analysis. However, in order to study the influences of the atmospheric depositions in the plant heavy metals uptake, the leaf was divided into washed and unwashed. Heavy metals determination was done using air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. Data collected was further analysed statistically.

Results and Discussion

In this study, the heavy metals concentrations (µg/g, dry weight) ranging from 0.02 – 1.44 for Cadmium, 3.81 – 33.14 for Cu; 17.78 – 1359.862 for Fe; 0.03 – 3.90 or Ni; 0.18 – 14.43 for Pb; and 9.18 – 135.35 for Zn.

The plant samples gathered in Sg. Kembung Landfill site was found generally to be higher metal concentrations as compared with other sites except for Fe concentration in root collected from Kg. Ayer Hitam (849.66 µg/g), Pb concentrations

Table 1: Heavy metal concentrations of plant samples by parts (Dry, µg/g).

Place	Organs	Cd			Cu			Fe			Ni			Pb			Zn		
		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM	
Kg. Ayer Hitam		0.09	0.06	a	9.64	0.46	b	18.10	0.32	a	0.70	0.20	a	0.63	0.19	a	10.12	0.29	a
Sg. Kembung	Pericarp	0.04	0.01	a	11.75	0.52	c	31.57	2.78	b	1.85	0.19	b	0.30	0.12	a	15.35	1.28	b
Chukai/Kemaman		0.93	0.05	b	6.80	0.16	a	26.17	1.69	ab	0.40	0.07	a	1.12	0.26	b	9.84	0.66	a
Kg. Ayer Hitam		0.10			17.10			21.80			3.90			1.90			25.30		
Sg. Kembung	Seed	0.04	0.01	a	21.75	1.58	b	33.89	1.71	a	0.50	0.45	a	1.21	0.54	a	34.74	1.02	b
Chukai/Kemaman		1.05	0.06	b	12.86	0.05	a	26.30	0.12	b	1.48	0.59	b	1.75	0.11	b	26.26	0.30	a
Kg. Ayer Hitam		0.19			9.27			30.29			1.10			2.53			24.45		
Sg. Kembung	Stalk	0.17	0.06	a	10.92	0.26	b	64.79	4.65	a	0.87	0.18	a	1.97	0.42	a	31.37	2.35	b
Chukai/Kemaman		1.25	0.10	b	4.05	0.24	a	55.81	6.60	b	0.78	0.13	b	2.01	0.72	b	19.89	0.22	a
Kg. Ayer Hitam		0.03	0.00	a	24.39	0.01	a	849.66	22.53	b	0.63	0.16	a	4.98	0.21	b	55.56	1.34	a
Sg. Kembung	Root	0.07	0.01	b	20.34	1.46	b	201.99	15.57	a	1.15	0.19	b	4.58	0.24	b	91.40	6.97	b
Chukai/Kemaman		0.03	0.00	a	11.53	0.31	c	196.94	1.51	a	1.41	0.24	b	2.43	0.16	a	65.41	1.81	a
Kg. Ayer Hitam		0.53	0.00	a	8.65	0.23	a	38.82	1.36	a	1.06	0.48	b	2.03	0.04	a	33.70	0.45	a
Sg. Kembung	Stem	0.52	0.03	a	10.02	0.23	b	81.08	2.14	b	1.00	0.13	b	2.42	0.73	a	52.61	0.15	b
Chukai/Kemaman		0.69	0.02	b	6.36	0.00	c	54.89	0.46	c	0.23	0.21	a	2.76	0.88	a	51.68	0.25	b
Kg. Ayer Hitam		0.03	0.00	a	12.83	1.59	a	83.89	9.16	a	1.54	0.04	a	7.95	0.66	a	29.93	3.01	a
Sg. Kembung	Leaf	0.92	0.02	b	16.18	0.66	b	287.93	11.68	b	3.25	0.57	b	6.08	0.50	a	51.36	0.36	b
Chukai/Kemaman		0.18	0.09	a	9.29	0.48	c	160.38	17.93	c	1.17	0.52	a	9.76	1.43	a	39.78	2.62	a

Note: Same alphabet at the side of the value indicates that there is no significant difference between sites of the similar parts.

in leaf collected from Chukai (9.76 µg/g). This indicates that plants samples from landfill generally have higher concentrations of heavy metals than the other sites. We speculated that this was due to the uptake of heavy metals from soil contaminated by heavy metals.

Based on Cd accumulation patterns, leaf generally showed lower concentrations than the other parts except for Sg Kembung landfill. Plants accumulation patterns showed that flowering and fruiting body have lesser Fe, Pb and Zn uptake than leaf, root, and stem in general. Cu accumulations patterns maintain consistent patterns of Root > Leaf > Stem for all sites. Sg. Kembung's plant samples displayed higher heavy metals concentrations (Cd, Fe, Ni, and Zn) in leaf than other parts, which was found comparatively different from the other sites. Evidently, Sg. Kembung was found to have the highest heavy metals pollution in soil. The plant may have redistributed heavy metal contaminants to the leaf parts in order to adapt to the high metal enrichment in their surrounding habitat (Peralta-Videa *et al.,* 2009).

Chukai and Sg, Kembung were found to have higher leaf to stem ratio in Ni concentration compared with Kg. Ayer Hitam. This was due to the Chukai plants were sampled near the busy traffic road. Thus, we speculated that vehicular activities cause the increased surface dust deposition on leaves. Therefore, it contributed to the uptake of the Ni into the leaves since Ni is one of the main component in car (Meza-Figueroa *et al*., 2007). Ni concentration in stem was found very low compared with other parts. Hence, translocation of Ni may not be the main pathway of the Ni uptake in Leaf. However, Sg. Kembung plant samples indicated that the main pathway of Ni uptake in leaf was due to translocation of Ni from root (Bu-Olayan and Thomas, 2009). Stem and soils collected from Sg. Kembung were found to have higher Ni concentrations.

Conclusion

Plant samples from Sg. Kembung are found to have high heavy metals concentrations due to the contaminations from the landfill. Where, plant samples from Chukai have higher concentrations of heavy metals in leaf due to the uptake of heavy metals from surface dust depositions from vehicular activities. In general, *A. gangetica* can be very useful to monitor the heavy metals concentrations in the surroundings area. This plant is a good potential candidate as a biomonitor of urban pollutions. However, further studies still needed.

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Interlinkage among primary production, phytoplankton abundance, chlorophyll and environmental conditions of a mangrove estuary in Sarawak, Malaysia

*Saifullah A. S. M., Abu Hena Mustafa Kamal, Mohammad Hanafi Idris and Amy Halimah Rajaee

[1]Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road , P.O. Box 396,Bintulu 97008, Sarawak, Malaysia

**Corresponding author. email address: saifullahasm@yahoo.com*

Introduction

Mangrove estuaries are claimed to be productive and also important for breeding and nursery ground of fishery resources. There exists a broad relationship between the productivity and the availability of resources like phytoplankton in the estuarine ecosystems (Saifullah *et al*., 2014). This paper aims to ascertain the existing links among primary production, phytoplankton abundance, chlorophyll *a* content and environmental parameters in the Kuala Sibuti (KS) mangrove estuary. The outcome of this study would be able to unveil another dimension for appraising ecological pathway of a mangrove estuary in tropical region.

Materials and Methods

The Kuala Sibuti (KS) mangrove estuary is located in tropical region of Malaysia. The mangrove estuary is margined both sides by *Rhizophora apiculata* and *Nypa fruticans*. Samplings were done from January 2013 to December 2013 in three seasons *viz.* intermediate (January to April), Dry (May to August) and wet (September to December) in three stations named stations 1, 2 and 3 located at 3°59´25.76´´ N and 113°43´51.6´´E; 3°58'53.5´´N and113°44'12.12´´ E and 3°59'22.24´´ N and 113°44'35.33´´E, respectively. *In situ* observation of environmental parameters (pH, salinity, TDS and dissolved oxygen) were done using a water quality meter (WQC-24) with three replicates of readings for each parameter. Water transparency was measured using the Secchi disc and light attenuation coefficient was calculated. Primary production was estimated using light and the dark bottle method (Strickland and Parsons, 1972). Water samples were collected in triplicate for chlorophyll *a* estimation (Coombs and Hall, 1982), water nutrients viz. nitrate, phosphate and ammonium were determined following Parsons *et al*. (1984), Kitamura *et al*. (1982) and Weatherburn (1967), respectively. Sampling of phytoplankton was carried out from the surface water by towing a phytoplankton net (mesh size of 20µm; mouth diameter 0.35m) and numerical analysis was done using a Sedgwick rafter cell counter under a compound microscope. Statistical analyses were conducted using CANOCO version 4.50 SAS 9.1.

Results and Discussion

Physical, chemical and biological parameters of this estuarine water showed a seasonal rhythm (Figure 1 a-k). Surface water temperature ranged from 27.4°C to 32.2°C with a range of salinity from 0.70 PSU to 27.10 PSU followed by Dissolved

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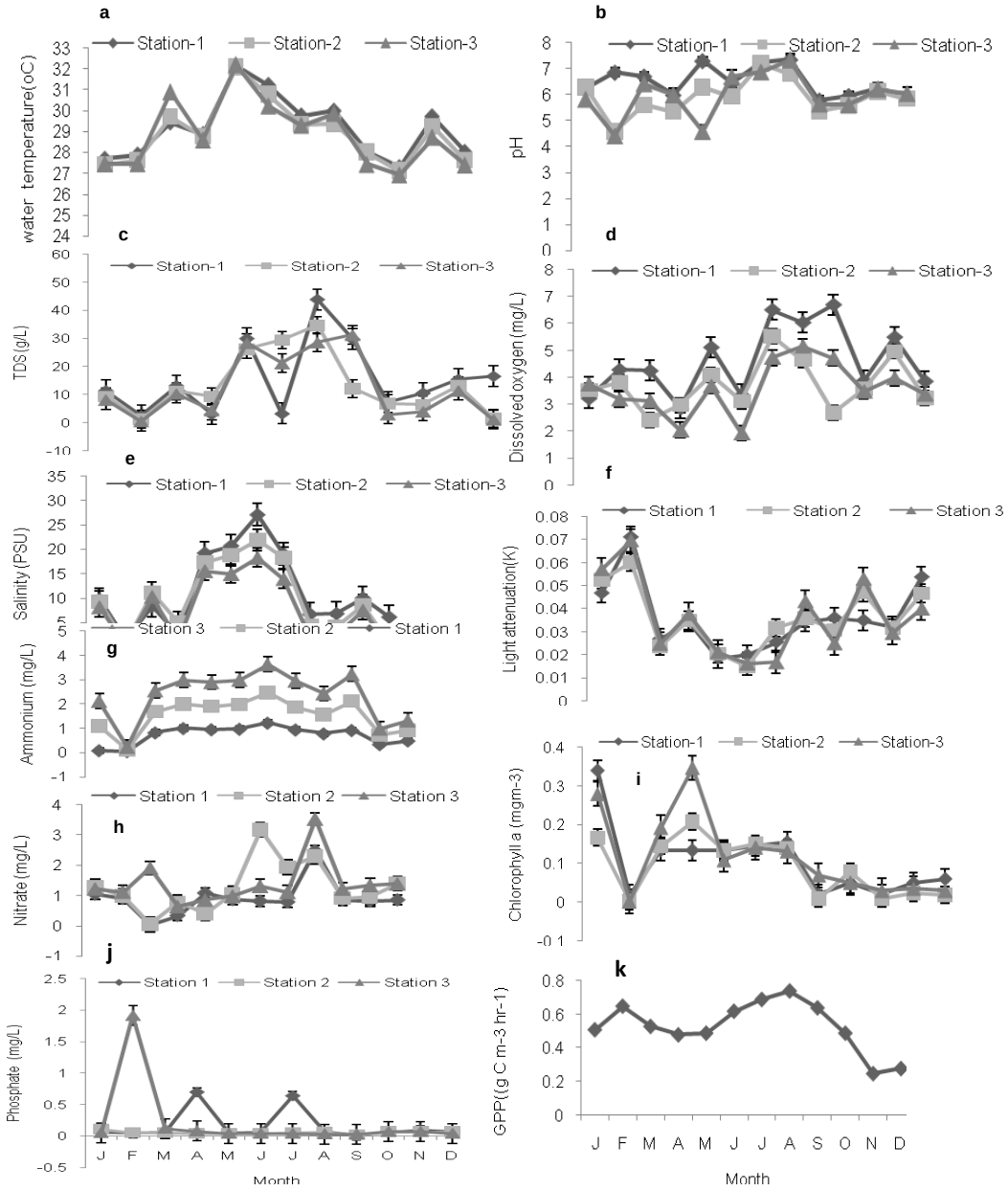

Figure 1 (a-k): Seasonal variation (±SD) of (a) water temperature; (b) pH; (c) TDS; (d) DO; (e) salinity; (f) Light attenuation; (g) ammonium; (h) nitrate (i) chlorophyll a (j)Phosphate and (k) GPP in Kuala Sibuti mangrove estuary.

Oxygen (DO) from 1.94 to 6.71 mgL^{-1}. The concentration of chlorophyll *a* ranged from 0.02 to 0.16 mg m^{-3}. Nitrate, phosphate and ammonium concentrations ranged from 0.40 to 3.53 mgL^{-1}, 0.01 to 1.92 mgL^{-1} and 0.06 to 1.24 mgL^{-1}, respectively. The gross primary production (GPP) showed little seasonal variation with the highest peak in dry season (Figure 1 k) with the mean of 0.52±0.14$gCm^{-3}hr^{-1}$. The mean abundance of phytoplankton was found 53240 $cellsL^{-1}$ with significant seasonal variation and highest abundance was found in the dry season (75400 $cellsL^{-1}$). Chlorophyll *a* concentration and gross primary production showed a positive

correlation (r=0.73) and phytoplankton abundance showed a positive correlation (r=0.78) with GPP.

Pearson correlation coefficients showed significant positive correlation between nitrate and TDS (r=0.83), nitrate and pH (r=0.99), ammonium and salinity (r=0.60), water temperature and salinity (r=0.74), temperature and TDS (r=0.71), phosphate and light attenuation (r=0.58). ANOVA showed the seasonal variation among environmental parameters, nutrients and Chlorophyll *a* content. The concentration of chlorophyll *a* that found in KS mangrove estuary showed similar value that of Kaduviyar estuary in India (Perumal *et al*., 2009).

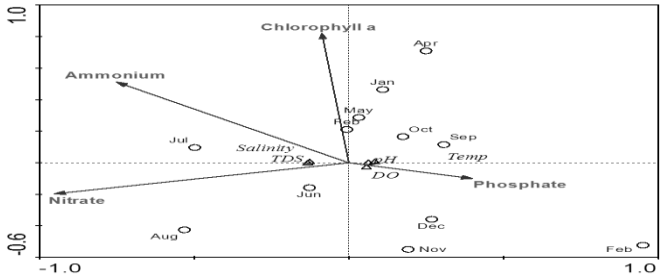

Figure 2: Canonical correspondence analysis (CCA) of the physico-chemical parametersalong with chlorophyll *a* and nutrients at KS mangrove estuary.

Canonical correspondence analysis (CCA) showed a significant interaction between ammonium distribution and TDS; salinity and phosphate distribution showed a poor relation with DO, whereas, chlorophyll *a* showed a poor relation with salinity and TDS. The association of nutrients and chlorophyll *a* distribution in the KS mangrove estuary agreed with the findings of Perumal *et al*. (2009). The KS was found mesohaline in nature (Van Damme *et al*., 2005) considering its salinity gradients.

Conclusion

The KS mangrove estuary was found productive in terms of nutrients content and chlorophyll *a* biomass. There was interactive relation among nutrient content, phytoplankton abundance, chlorophyll a content and primary production in the estuary with some seasonal variation.

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Greenhouse gas emissions during the agricultural stage of palm oil-based biofuel production associated with land use changes

*Faradiella Mohd Kusin[1,2], Nurul Izzati Mat Akhir[1], Wong Siew Yien[1], Ferdaus Mohamat-Yusuff[1,2], Muhamad Awang[3], Farahana Hamka[1]

[1]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang*
[2]*Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
[3]*SEGi University, Kota Damansara, 47810 Petaling Jaya, Selangor, Malaysia*
**Corresponding author.Tel. +6038946 8596; email address: faradiella@upm.edu.my*

Introduction

The environmental aspect and impact associated with the process and utilisation of biofuel production to support the sustainability of agro-based energy industries have become the interest of many relevant sectors. Biofuel production from palm oil has been promoted and initiated in Malaysia since a few years back so as to sustain energy production using alternative source of bio-energy and to reduce the dependency on fossil fuel. Environmental impacts in terms of energy balance and GHG balance would be of great concern when addressing this issue with regard to sustainability of palm oil-based biofuel production (Siangjaeo *et al*., 2011). Generally, it is known that land use changes (often related to carbon stock changes), oil palm plantation (agricultural stage) and palm oil extraction phases significantly contribute to the emissions of greenhouse gases over the life cycle of palm oil production. The GHG emissions from palm oil production have generally been categorised as the emissions arising from operations during oil palm growing and fresh fruit bunch (FFB) processing (i.e. emissions related to the use of fertiliser, use of fuel for internal transportation, use of fuel in palm oil mill and emissions from palm oil mill effluent), and the emissions arising from carbon stock changes (i.e. during the development of new plantation and during the operations of plantations (Brinkman Consultancy, 2009; Klaarenbeeksingel, 2009). In this study, we focused on the emissions arising during the agricultural stage of palm oil production (primarily due to synthetic fertiliser application) by experimentally determining the contribution of fertiliser-related emissions.

Materials and Methods

The study was undertaken at three different sites according to the land use changes of the oil palm plantation. The soil samples were collected from Kempas Estate (transformed land use, large-scale), UPM oil palm plantation (transformed land use, small-scale) and Chepor Estate (logger-over forest). The soil sampling was conducted for the three estates according to the age of the palms. Soil samples were collected for analysis of N, P, K contents and soil organic carbon (and organic matter). The emissions from oil palm plantation are determined from relevant substances, i.e. the N, P and C over the life cycle of oil palm (Schmidt, 2010). In this study, the emissions are expressed in unit kg CO_2-eq/ha for ease of comparison. The amount of nitrogen fertiliser applied was used to calculate direct

N_2O emission based on the model described in the Intergovernmental Panel on Climate Change (IPCC) incorporating peat soil which is relevant in Malaysia and Indonesia (Schmidt, 2007). The N_2O emissions were converted into CO_2-eq using the Global Warming Potential of the gas.

Results and Discussion

The major inputs of GHG emissions during agricultural stage were the emissions from the fertiliser application (i.e. N and P-related emission) and the emission from fuel use, i.e. transportation use within the estate. The emission from fuel was estimated based on the observed types of tractors used during the transportation of oil palms within the estate, i.e. fuel capacity, distance travelled and fuel use. 249.24 kg CO_2 and 289.5 kg CO_2 emission have been estimated from the fuel use at Chepor Estate (logged-over forest) and UPM plantation (transformed land use), respectively. The contribution of CO_2-eq emission from fertiliser application was taken as the average emission from oil palms of different ages. The contributions of CO_2-eq emission from major inputs of GHG emissions during plantation stage of palm oil production are shown in Figure 1. The overall CO_2-eq emission from fertiliser use, i.e. the N and P-related emissions for both types of land use change scenarios contribute to more than 80% of the plantation GHG emissions; although P-related emission contribution is only about 1%. The contribution from fuel CO_2-eq emission accounts for 16-19 % of the plantation GHG emissions. In total, relatively higher CO_2-eq emissions were emitted from the logged-over forest compared to the transformed land use estate.

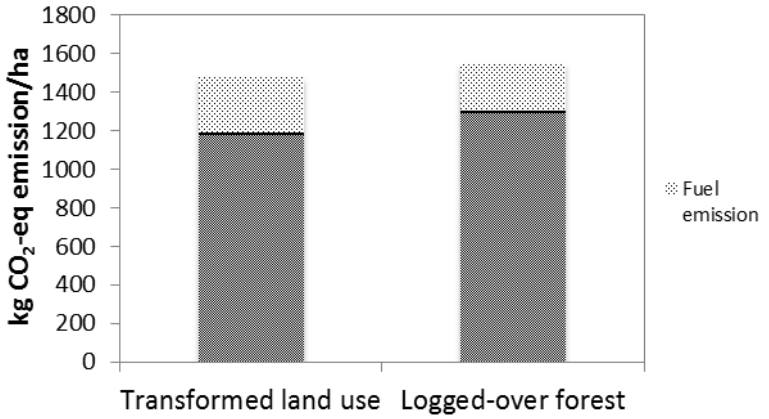

Figure 1: Contribution of CO_2-eq emission from major inputs of GHG emissions during agricultural stage of palm oil production for different land use changes The results obtained from this study were compared to those of the reported values for Malaysian oil palm plantation in general (e.g. Schmidt, 2010). In this study, we found 249-289 kg CO_2 emission from the internal fuel use, and 1027-1430 kg CO_2-eq/ha from artificial fertiliser use within the plantation.

Conclusion

The results from this study have highlighted the contribution of GHG emissions from oil palm plantation of different category of age and with associated land use changes. The findings could be of useful contribution to site-specific cases of GHG emissions

with regard to oil palm plantation development for biofuel production. Field verification was conducted wherever possible to help strengthen the findings on GHG emissions and the carbon stock changes at respective sites. It was noted that contribution from field nitrous oxide emission constitutes the largest portion of GHG emissions among the major inputs of GHGs during the agricultural stage of oil palm development. Generally, it was found that the conversion of tropical forest into palm plantation has resulted in relatively higher GHG emissions compared to transformed land use for oil palm development. Additionally, the N and P-related emissions also correspond with the types of land use change, i.e. greater amount of the emissions were found from the logged-over forest compared to the transformed land use. Therefore, in support of sustainable biofuel production from such a developing country, future development may need to incorporate the types of land use changes prior to plantation development.

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Elemental speciation, bioavailability and risk assessment of selected heavy metals in the intertidal surface sediments of Sungai Puloh mangrove, Malaysia

Bede Emeka Udechukwu, *Ahmad Ismail, Syaizwan Zahmir Zulkifli and Hishamudin Omar

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

**Corresponding author.Tel.:+6012 2033727; email address: aismail@upm.edu.my*

Introduction

The mangrove ecosystem is one of the world's major productive ecosystems because they are key ecological habitats that link terrestrial and marine environments (Vane *et al*., 2009). The intertidal mud flats of this ecosystem sustain a good diversity of marine organisms including mudskippers, gastropods, crabs, barnacles, rodong shell, mullet, mussels monitor lizard, migratory shorebirds and aquaculture products (principally fish and prawn) which are commonly consumed in South-East Asia (Bayen *et al*., 2005; Hashim *et al*., 2010).

However, today's mangrove ecosystem has been subjected to intense and continuous chemical anthropogenic inputs resulting from increased urbanization and industrialization. Among the major chemical contaminants from anthropogenic inputs are heavy metals (MacFarlane, 2002). Therefore this study is aimed to (1) quantify the speciation of Cd, Ni, and Zn (2) identify the metals with higher percentage of anthropogenic origin and evaluate the possible bioavailability in the food web of Sungai Puloh intertidal mangrove ecosystem.

Materials and Methods

The study area (Sungai Puloh: N 03° 04.786′, E 101° 23.903′) is located in the state of Selangor, west coast of Peninsular Malaysia. It stretches about 6.87km in length. The industries within the vicinity of this mangrove involve various types such as scrap metal yards, recycling sectors, power plant, automobile workshops, oil palm mills and aquaculture.

Surface sediment samples were collected in March, 2012 from 14 sampling stations which were categorized into four site groups (I, II, III, and IV) based on possible anthropogenic activities along the intertidal mangrove area.

The Samples were dried by using air-circulating oven to a constant dry weight at 80°C, and the Aqua regia method as described by (Ismail,1993; Ismail and Ramli, 1997) was used for pseudo total sediment digestion. Metal speciation was investigated by using Sequential Extraction Technique (SET) (Table 1) as described by Badri and Aston (1983) and Tessier and Campbell (1987).

After digestion and filtration, the samples were analyzed by using an air-acetylene flame Atomic absorption Spectrophotometer (Analyst 800 model, by Perkin-Elmer) and the data are presented in dry weight basis (µg/g dry weight). The statistical analyses were performed using Statistical package for Social Science (SPSS) version 20.

Table 1: Extrancts used in each extraction stages and various phases of sediment in the sequential extraction scheme.

Extraction Stages	**Extrctrants**	**Sediment target phase**
Fraction 1	1.0 M NH_4CH_3COO, pH 7.0	Exchangeable
Fraction 2	0.2 M $NH_2OH.HCl$, pH 2.0	Acid- reducible
Fraction 3	H_2O_2 (35%), 1.0M NH_4CH_3COO, pH 2.0	Oxidizable - organics
Fraction 4	HNO_3 (69%), HClO4 (60%)	Residual

Results and Discussion

Metal Speciation patterns

Among these metals, the least residual fraction was observed in Zn (12.24%), followed by that of Cd (46.82%), and then Ni (48.32%). The speciation profile also revealed that Zn is more dominant in exchangeable (15.90%), and acid reducible (56.23%) fractions compared to other metals, Ni (36.76%) is more dominant in oxidizable organics fraction compared to other metals (Table 2). This suggests that the rate of bioavailability and remobilization is much higher for Zn, and Cd, than for Ni. Therefore, the rate of chances of remobilization for these heavy metals in this intertidal mangrove ecosystem is Zn > Cd > Ni.

Table 2: Mean percentages of four chemical speciation fractions for heavy metal in Sg. Puloh intertidal sediment.

Metals	**%EFLE**	**%Acid reducible**	**%Oxidizable organics**	**%Resistant**
Zn	15.9	56.23	15.62	12.24
Ni	4.05	10.87	36.76	48.32
Cd	11.94	18.65	22.59	46.82

The role of TOC and pH on metal partitioning and bioavailability

The relationship between phase distributions of metals, TOC, and pH are determined and shown in Table 3. For the exchangeable fraction the phase distribution of Ni (r = 0.899, $p < 0.01$, and Zn (r = 0.684, $p < 0.01$) were strongly correlated with TOC, while Cd (r = 0421, $p > 0.05$) showed weak positive correlation. Similarly, for acid – reducible fraction, all the examined metals showed strong positive correlation with TOC except for Zn (r = -0.15, $p > 0.05$) in this distribution phase. This result indicates that in the acid – reducible fraction, the distribution of Zn may not be affected by TOC. However, for the oxidizable – organics fraction, the distribution of all the

studied metals revealed significant strong positive correlations Cd (r = 0.836, p < 0.01), Ni (r = 0.938, p < 0.01), and Zn (r = 0.069, p < 0.01) with TOC.

The relationship between pH and the phase distributions of all the studied metals revealed that there is no significant correlation between the pH and the metals in all the fractions. This is suggestive that TOC has a more significant effect than pH on the distribution of elements among phases. It is also in agreement with that of Yuan *et al*. (2004) who concluded that the effect of pH on the distribution of elements in real sample analysis was not obvious.

Table 3: Correlation of non-resistant fractions of metal speciation with TOC and pH in Sg. Puloh surface sediment.

		Cd	**Ni**	**Zn**	**TOC%**	**pH**
Exchangeable fraction	Cd	1				
	Ni	0.552[b]	1			
	Zn	0.706[a]	0.811[a]	1		
	TOC	0.421	0.899[a]	0.684[a]	1	
	pH	-0.390	-0.358	-0.279	-253	1
Acid- reducible fraction	Cd	1				
	Ni	0.504	1			
	Zn	0.389	0.200	1		
	TOC	0.682[a]	0.820[a]	-0.15	1	
	pH	-0.704	0.182	0.477	-0.253	
Oxidizable- organics	Cd	1				
	Ni	0.792[a]	1			
	Zn	0.562[b]	0.670[a]	1		
	TOC	0.836[a]	0.938[a]	0.692[a]	1	
	pH	-0.201	-0.279	0.429	-0.253	1

[a]*correlation is significant at the 0.01 level;* [b] *correlation is significant at the 0.05 level*

Risk Assessment

The risk assessment code (RAC) as shown in Table 4 indicate that surface sediments which can release in exchangeable phase (i.e. fraction 1) less than 1% of the total metal will be considered safe for the environment. However, surface sediment releasing more than 50% of the total metal in the same fraction is considered to be of extreme risk to the environment and can easily be transferred from one trophic chain to another (Davutluoglu *et al*., 2010). The metal speciation revealed that the percentage content of metals in the exchangeable phase ranged from 5.14 - 23.46% for Cd, 1.50 – 6.33% for Ni, and 1.29 – 38.02% for Zn. The RAC was applied to this study to determine the element/s that may pose a threat to food

chain in this mangrove ecosystem. An average percentage content of and 4.05% for Ni exist in the exchangeable fraction. Therefore, nickel falls into the low risk category which means that there is a low possibility that Ni may enter the food chain. Finally, an average percentage metal content of 11.94% for Cd and 19.90 for Zn exist in the exchangeable fraction. Therefore, cadmium and zinc come under the medium risk category, based on the RAC, it suggests that these metals may have a high possibility to be remobilized and thus becoming available to aquatic biota. Cadmium is a non-essential element which can posed serious problem to any ecosystem (Davutluoglu *et al*., 2010), therefore any trace of it in the exchangeable fraction should be of ecological concern.

Table 4: Risk assessment

Risk assessment Code (RAC)	**Criteria (%)**
No risk	<1
Low risk	1-10
Medium risk	11-30
High risk	31- 50
Very high risk	>50
Metal	**Exchangeable fraction (%)**
Cd	5.14 - 23.46
Ni	1.50 – 6.33
Zn	1.29 – 38.02

Conclusion

Based on the speciation profile, the probability of bioavailability and remobilization of these metals in Sg. Puloh mangrove surface sediments follow the following pattern: Zn > Cd > Ni. This study further revealed that the effect of TOC on the distribution and partitioning of heavy metal is more pronounced than that of sediment pH. Risk assessment showed that there is low possibility for Ni to enter into the food chain. However, there is a medium to high risk that Cd and Zn may enter the food chain in Sg. Puloh mangrove ecosystem.

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Biomass and horizontal structure of supervised-logged-over hill dipterocarp forest in Ulu Muda forest reserve, Kedah, Malaysia

*Seyed Mousa Sadeghi[1,2], Faridah Hanum Ibrahim[1], Wan Razali Wan Mohd[1], Kamziah Abd. Kudus[1] and Khalid Rehman Hakeem[1]

[1]*Faculty of Forestry, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia*
[2]*Natural Resources and Agricultural Research Center of Bushehr Province, I.R.Iran*
**Corresponding author. email address: smbooraki@gmail.com*

Introduction

Hill dipterocarp forests (HDF) of the peninsular Malaysia are distinguished from other fifteen types of tropical rainforest by dominancy of emergent trees in Dipterocarpaceae (Symington *et al*., 2004). Within the country, Ulu Muda Forest Reserve (UMFR), Kedah, Malaysia is regarded as the eco-socio-environmental center for wood products, biodiversity conservation, fresh water and soil preservation and ecotourism (Latiff and Faridah-Hanum, 2005). Previous forest studies at UMFR indicated high level of species diversity and biomass of primary HDF (Saiful, 2002).

Immediate effects of logging operations on forest structure and biomass of HDF was reported. The tree density, basal area and above-ground biomass (AGB) decreased 50%, 58% and 66.7%, respectively (Saiful, 2002). Understanding the changes in forest structure and biomass after logging are important because: (1) fundamental significance of biomass in the site yield, forest structure and forest condition (MacKay *et al*., 2011). (2) Biomass estimating is the basic level of sequestered carbon calculating.

Effects of supervised logging on forest structure and biomass of HDF at UMFR after intermediate period of time (12 years) was investigated and comparison was made between primary and logged-over HDF to analyze the changes between them.

Materials and Methods

The research was carried out at compartment 25 A in Ulu Muda Forest Reserve, Kedah, Malaysia (5°, 50' N & 100°, 55' E). The elevation ranged from 419 to 555 m above sea level. In the period of 2000-2001 the forest was logged under supervised logging (SL) operations.

In the field work, 20 sampling units (50 m × 20 m) with systematic sampling layout (100 × 100 m) were inventoried. In each plot all trees with Diameter at breast height (DBH) ≥ 1cm were enumerated, measured and identified. To estimate tree density (D), we counted the trees and calculated tree density as follow:

Equation 1: $D = (\text{Number of total individuals}/\text{Total surface of sampling unit } (m^2)) \times 10000$

Basal area (BA) was calculated by using the following equation:

Equation 2: $BA\ (m^2) = (DBH^2 \times \pi)/40000$

To estimate AGB, the following equations were used. Below-ground biomass (BGB) was estimated as ratio of above-ground biomass (0.18), (Niiyama *et al*., 2010):

Equation 3: $1/H = 1/(a\ dbh) + 1/H_{Max}$
Equation 4: $M_{sb} = a\ (dbh^2\ H)^b$
Where M_{sb} is stem plus branch mass (Kg per tree), dbh is diameter at breast height of tree, H is height of tree, (a) and (b) are coefficients, H_{max} is upper limit of tree height which is 69 m.

M_{sb} was used to calculate leaf mass:
Equation 5: $1/M_l = 1/aM_{sm}^b + 1/M_{l\ Max}$
Where M_l is leaf mass (Kg per tree), M_{lmax} is upper limit of leaf mass which is 105.

Results and Discussion

In term of tree density, 1048 trees ha^{-1} were identified. Five top families provided 44.4% of total density. The highest and lowest amount of stand density was related to Euphorbiaceae (21.2%) and Rutaceae (0.05%), respectively. The Total basal area (BA) was found as 19.6 m^2 ha^{-1}. Five top families contributed 47.9% of total BA production. Dipterocarpaceae and Rutaceae contributed the biggest and smallest value of BA which was 18.9% and 0.001%, respectively.

At the species level, five top species contributed 9.1% of total individuals ha^{-1}. *Croton argyratus* Blume provided highest level of tree density which was 2.1%. Because majority of young trees was member of Euphorbiaceae. In relationships with BA, five top species contributed 20.1% of BA. *Shorea macroptera* Dyer contributed biggest amount which was 6.4%.

In relationship to biomass, AGB, BGB and total biomass (TB) was 313.3 × 10^3, 56.4 × 10^3 and 369.7 × 10^3 kg ha^{-1}. Five top families contributed 51.8% of TB ha^{-1}. Dipterocarpaceae provided highest level of TB (25.1%). The minimum value of biomass was from Rutaceae (0.0001%). At the species level, five top species contributed 26.4% of TB. *Shorea macroptera* Dyer contributed highest level which was (8.4%). The minimum value of biomass contribution was from *Melicope glabra* (Blume) T.G. Hartley (Rutaceae), (0.0001%). Although, Euphorbiaceae contributed the highest level of tree density, in terms of BA and TB, it was at second level. Due to majority of individuals were young tree.

At the species level, even though, majority of tree density was from Euphorbiaceae (four species amongst 5 tops species) they were not among five top species with regards to BA. Because those heliophile young trees contributed in the process of forest regeneration after logging. In term of tree density SL area was richer than CL site at UMFR (DBH ≥ 1 cm) which was 1048 and 722 tree ha^{-1}, respectively (Mardan *et al*., 2013). Because the logging damage was higher in CL area than SL site. So, the forest at CL area could not recover up to SL site level. As a comparison in a logged-over HDF, Tekai Tembeling Forest Reserve (TTFR), Malaysia, 1307 trees ha^{-1} was identified (Kamziah *et al*., 2011). The stand density of TTFR was higher than logged-over UMFR. This may be because of site fertility or different logging intensity. The forest density at primary HDF, UMFR (tree > 5 cm) was 1027 tree ha^{-1}. But that value reduced sharply after CL from 1027 to 513.3 tree ha^{-1} (Saiful 2002). Our research showed 572 trees ≥ 5 cm per hectare. We can conclude that in term of tree density, SL site recover 11.4% in comparison with after logging data. But in comparison with pre-felling data, there was a big gap from 572 to

1027 tree ha^{-1}. With regards to BA, the estimated BA of primary forest at UMFR for trees with DBH ≥ 5 cm was 35.4 m^2 ha^{-1} (Saiful, 2002). The CL operations reduced the BA from 35.4 to 14.9 m^2 ha^{-1} (58%), (Saiful, 2002). Our results showed that the mean BA for tree ≥ 5 cm was 19.2 m^2 ha^{-1}. Inconclusion, the supervised-logged-over site (Compartment 25 A) could recover up to 30.9% after 12 years in comparison with post-felling data.

In relationship with AGB, the SL site was richer than CL area which was 313.3 × 10^3 and 190.3 × 10^3 kg ha^{-1} (Mardan *et al*., 2013), respectively. Since, Higher logging intensity in CL area could affect the mature trees and biomass was lower than SL area (Saiful, 2002). The AGB of primary HDF at UMFR was 455.19 × 10^3 kg ha^{-1} (trees > 5 cm), (Saiful, 2002). The AGB reduced from 455.19 to 303.6 × 10^3 kg ha^{-1} After CL (66.7%). Our data showed that mean value of AGB of SL area (DBH ≥ 5 cm) was 312.3 × 10^3 kg ha^{-1}. Consequently, SL area could recover 2.9% after logging from 2000-2013 in comparison with CL area.

Conclusion

Results showed that dipterocarp trees were dominated in terms of size but in terms of frequency non-dipterocarp trees were abundant. As a comparison with primary HDF at UMFR, the supervised-logged-over site recovered In terms of tree density, BA and Biomass which was 11.4, 30.9 and 2.9% in the period of almost 12 years. But a big gap was revealed for forest recovery when compare our data with primary HDF data.

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Acute test of the antifouling biocide Zinc Pyrithione on Javanese Medaka (*Oryzias javanicus*)

Fatin Zahidah Abdul Aziz[1], *Syaizwan Zahmir Zulkifli[1], Ahmad Ismail[1] and Ferdaus Mohamat-Yusuff[2]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding author.Tel.:+603 89466615; email address: syaizwan@science.edu.my*

Introduction

The use of substitute booster biocides in antifouling paints has been established by International Maritime Organization because of the recent ban on tributylin (TBT) (Konstantinou and Albanis, 2004).The effectiveness of Zinc pyrithiones (ZnPT) against bacteria, fungi and algae make them as one of the organic biocides replacements for organotin compound in antifouling paints. Besides that, ZnPT also widely used in antidandruff shampoos or as additive in cosmetics and dermatitis treatment (Shuster, 1984). High toxicity of antifouling biocides to a large diversity of aquatic organisms such as algae, barnacles and mussel embryo is a main feature desired by the user. However, in broad spectrum of target organisms many species in coastal marine ecosystems may become unintended targets of the biocide through the antifouling treatment. Besides, ZnPT was assumed to be environmentally neutral and degrade rapidly under sunlight due to photolysis (Marcheselli *et al*., 2010). However, the area that limited sources of light such as in water and sediment under parking vessels in marine and harbors still have a potential of persist and contamination (Maraldo and Dahllof, 2004).

A lot of studies have been done on the study of ZnPT characteristics, but little is known about it's hazardous to marine organisms (Kobayashi and Okamura, 2002). Javanese Medaka (*Oryzias javanicus*) was chosen to find out the correlation between the effect of ZnPTtowards it physiological changes. *O. javanicus* fish is a type of small bony fish which can be found in a diverse group distributed around Asia. They live in brackish water, freshwater and also the saltwater. In the same genus with *O. javanicus*, Japanese Medaka (*Oryzias latipes*) isone of the most established species where they are widely used in experimental of vertebral biology for many years (Ismail and Yusof, 2011). Based on Koyama *et al*., (2006), *O. javanicus* is commonly found in estuarine waters of southern to eastern Asia. Nevertheless, Ismail and Yusof (2011) had stated that this species of fish is one of the most suitable type which easily being reared in the laboratory. They have a short life cycle and life span with wide geographical range and availability. Nevertheless, suitable animal used as bioindicator of pollutant must be abundant, sufficiently long-lived, reasonable size and are able to adapt well with laboratory surrounding. It was also determined that they have a fast growth rate, hardy species, easily to identify and to be cultured. *O. javanicus* serves as suggested excellent fish model where at

its early life stage is sensitive to toxicants and endocrine disrupting chemicals (Zha and Wang, 2005). It is desirable that the full life cycle of Javanese medaka to assess the effects of ZnPT chemicals, therefore this studies is a beginning for range finding of NOEC (no observe effect level) and LC50 (median lethal concentration for 50% of sampled animals) in a short-term exposure.

Material and Methods

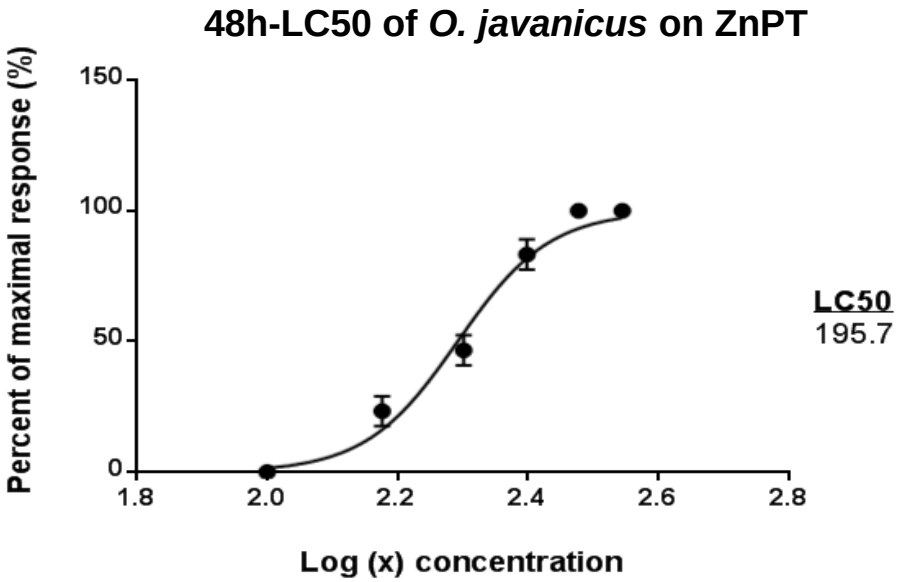
Stock solutions were prepared by dissolving zinc pyrithione in a non-toxic organic dissolvent, dimethylsulfoxide (DMSO) approximately 1hour before the start of experiments. The experimental solutions were static conditions and the quality parameters (pH, temperature, and dissolved oxygen) were maintained and measured daily. The experiments carried out in 5-L glass aquaria were kept 27^{o}C, with constant light-dark condition (14:10 h). No food provided for the fish. Adult fishes (> 2.5 cm) were collected from a natural environment (Sungai Pelek, Sepang, Selangor) to perform the exposure experiments. All fishes were transported to the laboratory and maintained in glass aquaria for 2 weeks acclimatize. After acclimatization, the fishes were used for the exposure experiments (acute toxicity tests and biological fates experiments). Since no data were available on ZnPT toxicity to Javanese medaka, 48h range finding in dark conditions was performed to determine the appropriate concentration ranges for the test chemical. The tested concentrations were 20, 40, 60, 80, 100, 150, 200, 250 and 300 µg/l. Each experimental concentration consists of 30 individuals and replicates into 3 difference glass aquaria. Animals were not fed during the short-term exposure. The number of dead specimens was recorded daily in order to calculate 48-LC50 (median lethal concentrations for 50% of sampled animals).

Results and Discussion

Zinc pyrithione functioning as a bactericide and fungicide in active ingredients in anti-dandruff shampoos or as additive in cosmetics and dermatitis treatment and has recently been used as an alternative antifouling booster biocide to old coatings containing organotin compounds *(*Voulvoulis *et al*., 1999).In the present study we investigate the acute toxicity level of Javanese medaka (*O. javanicus*) on the ZnPT. A major difference between ZnPT and tributyltin (TBT) is in the length of their half-lives in sea water. ZnPT are very light sensitive (Turley *et al.*, 2000; Harino *et al.*, 2005).Under natural light conditions, the half-life of PTs is approximately 8 min (Maraldo and Dahllöf, 2004) whereas that of tributyltin oxide exceeds 89 days (Maguire *et al.*, 1983).

Toxic effect of ZnPT resulted significant increase the no of lethal fish with the increasing of concentration. The NOEC (no observed effect concentration) and 100% survivor of *O. javanicus* is at 100 µg/L and 100% dead starting at 250 µg/L. The Sigmoidal dose-response (Figure 1), it shows response as a function of the logarithm of concentration. This curve allows the prediction about what proportion of population of subjects' responses to the given toxin. In general, the LC50 values from the experimental (180 µg/L) were not diverged different to the predicted LC50 (195.7). From acute toxicity tests conducted to US EPA Guidelines, Environment Australia concludes that ZnPT is very toxic (LC50 < 100 µg/L) to highly toxic (LC50 = 100-1000

µg/L) to fish. Therefore, finding range of LC50 from this study shows that ZnPT still highly toxic to *O. javanicus* population.



Log (x) concentration

Figure 1**:** Percentage survival of *O. javanicus* after 48h exposure to different Log(x) concentration of ZnPT.

Conclusion

Therefore, with the LC50 less than 1000µg/l, ZnPT considered highly toxic and threat to *O.javanicus* and others aquatic organisms. If we take account the photolysis the probability of toxic effect will be low, however ZnPT still have a chances to persist in the marine or freshwater environment where the influence of light is limited.

Acknowledgements

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Comparisons of As and Cu in the *Corbicula javanica* and sediments collected between Pangsun and Kajang sampling sites

[*]Koe Wei, Wong[1], Chee Kong, Yap[1], Rosimah, Nulit[1], Ahmad Zaharin, Aris[2] and Mohd Suhaimi, Hamzah[3]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[2]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*
[3]*Waste and Environmental Technology Division, Malaysian Nuclear Agency, Bangi 43000 Kajang, Selangor, Malaysia*
**Corresponding author. email address: wongkoewei@gmail.com*

Introduction

Langat River is a river in Selangor. The Langat River is 120 km long and originates from the Titiwangsa Range in Gunung Nuang. It drains westward to the Straits of Malacca. The major tributaries of Langat River are the Sungai Semenyih and Sungai Labu. The water flows from the upstream area in Hulu Langat to Kuala Selangor at the downstream area where the Langat River becomes the main river in the basin which flows in southwest direction and drains into the Straits of Malacca. The river basin has an area of about 2423 m^2 (Azrina *et al.,* 2006). Due to the river's importance, there is necessity to constantly monitor the water quality of this important river.

Corbicula javanica (C. javanica) is a species of freshwater clam that are indigenous in various countries within the Southeast Asia. There are reports of its presence in Malaysia (Berry, 1974; Yap *et al.*, 2003; Yap *et al.*, 2011), Indonesia (Java: Wardani *et al.*, 2012; Salamah *et al.*, 2012; Sumatra: Setiawan, 2010) and Thailand (Krailas *et al.*, 2012).

According to Yap *et al.* (2004), the ideal biomonitor species should fulfil the 8 criteria which are (1) have wide geographical distribution; (2) undergo stationary or low mobility life; (3) abundant and hence enabling easy sampling; (4) have simple correlations between the metals concentration levels in the mussels and those in their environments; (5) Low variability with species enabling easy identification; (6) Capacity to accumulate pollutants in the tissue of the mussels; (7) Tolerant but relatively sensitive to chemical pollutants; (8) Commercially important as it could easily generate public concern from the health point of view. Table 1 shows the comparison of the criteria of an ideal biomonitor (Yap *et al.*, 2004) and the relative relevance of *C. javanica* as a biomonitor. The freshwater clam *C. javanica* has hypothetically fulfilled criteria 1, 2, 3, 5. The suitability of *C. javanica* fulfilling criteria 4, 6, 7, shall be determined by further study. It failed to fulfil the criterion 8 as *C. javanica* poses less economic significance as less Malaysian treat this freshwater clam as food source.

As the freshwater clam *Corbicula javanica* fulfilled some of the 8 criteria of ideal biomonitor, it has the potential to become a good biomonitor according to those criteria established by Yap *et al.* (2004)

The aim for this study are to determine the relationship between the level of As and Cu in the soft tissue as well as the shell of transplanted C. javanica and those in the sediment collected across the site studied by the perspective of total digestion and fractionation of As and Cu.

Materials and Methods

The upstream site of Pangsun located at Kampung Kuala Pangsoon, it is the first habited village of the Langat River, lack of industrial activities make it a candidate of unpolluted site for this study. While the polluted site of Kajang, a township with plenty of human activities, are chosen as the comparison against the unpolluted site of Pangsun.

The *C. javani*ca were captured at the upstream site of Pangsun and then transferred to Kajang site for exposure at the same day by cage. Some individuals were also collected at the Pangsun site for background analysis. Surface sediments were also collected at both side to determine the content of Cu and As in the study area. The transplanted *C. javanica* at Kajang site was then collected after 3 days. The collected *C. javanica* and surface sediments from both sites were stored at -10°C refrigerator until analysis.

During analysis, the *C. javanica* were thawed at room temperature then carefully dissected into Shell and soft tissues, each dissected parts were pooled together to make up the amount required for the analysis. The samples were then dried under 60°C for 72 hours until constant weight. Sediment samples were dried until a constant dry weight. Afterwards, the samples were sieved through a 63µm stainless steel sieve and shaken vigorously to produce homogeneity (Yap *et al*., 2002). 0.5g of the soft tissue and shell of *C. javanica* were digested in 7 ml of 65% HNO_3 and 1 ml of 30% H_2O_2 in a microwave digester at 200°C for 25 mins. 0.5g Sediments were digested in 6 ml of 65%HNO3, 1 ml of 30% H_2O_2 and 1 ml of 65% HClO4 in microwave digester at 200°C for 30 mins. The digested samples were then diluted to a certain volume with double distilled water (DDW). The sample was then filtered through Whatman No. 1 filter paper and the filtrate was stored until metal determination.

The determination of Cu and As for all digested samples were done by using ICP-MS (Perkin elmer ELAN 6000) at Agensi Nuklear Malaysia. The data are presented in micrograms per gram dry weight basis.

Results and Discussion

The concentration of Cu and As in soft tissue, shell and Soil are shown in Table 1. The sediment of Kajang are shown slightly higher in Cu than Pangsun with 16.26 in Kajang and 12.13 in Pangsun. The sediment of Kajang also show significantly higher concentration in As with 61.35 compared with 16.89 in Pangsun. These shows that Kajang site are more polluted compared with Pangsun.

Table 1: Mean Cu and As concentrations (mg/g dry weight) and Standard Deviation of mean (SD) in Soft Tissue and Shell of *C. javanica* and Soil.

	Cu	Mean Soft tissue	SD	Mean Shell	SD	Mean Soil	SD
Cu	Pangsun 120726	5.01	1.07	2.42 (2.54-2.30)	1.07	12.13 (12.12-12.14)	1.97
	Kajang 121129	5.35 (3.6-6.72)	1.07	0.66 (0.57-0.74)	0.22	16.26 (16.16-16.36)	0.14
As	Pangsun 120726	2.96 (2.35-3.57)	0.86	0.20 (1.09-1.37)	0.07	16.89 (16.50-17.28)	0.55
	Kajang 121129	6.34 (5.41-7.66)	1.18	0.39 (0.31-0.51)	0.15	61.35	0.00

After the 3 days exposure, the Cu content in the soft tissue increases from 5.01 measured by Soft tissue in Pangsun as background to 5.35 in Kajang, while As increases significantly from 2.96 in Pangsun to 6.34 in Kajang. However, the Shell shows opposite pattern of soft tissue and soil, its Cu content decreases from 2.42 in Pangsun to 0.66 in Kajang while As in Kajang showed a slight increasing pattern, the As content increased from 0.2 in Pangsun to 0.39 in kajang. It shows that the soft tissue of *C. javanica* shows capability to accumulate both Cu and As from environment but the shell cannot effectively accumulate Cu but able to slightly accumulate As as shown by the slight increase of As level in shell after transplanted to Kajang.

Conclusion

Higher Cu and As concentration in Kajang than Pangsun shows that Kajang sites are more polluted in term of both Cu and As. The Cu and As concentration in soft tissue of *Corbicula javanica* shows increasing pattern along with the transplantation indicates that the soft tissue are able to accumulate Cu and As. This showing the potential of soft tissue of *C. javanica* to act as a biomonitor for Cu and As pollution. To confirm the capability of *C. javanica* to act as a biomonitor for Cu and As pollution, further study are required to determine the tolerance limit for this clam for Cu and As.

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Heavy metal concentrations in fish organs from upper stream area of Kelantan River, Malaysia

*Zarith Sufiani Baharom and Mohd Yusoff Ishak

Department of Environmental Management, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia
**Corresponding author. email address: zarithsufianibaharom@yahoo.com*

In the present study, level of heavy metal concentrations on fish organs in the Kelantan River basin was assessed using the utility of different organs and tissues as indicators of heavy metal pollution. The concentrations of Cu, Zn, Pb, Ni and Mn were detected in muscle tissue, liver and gills of ten native fish species caught using gill nets. The fishes were sampled at three different sites in the upper stream of the Kelantan River, namely Gua Musang, Kuala Geris and Limau Kasturi, isolated from each other by different local riverine characteristics. Although heavy metal pollution is believed to be one of the main threats to the fish population in the area, there is a lack of knowledge of the exact level of heavy metals in their tissues. Fish and water samples were analyzed by means of Inductively Coupled Plasma- Mass Spectrometry (ICP-MS). The initial results for both samples at all locations showed that the concentrations of Pb were higher than the permissible limit set by FAO/WHO 2004 as well as Food Act 1983 and Malaysian Food Regulations 1985. The level of heavy metal concentration in water and fish organs shall be considered as an important matter as the locals are consuming these fishes and it represents the quality of the river itself. The next step would be to determine whether these heavy metals are potentially harmful for human health as the freshwater fish are among the main in their diet.

Keywords: Heavy metals, fish organs, Kelantan River

Radioactivity Levels of ^{234}Th and ^{210}Po in the Green Mussel (*Perna viridis*) at the Straits of Johor) and the Estimated Accumulations to Human Body

Mei Peng Lim[1], Asnor Azrin Sabuti[1], Khairul Nizam Mohamed[2], Ferdaus Mohamat-Yusuff[2], Wan Lutfi Wan Johari[2], Ahmad Zaharin Aris[2], Syaizwan Zahmir Zulkifli[3], Mohamad Pauzi Zakaria[2], Ahmad Ismail[3] and *Che Abd Rahim Mohamed[1]

[1]School of Environmental and Natural Resource Sciences Faculty of Science and Technology,Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor
[2]Department of Environmental Sciences Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor
[3]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor
**Corresponding author. email address: carmohd@ukm.my; profcar6566@yahoo.com*

Introduction

Straits of Johor are well known with the production of green mussels because of the availability of spat fall in this area. The existence of radionuclides contaminant in the seafood might possess certain level of health problems to consumer. ^{210}Po have been widely investigate by most researches on the relationship between ^{210}Po with marine organisms because it is the most important source of internal radiation dose for most of the marine organisms which emits alpha radiation (Carvalho 1988; Cherry and Heyraud 1982; Mcdonald *et al*., 1996). In contradiction to ^{234}Th, information is barely found for the interaction among ^{234}Th and marine organisms (Fisher *et al*., 1987). The objective of this research is to review the activity of ^{210}Po and ^{234}Th level in green mussels sample from water of Straits of Johor, Malaysia.

Materials and Methods

Sampling was conducted 25th March of 2014 and 1st June of 2014 at the Straits of Johor, Malaysia. Samples were collected from cages, wild and market and measured its shell length and width. The edible part of the green mussels was dissected into stomach and others edible organ and dried up at 60 °C. Samples were spike with ^{209}Po (12.17 dpm/ml) and ^{229}Th (36.23 dpm/ml) prior to the digestion with HNO_3, $HClO_4$ and H_2O_2. Then the solution was filtered and evaporated to dryness. The samples were dissolved with 50 ml of 0.5 M HCl and ^{210}Po was spontaneously deposited on silver disc for 2-3 hours. Activities of ^{210}Po were counted with the Alpha Spectrometer, model 7401. For ^{234}Th analysis, the solution used for the deposition was added with HNO_3 and H_2O_2, and then precipitated with the $FeCl_3$ and NH_3. This step was repeated twice and the precipitate was centrifuged. All the coprecipitate were collected by dissolve with 50 ml of 7.5 M HNO_3 and continued with anion exchange methods. The eluted sample was dried up and undergoes electrodepositing. Activities of ^{234}Th were counted with Alpha/Beta Counting System.

Results and Discussion

The average activity concentrations of ^{234}Th and ^{210}Po of green mussels sampling from various area at Straits of Johor are listed in Table 1. As comparing between the activity concentrations of both natural radionuclides in stomach and other tissue,

stomach have shown a remarkable higher activity concentration of ^{234}Th (388 ± 315 Bq kg^{-1}) and ^{210}Po (150 ± 65 Bq kg^{-1}). The distributions of radioactive materials which transform and incorporated into the body can be divided into few parts. According to the ICRP recommended in publication 30, ingested food will first get into stomach followed by small intestine (SI) and there could be 50% from the total amount activity concentration in SI to transfer into blood and distribute to other part of tissue (ICRP 1979). Although the main function of absorption is responsible by small intestine but there are chances for the radioactive materials to associate and get involved in metabolism with the stomach tissue upon the ingestion and digestion process. The activity of ^{234}Th had shown to have a higher level of concentration which is more than double the activity of ^{210}Po (Table 1). Among the activity of natural radionuclides in sediment, thorium is much abundance than others. In addition, green mussel is a strong filter feeding organism where its ingestion is control by the automatic water pumping and filtration. Water bodies consist of suspended particle and plankton will ingested into the esophagus and enter the stomach (Jorgensen, 1996).

Table 1: Average ^{234}Th and ^{210}Po activity concentration of green mussels in stomach and other tissue.

	Stomach		**Others**	
	^{234}Th average activity (Bq kg^{-1})(± 1 SD)	^{210}Po average activity (Bq kg^{-1})(± 1 SD)	^{234}Th average activity (Bq kg^{-1})(± 1 SD)	^{210}Po average activity (Bq kg^{-1})(± 1 SD)
Wild	236 ± 121	102 ± 33	81 ± 79	37 ± 12
Cage	641 ± 509	257 ± 62	260 ± 166	70 ± 18
Market	387 ± 164	68 ± 11	74 ± 48	40 ± 6
Mean	388 ± 315	150 ± 65	131 ± 94	48 ± 21

Figure 1 and Figure 2 present the activity concentration of ^{234}Th and ^{210}Po associated with green mussels from the origin of cage, wild and market. Generally, green mussel recruit from wild showed higher of ^{234}Th (mean: 641 ± 509 Bq/kg in stomach and 260 ± 166 Bq/kg in other tissue) and ^{210}Po (mean: 257 ± 62 Bq/kg in stomach and 70 ± 18 Bq/kg in other tissue). Production of wild green mussels in analysis sample are smaller in size than both cage and market. Variation in size has been shown to influence the ^{234}Th and ^{210}Po activity concentration in green mussels. Literally, bioaccumulation and bioconcentration of contaminant will occur in one individual, the level of contaminant will eventually increase as the individual grow. However, from this study, the accumulation of radionuclides has shown is not size-dependent and similar result has reported by Alam and Mohamed (2011). As a result, it can be infer that the juvenile green mussel tends to have more frequent feeding rate for growth in order to corps with the fast rate of metabolism compared to the adult green mussel. As an implication to this, higher accumulation level of ^{234}Th and ^{210}Po are seen for wild sample. An overview onto the result in this study, we hypothesize that caution should be taken on the green mussels in the Straits of Johor. It is due to the slightly higher risks of radiation might contribute to the consumer of seafood as the level are higher than the permitted levels recommended by the USEPA with estimated effective radiation dose of ^{234}Th (between 0.44×10^{-2} to 9.72×10^{-2} mSv/year) and ^{210}Po (between 2.61 to 11.54 mSv/year).

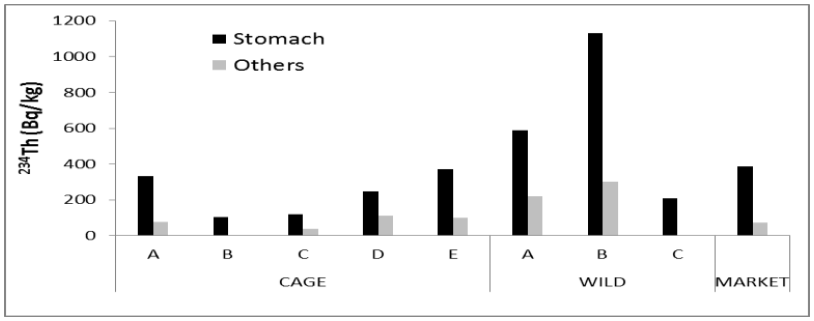

Figure 1: ^{234}Th activity concentration from various origins

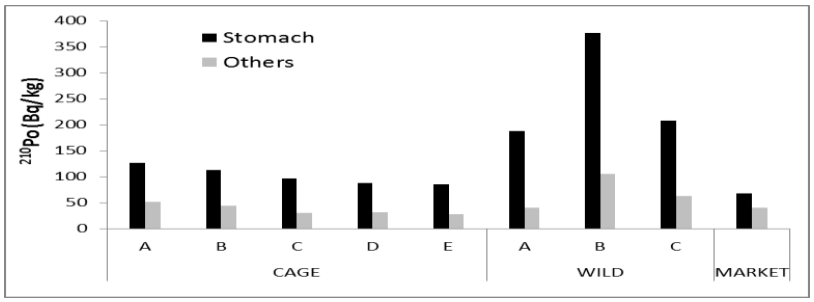

Figure 2: ^{210}Po activity concentration from various origins

Conclusion

This study has delivered a supportive idea on the capability of green mussel as an effective in-situ bio-indicator for radioactive materials instead of reportedly the contaminations of heavy metals. Based on the result of this study, it can be proposed that Straits of Johor might liable to certain levels of radionuclides contamination from the exiting natural radionuclides and inputs of anthropogenic activities.

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Availability and suitability of Kuala Gula mangroves for Milky Stork Reintroduction Program in Malaysia: a re-evaluation

Faid Rahman and *Ahmad Ismail

Department of Biology, Faculty of Science, Universiti Putra Malaysi, 43400 UPM Serdang, Selangor, Malaysia.
*Corresponding author.Tel.:+603 89466617; email address: *aismail@upm.edu.my*

The Milky Stork population is endemic to a small part of the Southeast Asia region and is considered as an endangered species as its number has gone considerably low throughout its home-range. In Malaysia alone, the species numbers have dwindled significantly and the last group of the wild population was last recorded in 2010. Hence, the work to repopulate the species is currently being done in Kuala Gula, Perak as the location is still considered as pristine and of high importance to large numbers of resident and migratory birds. However, the recent increase of anthropogenic activity in the area has raised considerable attention among environmentalist and scientist as the mangroves along Kuala Gula coast are being reclaimed excessively to give way to the aquaculture industry. Accordingly, this study was undertaken to re-evaluate the availability and suitability of Kuala Gula as nesting and foraging grounds for the reintroduction of the Milky Stork population. Important nesting and foraging areas were surveyed and data on the habitat, including size, types, forest composition and structure were recorded. In general, at least one nesting site and two foraging areas have been affected and could no longer benefit the waterbirds population. The findings suggest that urgent attention be given to protect remaining mangroves from further development and reclamation activity to ensure the success of the reintroduction program in the future.

Keywords: Milky Stork, reintroduction, habitat availability, suitability, Kuala Gula, mangroves

Co-toxicity of mercury and beach plastic litter to *Portunus pelagicus* in the marine environment

*AH Bu-Olayan and BV Thomas

POB 5969, Department of Chemistry, Kuwait University, Safat 13060, Kuwait
**Corresponding author.Tel.:+965 66040903; email address: buolayan@yahoo.com*

Plastic materials disposed along the seacoast from various sources witnessed marine pollution, recently. Our studies revealed total mercury (T-Hg) in plastic litter causing toxic effect to marine crustaceans. Unlike common methods and instrumentation that indicated analytical limitations, precise and reproducible results were achieved using a direct mercury analyzer (DMA-80) with the least detection limits of 0.0015ng.g-1. In the present study, the inclusion of common beach plastic litter to crustacean *Portunus pelagicus* revealed significant T-Hg concentrations as well as bioaccumulation factor (BAF)>1 when they were exposed for 180d and indicating the effects of litter in scenic beaches.

Keywords: Blue crab, bioaccumulation, beach pollution, mercury, Kuwait

Distribution of rare earth elements in sediment cores of Sedili and Tanjung Pelepas, Johor as pollution indicator

Wai Seong Koh[1], Asnor Azrin Sabuti[1], Abdul Hafidz Yusoff[1], Syaizwan Zahmir Zulkifli[2], Ahmad Ismail[2] and *Che Abd Rahim Mohamed[1]

[1]*School of Environmental and Natural Resource Science, Faculty of Science and Technology University Kebangsaan Malaysia, 43600 bangi, Selangor*
[2]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor*
**Corresponding author. e-mail address: carmohd@ukm.my; profcar6566@yahoo.com*

Introduction

Rare earth elements (REEs) are unique metal elements that have similar chemical and physical properties. REEs have been used extensively to trace the natural processes that happen in marine environment and also used as an indicator to trace anthropogenic disturbance in marine environment (Åström, 2001; Borrego *et al*., 2004; Davranche *et al*., 2005; Elderfield and Sholkovitz, 1987; Haley *et al*., 2004; Olmez *et al*., 1991). Accumulation of light rare earth elements (LREE) are enriched by industrial processes such as petroleum catalytic cracking can yield anomaly REE concentration in river and marine sediments. Objective of this research is study the distribution of REEs concentration in sediment cores at harbor of Sedili and Tanjung Pelepas.

Materials and Methods

The sediment cores about 70 cm were collected with a gravity corer at the harbor of Sedili and Tanjung Pelepas, Johor. The sediment core was then sub-sampled at 3 cm intervals. The coordinate of the sampling location for harbor of Sedili and Tanjung Pelepas are (N 01˚ 55' 54.3'', E 104˚ 06' 39.8'') and (N 01˚20' 17.5'', E 103˚ 32' 46.4''). The sediment samples were crushed into homogenized powder using mortar and pestle and filtered through 63 µm sieves. About 0.5 g of dried sample was digested in a mixture of $HClO_4$:HNO_3 (1:5 v/v) for 2 hours. Then 10 ml of hydrofluoric acid was added and further digest until get a pastel form. The digested samples were dissolved in 2% HNO_3 up to 30 ml. Similarly, blank samples were prepared by the same procedure. The solution were then analyse for REE elements and by using ICP-MS (Perkin-Elmer Elan 9000).

Results and Discussion

Generally, the REEs concentration at Harbor of Sedili is higher than Tanjung Pelepas (Table 1). Based on the result that observed, LREE is more abundance than HREE (heavy rare earth elements) shows in both stations. The REEs uptake sequence is LREE > HREE, and REEs concentration shows a decreasing trend with the increasing of depth. Organism behavior such as bioturbation might increase the concentration of metals at the surface sediment. Besides that, anthropogenic activities also raised the loading of metals to the environments. Element Lanthanum (La) chosen as representative for LREE. Enrichment factor (EF) > 2.0 suggest that

anthropogenic sources whereas EF < 2.0 is natural origin. EF value for La at Sedili Harbor and Tanjung Pelepas Harbor range from (1.2832 – 5.4848) and (0.2274 – 2.3735) respectively and is suggest that the La in Sedili Harbor may enrich by anthropogenic sources.

Ce element occur more abundance in oceanic ferromanganese compare to seawater. Marine manganese nodule will have positive Ce* anomaly due to Cerium easy hydrolyses and co-precipitates with manganese. Negative Ce* anomaly (<1) shows that the environment is oxic condition, reduction process occur and the dissolution of insoluble Ce (IV) is reduce to soluble Ce (III). Liu 1987 suggest that high biogenic and chemical deposition will have negative Ce* anomaly.

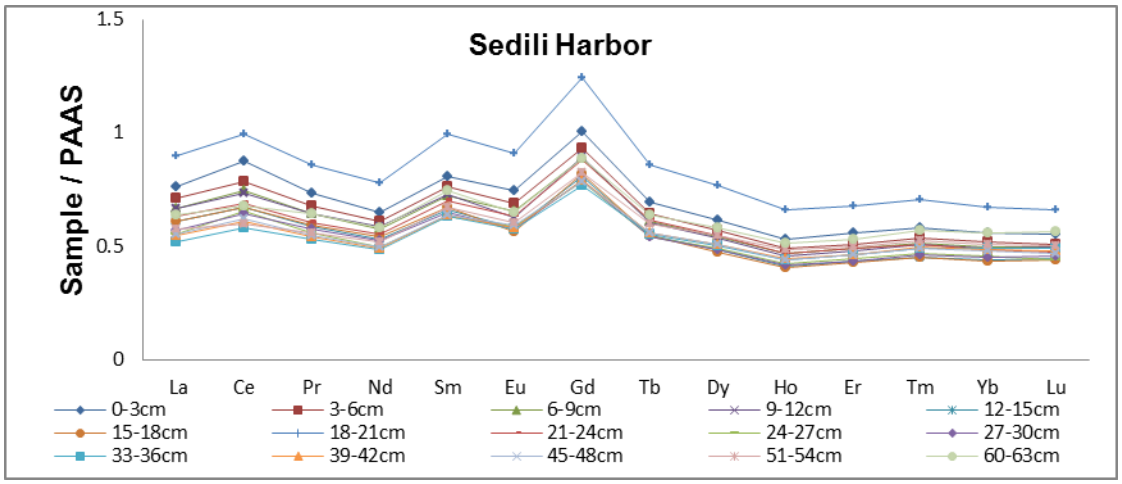

Figure: 1 The PAAS normalized REE Distribution Pattern at Sedili Harbor.

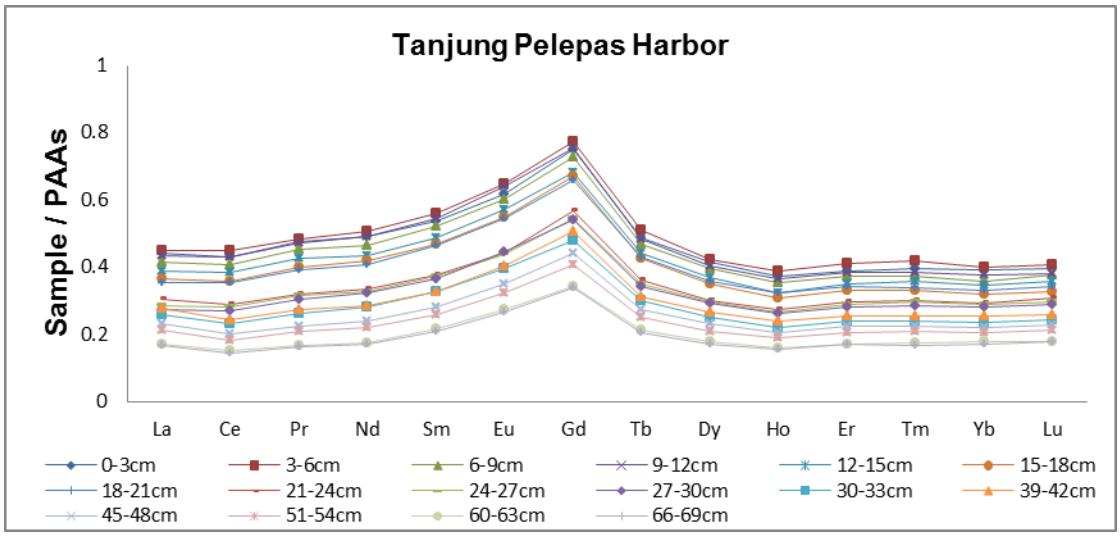

Figure 2: The PAAS normalized REE Distribution Pattern at Tanjung Pelepas Harbor.

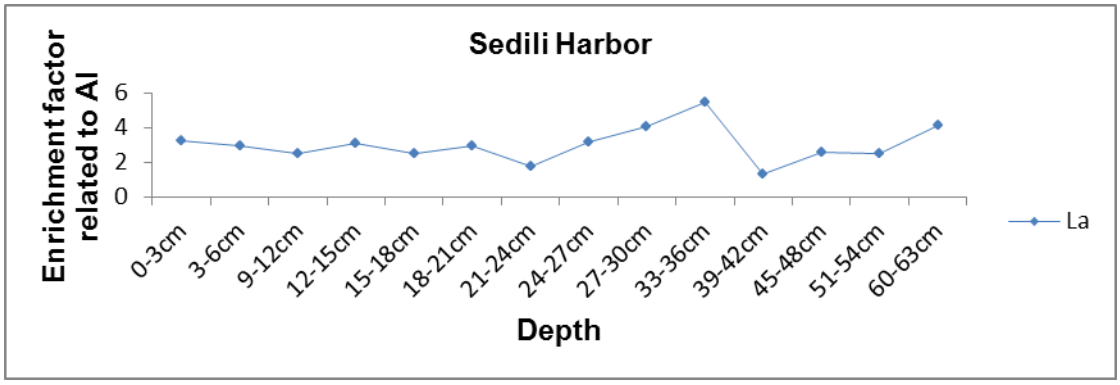

Figure: 3 Aluminium normalized enrichment factor for La as a function of depth in Sedili Harbor.

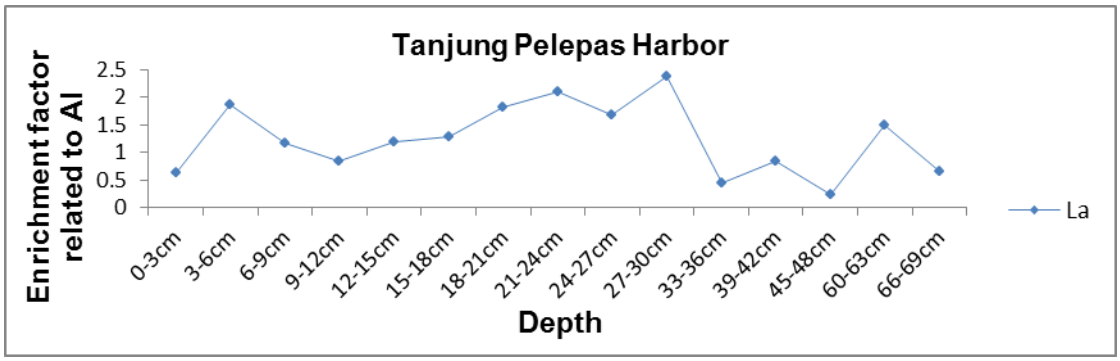

Figure 4: Aluminium normalized enrichment factor for La as a function of depth in Tanjung Pelepas Harbor.

Table 1: The Distribution of ∑LREE, ∑HREE, ∑(LREE?HREE), $(Ln/Yb)_n$, Ce* and Eu* in harbor of Tanjung Pelepas and Sedili.

Depth/cm	Location	∑LREE	∑HREE	∑(LREE/HERE)	$(La/Yb)_n$	Ce*	Eu*
0-3	TPP	75.65	8.776	8.62	1.128	0.940	0.968
	SDL	132.2	12.33	10.72	1.356	1.17	0.825
3-6	TPP	78.01	9.066	8.605	1.125	0.958	0.982
	SDL	121.2	11.40	10.63	1.37	1.129	0.820
6-9	TPP	71.56	8.383	8.536	1.157	0.938	0.976
	SDL	114.5	10.88	10.528	1.35	1.136	0.820
9-12	TPP	75.52	8.669	8.712	1.16	0.953	0.998
	SDL	114.4	10.80	10.588	1.359	1.123	0.777
12-15	TPP	67.08	7.869	8.524	1.129	0.939	0.987
	SDL	104.3	9.760	10.684	1.377	1.129	0.797
15-18	TPP	63.51	7.55	8.412	1.147	0.94	0.976
	SDL	105.0	9.732	10.79	1.402	1.119	0.758
18-21	TPP	62.04	7.636	8.125	1.07	0.944	0.985
	SDL	153.95	15.20	10.131	1.335	1.131	0.818
21-24	TPP	51.38	6.571	7.819	1.038	0.921	0.960
	SDL	107.6	10.84	9.92	1.311	1.114	0.802
24-27	TPP	49.77	6.38	7.801	0.996	0.934	0.972
	SDL	99.37	9.784	10.156	1.214	1.179	0.837

27-30	TPP	47.93	6.283	7.628	0.976	0.929	0.998
	SDL	100.05	9.736	10.276	1.260	1.128	0.806
30-33	TPP	42.44	5.428	7.82	1.09	0.895	1.000
	SDL	91.22	9.963	9.156	1.058	1.107	0.831
39-42	TPP	44.08	5.768	7.643	1.088	0.883	0.994
	SDL	95.04	10.09	9.419	1.123	1.121	0.817
45-48	TPP	36.88	5.033	7.327	1.052	0.886	0.991
	SDL	96.32	10.02	9.610	1.159	1.125	0.834
51-54	TPP	33.75	4.629	7.292	1.022	0.872	0.994
	SDL	96.47	10.68	9.031	1.144	1.048	0.813
60-63	TPP	27.59	3.92	7.039	0.967	0.895	1.000
	SDL	108.6	11.46	9.471	1.140	1.059	0.800
66-69	TPP	26.58	3.824	6.949	0.978	0.871	0.998
	SDL	-	-	-	-	-	-

A positive Ce/Ce* anomalies (1.10-1.18) result in Sedili harbor whereas Tanjung Pelepas showed negative Ce/Ce* anomalies (0.87-0.96). Negative Ce* anomalies in harbor of Tanjung Pelepas suggest high biological activity occurred at the study area. Both stations showed the weak positive Eu/Eu* anomalies value which are 0.76-0.84 (Sedili Harbor) and 0.96-1.0 (Tanjung Pelepas Harbor). Roberta (1991) suggested that positive Eu* anomaly may due to input of hydrothermal vents or lithogenic sources. Hydrothermal vent is absent in Malaysia water, thus positive Eu* anomaly in both station may yield from the lithogenic sources. High La/Yb_n ratio indicate high erosion rate (Anawar & Freitas 2010). The La/Yb_n ratio for harbor of Tanjung Pelepas and Sedili are varies from 0.967 to 1.160 and 1.058 – 1.402 respectively thus the erosion rate is higher at harbor of Sedili compared to Tanjung Pelepas.

Conclusion

Based on the result, this objective of this study had successful delivered. REEs are suitable to use as pollution indicator due to its unique characteristic. The concentration of REEs at Sedili Harbor is higher compared to Tanjung Pelepas Harbor. Al normalized enrichment factor of for La at Sedili Harbor suggests that sources of lanthanum come from anthropogenic sources. Negative Ce/Ce* anomalies value show in Tanjung Pelepas Harbor are due to high biogenic activity.

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The authors would like to thanks the laboratory and individuals in deliver a helping hands in this research and also thanks the financial support from the Ministry of Science, Technology and Innovation (Grant no. 04-01-02-SF0801) during conducting this project. Finally many thank the laboratory staffs of School of Environmental and Natural Resource Sciences, for their technical supports

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Effect of cadmium on DNA changes of *Lpomoea aquatica* Forssk

*Tawatchai Tanee[1,2], Lamyai Neeratanaphan[2,3] and Penkhae Thamsenanupap[1]

[1]*Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand*
[2]*Genetics and Environmental Toxicology (GET) Research Group, Khon Kaen University, Khon Kaen 40002, Thailand*
[3]*Department of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
** Corresponding author.Tel.:+66 80 4729815; email address: tawatchai5@hotmail.com*

Introduction

Cadmium (Cd) is one of the heavy metal that has been used extensively as a material in the agriculture and chemical industry such as inorganic fertilizers, pesticides, paintings, etc., and it can be released into the soil and water. Cd contamination in agricultural soils is unlikely to affect plant growth, but Cd is easily transferred to human food chains from the soils. Moreover, an excess of toxic heavy metal ions induces several cellular stress responses and damage to different plant cellular components such as membranes, proteins and DNA (Manahan, 2003; Liu *et al*., 2005). *Ipomoea aquatica* is one of the popular vegetables in northeastern Thailand, and other Asian countries. The aims of this study were to determine the contents of Cd in different tissues, *viz.* roots, stems, and leaves, and to detect DNA damage in the plant induced by Cd using random amplified polymorphic DNA (RAPD) markers.

Materials and Methods

The plant was grown in the soil supplemented by Cd at 0 (control), 15, 30, 60 and 120 mg/kg. After 21 days, the plant height was measured and the leaves were collected for DNA analysis. All of the plants and soil were taken for determination of accumulated Cd. Accumulations in the roots, stem and leaves, were analyzed using Atomic Absorption Spectrophotometer (AAS) (Tanee *et al*., 2013), then the bioconcentration factor (BCF) and translocation factor (TF) were calculated (Malik *et al*., 2010). DNA changes were accessed by RAPD technique with genomic template stability (GTS) test (Liu *et al*., 2007).

Results and Discussion

The height ranged from 1.6 to 9.7 cm. Before the experiments, the field soils contained 14.92±1.18 mg/kg of Cd. The amount and distribution of Cd accumulated in the *I. aquatica* tissues treated with the different concentrations (0, 15, 30, 60, 120 mg/kg) are shown in Table 1. The Cd accumulation in the each plant part ranged from 0 to 12,333 mg/kg, 0 to 5,909.27 mg/kg, and 0 to 1,653.26 mg/kg, respectively. The BCF and TF values ranged from 0 to 21.15 and 0 to 1.21, respectively. Cadmium accumulation in edible plants is a serious problem, not only because it could reduce the yield of crops, but also it is a hazard to human health through food chains. *I. aquatica* is one the popular vegetables that are used for Thai food. From

this research, it was observed that the accumulation in the shoots increased with increasing Cd concentrations in the soil. These results are concordant with previous research that shows that the plant can accumulate Cd from the soil (Qin *et al*., 1994; Yan *et al.*, 2009).

Table 1: Cadmium accumulation, bioconcentration factor (BCF), translocation factors (TF) and genomic template stability (GTS) of *Ipomoea aquatica* under Cd treatments.

Cd conc. (mg/kg)	Accumulated Cd (mg/kg) in the plant tissues (mean ± SD)			BCF	TF	GTS (%)
	Root	Stem	Leaf			
0 (control)	0	0	0	0	0	-
15	1,873.55±918.20	1,612.50±825.62	491.16±266.53	12.58	1.12	91.1
30	2,950.83±1,817.87	2,647.13±1,573.62	913.23±448.51	15.85	1.21	76.6
60	6,937.45±3,003.51	2,289.40±1,235.15	1,256.13±643.78	21.15	0.51	55.5
120	12,333.00±6,413.73	5,909.27±2,854.82	1,653.26±748.66	16.84	0.61	52.3

From RAPD fingerprints, the example of RAPD banding patterns are shown in Figure 1. The RAPD profiles showed substantial differences between the control and the treated plants with apparent changes (disappearance and/or appearance) in the number of DNA bands produced by each primer. The GTS values ranged from 52.3 to 91.1% (Table 1). At the highest concentration of Cd supplemented (120 mg/kg), the DNA resulted in the highest changes (GTS = 52.3%). The results indicate that the GTS value in *I. aquatica* was affected by Cd exposure. These results correlated with previous data suggested that Cd could induce DNA changes in plant species (Liu *et al*., 2005; 2009; Duman *et al*., 2014).

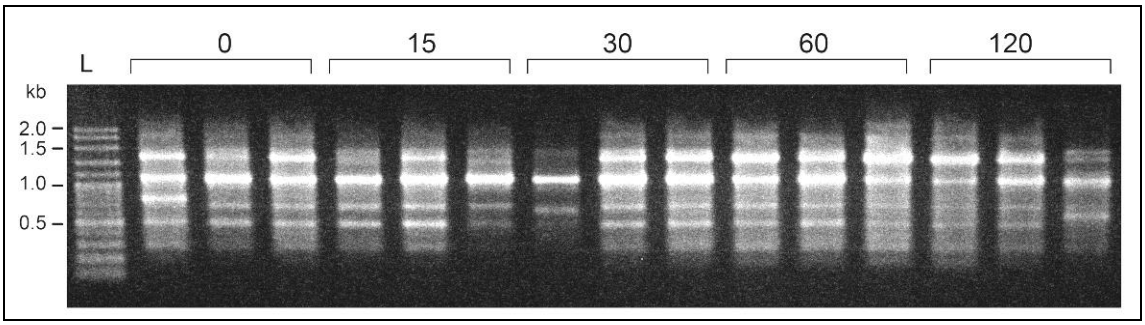

Figure 1. RAPD profiles of *Ipomoea aquatica* exposed to different concentrations of Cd, 0 (control), 15, 30, 60, 120 mg/kg, from primer CGTGGGCAGG.

Conclusion

Cadmium accumulation in plant species should be concerned, not only because of Cd potentially affects the consumers but also causes the DNA damages of *I. aquatica* as shown by RAPD assay. The plant can accumulate Cd at the concentration higher than 100 mg/kg, whereas, the normal level of Cd in most plants is only 0.1 mg/kg. This accumulation level as well as the BCF and TF values, therefore, suggested that consuming the plants growing in the Cd-polluted area is a health risk. The results of this research should be broadcasted to the public for proper consideration to be taken about consumption of edible plants. Guidance for

farmers and others should be provided, especially concerning the instructions for use of pesticides, herbicides, or fertilizers, for food safety in the future.

Acknowledgments

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Efficient bacterial species for arsenic bioremediation of gold mining soil

*Lamyai Neeratanaphan[1, 2] and Tawatchai Tanee [2, 3]

[1] Department of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2] Genetics and Environmental Toxicology (GET) Research Group, Khon Kaen University
[3] Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand
**Corresponding author.Tel.:+66 899175242; email address: hlamya@kku.ac.th*

Introduction

Arsenic (As) has become a major problem of gold mining because the concentrations in the soil are high and cannot be reused without being decontaminated, generating an environmental health problem. The concentrations of As in non-contaminated soils are typically well below 10 $mg \cdot kg^{-1}$. Its presence at elevated concentrations in soils is due to both anthropogenic and natural inputs (Shahedur *et al*., 2014). Microbial based technologies for metal extraction have become attractive because they are cost effective compared to chemical methods and might be applicable for a large number of inorganic pollutants (Hutchins *et al*., 1986). Bioremediation of As by microorganisms has been widely applauded because of the potential advantages of providing a cost-effective technology and an environmentally friendly method for heavy-metal removal (Valls and Lorenzo, 2002). In this work, the authors aim to study As contamination level in soil and to identify the species of microorganisms present in the soil and their effectiveness in As treatment for further use near gold mining areas.

Materials and Methods

Sampling sites and As soil contamination measurements

The sampling sites are located near the gold mine and are defined as the affected area, located in Loei province of Thailand. As concentrations in the soil samples were determined with an atomic absorption spectrometry (AAS) model Analyst 300, Perkin-Elmer (Correira *et al*., 2003).

Microorganism screening and species identification

The microorganism strains were isolated from the soil sample observed to have the highest As concentration (0.72 $mg \cdot kg^{-1}$). The medium used for isolation was nutrient agar (NA) containing 10 $mg \cdot l^{-1}$ of sodium arsenate (Suresh *et al*., 2004). The fungi and bacteria obtained from the microorganism screening were tested to determine if fungi or bacteria were more As resistant in NA supplemented with 10, 100, 500, 1,000, 1,200, 1400, 1600 and 1800 $mg \cdot l^{-1}$ of As. Finally, two bacteria, B109 and B204, were observed to be more tolerant than fungi and were identified by 16S rRNA gene sequencing with dendrogram construction using MEGA5 program (Tamura *et al*., 2011). Additionally, morphological characteristics were examined by scanning electron microscopy (SEM) using a LEO 1450VP (Lyman *et al*., 1990).

Bioremediation of As in Gold Mining Soils

In gold mining soils, As can be found at concentrations as high as 0.72 $mg \cdot kg^{-1}$. Therefore, this concentration was selected for As remediation by the isolated bacteria at 0, 24, 48, 72, 96, 120, 144 and 168 h. After As remediation, the remaining concentrations of As in the soil samples were measured by AAS and the growth of bacteria in the soil samples was detected with the PEG-DOG method (Trung *et al*., 2011).

Results and Discussion

As soil contamination measurements at the five sampling sites

Concentrations of As in soils from the five sample sites ranged from the lowest, 0.04 $mg \cdot kg^{-1}$ to the highest, 0.72 $mg \cdot kg^{-1}$. The average concentration of As detected from three replicates at sites 1-5 was 0.72±0.0058, 0.36±0.0115, 0.57±0.0115, 0.30±0.0577 and 0.04±0.0100 $mg \cdot kg^{-1}$, respectively.

Microorganism screening and species identification

Colonies from the strain B109 were cream, raised, smooth, and convex on nutrient agar, and B204 colonies were cream, circular, and convex on nutrient agar. The photos of the strains B109 and B204 from SEM revealed the rod and coccus morphology. Sequence alignment and dendrogram construction from 16S rRNA gene sequence analysis showed the genetic relationship of these two strains compared to other bacteria from GenBank of the National Center for Biotechnology Information (NCBI). When all of the data were examined, including the morphological characteristics assessed by SEM photos and 16S rRNA sequence, these isolates were concluded to be *Brevibacillus reuszeri* and *Rhodococcus* sp.

Bioremediation of As in Gold Mining Soils

The percentage of As removal using *B. reuszeri* was higher than *Rhodococcus* sp. and was observed as 15.28, 68.10, 79.17, 90.69, 91.67, 94.45, 95.14, and 96.67% compared to 2.78, 48.62, 69.45, 75.00, 80.56, 88.89, 93.06 and 94.17%. *B. reuszeri* has a larger capacity for As treatment than *Rhodococcus* sp. The number of *Rhodococcus* sp. was higher than *B. reuszeri* during hours 0-144, whereas after 144-168 h of incubation, the number of *B. reuszeri* bacteria was much higher than the *Rhodococcus* sp. These results suggest that *B. reuszeri* could be adapted for As treatment.

Conclusion

Microorganism strains isolated from the gold mining soil samples were tested for As removal capacity. Two bacterial isolates were identified by 16S rRNA gene sequence analysis and morphological characteristics as *Brevibacillus reuszeri* and *Rhodococcus* sp. Both species have the capacity to remove As, but *B. reuszeri* shows improved growth compared to the *Rhodococcus* sp. *B. reuszeri* might be suitable for adaptation and use in As treatment.

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Estimation of phytomass at highly degraded area and logged over forest of oil palm plantations

Nurul Athirah Liyana Abdul Rashid, *Ferdaus Mohamat-Yusuff, Faradiella Mohd Kusin and Muhamad Awang

Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia
**Corresponding author.Tel.:+603 89468023; email address: ferdius@upm.edu.my*

This study was conducted to determine the aboveground biomass in different scales of oil palm plantation covering biomass of the non-woody plants or known as phytomass such as grass. Sampling sites chosen for this study were an oil palm plantation at Chepor, Perak which was categorized as a logged-over forest area and second site was at Universiti Putra Malaysia (UPM) which was categorized as highly degraded area. Four different identical ages (1992, 2009 and 2011) of palm oil cultivation were chosen at both sites, except for the oldest palm oil tree in UPM, planted in 1973 while in Chepor, planted in 1986. The objectives of this study were to identify the phytomass present in different cultivation ages and in different land usage history, and to determine the contribution of phytomass for carbon sequestration and net carbon balance in the soil of oil palm plantation. The amount of phytomass of four different oil palm ages in UPM were higher in very old and very young cultivation while phytomass at Chepor plantation were at uniform amount at all four plots. In comparing phytomass at Chepor and UPM, the amount of phytomass at Chepor, Perak is 517.95 tonnes per hectare far less than the phytomass at UPM which is 1178.8 tonnes per hectare. The results indicated that history of the land usage has no influence on the amount of phytomass instead of the plantation management. In comparison with the soil carbon stock, this study found that soil in UPM plantation contain higher organic carbon compared to the soil in Chepor plantation. For the carbon storage, it was said that the carbon sequestration is assumed to be about 50% from the biomass amount. In conclusion, phytomass in oil palm plantations influence soil's carbon storage. Soils in highly degraded area in UPM act as effective carbon storage compared to logged-over forest at Chepor plantation.

Keywords: Phytomass, carbon stock, highly degraded area, logged-over forest, carbon sequestration

Gonadal histology of tiny scale barb, *Thynnichthys thynnoides* Bleeker 1852, during mass migration season in Rui River, Perak

Nurrul Shaqinah Nasruddin[1], *Mohammad Noor Amal Azmai[2], and Ahmad Ismail[2]

[1]*Faculty of Veterinary Medicine, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding author.Tel.: +603 8946 6644; email address: mnamal@upm.edu.my*

This study was conducted to evaluate the gonadal histology of tiny scale barb or locally known as "ikan loma", *Thynnichthys thynnoides* Bleeker 1852, during the mass migration season in Rui River, Gerik, Perak. A total of 62 (46 males: 16 females) and 60 (44 males: 16 females) fish were randomly collected along the Rui River in Kampung Kuala Rui (downstream of Rui River) and Kampung Kerunai (midstream of Rui River), from October to December 2013. The collected fish gonads were separated, fixed in 10% formalin and processed for H&E staining. The results showed that female samples collected at Kampung Kuala Rui and Kampung Kerunai was at the stage of ripe and running period which indicated by higher percentage of vitellogenic oocytes compared to other stages of oocytes. Gonads collected from Kampung Kuala Rui demonstrated only massive number of vitellogenic oocytes than other type of oocytes with no presence of atretic oocytes, while all samples from Kampung Kerunai showed the presence of atretic oocytes with the mean at 9±3% from overall oocytes counted, indicating that the fish already released their eggs. The atretic oocytes are characterised by thick ovarian wall, non-oval, shrank and wrinkled shape with less yolk granules in the oviplasm. For male gonads, 100% of samples taken from Kampung Kuala Rui demonstrated ripe and spawning stage which indicated by compacted spermatozoa in seminiferous tubules. However, at Kampung Kerunai, 57% of the samples showed ripe and spawning stage while the other 43% demonstrated spent stage or post spawning characteristics which indicated by flaccid with hollow appearance of seminiferous tubules with little amount of spermatozoa in the tubules. As a conclusion, the results revealed that *T. thynnoides* migrated from Perak River into Rui River in order to spawn in the area between Kampung Kuala Rui and Kampung Kerunai.

Keywords: Gonadal histology, *Thynnichthys thynnoides*, mass migration, Rui River

Identification of chlorophyll-a and biogenic silica from Western Street of Johor during Southwest Monsoon

Nor Faizah Noorulhudha and *Che Abd Rahim Mohamed

School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Malaysia
**Corresponding author. email address: carmohd@ukm.edu.my*

Chlorophyll-a (chl *a*) is a pigment used by photosynthetic organisms in the production of energy. While, biogenic silica (BSi) consists of marine phytoplankton that depend on silicon for the formation of the cell wall. In this study, concentration of nitrite, orthophosphate, dissolved silica an BSi have been identified as to explain the distribution of chl a. Samples were collected at western Street of Johor during the southwest monsoon which on June 2014. Published procedure of SCOR/UNESCO was used in order to obtain a concentration of chl *a*. While determination of nitrite, orthophosphate and dissolved silica conducted based on detection of colour formed. W*et al*kaline digestion followed by colour formation was used to detect BSi. Results shown that nutrient input from the river caused the highest concentration of chl *a* at J6 (13.02 µg/L) correspond to the highest concentration of dissolved silica (12.81 µM) and BSi (33.23 mg/g). In contrast, at J1, the lowest concentration of chl *a* (0.9145 µg/L) yet the highest concentration of BSi (28.02 mg/g) was recorded. This may suggest that BSi in this area consists of disintegrated diatom. Low concentration of orthophosphate (0.44 – 2.88 µM) compare to nitrite (1.56 – 7.29 µM) and dissolved silica (5.85 – 12.81 µM) suggest that orthophosphate as the limiting factor for the growth of phytoplankton. The unparallel concentration of BSi and chl a suggest that BSi is less suitable in explaining phytoplankton distribution.

Keywords: Chlorophyll a, biogenic silica, nitrite, orthophosphate

Imposex study on *Thais bitubercularis* in Merambong Shoal, Johor

Noor Farah Elinda Mohd Taib[1], *Ferdaus Mohamat-Yusuff[1,2], Ahmad Ismail[3] and Syaizwan Zahmir Zulkifli[3]

[1]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding author.Tel.:+603 89468023; email address: ferdius@upm.edu.my*

Imposex in *Thais bitubercularis* was observed in Merambong Shoal. About 18% from female individuals of the species were found to have male sexual organs, which is imposex at various stages. Stage 2 is the highest level of imposex discovered. The imposex occurrence was determined by calculated the proportion of females with imposex to the total number of female in sample and the degree of imposex was assessed using the vas deference sequence index and the percentage of females possessing the imposex characteristics. There are no significant relationship between the shell height and the number of spines between the degrees of imposex occurrence in *T. bitubercularis*. The present study also provided an overview of the present condition of the population health of *T. bitubercularis* at Merambong Shoal. Based on the male to female ratio, the population in Merambong Shoal showed in normal ratio which is 5:7 and this ratio is closer to normal ratio condition which is 4:7. The imposex incidence in the population reveals that *T. bitubercularis* in Merambong shoals are no exception from being exposed with the pollutant that can promote imposex occurrence. Tributyltin (TBT) which was recorded as the most suspected pollutant causing imposex in gastropod is expected contaminating the Johor Straits. However, the low incidence of imposex can be explain as the sample was collected from a shoal that receive high water current. The condition causing very low suspended particles or organic matter in the sediment resulting low TBT deposition in the sediment, then automatically low uptake by the biota living in the shoal.

Keywords: Imposex, *Thais bitubercularis*, tributyltin (TBT), Johor Straits

Metal (Cu, Fe, Mn, Zn) pollution assessment of surface sediments in Miri River estuarine mangrove, Sarawak, Malaysia

*Md. Masum Billah, Abu Hena Mustafa Kamal, Mohd Hanafi Idris, Johan Ismail and Nur Anisah

Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia
**Corresponding author.Tel.:+6017 8734166; email address: masum_imsf07@yahoo.com*

Introduction

Miri river criss-crosses the Miri city in Sarawak and finally meet with South China Sea. This estuary is endowed with small fringes of naturally growing mangroves. This region is renowned for its several timber and palm oil industries. Like some other estuaries, this estuary is heavily used for anchorage of seagoing vessels, cargoes and fishing boats, which eventually may contaminate its adjacent ecosystems. Besides, this area may receive organic and inorganic contaminant derived mainly from various urban and industrial runoffs.

Heavy metal contamination is an important urban problem now a day. These metals are non biodegradable, can be shifted to higher trophic level by accumulation and harmful for organisms beyond certain concentrations. Although a number of studies describe the heavy metal pollution status of coastal area of Peninsular Malaysia, comparatively few studies are found related to the metal pollution level of the coastal environment of Sarawak (Ismail, 1993 and Nagarjan *et al*., 2013). Therefore, present study examined heavy metal pollution status and their ecological risk in Miri river estuarine mangrove area using USEPA sediment quality guidelines and the contamination assessment methods (pollution load index, contamination factor as well as enrichment factor).

Materials and Methods

The study area is located in Miri river estuary, Sarawak (04º 24' 15.8" N and 113º 59' 20.1" E). Three sampling stations were selected approximately 1 km interval from the mouth of the estuary to the upper reach. Surface (< 5 cm) mangrove sediment samples from each station were collected randomly with three replicates. All soil samples were air-dried in the laboratories for 3 weeks and grinded and sieved (250 µm mesh). Two (2) g of sieved sediments samples were digested with 20 ml aqua regia solution (a 3:1 mixture of HCl and HNO_3) before metal analysis (Nagarjan *et al*., 2013). Acid extracted metals were measured using AAS (Atomic Absorption spectrophorometry. Ecological risk of metal pollution in Miri river estuary was evaluated by USEPA sediment quality guideline (Perin *et al*.,1997), pollution load index (PLI), contamination factor (CF) as well as by determining enrichment factor (EF). PLI is a simple and comprehensive way of determining metal pollution status of a site. PLI, CF, and EF were determined by the following formulas:

$$PLI = \sqrt[n]{CF_1 \times CF_2 \times CF_3 \times CF_4 \ldots\ldots\ldots \times CF_n}$$

$$CF = C_{sample}/C_{background}$$

$$EF = (X/Fe)_{sediment}/(X/Fe)_{background}$$

Where, C_{sample} is the mean metal concentration of target metal, while $C_{background}$ is the background concentration of that metal, in this study average values of world rock surface was used for background concentration of each metal studied (Table 1). $(X/Fe)_{sediment}$ is the ratio of mean concentration of target metal and Fe concentration in a given site, whereas the $(X/Fe)_{background}$ is the ratio of natural background level of the target metal and Fe. The letter "n" is the total number of metal studied.

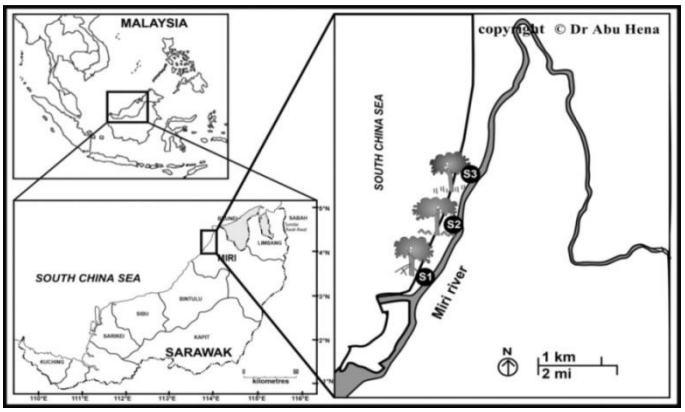

Figure1: Location of study area showing three (3) sampling stations.

Results and Discussion

The soil texture was 66-72% for sand, 1.8-5.8% for silt and 20.2-31.2% for clay with slightly acidic pH value of 5.73-6.36. Comparatively, higher proportion of sand particles suggesting the charactersitics of high energy area of estuarine environment (Essien *et al*., 2009). On average, higher Fe (7664.30 µg/g) and Zn (26.68 µg/g) concentration were found compared to Mn (14.33 µg/g) and Cu (13.43 µg/g; Table 1). Two main groups of metal concentrations were found in the mangrove sediment of Miri river estuary (Figure 2). The concentrations of Fe, Cu and Zn were found to be higher in the present study compared to other studies elsewhere (Ismail, 1993; Sany *et al*., 1993; Santhiya *et al*., 2011), suggesting probable industrial discharge and urban runoff in this area. In comparison to USEPA sediment quality guideline, the concentration of Fe was found two hundred folds higher, while the concentrations of Cu, Mn and Zn were below the guideline. The measured pollution load index of Miri river estuary was 0.13 (<1), which indicates that this estuarine mangrove sediment is considered to be of less metal pollution concern. The enrichment factors were found to be 1.99 for Cu, 0.98 for Zn and 0.09 for Mn (Table 1). Based on enrichment factor, the study area was enriched with Cu (<3), however, deficiency to minimal enrichment was found with Zn and Mn (<1), suggesting that this estuarine mangrove sediment is not contaminated by Zn and Mn by anthropogenic activities (Birth, 2003). The metal

concentrations in the mangrove sediment of Miri river estuary did not reflect the likely effect of toxicity to the aquatic biota. Therefore, future research is needed to be carried out to determine the likely effect of metal contamination in this aquatic ecosystem using suitable biomonitor organisms.

Table 1. Range, mean metal concentration, USEPA guideline concentration factor, enrichment factor and pollution load index of the studied metal (n =18).

	Cu	Zn	Fe	Mn
Range (µg/g)	4.42-35.15	15.25-45.3	2290-17360	9.02-21.25
Mean±SE(µg/g)	13.43±2.50	26.68±1.63	7664.30±1022.4	14.33±0.8
USEPA(µg/g),	16	110	30	36
World rock surface average (µg/g),	32	127	35900	750
CF	0.420	0.210	0.213	0.019
EF	1.966	0.984	1.000	0.090
PLI	0.13			

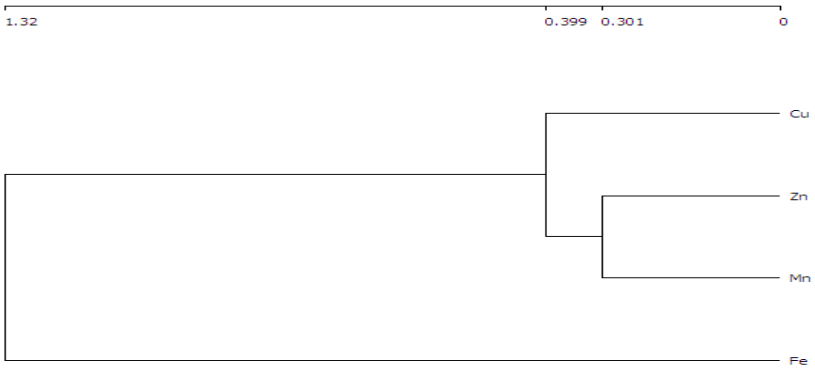

Figure 2: Hierarchical cluster analysis based on Fe, Mn, Cu, Zn concentrations (log10 x + 1) in the surface sediment of Miri river estuary.

Acknowledgements

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Natural thorium isotopes and recent sedimentation rates in Port of Tanjung Pelepas (PTP), Johor, Malaysia

*Abdul Hafidz Yusoff[1], Asnor Azrin Sabuti[1], Khairul Nizam Mohamed[2], Ahmad Ismail[3], Syaizwan Zahmir Zulkifli[3] and Che Abd Rahim Mohamed[1]

[1]Pusat Pengajian Sains Sekitaran, Fakulti Sains dan Teknologi, Universiti Kebangsaan Malaysia 43600 Bangi, Selangor
[2]Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
[3]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
** Corresponding author.Tel.:+618-2844201; email address: hafidzyusoff@siswa.ukm.edu.my*

Introduction

The Port of Tanjung Pelepas (PTP) of the Sungai (river) Pulai located in Malay Peninsula's most southern tip in the State of Johor, close to the new Malaysia-Singapore Second Crossing. The port development at Tanjung Pelepas transformed the river and mangrove area in 1998 into one of the world's most equipped container port. The port has stimulated rapid development in the region stretching from state capital Johor towards the west along the Johor Straits; it has changed the region to developed area with excellent infrastructure, housing facilities and new areas for industrial. The geochemical behaviors of thorium and ^{210}Pb in sediment have received extensive study due to the importance of these radionuclide as geochronology indicator and application in environmental monitoring (Handerson and Anderson, 2003; San Miguel *et al*., 2004). The ^{230}Th introduced to marine environment mostly by the in situ decay of dissolved ^{234}U. The long-lived ^{232}Th isotopes enter the marine ecosystem in continental detritus and hence enriched the activity of ^{232}Th in area adjacent to continental margin and in high dusk flux zones (Anderson *et al*., 1995). Previous study done by Hafidz *et al*. (2014) show Labuan port which is located at the east Malaysia was dominated with lithogenic origin of thorium isotopes.

Studies on naturally occurring radionuclides ^{210}Pb as geochemical tracers are poorly known in Malaysia. Teng *et al*. (2003) was determined the sedimentation rate at coastal water of Sabah by using ^{210}Po and ^{210}Pb method. The sedimentation rates estimated from ^{210}Pb at coastal water of Sabah were in the range 0.003 to 0.049 cm/year. Another study was conducted by Zal Uyun *et al*. (2011), they reported the sedimentation rate occurred at the Sabah and Sarawak coastal water by using ^{210}Pb methods and found high sedimentation rate occurred at these area 0.24-0.48 cm/year. To the author's knowledge, no radionuclide depth profiles from marine port were studied or used for estimating recent sedimentation rates up to this date. The main aim of this work is to provide the current activity of natural radionuclide such as thorium and lead isotopes at the study area. This work also investigates the sedimentation rate occurred at PTP port by using ^{210}Pb methods. Since the core sediments act as sink for different anthropogenic pollutants, a study of

geochronology in sediments cores can reveal pollution history and its effect to the marine environment.

Materials and Methods

The sediment cores of 63 cm long were obtained from the PTP, Johor (Figure 1). The coordinate of the exact sampling location was recorded using Geographical Positioning System (GPS). The location are Lat. 01° 20'17.5"N Long. 103°32'46.4"E. The core sediment was collected using the gravity corer and sediment was sliced every 3 cm.

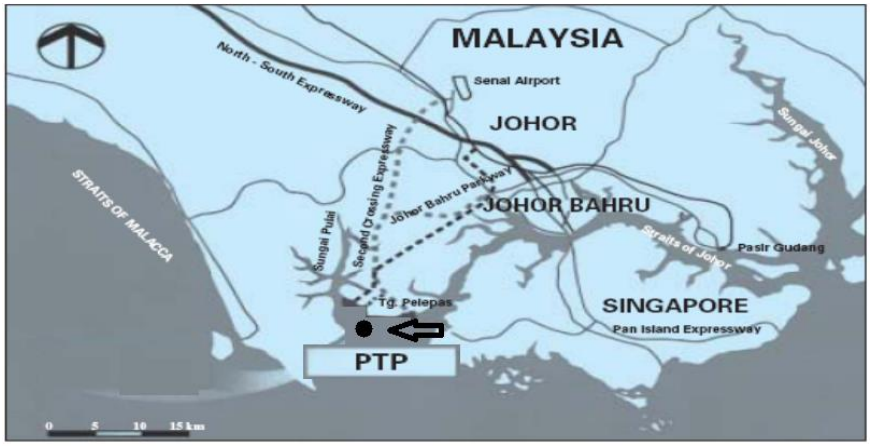

Figure 1: Map of the Port of Tanjung Pelepas (PTP).

The analytical separation techniques such as extraction, column separation and detection of thorium were followed from the procedure publish by Hafidz *et al*. (2014) while for ^{210}Pb the procedure were followed from the paper publish by Zal Uyun *et al*. (2011).

Results and Discussion

Profile of ^{232}Th and ^{230}Th

The result for the activities of ^{232}Th, ^{230}Th and ^{230}Th/^{232}Th at PTP were summarized in Figure 2. The activity of ^{232}Th in core sediments collected from the PTP show the consistent profile from bottom to the top of sediment core with the range activity between 3.13±0.46 to 5.52±0.55 while the activity of ^{230}Th decreased from bottom the top area with the range activity from 2.42 ± 0.37 dpm/g to 3.99 ± 0.51 dpm/g. High activity of ^{232}Th and ^{230}Th detected at the 31.5 cm core might be effected by physical factors occurred at the study area. Table 1 shows the comparison data of thorium isotopes from PTP and others area. The activity of ^{232}Th detected at the PTP is within the same order of magnitude with the activity detected at the Thailand gulf as reported by Srisukawad *et al*. (1997) and Northeastern Taiwan as reported by Chung and Chang (1996). However, the activity of ^{232}Th at PTP is more higher compared to Labuan port as published by Hafidz *et al*. (2014). High activity of ^{230}Th detected at the study area might be due to high organic input such as mangrove forests which are carrying more ^{230}Th to the study area.

The ratio of $^{230}Th/^{232}Th$ has been widely used in determining the geochemistry of Th in marine environment. They may provide clues to their sources as well as their pathways. The normal value of $^{230}Th/^{232}Th$ ranges between 0.8 to 1.5 (San Miguel *et al*., 2004). The activity ratio of $^{230}Th/^{232}Th$ detected at the PTP is in the range 0.55 to 1.58. Most of the upper layers (0-19.5 cm) show the ratio < 1 while lower layer > 19.5 cm show the ratio > 1. These results suggest the decreasing input of ^{230}Th at the recent time to the study area. Much of the mangrove at this area is being degraded due to tidal effects from the two open waters of the South China Sea and the Straits of Malacca which affect the shoreline. Furthermore, development at Tanjung Pelepas was transformed the river and mangrove area into one of the world's most equipped container port. The degradation of mangrove forest at this area leads to decreasing the activity of ^{230}Th from bottom to the top area of core sediments. The ^{232}Th enters the marine ecosystem in continental detritus (Bacon and Handerson, 1982) and hence enriched the activity of ^{232}Th adjacent to the continental margin. The high input of ^{232}Th at the surface area of PTP might be from the igneous rock which is abundant adjacent to Kulai River (Yii *et al*., 2008).

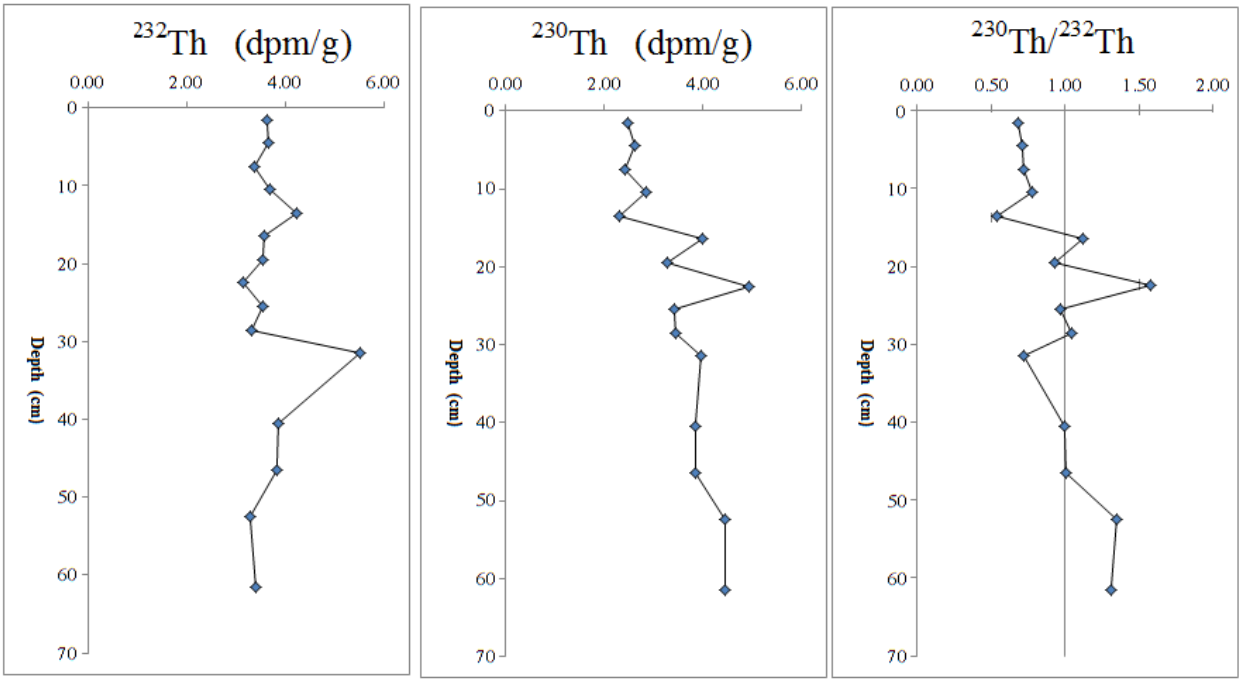

Figure 2: Profile activities of ^{230}Th, ^{232}Th and ratio of $^{230}Th/^{232}Th$ in core sediments from PTP.

Table 1: Comparison of ^{230}Th and ^{232}Th in PTP port with other areas in Southern South China Sea.

Thorium Isotopes	**Labuan Port (Hafidz *et al*., 2014)**	**Thailand Gulf (Srisukawad *et al*., 1997)**	**Northeastern Taiwan (Chung and Chang, 1996)**	**PTP port (this study)**
^{230}Th	1.39 - 9.84	1.33-3.77	1.5-2.30	2.42 - 3.99
^{232}Th	1.31 - 2.87	1.70-4.92	2.72-3.64	3.13 - 5.52

Sedimentation rate

The sedimentation rate of core sediment at PTP was successfully estimated by using the ^{210}Pb methods. The sedimentation rate and age of sediment was estimated from the slope of Ln ^{210}Pb (excess) at the decay zone (Figure 3) and calculated by using equation 1 and 2.

$A = A_0 e^{-\lambda(t)}$ …… (a)

$t = z/s$ ……(2)

Where A_0 and A are the activities of radionuclides at the surface and depth z, respectively, λ is the decay constant, s is the sedimentation rate (cm/year) and t is the age of sediment at depth z. The result show the sedimentation rate is 0.28 cm/year. The sedimentation rate detected at the PTP is within the same order of magnitude with the sedimentation rate detected at the coastal water of Sabah and Sarawak (0.24-0.48 cm/year) published by Zal Uyun *et al*. (2011) and Kuala Selangor, Malaysia (0.1-0.2 cm/year) as published by Choy *et al*. (2007).

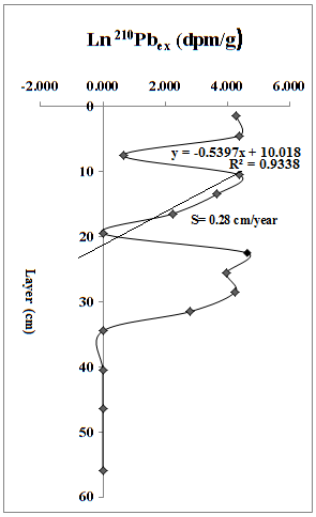

Figure 3: Sedimentation rate estimated from the slope of Ln ^{210}Pb$_{ex}$ at the PTP.

Conclusion

The activity of ^{232}Th in core sediments collected from PTP show the consistent profile from bottom to the top of sediment core while the activity of ^{230}Th decreased from bottom layer to the top area. Most of the upper layers show the ratio < 1 while lower layer show the ratio > 1 suggest the decreasing input of ^{230}Th at the recent time to the PTP. Much of the mangrove at this area is being degraded due to tidal effects from the two open waters of the South China Sea and the Straits of Malacca which affect the shoreline. Furthermore, development at Tanjung Pelepas was transformed the river and mangrove area into one of the world's most equipped container port. Thus, the degradation of the mangrove forest at this area leads to the decreasing of the ^{230}Th activity from bottom to the top area of sediment core. The sedimentation rate of core sediment from PTP was successfully estimated by using ^{210}Pb model. The result show the sedimentation rate is 0.28 cm/year. Well dated sediment core

can be used in forthcoming research to investigate the historical profile of anthropogenic activities affecting the PTP port.

Acknowledgements

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Population characteristics, length–length and length–weight relationship of *Cherax quadricarinatus* (Von Martens 1868) from natural and man–made habitats in Malaysia

Nazirah Omar, *Johan Ismail, Mohd. Hanafi Idris and Abu Hena Mustafa Kamal

Department of Animal Science and Fishery, Faculty of Agriculture and Food Sciences Universiti Putra Malaysia, UPM Bintulu Campus, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia

**Corresponding author.Tel.:+6086 855368; email address: ijohan@upm.edu.my; joismail@gmail.com*

Introduction

The freshwater crayfish *Cherax quadricarinatus* originated from Northeastern Australia and Papua New Guinea have been translocated by human all over the world (Ahyong and Yeo, 2007). Suitable biological attributes such as fast growing and omnivorous feeding habits have contributed to the successful establishment of *C. quadricarinatus* in many countries. In Malaysia the first record of wild populations of freshwater crayfish *C. quadricarinatus* were recorded in Sarawak (Johan *et al.,* 2012). The objectives of this study are to determine the population characteristics, length-weight and length-length characteristics of *C. quadricarinatus* from Similajau River, Sri Rajang Stream and Parit Sulong Waterway.

Materials and Methods

The crayfish were collected using baited traps in the months of May 2013 until February 2014 from three different locations, Similajau River, Parit Sulong and Sri Rajang. Data collected consisted of total length, carapace length, total weight and sex. Length-weight relationship was measured using total length and total weight of 413 samples from the locations. Length-weight and length-length relationship were established according to Nurul Amin *et al.* (2008). Length frequency was analyzed based on the frequency of total length (TL) of the samples for each location. The number of sample was considered as dependent variable of total length. Length frequency analysis was done using SPSS Statistic version 20.0. The sex ratio of *C. quadricarinatus* was determined based on female to male.

Results and Discussion

Systematic and Distribution

Collected crayfish were examined to determine the species based in keys provided by Holdich (2002). The species caught were identified as *Cherax quadricarinatus* (Von Martens 1868). The key identification characteristics of the species are as follows: 1) Redclaw crayfish are typically blue in colour, often they are marketed as blue lobster. 2) The male have a red patch on the outside the claw, it become more prominent as the age. 3) The head of *C. quadricarinatus* has four long and distinct ridges on the top. The inner ridges are longer and have three pairs of lateral spines (Ahyong and Yeo, 2007).

Table 1: Length-weight relationship parameters of *C. quadricarinatus* in Similajau river, Sri Rajang stream and Parit Sulong Waterway.

Sex	N	TL (mm)	Weight	a	b	r^2	Relationship
Female	182	61.4 -167.5	6 – 98g	0.000070	2.71	0.899	Allometric
Male	231	60.6 -181.0	6 – 155g	0.000025	2.986	0.850	Allometric

N: Sample size; TL: Total length; a and b: Parameters of the length-weight relationship; r^2: coefficient of correlation.

Length-weight relationship

The calculated equations for length-weight relationship for males and females were $W = 0.000025L^{2.9896}$ or $\log W = - 4.6009 + 2.9896L$ ($r^2 = 0.850$) which is perform in $W = aL^b$ equations (Figure 1). The log-log transformed data were derived from $\log_{10} W = \text{Log}_{10}a + b\ \text{Log}_{10}L$. The equations for female *C. quadricarinatus* for length-weight equations were $W = 0.000070L^{2.751}$ or $\log W = 2.751L - 4.1527$ ($r^2 = 0.899$).

The values of the growth coefficient b (exponents) obtained for male and female *C. quadricarinatus* was 2.71 for female and 2.98 for male. In this case, the value of b lies between the values of 2.50-3.50 that was reported for most aquatic organisms such as *Acetes vulgaris* (Arshad *et al*., 2008). So in the study, the growth of body parts were allometric indicates that the growth was slower. Parameter of this condition was affected by number of factors including sex, health and environment. Impact on environment may reduce the population of crayfish (Nzeh *et al.,* 2007). The estimated coefficient correlation (r^2) between total length and total weight was 0.899 (female) and 0.850 (male). This indicated that the relationship between length and weight was highly significant ($p<0.05$).

Length-length relationship

The relationship between carapace length and total length was tested according to locations and was found to be highly significant ($p<0.05$) in all three locations (Table 2).

Table 2: Population characteristics and morphometric relationship between total length and carapace length of *C. quadricarinatus* in Similajau River, Sri Rajang Stream and Parit Sulong waterway.

Parameters	Similajau river	Parit Sulong	Sri Rajang
N	282	107	24
L-L	CL = 1.455 TL + 35.49	CL = 2.105 TL + 4.42	CL = 2.051 TL – 0.35
r^2	0.734	0.939	0.806
Mean CL	54.10 mm[a]	52.76 mm[a]	42.07 mm[b]
Mean TL	114.181 mm[a]	107.83 mm[a]	92.89 mm[b]
Mean W	37.45 g[a]	32.83 g[a]	19.79 mm[b]
SR	1:1.2	1.0.9	1.11

N: Sample size; LL: Length-length relationship; CL: Carapace length; TL: Total length,
W: Weight; r^2: coefficient of correlation; SR: Sex ratio (female:male)

Population structure

Total number of crayfish collected for this study was 282 (Similajau river), 182 (Parit Sulong) and 24 (Sri Rajang) (Table 2). The higher population number of crayfish in Similajau River was due to more sampling attempts in the location. The mean carapace length, total length and weight of crayfish from Similajau River was more similar with Parit Sulong compared to Sri Rajang Stream and the difference between means was significant ($p<0.05$). The differences can be due to the sample size in Sri Rajang that was very small.

Sex ratio

A total 413 samples were examined. The sex ratio was found to be 1:1.27 (females: males). Study indicated that the number of males relatively higher than females.

Conclusion

The population structure of *Cherax quadricarinatus* were described for the first time from Similajau River and Sri Rajang stream in Sarawak and Parit Sulong waterway in Johor. The study showed that the length-weight and length-length relationship were significant. Results indicated that *C. quadricarinatus* exhibited allometric growth pattern. The sex ratio was found to be 1:1.2 (females:males). The population of the crayfish in Similajau River and Parit Sulong waterways were more similar compared to Sri Rajang Stream.

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Preliminary studies of ligament lines: is it suitable to determine the age of cockle (*Anadara granosa*) in tropical area?

Amirul Azuan Md Joni[1], Ferdaus Mohamat-Yusuff[1], Syaizwan Zahmir Zulkifli[2] Aziz Arshad[3] and Mad Nasir Shamsudin[4]

[1]*Environmental Forensics Research Center, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor*
[2]*Department of Biology, Faculty of Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor*
[3] *Departments of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor*
[4]*Department of Agrobusiness and Information Systems, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor*
**Corresponding author.Tel.:+603 89468023; email address: ferdius@upm.edu.my*

Introduction

Cockle culture is extensively operated in Malaysian estuary especially at the Straits of Malacca. Not only it has a major important economic value, this animal is a benthic organism and has very limited mobility which makes it as a good bioindicator especially for the wild breed. However, a method to estimate the age of wild cockle is important to obtain the actual data representing the period of pollution exposure. For that reason, this study has been conducted to identify the age of cockle *Anadara granosa* by observing the ligament lines. In most common research, the method in determining the age of bivalves is to count the annual rings on the surface of the shells (Seed and Brown, 1978; Richardson, 1987). However, it is not suitable method in tropical region as annual ring only formed in the temperate region due to the present of winter season. Since *Anadara granosa* are present only at tropical region, the methods that are based on annual rings were practically impossible. As for that, new approaches have to be developed as an alternative method in order to determine the age of this particular species.

Materials and Methods

The fresh specimen were taken from the area of Kong Kong Laut, Johor and been further analyzed in the laboratory. The content of the cockles were removed and the shell were being washed while the hinge side were carefully scrubbed until the ligament lines can be seen clearly while the hinge side of the shell were then been observed under a compound microscope. The shells were then and been separated according to their ligament lines. The width, height, and length for each of the cockle were measured in order to compare with the number of ligament lines. Scatter graph had been plotted to see the trend of the increment of both ligament lines (x-axis) and the length of the cockles (y-axis). Regression variables were calculated to explore the predictive ability and also to determine the strength of the correlation between the ligaments lines and the length of the cockles.

Results and Discussions

There were about 23 samples of *A. granosa* had beed collected along the mudflat with the minimum length of at the Kong Kong Laut. The ligaments lines were vary among the cockles, and several groups were formed based on the numbers of their ligament lines.

Table 1: Several group and individuals were separated according to the number of their ligament lines.

No. of Cockles	No. of Ligaments	Mean of the cockle's length
12	1 - 2.5	56.86
8	3 - 4.5	67.67
3	5 - 8.5	72.47

The treadlines values from the scatter graph shows the value of R^2=0.4439. It shows that the chances of the number ligaments increase as the length increase is only about 44%, which can be considered to be slightly deviated from the Ubakata (2003) statement, may due to heavy storms and unfavorable sea water salinity. However, the Pearson correlation test shows that both of the variables have strong correlations with the significant value of ($p<0.01$). Regression analysis reveals that every single increase in ligament number will cause the increment of (b=3.971) as Y= cockle length, a= constant value, b= ligament unit value, and x= number of ligament, according to the linear regression formula, Y=a+b*x. Thus, with these data, we can predict the increment of the cockle length by using the cockle's ligament number (x) as the base of the calculation.

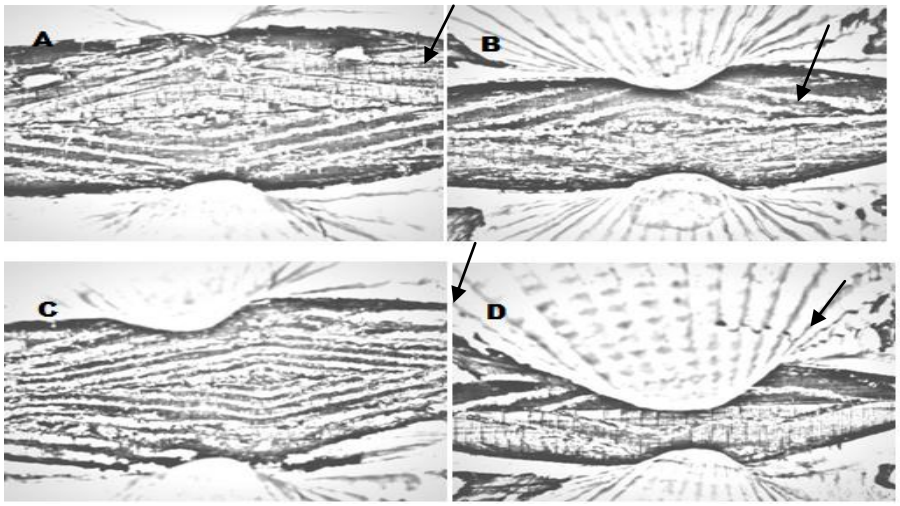

Figure 1: Sketch picture shows the difference in the number of ligament lines (shown by the arrow) among the samples that had been collected. **A.** 5 ligaments, **B**. 3 ligaments, **C**. 7 ligaments, **D**.1.5 ligaments.

Ligament serves to open the valves while the adductor muscles relax, thus it is considered to be highly adaptive structures in Bivalves (Seilacher, 1984). According to Ubukata (2003), as shell increase in size, it will cause the ligament layer increase its length along the hinge axis and extends amphidetically while new fresh material is continuously added at the ventral margin of the ligament. In other words, as the shell increase in length, the number of the ligament lines will increase and extend outwards.

Generally, there several types of the arrangement of cockle's ligament which are parivincular, planivincular, alivincular, multivincular and duplivincular. For *A.granosa*, it has the arrangement of duplivincular ligament. Thomas (1976) stated that increasing number of the ligament for duplivincular is highly necessary due to increase in shell's size in order maintaining the relationship between the animal weight and the strength of ligament, thus support Ubukata's statement.

Conclusion

The method for ligament lines are proven to be simpler, practical, time-saving and also can be done at the field since it does not require any complex machine to count the ligament line of the cockle. However, further studies have to be made due to the period for a single ligament line is yet to be determined.

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Preliminary study of selected heavy metal contamination in surface sediment of Merambong Shoal Seagrass Bed

*Syaizwan Zahmir Zulkifli, Azizul Aziz, Ahmad Ismail and Mohammad Noor Amal Azmai

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

Corresponding author:Tel:+603 89466627; email address: syaizwan@science.upm.edu.my

Surface area of Merambong Shoal Seagrass Bed (MSSB) is an important area for aquatic organisms as well as human communities for fishing activity. Present study was conducted to evaluate the current status of selected heavy metals contamination in surface sediments collected from the MSSB. Samples of surface sediments were collected from multiple locations along MSSB. The heavy metal concentrations of the present study were compared with previously conducted studies in similar location, as well as other location in Malaysia. Geofractionation of heavy metals were also determined. Based on the obtained mean concentration of each metal, a few indices were used to demonstrate the present level of contamination in the surface sediment of MSSB. The findings of this research are important for monitoring heavy metals pollution and conservation management.

Keywords: Heavy metals, surface sediment, pollution level, Merambong Shoal Seagrass Bed

Profile of Heavy Metals Level in Catfish (Hexanematichthys sagor) and Green Mussel (Perna viridis) from Kong Kong Laut, Johor Straits

*Ferdaus Mohamat-Yusuff[1,2], Liong Swee Yun[1], Eddie Cheah Kee Wan[1] and Syaizwan Zahmir Zulkifli[3]

[1]Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Environmental Forensics Research Centre, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
**Corresponding author.email address: ferdius@upm.edu.my*

Introduction

High heavy metal concentrations in the environment are normally related to human activities. They are accumulated in estuaries environments including water, sediments and marine organisms, and subsequently transferred to human in the food chain. Therefore, heavy metal contamination has become worldwide concern due to their toxicity, prolonged persistence, bioaccumulation in organisms, and ultimately their effect on the aquatic environment and human health (Zulkifli *et al*., 2010). Fish is the important protein supply for Malaysian. Fish is almost the highest trophic level in the aquatic ecosystem and may contain higher concentration of pollutants due to bioaccumulation and biomagnification process. Hence the determination of heavy metal in fish is necessary to ensure the fish is safe to consume by human. Green mussel has been used as biomonitoring in Asia-pacific countries due to its best accumulator of heavy metal (Yap *et al*., 2006). Besides that, green mussel is a commercial fisheries product of the estuaries, bays and coastal. Thus, analysis of green mussel is important to determine the heavy metal contamination at the study area and the product is safe to consume by human. The objectives of this study are to investigate the heavy metals concentration in tissues of catfish and green mussel and estimate the potential human health risk by consuming these organisms.

Materials and Methods

The samples of catfish and green mussel were collected from Kong Kong Laut. All samples were stored at low temperature in an ice box and transported to the laboratory. The total length and weight of the catfish are recorded. Dissection was conducted to obtain flesh tissues for heavy metals analytical procedures. Extraction of heavy metals from biological tissues was based on description by APHA (1999). Each category of samples was prepared in triplicate and digested by acid digestion. The digested samples were diluted with Milli-Q water before analysed by a flame atomic absorption spectrophotometer (AAS) Shimadzu Model AA-6800. The instrument calibration standards were made from diluting stock solution (1000ppm). The procedural blanks were prepared with the same procedure without sample addition. Prior to any analysis, all glassware and apparatus were acid-washed overnight. The results are expressed as µg/g of dry weight. Two-way ANOVA and linear regression analysis test are used to determine the significance of the results. The result also used to conduct potential human health risk by Estimation Dietary Intake and Target Hazard Quotients (Yi *et al*., 2011).

Results and Discussion

Heavy metals concentration in different catfish tissues from Kong Kong Laut are shown in Table 1. The concentration of the heavy metals decreased in the following order Fe > Mn > Cu > Pb > Cr in the fish. The concentrations of heavy metals were significantly different according to ANOVA test in different tissues of the fish. The differences in heavy metal concentration of the tissue might due to their capacity to induce metal-binding proteins such as metallothioneins (Bashir *et al*., 2012). The result also showed the total heavy metal concentrations in liver and gills are about the same. This indicates that large amount of metallothioneins induction occurred in the liver tissue of the fish (Bashir *et al*., 2012). The total metal level in the gill is expected caused by the adsorption of metals onto gill surface (Bashir *et al*., 2012).

Heavy metal concentrations for different part of tissues in different sizes of catfish are shows in Table 1. In comparing the heavy metals concentration with the sizes of the fish shows that there are significant positive relationships found between the catfish length with Fe ($P<0.1$) and Mn ($P<0.05$) concentration in the catfish muscle tissues, and also between the weight with the Fe concentration in the catfish muscle ($P<0.05$) tissues. This may due to the ability of Fe to accumulate in fish muscle with the growth of the fish since Fe is an essential element for red blood cell formation. The positive relationship between Mn with length of fish is probably Mn is accumulated at higher rate compared to rate of excretion as the fish grows (Ansari *et al*., 2006). No significant relationship between concentration of Cu, Cr and Pb with fish sizes may due to the ability of cat fish to regulate and maintained these heavy metals concentration in their body by metabolic activity (Jezierska and Witeska, 2006). Another possible explanation is the difference in metabolic activity between younger and older fish (Canili and Atli, 2003).

Table 1: Heavy metal concentrations for different part of tissues in different sizes of catfish. (Mean±Standard of error, in µg/g as dry wt. by AAS (n=4).

Metal	Organ	Fish Sizes					
		Small		Medium		Large	
Cu	Muscle	1.66	±0.26	1.76	±0.43	1.94	±0.52
	Gills	2.45	±0.75	2.67	±0.68	2.19	±0.19
	Liver	7.34	±0.44	14.45	±0.44	7.07	±0.85
Cr	Muscle	1.47	±0.18	2.38	±0.63	2.29	±1.00
	Gills	1.73	±0.12	1.68	±0.26	2.30	±0.68
	Liver	1.65	±0.34	1.72	±0.81	3.02	±1.56
Mn	Muscle	2.10	±1.52	3.40	±1.66	4.05	±3.25
	Gills	5.43	±2.27	7.88	±4.48	7.02	±3.50
	Liver	3.57	±1.73	6.92	±4.13	6.24	±3.62
Pb	Muscle	1.47	±0.52	0.89	±0.41	1.74	±0.72
	Gills	7.79	±0.80	9.45	±0.58	13.11	±2.78
	Liver	1.49	±0.50	1.91	±0.64	0.51	±0.18
Fe	Muscle	32.69	±8.88	36.69	±11.46	39.42	±12.45
	Gills	316.62	±23.21	112.87	±11.18	142.68	±17.21
	Liver	171.87	±15.13	228.54	±24.87	166.75	±10.40

Concentration of heavy metals in green mussel (*Perna viridis)* in this study is shows in Table 2 along with reported level from other studies in Malaysian. The result of the present study is within the ranges of reported level from previous study in Malaysia. Therefore, the heavy metal concentration in green mussel from Kong Kong Laut is found not to be serious. Yap *et al*. (2006) reported that the contamination of heavy metal along the Johor Straits is resulted from the discharge of effluents from domestic, industrial source and shipping activity such as some petrochemical plants, port activity. High level of Cu has been reported due to leachate from antifouling paints of boats Yap *et al*. (2006). Furthermore, the semi-enclosed ecosystem of the strait has enhanced the pollution problem in the Johor Straits.

Table 2: Compilation of Cu, Pb and Fe concentrations (ug/g dry weight) in the soft tissue of *P. viridis* reported from previous regional studies along with present study.

Location		**Cu**	**Pb**	**Fe**	**References**
Kong Kong Laut		6.15±1.4	2.55±0.37	122.5±19.72	Present study
Kg. Masai		12.2±1.36	NA	NA	Yap *et al*., 2012
Kg. Sg. Melayu		5.52±0.55	NA	NA	
Johor Straits (8 sites)	Gill	11.2±1.11	11.9±2.28	453±112	Yap *et al*., 2006
	Gonad	8.28±0.56	10.9±1.92	133±37.4	
	Foot	8.07±0.47	11.8±2.67	117±27.7	
	Muscle	6.98±0.84	10.2±1.84	89.8±15.7	
	Mantle	9.16±0.96	15.0±4.05	19.5±82.3	
	Remainder	11.5±1.17	14.4±2.64	448±222	
East Coast of Peninsular Malaysia	Nenasi	3.84	8.84	NA	Yap *et al*., 2005
	Kuala Pontian	10.34	7.95	NA	
West Coast of Peninsular Malaysia	Pulau Aman, Penang	10.8	4.76	NA	Yap *et al*., 2004
	Bagan Lalang, Selangor	8.2	3.41	NA	
	Kuala Linggi, N. Sembilan	9.14	7.98	NA	
	Pantai Lido, Johor	9.39	4.03	NA	
	Sebatu, Malacca	11.20	7.59	NA	
	Kuala Dinding, Perak	7.76	2.51	NA	
	Muar Estuary	8.96	2.28	NA	Kamaruzzaman *et al.*, 2008

Estimated daily intake (EDI) of heavy metals through consumption of catfish (except for Cr) and green mussel (except for Cr and Pb) are below the RfDs as shows in Table 3. This indicating that normal consumption of catfish would not result in health risk of the three heavy metals. The EDI above the RfD such as Cr in catfish, and Cr and Pb in green mussel would possible expose to high level of the heavy metal through excessive consumption. the risk of non-carcinogenic effects of the fish and mussel consumption is express by Estimate Target Hazard Quotients (THQ). THQ for Fe is unavailable due to unavailable RfD from USEPA, 2009. The estimated THQ for each metal in catfish and green mussel from Kong Kong Laut decreased in following sequence: Pb > Cu > Mn > Cr. The THQ of heavy metal in catfish and green mussel (except for Pb) is less than 1, indicates that people would not experience significant health risk from intake of individual metals (Table 5). THQ

higher than RfD may experience a certain degree of adverse health effects related to high intake of the individual heavy metal (Yi *et al*., 2011).

Table 3: Calculated EDI and THQ of Cu, Cr, Mn, and Pb of catfish and green mussel from Kong Kong Laut.

Metals	**Cu**	**Cr**	**Mn**	**Pb**	**Fe**
RfD /(µg/kg/day)	40	3	140	3.5	NA
EDI in catfish / (µg/kg/day)	4.58	**10.13***	8.17	3.48	NA
EDI in green mussel /(µg/kg/day)	15.73	**6.90***	99.11	**6.52***	NA
RfD/(mg/kg/day)	0.04	1.5	0.14	0.004	NA
THQs (catfish)	0.12	0.007	0.06	0.9	NA
THQs (green mussel)	0.40	0.0047	0.73	**1.68***	NA

Conclusion

The study concluded that heavy metals concentrations are in the moderate level in the catfish and green mussel. However, a potential changes may occur in the future depending on the pollution sources. Overall, the objectives of the study have achieved. The study may be taken as a base line study at this study area.

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Safety consumption of heavy metals (Cd and Pb) in edible tissues of paddy eel, *Monopterus albus*, from paddy cultivation areas

Sow Ai Yin, Ahmad Ismail*, Syaizwan Zahmir Zulkifli and Mohammad Noor Amal

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Introduction

Fish is an important protein source and also rich in other nutritional values for human. Paddy eels are reported to have high nutritional values and said to be on par with several fishes such as mackerel and scad. Moreover, consume of fish could provide two types of omega 3 poly unsaturated fatty acids, which are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and other nutrients (Castro-Gonzalez, 2002; Clarkson, 2002; Dominogo *et al*., 2007). In Kelantan, paddy eel can be found in temperate and freshwater system. It also can be found in canals, swamps, rivers, and paddy fields (Sow *et al*., 2013).

Due to the regular application of agrochemical fertilizers and pesticides in paddy cultivation areas throughout the paddy seasons, the accumulation of heavy metals in paddy soil are elevated. This situation could happen when there are presence of large population of apple snails and paddy rats in the paddy fields. Different soil management during paddy seasons could contribute an increasing of pollutants. This could be observed during the plowing season, where the plowing process could potentially resuspend the pollutants that exist from previous paddy cycles in the deep soil layers (Zulkifli *et al*., 2010; Sow *et al*., 2013).

When heavy metals and metalloids occur in higher concentrations, the aquatic organisms, particularly paddy eel, are subjected to severe toxins and contamination. At the same time, the contaminated paddy eel can cause a potential health risk and problem to top level receptors, including humans (Burger and Gochfeld, 2005). In this study, the muscle of paddy eel was selected to determine the potential human risk of consuming paddy eel as one of their foods.

Materials and Methods

Tukil is a tool that had been used by eel collectors to trap the swamp eel. Prior catching the swamp eel, Tukil was placed horizontally in the selected positions for overnight and swamp eel was collected for the next day. The baits, particularly, small well-cooked fish were inserted in the Tukil to attract the swamp eel. The caught swamp eels were placed in the polyethylene plastic bag and transported back to the laboratory and stored in the freezer at -20ºC for further analysis. Sample digestion was conducted based on method described by Ismail (1993) and Ismail and Ramli (1997). Prior to any analysis were carried out, all apparatus were acid-washed with 5% nitric acid by dipping for 16 hours and then rinsed with distilled water. The eel samples were dried in air-circulating oven for 72 hours at 60°C to obtain a constant dry weight (dw). Afterwards, 1 g of each sample (muscle and skin) was digested in 10 mL concentrated nitric acid (AnalaR grade, BDH 69%). Then, the

digestion tubes filled with samples were placed on a digestion block at 40°C for 1 hour and was increased to 140°C for 3 hours (Yap *et al*., 2002). After digestion was finished, all digested samples were allow cooling to room temperature and were diluted with distilled water (DW) to a fixed volume (40 mL). The samples were then filtered by using filter papers (Whatman No.1) and the filtrates were stored until metal determination carried out. Cd and Pb in all digested samples were analyzed by using an Air-Acetylene Flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 880. The data obtained from analyzing were converted into µg/g (dry or wet weight) basis. The daily intakes doses were calculated based on equation established by the standard US EPA (1992) as below:

$$Dij = (Ci * Ij) / W$$

where:

Dij estimated dose (µg/kg body weight/day) for chemical i at ingestion rate j
Ci concentration of chemical i on fish
Ij ingestion rate for jth percentile of population
W assumed human body weight (50kg)

Results and Discussion

Table 1:Mean concentrations (µg metal /g d.w.) and standard deviations of Cd and Pb in the tissues of *M. albus* and comparison of different seasons for 2 years completed paddy cycles.

Year	Season	n	Skin (Cd)	Muscle (Cd)	Skin (Pb)	Muscle (Pb)
2011	Ploughing	38	2.184±1.40[a]	1.26±0.85[ab]	19.23±19.8[a]	11.58±10.99[a]
	Seedling	9	0.076±0.13[b]	0.08±0.11[c]	17.33±11.27[a]	11.65±5.94[a]
	Growing	24	1.363±1.02[a]	0.58±0.36[b]	18.51±12.53[a]	9.86±5.02[a]
	Harvesting	11	2.155±0.91[a]	1.14±0.21[a]	9.128±4.84[a]	4.72±1.07[a]
	Mean		1.44	0.77	16.05	9.46

*Post-hoc: Mean metal concentrations of liver, muscle and skin sharing a common letter for a particular metal are not significantly different, $p > 0.05$. a: bigger value; b: smaller value

Table 2: Daily intake of Cd and Pb (µ/kg/day) in paddy eel muscle for a person (50kg) in Malaysia.

Year	Season	n	Cd	Pb
2011	Plowing	38	0.8	7.32
	Seedling	9	0.053	7.363
	Growing	24	0.37	6.23
	Harvesting	11	0.721	2.993
Reference Doses (RfDo) in µ/kg/day unit			1[a]	3.57[b]

Remark: [a] USEPA, 2005; [b] Agusa *et al*., 2007

Based on Table 1, muscle and skin tissues accumulated the lower levels of Cd concentration than Pb in skin and muscle in year 2011. Based on Le *et al*. (2009), the Cd levels in the muscle of the tropical eel, *Anguilla marmorata* ranged 0.02 to 0.13 µg/g dw, which was much lower than in the present study (0.08 to 1.41 µg/g

dw). In comparison with study conducted by Yap *et al*. (2004), the Cd concentrations were slightly above the maximum allowable concentration in fish flesh for human consumption in Malaysia (1.0 µg/g) and daily intake of Cd metal is almost to 1 µg/day (Table 2) during the plowing and harvesting seasons. These results suggest that potential pollution of Cd has occurred in paddy cultivation areas of Kelantan. Furthermore, the intensive use of rock phosphate in the paddy cultivation areas as fertilisation to the paddy has gradually increased the Cd contents, which might lead to the eel population being affected. In this study, Pb levels in the muscle of the paddy eel were 8 times higher than permissible limits set by Malaysian Food Regulation (2.00 µg/g) and above the guidelines values of 3.75 µg/day (Table 2) for all paddy seasons except harvesting. This reflected that, the muscle of the paddy eel is highly contaminated by the Pb and Cd pollution. Yet, these results also suggest that, more precaution steps should be taken to prevent Cd and Pb toxicity.

Conclusion

Consumption of *M. albus* collected from this area should be reduced to avoid adverse effects on consumers.

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The efficiency of using manganese dioxide (Mno_2) and limestone in the removal of manganese from acid mine drainage

*Mohd Syakirin Md Zahar[1], Faradiella Mohd Kusin[1], Siti Nurjaliah Muhammad[1] and Shamsul Kamal Sulaiman[2]

[1]Department of Environmental Science, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang
[2]Mineral Research Centre, Minerals and Geoscience Department Malaysia, 31400 Ipoh
**Corresponding author.Tel.:+013 2963664; email address: mohdsyakirin_mz91@yahoo.com.my*

Introduction

Manganese is a common toxic metal that is found in the effluents of many industries, as well as in mine waters, either in alkaline or acidic mine drainage (Silva *et al*., 2012). The toxicity of Mn is related to kidney, lung and instetinal damage and its compound may reasonably be anticipated to be carcinogens (Dal Bosco *et al*., 2006). The removal of Mn is difficult because the interactions among Mn, Fe and others metal are very complex, affecting Mn solubility. The application of manganese dioxide in order to remove manganese from mine water had been studied. There is a sharp decrease in the metal concentration when the manganese dioxide solid is added to a solution containing Mn^{2+} ion (Aguiar *et al*., 2010). This study will provide an alternative method to remove manganese from mine water so that it could reach the limits that are allowed by the authorities in our country especially when discharged to the stream

Materials and Methods

A synthetic acid mine drainage (AMD) contain heavy metal of manganese were be prepared to provide the source of mine water to be treated. It is a consistent way to monitor the effects of treatment and to reduce the effects of possible interference by conflicting pollutants (Cheong *et al*., 2010).The concentration of manganese prepared in this study was 8.5 mg/L.

The substrates used included the limestone (calcite, dolomite) clamshell and pyrolusite contains manganese dioxide. The calcite and was collected at Simpang Pulai while the pyrolusite was collected from Felda Aring, Kelantan. The limestone and pyrolusite was prepared at a particle size range between 1 and 2 cm. 12 beaker of 1.5 L were prepared with different proportion. The proportion of every beaker was shown in Table 1 below. The liquid to solid ratio use in this experiment was 3.3.

For first 15 h, every beaker was sealed for 15 hour to maintain an anaerobic environment (Genty *et al*.,). After this period, the beaker than was then transferred into an open recipient to allow contact with oxygen. Parameters such as pH, alkalinity were monitored throughout the entire testing period of 45 h (at 0, 2, 4, 8, 15 and 45 h). The samples than were then filter with 0.45µm filter and analyzed for manganese concentration using ICP-MS.

Table 1: The proportion of beaker.

Beaker	Proportion
1	100% calcite
2	100% dolomite
3	100% clamshell
4	100% manganese dioxide
5	50% calcite + 50% manganese dioxide
6	50% dolomite + 50% manganese dioxide
7	50% clamshell + 50% manganese dioxide
8	30% calcite + 70% manganese dioxide
9	30% dolomite + 70% manganese dioxide
10	30% clamshell + 70% manganese dioxide
11	70% calcite + 30% manganese dioxide
12	70% clamshell + 30% manganese dioxide

Results and Discussion

The lowest concentration (0.2 mg/L) was achieved after 45 hours treated time at beaker number 10 (30% clam shell + 70% manganese dioxide) with pH of 6.5. It means 97% manganese concentration was reduced from the initial concentration which is 8.5 mg/L. The figure 1 shows the constant reduction of manganese concentration of beaker 10 within the increase of time. The beaker 11 (70% calcite + 30 % manganese dioxide) reduce 82% of manganese concentration after 45 hours treated time with the concentration of 1.45 mg/L and pH of 6.8.

The clam shell and limestone (calcite and dolomite) act as alkalinity generation to enhance the oxidation of manganese from the water. The proper and suitable pH is required to achieve the complete oxidation of manganese. The removal of this manganese from this experiment could be achieved by oxidation and also adsorption.

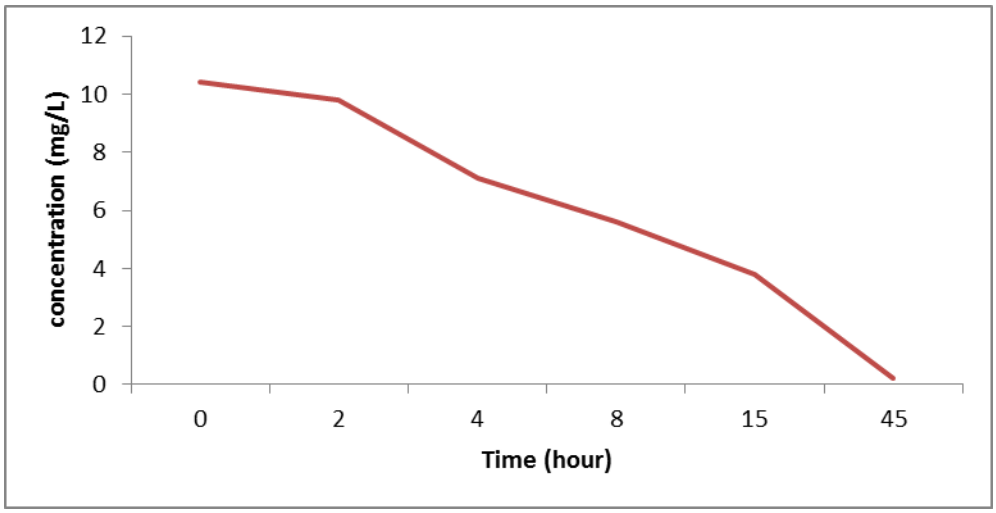

Figure 1: Concentration of manganese in beaker 10 within time.

Conclusion

Pyrolusite contain manganese dioxide are potentially to be used as substrate to remove manganese from water. Limestone and clamshell as alkanity generation help to increase and maintain pH need for oxidation and adsorption of manganese from mine water.

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The potential use of mixed substrates in passive treatment of acid mine drainage: batch experiments

*Siti Nurjaliah Muhammad[1], Fardiella Mohd Kusin[1], Mohd Syakirin Md Zahar[1] and Shamsul Kamal Sulaiman[2]

[1]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor*
[2]*Mineral Research Centre, Department of Minerals and Geoscience Malaysia, 31400 Ipoh, Perak*
**Corresponding author.Tel.:+6017 2761312; email address: nurjaliah@gmail.com*

Introduction

Acid mine drainage (AMD) is an acidic solution which contains high concentration of heavy metals. AMD is a worldwide problem which can lead to ecological destruction in watersheds and the contamination of human water sources by sulfuric acid and heavy metals such as arsenic, copper, and lead. Once acid-generating rock is crushed and exposed to oxygen and the surface environment, acid generation is very difficult to contain or stop, and can continue for tens or thousands of years until the available sulfide minerals are exhausted (Mayes *et al*., 2011). AMD forms when sulfide minerals in rocks are exposed to oxidizing conditions in coal and metal mining that produce acidity and sulfates. The weathering reactions are accountable for releasing heavy metals into both groundwater and surface water (Kruse *et al*., 2012). Passive treatment is an economical and low maintenance of AMD treatment in terms of biological remediation (Johnson and Hallberg, 2005). In laboratory-scale, batch tests are generally used as a preliminary procedure of acid mine drainage (AMD) treatment. This test is to evaluate the efficiency of each treatment medium and the potential use of mixed substrates as treatment media for AMD. Most SRBs need almost-neutral pH, an appropriate nutrient source, a solid matrix on which they can develop and survive in critical conditions. A suitable treatment medium for bioreactor is a medium with large pore spaces, low surface area, and a small void volume. The characteristics of the medium are preferred because it reduces the clogging of the bioreactors which is an essential operational problem (Cheong *et al*., 2010). SRB may use a wide range of substrates as an electron donors and carbon sources, which oxidize incompletely (to acetate) or thoroughly (to CO_2). These substrates are generally organic compounds composed of activated sludge, wood chips, farm manure, sawdust, mushroom compost, and other agricultural wastes (Luptakova and Macingova, 2012).

Materials and Methods

The batch test is performed by using different treatment media such as limestone (crushed and uncrushed), activated sludge (AS), and spent mushroom compost (SMC) in synthetic mine water. The synthetic mine water is prepared in acidic condition with sulfate-containing and high concentration of heavy metals. In addition, different ratios of all treatment media combined as mixed substrates are also being

tested. The best proportion will be selected to be used in column experiment. All of treatment media would be single-tested in different 1500ml beakers with liquid to solid ratio of 3.33 including the ratios for treatment media. The ratios are being divided for two different types of limestone for example crushed limestone with size of 1-2cm and uncrushed limestone with size of less than 5cm (<5cm). For ratio 1, the treatment media that have been combined are 50% limestone, 20% SMC, 20% AS, and 10% woodchips; while 40% limestone, 30% SMC, 20% AS, and 10% woodchips for ratio 2. The beakers are sealed with parafilm to maintain the anoxic condition. Parameters conducted are pH, temperature, conductivity, redox potential (Eh), total dissolved solids (TDS), dissolved organic carbon (DOC), alkalinity, and heavy metals (Zn, Pb, Cu, Al, Fe, Mn) throughout 0h, 7h, 12h, 24, 72h, and 120h. Basic parameters such as pH, conductivity, total dissolved solids (TDS), and redox potential (Eh) have been analyzed by using Myron Ultrameter; dissolved organic carbon (DOC) has been analyzed by using TOC analyzer; heavy metals are analyzed by using ICP-OES; sulfate is tested using HACH spectrometer while chloride is analyzed using titration method.

Results and Discussion

From the analysis, the basic parameters shows that the best treatment medium for single batch test is spent mushroom compost (SMC). It shows that SMC can generate alkalinity and reduce sulfate at the same time. It generates alkalinity much faster than any limestone tested. The pH value is changing from 3.17 to 7.54 after 7 h and it constantly increase until 24 h. But, the pH value is dropped at 72 hours and increased back to 7.52 when the period is 120 h. The conductivity, total dissolved solids (TDS), and redox potential (Eh) are increased from 3094 to 3802 µS, from 2246 to 2810 ppm, and decreased from 384 to 91mV respectively. Moreover, the alkalinity and DOC are increased gradually from 0 to 1190 mg/L as $CaCO_3$ and from 8.201 to 1403 ppm each other. Finally, the sulfate indicates sharp decreased from 1500 mg/L at 0h to 80 mg/L a 120 h. The SMC shows better results compared to other treatment media.

In addition, the suitable proportion of mixed substrates which is ratio 2 for uncrushed limestone (<5cm) has been selected with limestone (40%), spent mushroom compost (30%), activated sludge (20%), and woodchips (10%). The pH value is increasing from 3.17 to 7.19 at 120h, similar to the alkalinity which it is increasing from 0 to 663 mg/L as $CaCO_3$. pH and alkalinity are interrelated to each other because pH indicates broadly on acidity, alkalinity, and neutrality of the water solution while alkalinity can determine and control the pH by alkalinity equilibrium of carbon dioxide, carbonate, and bicarbonate in natural waters. Conductivity also increasing from 3094 to 3268 µS mostly because conductivity in water is affected by the presence of inorganic dissolved solids such as anions and cations. Total dissolved solids (TDS) composed of dissolved solids, suspended and settleable solids in water which it is increasing from 2246 to 2383 ppm. This is due to dissolved solids that consist of calcium, chlorides, nitrate, phosphorus, iron, sulfur, and other ions particles while the suspended solids that are coming from silt and clay particles, fine organic debris, and other particulate matter from the mixed substrates. On the other hand, the redox potential (Eh) is reducing from 384 to 113 ppm within 120 h.

Even though the water solution is in positive state, the Eh has been reduced which means it has lose electron and it has been oxidized by reducing new species in the solution. Moreover, the total organic carbon (TOC) is increasing from 8.201 to 508.4 ppm. The TOC is high during 120h because the water receives wastes or is highly coloured due to natural organic material from spent mushroom compost and woodchips. Last but not least, the sulfate reading at initial time is 1500 mg/L and it decreases to 400 mg/L. The reduction might because of the microorganisms from other treatment media that help the sulfate to be reduced.

Conclusion

As a conclusion, the batch test aims to evaluate the efficiency of each treatment medium and the potential use of mixed substrates as treatment media for AMD. The media are tested for various parameters to ensure clear reduction in acid mine drainage (AMD). This experiment has been done in anoxic condition which allows limited oxygen. The potential use of mixed substrates in passive treatment of acid mine drainage from the batch experiments is ratio 2 for uncrushed limestone which is less than 5 cm (< 5 cm). The combination of the treatment media has shown it is the best mixed substrates to be used in column experiment.

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Tracing carbon and nitrogen fluxes in soil of log-over forest and highly degraded area of oil palm plantations using stable isotope analysis

Fathia Alaswad[1], *Ferdaus Mohamat-Yusuff[1], Ahmad Ismail[2], Faradiella Mohd Kusin[1], Syaizwan Zahmir Zulkifli[2] and Muhamad Awang[3]

[1]*Environmental Forensics Research Center, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang*
[2]*Department of Biology, Faculty of Sciences, University Putra Malaysia, 43400 Serdang, Selangor*
[3]*SEGI University College, No. 9, Jalan Teknologi, Taman Sains Selangor, Kota Damansara, PJU 5, 47810 Petaling Jaya, Selangor.*
**Corresponding author.Tel.:+603 89468023; email address: ferdius@upm.edu.my*

The role of soil organic matter in the world's climate has become the focus of recent studies, particularly with suggestions by the Kyoto protocol that soils may act as a potential sink for CO_2. With approximately 1500 Gt C contained in the upper meter of the world's mineral soils. Changes in climate and land-use will have significant effects on the carbon budget, particularly with respect to the turnover rate of soil carbon. Conversions of tropical forests in to oil palm plantations may effects on the amount of organic carbon in soil and consequently release the carbon into the atmosphere as carbon dioxide (CO_2). For this reason, this study will focus on use of natural abundance level of stable carbon and nitrogen isotopes as tracer of soil organic matter and nitrogen cycling in oil palm plantation with different land history. Furthermore, this work will include identification of relationship between C^{13} and N^{15}abundance in vertical soil profile and also the soil C and N concentration. Later the relationship of stable isotopes and heavy metals concentration which used extensively in this area will also be determined. At the end of this study, the selection of best land for oil palm plantation will be established for better environmental justice.

Keywords: Stable isotopes, Organic carbon, heavy metals, Oil palm plantation, CO_2 emission.

Workshop (Domain Ecology)

Integrated approach to solve pollution and biodiversity conflicts in Malaysia

Ahmad Ismail*

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Pollution and human activities affect biodiversity and it can be either direct or indirectly. A direct one usually occur through oil spill, acute chemical pollution, deforestation, overharvesting, etc. while indirect one often contaminate the environment affecting single or a group of organism within the food chains. Continuous chemical accumulation of hazardous chemicals not only disrupt target organism but also cause impairments or abnormalities in subsequent generations. For examples, there were many studies that highlighted the effects of heavy metals to waterbirds population including their young such as thinning of eggshells, deformities, low survival, etc. Many other cases relating to us humans are also well publicised as our health and life quality deteriorates in conjunction with hazardous chemical pollution, overexploitation and habitat degradation. The important question remains, how to solve pollution problems? Integrated approach that considers fundamental sciences, land availability and its uses, social awareness and education background is very much needed to help solving this issue. Through public recognition and support, implementing a green policy would be easier. This process can be hastened by providing accurate and up to date evidence for the public to act upon. Hence, the ultimate goal of any good scientist is not only to obtain important information from our surroundings, but also to deliver the right message and help promote awareness, develop commitment and to seek balance between public's needs and ecosystem's stability.

Keywords: Pollution, integrated approach, public support, ecosystem stability

Bio-logging as a key technology to assess foraging behavior of marine top predators

*Yasuhiko Naito[1], Akinori Takahashi[1] and Hideo Sakachi[2]

[1]*National Institute of Polar Research, Midori-cho 10-3, Tachikawa, Tokyo 190-8518, Japan*
[2]*National Research Institute of Fisheries and Environment of Inland Sea, 2-17-5 Maruishi Hatsukaichi-shi, Hiroshima 739-0452 Japan*
**Corresponding author. email address: yashiko@dream.ocn.ne.jp*

Foraging behaviour is central to animal ecology and a key to understand the interaction between animals and their environment. Without our understanding on the foraging behaviour it is unable to understand how animals react to environmental and ecological changes. Marine top predators have potential impact on the ocean environment due to their large magnitude of foraging demand and thereby wider foraging range. Their foraging trait tends to accelerate the ecological changes through either top down or bottom up effect by disruption of the food webs. However information about such changes is very much limited, and therefore our understanding is still inadequate due to our poor inability in observing the prey predator interaction quantitatively and thus our studies have depended on the theoretical approaches to some extent using proxy of foraging metrics. Towards more complete understandings of the ocean ecosystems we recently developed a technique to observe foraging behaviour of the marine animals in situ in very fine scale by using animal-born instruments, i.e. bio-logging. Firstly we could measure feeding behaviour by counting jaw motion in seals, head strike motion in penguins and swimming motion in a fish. Secondly using algorithm of jaw motion movement we could develop long term recorders of jaw motion event and swim stroke event of seals which can record more than a half year. Thirdly we developed video system linked with feeding behavior.

Keywords: Bio-logging, foraging behaviour, ocean ecosystem, top predators

Determination of food web in merambong shoal, seagrass area: evidence from stable isotope analysis

Aqilah Mukhtar[1], Syaizwan Zahmir Zulkifli[1], Ferdaus Mohamat-Yusuf[2], Ahmad Ismail[1] and Nobuyuki Miyazaki[3,4]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5, Kashiwanoha, Kashiwa-shi, Chiba 277-8568, Japan Malaysia*
[4]*Ocean Policy Research Foundation, 1-15-16 Toranomon, Minato, Tokyo, 105-0001, Japan*
**Coressponding author.Tel.:+603 89466627; email address: syaizwan@upm.edu.my*

Introduction

The study on food web ecology in Merambong shoal seagrass bed is arise from the need to evaluate the structure and dynamic of ecosystem (Post *et al*., 2000; Post 2002). The concept of trophic level in food web process had been used successfully in modeling the bioaccumulation of contaminant from lower to the upper consumer (Rasmussen *et al*., 1990; Cabana and Rasmussen 1994; Vander Zande *et al*., 1997). Recently, human anthropogenic activities especially shipping activities, land reclamation for port facilities cause the sedimentation and burial of seagrass bed resulting in decline of primary producers and consumers in population. The biomarker of stable isotope analysis has complemented the conventional methods of dietary analysis for examine the trophic relationship and transport of items sources in food chain (Michener and Schell, 1994; Stowasser *et al*., 2012). A general approach in stable isotope analysis had suggested by ratios of bio-element, carbon (term $\delta^{13}C$) and nitrogen ($\delta^{15}N$).

Materials and Methods

The samples were prepared base on the method prepared by Nakamura *et al*., (2008) and Zulkifli *et al*., (2012) with some modification. Tissues samples were washed with Mili-Q water and dried in a dry freezer for at least 24 hours or until constant weight was obtained. Each dried sample was ground to a fine powder. Mixture of 3 ml chloroform and methanol were added (2:1 ratio) into samples for 3 hours to eliminate the lipid component. The mixture was centrifuge for 10 minutes using a high speed centrifuge (760 x g, 4˚C) before the supernatant was discarded and remaining pellet was fumed with 12M HCl for 10 hours to remove inorganic carbonates. The excess acid contained in the pellet was removed in vacuum desiccator with pellets of NaOH for 3 hours. The samples were dried in oven for 3 hours before the stable carbon and nitrogen ($^{13}C/^{12}C$, $^{15}N/^{14}N$) was analyzed using a continuous flow isotopic ratio mass spectrometer with elemental analyzer. Carbon and nitrogen isotope ratios are expressed in delta notation ($\delta^{13}C$ and $\delta^{15}N$) in units of parts per thousand (‰), where

$$\delta X\,(‰) = \left[\left(\frac{R_{sample}}{R_{std}}\right) - 1\right] \times 1000$$

Results and Discussion

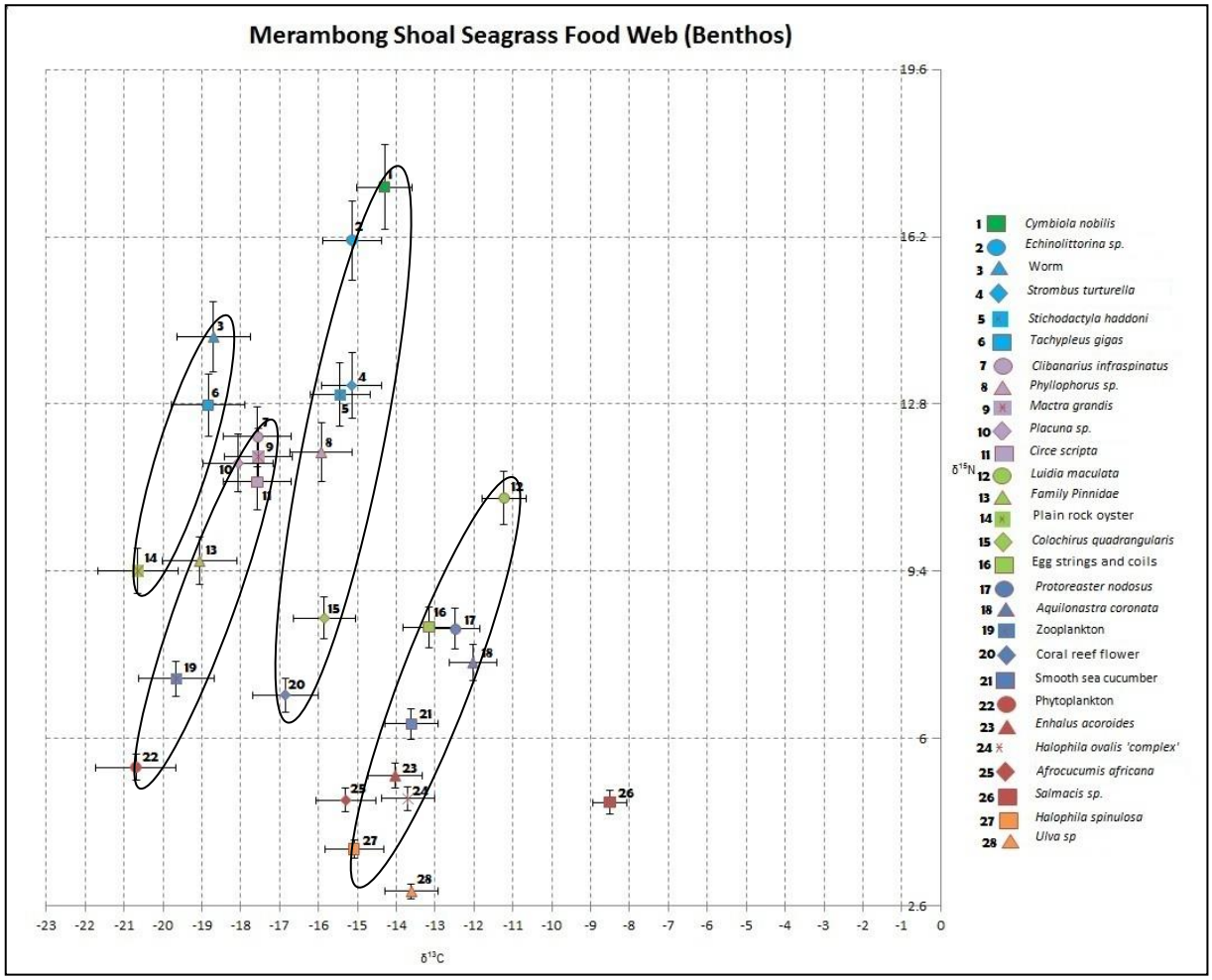

Figure 1: Food web structure in Marambang shoal, seagrass area

Stable isotope was analyzed in seagrass ecosystem to identify the carbon-13 ($\delta^{13}C$) and nitrogen-15 ($\delta^{15}N$) stable isotope ratios distribution in 26 taxa, comprise primary producer and invertebrate consumers include gastropod, bivalve and echinoderm. A cross the Merambong shoal seagrass area, there had about 4 food chains and 4 trophic levels above the presumed food base found on the seagrass bed (Figure 1). The smooth sea cucumber as a primary consumer grazing on marine fauna and small food items become the reference baseline. Higher trophic level had higher ratio value of $\delta^{13}C$ and $\delta^{15}N$ compare to the lower trophic level. *Cymbiola Nobiis* in the trophic level 5 become the top predator of seagrass food web. Consumers in trophic level 2 had ranged between 2.2-2.9 suggesting the herbivorous species. The TL 3 and 4 had values of ranged 3.4-3.9 and 4.0-4.9 indicated the omnivorous and carnivorous species, respectively. Overall, the majority (70%) of invertebrates are

above the group of herbivorous suspension feeders and between trophic level 2.2-5.2.

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Visual and behavioral evidence indicates active hunting by sperm whales

*Kagari Aoki[1,2,3], Masao Amano[4], Tsunemi Kubodera[5], Kyoichi Mori[6], Ryosuke Okamoto[7] and Katsufumi Sato[1]

[1]*Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan*
[2]*Present: School of Biology, University of St Andrews, Bute Building, St Andrews, Fife KY16 9TS, UK*
[3]*Present: Japan Society for the Promotion of Science, 5-3-1 Kojimachi, Chiyoda, Tokyo 102-0083, Japan*
[4]*Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, 1-14 Bunkyo, Nagasaki 852-8521, Japan*
[5]*Collection center, National Science Museum, 4-1-1 Amakubo, Tsukuba, Ibaraki 305-0005, Japan*
[6]*Department of Animal Sciences, Teikyo University of Science, 2525 Yatsusawa, Uenohara, Yamanashi 409-0193, Japan*
[7]*Ogasawara Whale Watching Association, Higashimachi, Chichijima, Ogasawara, Tokyo 100-2101, Japan*
**Corresponding author. email address: aokikagari@gmail.com*

Sperm whales are thought to employ active pursuit strategies for hunting their prey, mainly deep-sea squid at depth, but no visual evidence has been obtained to test this hypothesis. Using animal-borne cameras and accelerometers simultaneously deployed on 17 whales, we recorded the hunting behavior of sperm whales at depth. A total of 42.8 h diving data including 17715 images was obtained. Although the vast majority of the still images were empty water and uninformative (98.5 % of images), five classes of image with visible material were identified: (1) suspended possibly squid ink material (n = 17), (2) unidentified particles (n = 4), (3) possible animal body parts (n = 2), (4) other individual whales (n = 221) and (5) the sea bottom (n = 8). Class (4) images were only recorded at depths shallower than 339 m, suggesting that tagged whales swam alone while foraging at greater depth. In contrast, the other classes of image were only recorded at deeper depths (mean ± SD = 785 ± 140 m). Simultaneous use of the speed and image sensors revealed that class (1) images were associated with bursts of speed up to approximately twice (3.3 ± 1.0 m s–1, max. 6 m s–1) the mean swim speed (1.8 ± 0.4 m s–1). The bursts of speed linked with the images likely derived from prey support the hypothesis that sperm whales attempt to capture their prey by active hunting.

Keywords: Animal-borne camera, cetacean, data-logger, diving behavior, hunting, swim speed, *Physeter macrocephalus*

Natural radionuclides in Malaysian waters

*Che Abd Rahim Mohamed

Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

** Corresponding author. email address: carmohd@ukm.edu.my; carmohd@gmail.com*

South China Sea was boundary with many countries such as Malaysia, Indonesia, Thailand, Vietnam, Philippine, Singapore, Brunei, Taiwan and mainland of China. In general, the thickness of water column in the coastal area was less than 1000 m with heavily horizontal particles transport from the land. Studies on natural uranium-thorium decay series in marine Malaysian waters were well published by scientist from Universiti Kebangsaan Malaysia and Malaysia Nuclear Agency in waters, sediments, biota, suspended particles and marine aerosol. In east coast Peninsular of Malaysia e.g., coast of Mersing, the concentration levels of 210Po and 210Pb were varied between 0.76 to 2.24 mBq/L and 0.16 to 1.60 mBq/L, respectively. While the phosphorus concentrations include total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP) and dissolved organic phosphorus (DOP), were in the range of 6.06 to 23.31 µg/L, 2.24 to 13.42 µg/L and 0.47 to 16.10 µg/L. The concentration of TDP and salinity shows weak positive correlation (r = 0.39) might due to the shallow area of Mersing River. There are high positive correlation (r = 0.85) of 210Po activity and SPM concentration and moderate positive correlation (r = 0.59) of 210Po and TDP in water. While in other studies shows the distributions of 210Pb and 210Po activities in PM10 were varied between 162 to 881 µBq/m^3 (mean: 347 ± 170 µBq/m^3) and 85 to 1009 µBq/m^3 (mean: 318 ± 202 µBq/m^3), respectively. It is seen that the 210Po activity in Malaysia waters lies in a broader and/or higher than the global range, which contribute from external sources (i.e., biomass burning) injected to the local atmosphere. However in sediment cores the concentration levels of 234U was ranging from 1.66±0.12 dpm/g to 3.73±0.32 dpm/g, and 238U ranged from 1.79±0.32 dpm/g to 3.70±1.32 dpm/g with highest activity recorded at the top layer. But ratio value of 234U/238U, e.g., at upper layer of Kota Kinabalu and Labuan port was ≥1.14 and ≤ 1, respectively. Further discussion on the behavior and distribution of natural radionuclides in Malaysian marine waters will highlight during presentation.

Keywords: Natural radionuclides, seawater, sediment, trans-boundary, particulate

Physicochemical properties of sediment pore water in Merambong Seagrass Bed, Johor

*Nordiani Sidi and Ahmad Zaharin Aris

Department of Environmental Science, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor
**Corresponding author.Tel.:+603 8946755; email address: nordianisidi@gmail.com*

Introduction

Sediment Interstitial Water or Pore water is defined as the water occupying the spaces between sediment particles (USEPA, 2001). Bottom sediments in natural systems are formed by sedimentation process. Water is trapped and entrained in the sediment, forming the pore water or interstitial water (Batley and Giles, 1979). The chemical species that found in the water column and adsorbed to the suspended sediment will also be trapped in the bottom sediment. Due to diagenetic processes such as precipitation, adsorption, reduction, remobilization, biological degradation and biological uptake, the concentrations of nutrients in the pore water are higher then the overlying water (Bufflap and Allen, 1994). These processes will affect the availability of nutrients and toxic chemicals to biota. The aim of this study is to determine the physicochemical properties of sediment pore water in Merambong Seagrass Bed, Johor. The output of this study will help for better understanding of biogeochemical process involving pore water.

Materials and Methods

This study was conducted at Merambong Seagrass Bed ($1^0$20.137'N,$103^0$36.158'E) within the Sungai Pulai estuary, Johor Straits, Malaysia, in June 2013. Twenty random samples of surface sediments (0 – 10 cm) were collected using corers made up of PVC tubes. These samples were kept in acid-washed polyethylene bottles and transported to the laboratory at 4 °C.

In situ parameters (pH, temperature - Temp, electrical conductivity - EC, salinity - Sal, dissolved oxygen - DO, total dissolved solid - TDS) were measured immediately after sample collection by using YSI 556 Handheld Multiparameter Instrument.

Unfiltered water samples were used in bicarbonate and chloride analyses (APHA, 2005). On the other hand, analyses of sulphate and nitrate (HACH Method) were conducted using filtered water samples (0.45 µm cellulose acetate membrane filter, Whatman Milipores). Subsequently, the remaining pore water samples were acidified with a few drops of concentrated HNO3 to bring the pH below 2. Cations were analysed by using Flame Atomic Absorption Spectrometry (FAAS, Shimadzu AA6800).

Results and Discussion

The relative abundance of cations in the sediment pore water is in the order Na^+ > K^+ > Mg^{2+} > Ca^{2+} and while for anions is Cl^- > SO_4^{-2} >$HCO3^-$ > NO^{-3}. Na^+ and Cl^- are the dominant cation and anion respectively.

Table 1: Summary of the physicochemical parameter.

Parameter	Minimum	Maximum	Mean	Standard Deviation
pH	6.11	8.10	7.24	0.50
Temp (^{0}C)	20.57	30.21	27.52	2.78
EC (mS/cm)	34.03	48.19	44.77	3.31
Sal (ppt)	19.61	28.97	27.46	1.92
DO (mg/L)	0.69	4.46	2.48	0.84
TDS (mg/L)	20.55	29.08	27.82	1.76
Na (mg/L)	4000.00	31500.00	15158.33	6463.39
K (mg/L)	1150.00	11200.00	6233.50	1862.38
Mg (mg/L)	850.00	5830.00	1632.17	955.72
Ca (mg/L)	178.00	506.00	330.96	84.63
Cl^- (mg/L)	15395.23	21593.30	18962.45	1530.84
SO_4^{-2} (mg/L)	380.00	1130.00	797.83	168.27
$HCO3^-$ (mg/L)	73.20	190.32	130.95	31.54
NO^{-3} (mg/L)	1.20	2.70	1.96	0.32

The chemical composition of water samples from the study area is shown on the Piper diagram (Figure 1). In the cation plot field, the sample plot mainly towards Na^+ corner indicating Na-K type water. In the anion plot field, the samples 100% plotted towards the Cl^- indicating chloride type water. Principally, the sediment pore water samples plotted in the Na-Cl dominant of the diamond field.

Conclusion

The analysis of the physicochemical parameters of sediment pore water samples from twenty sampling points in Merambong Seagrass Bed, Johor shows that the dominant cation in the water is sodium whereas the dominant anion being chloride. From among the subtypes, the Na-K water type is dominantly found in this area. The probable influence of salinity may have contributed to the prevalence of this water type.

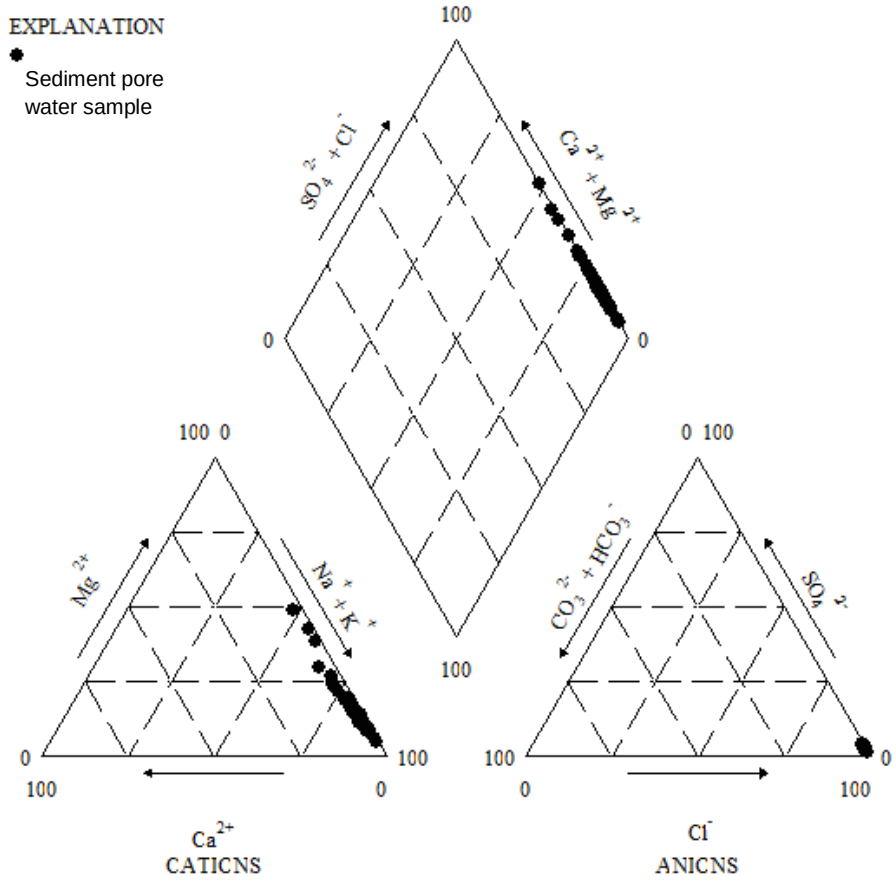

Figure 1: Piper diagram of sediment pore water samples of Merambong Seagrass Bed, Johor.

Aknowledgements

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Genetics

De Novo transcriptome sequencing, functional annotation and pathway analysis in callus culture of *Aquilaria malaccensis* Lam.

Siah Chai Har[1], Parameswari Namasivayam[2] and *Rozi Mohamed[1]

[1]Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Molecular & Cell Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
**Corresponding author.Tel.:+603 89467183; email address: rozimohd@upm.edu.my*

Introduction

With the reduction in the cost of next-generation sequencing (NGS) technologies, RNA sequencing (RNA-seq) has become wide spread because it enables the high-resolution characterization of transcriptomes. It not only provides rapid, cost-effective, and comprehensive transcripts analysis for model plants, but also provides opportunities to analyse non-model plants without a reference genome (Metzker, 2009; Wang *et al*., 2009), such as *Aquilaria*.

Aquilaria malaccensis is one of the main sources of agarwood. Due to its high demand, it is endangered and listed in the Appendix II of CITES (CITES, 2013). There are insufficient genomic and transcriptomic data in public database for understanding its molecular basis. *A. malaccensis* is a tree species which take long time to grow. In vitro culture of *A. malaccensis* has provide another alternative to study this species. Thus, transcriptome sequencing from *A. malaccensis* callus culture is helpful to generate comprehensive transcriptomic data and to understand its molecular mechanism under controlled environment.

Materials and Methods

Calli originated from leaf tissue of greenhouse-grown trees established by Jayaraman *et al*. (2014) were used. Healthy and stressed calli were collected separately, immediately frozen in liquid nitrogen and stored at -80˚C. RNA extraction was carried out using the RNeasy Plant Mini Kit (Qiagen, Germany). RNA quality and integrity were evaluated by nanophotometer (Implen, Germany) and Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). RNA samples were sequenced by the Michael Smith Genome Science Center, Canada using an Illumina Hiseq2000 platform.

Raw reads in qseq format were filtered and converted to fastq format using SAMtools (Li *et al*., 2009). Filtered reads were trimmed using FASTX-Toolkit (http://hannonlab.cshl.edu/ fastx_toolkit/index.html) before assembly using SOAPdenovo-Trans (Xie *et al*., 2014). The contigs and singlets obtained from SOAPdenovo-Trans were later re-assembled using TGICL (Pertea *et al*., 2003). The single-end reads from both samples were mapped against the assembled library using Bowtie (Langmead *et al*., 2009).

All assembled transcripts were blasted against the NCBI non-redundant database using BLASTX with a cut-off E-value of 10^{-5}. The Blast2GO program (Cones *et al*., 2005) was used to obtain gene ontology (GO) and Kyoto Encyclopedia

of Genes and Genomes (KEGG) annotations. The data were statistically analyzed using WEGO software (Ye *et al*., 2006).

Results and Discussion

The number of transcripts, defined as the number of contigs (consensus sequence obtained from assembled reads), and the number of singletons (unassembled reads), generated from the assembly process, are detailed in Table 1. After mapping, 70% and 75% of reads from healthy callus and stressed callus, can be mapped back into this assembled transcriptome library, respectively.

Table 1: Assembly Statistics of transcriptome analysis.

Descriptions	Healthy callus	Stressed callus
Sequencing reads	200,062,275	166,544,202
Contigs	21,572	14,580
Singletons	120,618	102,494
Total transcripts	259,264	

Of these transcripts, 107,593 (41.5%) showed significant BLASTX matches in the nr database. The top 5 species showing BLASTX hits were *Vitis vinifera* (25,899; 24.7%), *Populus trichocarpa* (22,296; 21.3%), *Ricinus communis* (22,032; 21.0%), *Glycine max* (10,533; 10.1%) and *Medicago truncatula* (3631; 3.5%) (Figure 1).

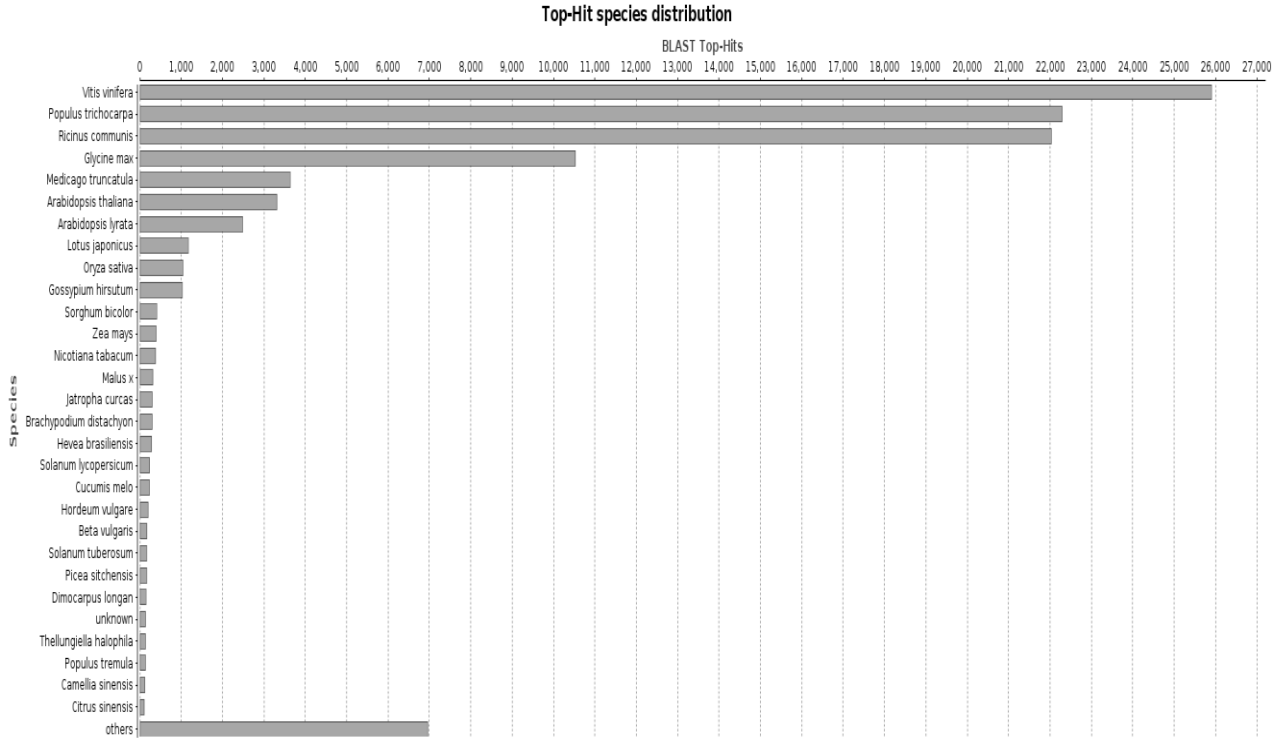

Figure 1: Top-hit species distribution in the BLASTX analysis against the nr database.

BLAST2GO was used to assign gene ontology (GO) annotation. In total, 96,743 transcripts were annotated and classified into the three main GO categories: biological processes (50.7%), molecular functions (24.0%) and cellular components (25.3%) (Figure 2). The sequences with corresponding ECs obtained from Blast2GO were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway database. We assigned 46,076 of the transcripts to a total of 144 KEGG pathways.

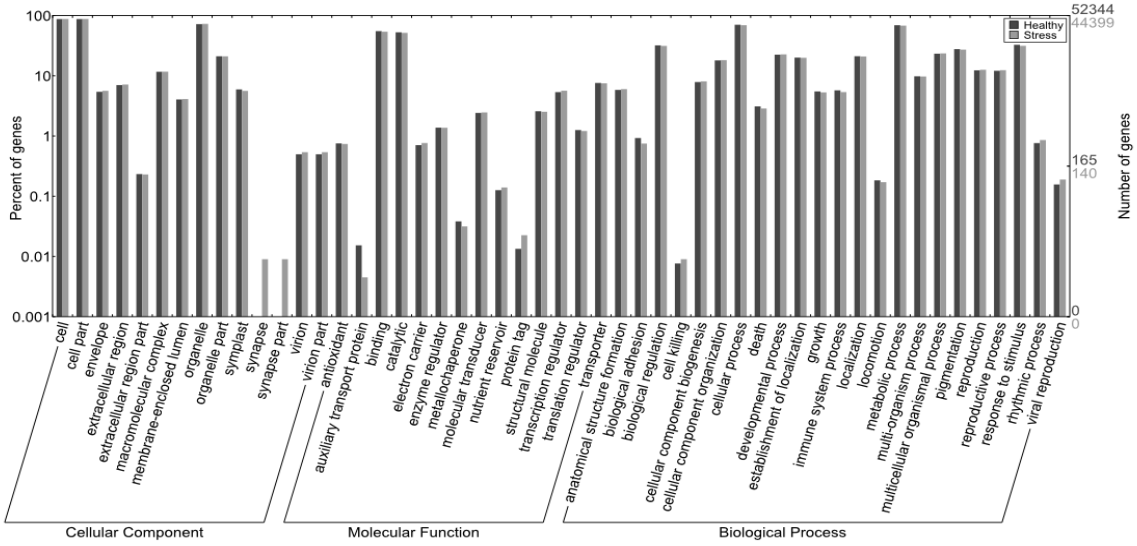

Figure 2: GO classification for the *A. malaccensis* callus transcriptome.

Conclusion

In this report, we present the sequencing, *de novo* assembly and functional analysis of the callus transcriptome of *A. malaccensis* using next-generation sequencing technology. The use of RNA-seq technology has allowed for a more comprehensive understanding of gaharu synthesis pathway and provided valuable sequence resources for future studies on *A. malaccensis*.

Acknowledgements

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Developing molecular marker for drought stress in soybean (*Glycine max* L. Merr)

*Estri Laras Arumingtyas[1], Evika Sandi Savitri[2] and Joni Kusnadi [3]

[1]*Department of Biology, Faculty of Science, Brawijaya University, Jl. Veteran, Malang, Indonesia*
[2]*Department of Biology, Faculty of Science and Technology, UIN Maulana Malik Ibrahim, Malang, Indonesia*
[3]*Agricultural Product Technology, Faculty of Agricultural Technology, Brawijaya University, Jl. Veteran, Malang, Indonesia*
**Corresponding author.Tel.:+62 8123308837; email address: laras@ub.ac.id; larasbio@gmail.com*

Introduction

One of environmental stress that gave high impact to plant performance is drought. Biochemical changes includes alteration of osmolite and specific protein caused by drought stress has been reported [1]. Changes in protein expression, accumulation and synthesis of protein during plant development has been observed in several plant species under conditions of drought stress during the growing [2]. Protein changes that occur both qualitatively and quantitatively detected during drought stress [3]. The changes includes increased or decrease the expression, and production of new proteins, however the mechanisms of plant resistance that caused by the synthesis of new protein is not yet known [4].

Late embryogenesis abundant (LEA) protein with a molecular weight of 10-30 kDa has been reported to have a role in the protection against drought stress on higher plants [5]. From the previous research proteins with molecular weight of 13 and 52 kDa which may relate to drought tolerance were identified [6,7]. The two protein bands, 13 kDa and 52 kDa were separated using 2D-PAGE and sequenced. The result shows that the 13 kDa protein band show high homology to auxin binding protein and germin like protein, which has enzymatic activity as detoxification enzyme *oxalate oxidase* and *superoxide dismutase.* These enzymes have a role in the drought tolerance mechanism.

In this research confirmation of the protein expressed in the field when plant was subjected to drought season was done in order to develop candidate marker for drought tolerance varieties.

Materials and Methods

Plant material and protein isolation

The plant materials used for the isolation of protein is the leaf of seven soybean varieties including: four drought tolerant varieties Tanggamus, Nanti, Seulawah and Tidar, two moderately tolerant varieties Wilis and Burangrang and one sensitive variety Detam - 1. Protein was isolated from leaves using acetone/trichloroacetic acid (TCA) method [8].The protein profile was identified using SDS - PAGE.

Results and Discussion

Because of some constraints, in this paper only the protein profile of tolerant variety Tanggamus and sensitive variety Detam which have been identified.

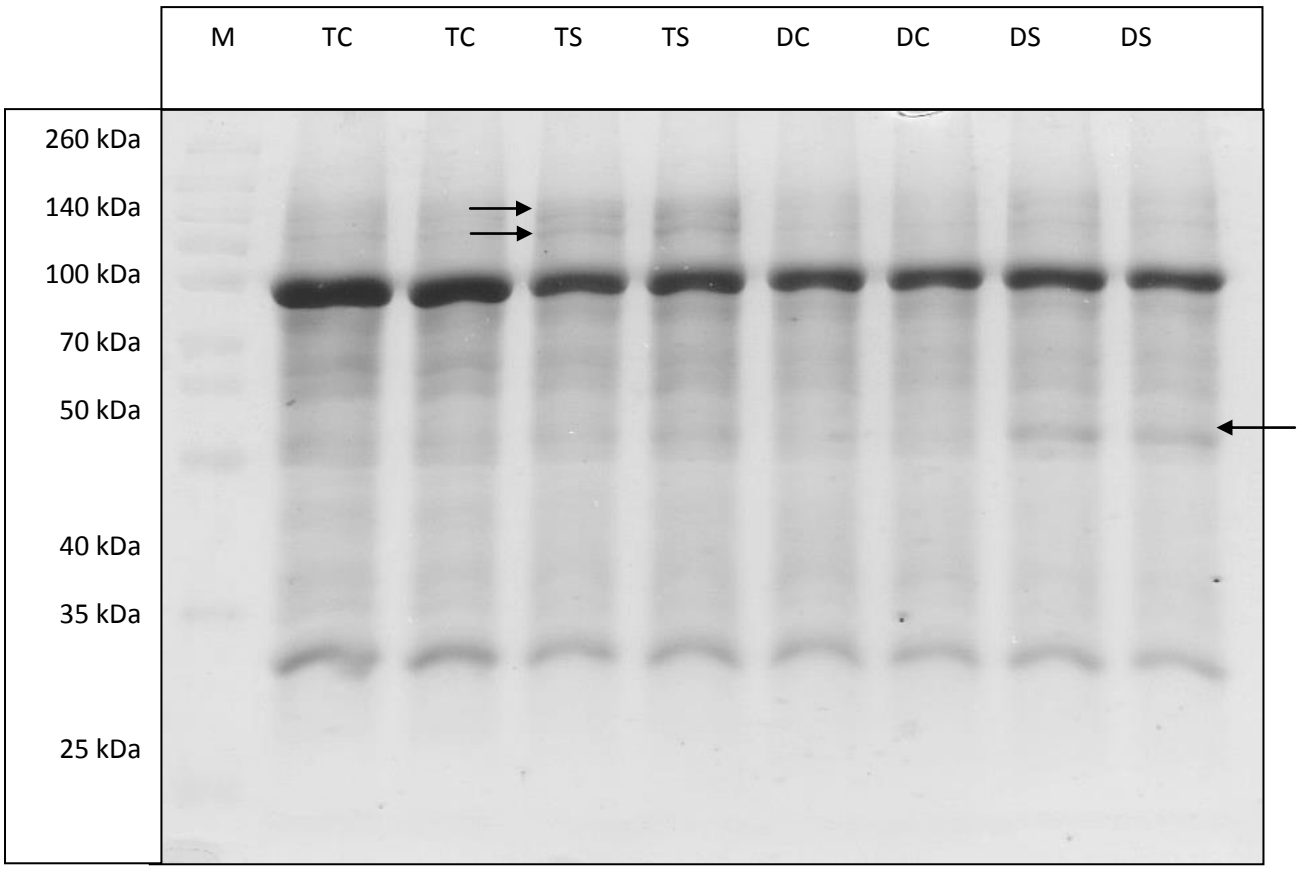

Figure 1: Protein profil of soybean varieties Tanggamus and Detam-1 in normal and stress condition. TC = Tanggamus control, TS = Tanggamus stress, DC = Detam-1 control, DS = Detam-1 stress .

Using SDS PAGE the protein profile of both soybean varieties in control and stress condition were different. No new protein was produced, however the thickness of protein band were different. In stress condition, drought tolerant variety, Tanggamus, showed thicker protein band of 70 kDa and 100 than that showed in normal condition. On the other hand the sensitive variety Detam showed no differences in thickness for both proteins but it showed thicker band of 25 kDa in stress condition compared to that in normal condition. It was reported that in stress condition other than the decrease of existed protein, there was also synthesis of new protein [5]. There are hundreds protein produced during drought stress condition as a response of plants toward the stress, however the mechanism of stress tolerance was remain unknown. A certain concentration of protein (10-70 kDa) increased during drought stress, some other protein decrease and new proteins was synthesized [4].

Based on those result, it is likely that drought stress given in this experiment induced the increase the production of 70 kDa and 100 kDa proteins in tolerant

variety but in sensitive variety, production of different protein (25 kDa) was induced. It seems that the stress did not severe enough to induce new protein.

This finding was different from the result of previous research. In the previous research, characterization of proteins using SDS-PAGE electrophoresis results showed the formation of new proteins with a molecular weight of 13 and 52 kDa in drought-tolerant varieties Tanggamus, Nanti, Seulawah and Tidar [7, 8]. This protein is not found in the moderate tolerant varieties Wilis and Burangrang and drought sensitive variety, Detam-1. Induction of new proteins in drought tolerant varieties showed the mechanism of these varieties to coupe with drought stress conditions. which has enzymatic activity as detoxification enzyme *oxalate oxidase* and *superoxide dismutase.* These enzymes have a role in the drought tolerance mechanism.

The differences of the results indicate that plant response to drought stress was varies. The protein expressed could be different in different condition, and a new protein was not always produced in response to drought stress.

Conclusion

The protein profile identified using SDS-PAGE show some differences between the tolerance and sensitive varieties. Plant response to drought stress was varies. The protein expressed could be different in different condition, and a new protein was not always produced in response to drought stress.

Acknowledgements

We are grateful to the Research Institute of Legumes and Tuber Crops, Kendalpayak, Malang, Indonesia, for pro- viding soybeans seeds. The research was supported by the Directorate General of Higher Education (DGHE)'s, Brawijaya University, Malang, Indonesia, BOPTN funding.

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Cloning an aluminum tolerance gene candidate from rice

*Miftahudin[1], Dewi Indriyani Roslim[1,2], Tri Ratnasari[1], Ahmad Zulkifli[1] and Tatik Chikmawati[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Kampus IPB Darmaga, Bogor 16680 Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau. Pekanbaru Riau, Indonesia
**Corresponding author. email address: miftahudinm@gmail.com*

Introduction

Rice is an important staple food in Indonesia. Extension of the rice cultivation area is considered as a solution to increase rice production. However, the extension of rice cultivation is limited by decreasing the area of fertile land due to the rapid conversion of such land into settlement and industrial uses. One of the alternative solutions is the use of marginal land for rice cultivation, such as acid soil. Unfortunately, cultivation of rice in acid soil could face aluminum (Al) toxicity due to high solubility of Al in acid soil that can be toxic to rice root.

It has been considered that the use of Al-tolerance varieties that are able to adapt to acid soil can solve the Al-toxicity problem in acid soil. There are various levels of Al-tolerance among cereal species, and rice is considered as the most tolerance species to Al toxicity. Al-tolerance trait in rice is controlled by many genes (Nguyen *et al*., 2003). Several genes that are believed to be correlated and resposible for Al-tolerance trait in rice have been isolated through many different approach (Yamaji *et al*., 2009; Tsutsui *et al*., 2011; Yokosho *et al*., 2011). Miftahudin *et al*. (2005) has been initiated to study microsyntenic relationship between rye (*Secale cereal* L.) and rice in the region of Al-tolerance locus. There are two flanking markers bordering the Al tolerance locus region in rye chromosome. Both markers are also present in rice chromosome spanning the region of 55 kb. Roslim (2011) has been identified a potential DNA fragment in between both markers in rice that can be an Al-tolerance gene candidate. The objective of the research is to clone an Al-tolerance gene candidate from an Indonesian local rice cv Hawara Bunar.

Materials and Methods

Primers were designed based on the sequence of the Al tolerance gene region in a rice BAC clone. The primers were then applied on RNA isolated from an Al-tolerant rice cv Hawara Bunar and an Al-sensitive rice cv IR64 to evaluate the gene expression. The positive primers combination producing up regulated gene expression by Al stress was then used to isolate the gene candidate. The gene was isolated from an Indonesian local rice cv. Hawara Bunar, and cloned in pGWB5 vector under strong promoter 35S. The recombinant plasmid was transformed into tobacco plant through *Agrobacterium* mediated transformation. After being selected, the transgenic tobacco was then used for gene expression and functional analysis in relation to Al tolerance.

Results and Discussion

Gene expression analysis

The gene expression analysis was carried out based on the semi quantitative expression using RT PCR technique. RNA was isolated form both Al-tolerant and -sensitive rice that previously stressed with 15 ppm Al. One of the primers combination used in the analysis showed the different expression level between Al-tolerant and –sensitive rice when Al-stressed. The gene was upregulated by Al stress and expressed higher in Al-tolerant rice cv Hawara Bunar than that of in Al-sensitive rice cv IR64. Based on this finding, we suggested that the gene was an Al tolerance gene candidate that should be isolated and need further confirmation.

Gene Isolation and cloning

The positive primer combination was then used to generate cDNA using RNA isolated form rice cv Hawara Bunar. The 573 bp length cDNA was successfully isolated and cloned into pGEM-T Easy vector (Promega, USA) and maintained in *Escherichia coli* DH5α. After blue-white selection, the positive clone was then plasmid isolated and transformed into *A. tumefaciens*. The clone was also sequenced. Bioinformatics analysis analysis showed that the gene candidate was a transcription factor with C2H2 like motif, which might be a regulator for abiotic stress tolerance gene such as Al tolerance gene. The gene candidate was then named as rice aluminum tolerance (*RALT*) gene.

Agrobacterium mediated transformation of tobacco

Tobacco (*Nicotiana tabacum* L) plant was transformed with *RALT* gene through *Agrobacterium* mediated transformation. Leaves of tobacco plant were infected with *A. tumefaciens* containing *RALT* gene and transgenic plants were then selected with hygromycin until T3 generation. Among the selected transgenic plants, there were several lines that showed consistently tolerant to hygromycin and homozygous for *RALT* gene. DNA analysis showed that the *RALT* gene was stably inserted into tobacco chromosome until T3 generation (Figure 1).

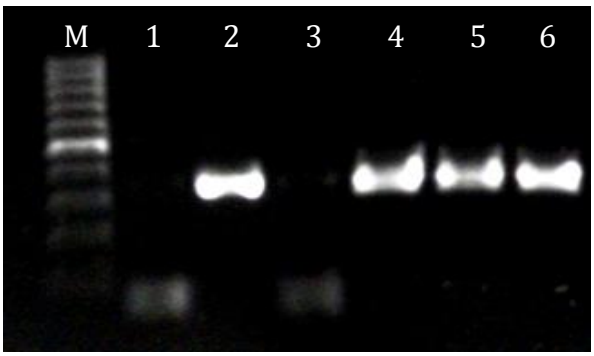

Figure 1: PCR analysis of T3 generation of transgenic lines using primers developed from *RALT* gene sequence. M: 100 bp DNA marker, 1: negative control, 2: positive control, 3: non-transgenic tobacco, 4-6: transgenic tobacco lines.

Al-tolerance analysis of the transgenic tobacco

To examine whether overexpression of the *RALT* gene increases plant tolerance to Al stress, the selected transgenic lines were then tested for the Al-tolerance using nutrient media containing 0, 300 and 555 μM Al. Since root elongation is the main target of Al toxicity (Kochian *et al*., 1995), the root length was measured after being stressed for 5 weeks. The result showed that transgenic tobacco had longer roots than that of the wild type, even under Al stress. Inhibition of root elongation increased with the increasing Al concentration (Figure 2). It was suggested that longer roots in transgenic tobacco was due to the role of the *RALT* gene in Al tolerance of transgenic tobacco.

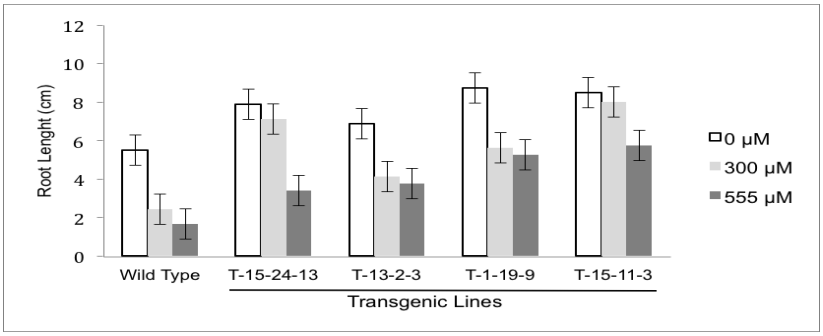

Figure 2: Effect of Al stress on root length of wild type and transgenic tobacco

Conclusion

Aluminum tolerance gene candidate, named *RALT* gene, has been successfully isolated and cloned from an Indonesian local rice cv Hawara Bunar. The gene has a role in Al tolerance in transgenic tobacco. It is expected that the gene has significant role in Al tolerant mechanism in rice. Further characterization of the gene is required in order to maximize the benefit of the gene for Al tolerance improvement in plant.

Acknowledgments

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Cross-species amplification of microsatellite markers in Malayan tapir (*Tapirus indicus*)

*Geetha Annavi, Fateen Khaliessa Mohd Arifin, Tengku Nor Diyana Tuan Mahmud and Shariza-Hanim Zainal Abidin

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang
**Corresponding author.Tel.:+603 89466621; email address: geetha@upm.edu.my*

Malayan tapir (*Tapirus indicus*) is listed as endangered species by IUCN due to population decline caused by habitat loss and fragmentation, and increasing hunting pressure throughout its ranges. Malayan tapir is the only one species of genus Tapiridae, distributed in the tropical rain forest of Southeast Asia. Tapirs are known as the key actors in forest dynamics as they are functionally important seed dispersers and seed predators. Despite their endangered status and functional role in ecosystems, little information is known about their social structure, mating system, population structure and dispersal pattern which are required for better conservation and management. Studies through traditional field methods are extremely difficult because tapirs are shy, cryptic, nocturnal, and prefer to inhabit deep tropical forest. Molecular genetics techniques now provide an alternative way to resolve ecological questions related to tapir. In this study, we tested cross-species amplification of 12 microsatellite markers designed for Lowland tapir (N = 9) and Bairdii tapir (N = 3) in Malayan tapir using fecal DNA. Result showed that all microsatellite markers failed to amplify in Malayan tapir. Poor quality and quantity of DNA, co-purification of PCR inhibitors, unsuitability of DNA extraction and preservation method and high genetic divergence are some of the factors contributing to this result.

Keywords: Malayan tapir, *Tapirus indicus*, microsatellite markers, non-invasive techniques, cross-species amplification

Spatial distribution pattern of *Cryptocoryne ×purpurea* Ridl. nothovar. *purpurea*, a natural aquatic plant hybrid of the Malay Peninsular

Rosazlina Rusly[1], Neils Jacobsen[2], Marian Ørgaard[2] and Ahmad Sofiman Othman[1]

[1]*School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*
[2]*Department of Plant and Environmental Sciences, Faculty of Sciences, University of Copenhagen, Rolighedsvej 21, DK 1958 Frederiksberg C, Denmark*
**Corresponding author.Tel.:+6012 4866882; email address: sofiman@usm.my*

Introduction

Cryptocoryne ×purpurea Ridl. nothovar. *purpurea* is a perennial aquatic plant species and can be found in the south of Malay Peninsula (Othman *et al*., 2009). It was noted that the pollen of *C. ×purpurea* nothovar. *purpurea* is completely sterile (Jacobson, 1977). One possible explanation could be the plant is of hybrid origin with most likely parents being *C. cordata* and *C. griffithii*. This assumption was based on the fact that the *C. ×purpurea* nothovar. *purpurea* possesses characteristics from the two species namely the broad collar zone of limb of the spathe (*C.cordata*) and the purple and rough limb (*C.griffithii*). The pollen sterility explains why fruits are unknown (Jacobsen, 1977). There is some variation in the colouration and also in the surface structure of the limb of the spathe in plants from different localities, an indication that the hybrid has arisen several times independently from different parental populations. The particular interest was to investigate the possible variations of clonal diversity and structure in different populations and how the population history affect them, which could provide some useful insights into the clonal structure and invasive history of this hybrid. The dynamics of clonal plants requires further genetic studies using powerful DNA markers such as AFLP (Amplified fragment length polymorphism). AFLP are dominantly inherited DNA markers and highly sensitive method for detecting polymorphisms in DNA. Nevertheless, because of the high amount of polymorphism that can be detected, this markers are the most efficient markers for this study; to identify individual genotypes at the landscape scale, in a species described to be highly clonal.

Materials and Methods

The primary study sites were in Kg Pulau Semut, Masjid Tanah, Melaka and Pos Iskandar, Tasik Bera Pahang. Patch margins and sizes were determined using a tape measure and compass in relation to a lattice of reference points that were established throughout the study area. This allowed for assignment of coordinates to each sampled ramet and patch size estimation. Genetic sampling was conducted using a 1M x 1M grid established within all patches. Ramets were then collected at grid intersection points, resulting in a total of 100 samples per population. Plant materials were immediately dried in silica gel and brought back to a laboratory for DNA extraction. Total genomic DNA was extracted from 0.3 to 0.5 g of dried leaves using the modified CTAB protocol outlined by Doyle and Doyle (1987).

Restriction-ligation reaction and preselective amplification of the AFLP procedure were carried out using the AFLP® Ligation and Preselective Amplification

Module for Regular Plant Genomes of 500-6000Mb (Applied Biosystems). Briefly, in the restriction-ligation reactions 500 ng of genomic DNA were digested and ligated to EcoRI- and MseI-adaptors in 11 µl volumes containing 1.1 µl 10X T4 DNA ligase buffer with ATP, 1 U MseI, 5 U EcoRI, 1 Weiss U T4 DNA ligase, 0.577 µl 1 mg/mL BSA (all: New England Biolabs), 1.1 µl 0.5 M NaCl, and 1 µl MseI- and 1 µl EcoRI-adaptors (Applied Biosystems). Restriction-ligation reactions were carried out for 2 h at 37°C in a thermal cycler. Afterwards the DNA was diluted 18.18-fold with $TE_{0.1}$ buffer. For the preselective amplification, 15 µL AFLP Core Mix, 1 µL AFLP preselective primer pairs (both: Applied Biosystems) and 4 µL diluted DNA prepared by restriction-ligation were combined in a PCR reaction tube. PCR amplification was carried out in a thermal cycler using the following program: 72°C for 2 min.; 20 cycles: 94°C for 20 sec., 56°C for 30 sec., 72°C for 2 min.; 60°C for 30 min. The product was diluted 20-fold with $TE_{0.1}$ buffer. For selective amplification, 3 primer pairs were used; (MseI-CAT/EcoRI-ACT (FAM), MseI-CAT/EcoRI-ACC (NED) and MseI-CAT/EcoRI-ACG (HEX)) for genotyping. For the PCR reaction 3 µl diluted product, 1 µl MseI-primer (at 5 µM), 1 µl fluorescent-labelled EcoRI-primer and 15 µl AFLP core Mix were mixed. The PCR conditions were: 94°C for 2 min.; 10 cycles: 94°C for 20 sec., 66°C – 1 °C/cycle for 30 sec., 72°C for 2 min., 20 cycles: 94°C for 20 sec., 56°C for 30 sec., 72°C for 2 min.; 60°C for 30 min.). Fragment analysis was conducted on an ABI 3730 capillary sequencer (Applied Biosystems). Bands between 50 and 700 bp were scored as being present (1) or absent (0). The number and frequency of multilocus genotypes was determined by GENEALEX 6.1 (Peakall and Smouse 2006). The clonal diversity was evaluated by the following indices (Ellstrand and Roose 1987): (a) number of genotypes, *G*; (b) the mean clone size, *Nc= N/G*, where *N* represents the sample size; (c) a modified version of the Simpson diversity index to measure clonal diversity within populations (Ellstrand and Roose 1987), $D = 1 - \sum\{[n_i(n_i - 1)]/ [N(N - 1)]\}$; where n_i is the number of samples of genotype *i* and *N* is the total number of the samples. The allelic frequencies data matrix was analyzed using a nested analysis of variance, AMOVA with GENEALEX 6.1 (Peakall and Smouse 2006) to estimate the components of variance within and among populations using 1000 permutations of the data at $P = 0.05$. Gene flow among populations was estimated from the F_{ST} statistics of Wright (1965), following the expression $M = Nm = (1– F_{ST} /4 F_{ST})$ with Arlequin 3.11 (Excoffier *et al*., 2005). To determine whether the matrix of genetic distances between *C.* ×*purpurea* nothovar. *purpurea* populations correlated with the matrix of geographic distances between locations, the Mantel test (Sokal 1979) using Tools for Population Genetic Analysis in (TFPGA) version 1.3 (Miller 1997) was performed for Nei's unbiased genetic distances matrix and the matrix of geographic distances.

Results and Discussion

Among all populations studied, a total of 198 ramets were sampled and assigned to 25 putative genets. The AMOVA analysis indicated that 12.0% of the total variation was due to within-population variation and 88.0% of the genetic variation was due to differences among populations. The pairwise F_{ST} values 0.415 revealing high values, with significant differentiation between the populations (indicating arisen from different parental populations). In support, gene flow was low among two populations

and several ramets within population (*Nm* < 1.00). The levels of clonal diversity in *C.* ×*purpurea* nothovar. *purpurea* was high (*D*=0.929). These results reflected a combination of very restricted distribution, somatic mutation and asexual propagation style of this hybrid. In addition, there is correlation between genetic differentiations among populations with geographical distance, revealed by Mantel Test. It was concluded that within *C.* ×*purpurea* nothovar. *purpurea* populations, clonality is a significant factor, but the spatial structuring of genetic variation suggests that both low levels of restricted gene flow and repeated recruitment of genets occured.

Conclusion

An understanding of clonality is critical for the implementation of the most appropriate conservation management of threatened clonal plants. These data can provide valuable information for conservation biologists because they allow for the estimation of population genetic differentiation between and within populations and support the design of sampling strategies for *ex situ* collections.

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Cloning of genes encoding for a key enzyme in gaharu synthesis, δ-Guaiene synthases from *Aquilaria malaccensis* Lam.

Azzarina Anor Basah[1], Mohd Nazre Saleh[2] and *Rozi Mohamed[1]

[1]*Forest Biotech Laboratory, Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding author.Tel.:+603 8946 7183; email address: rozimohd@upm.edu.my*

Introduction

Gaharu, also known as agarwood, is a fragrant wood from the genus *Aquilaria*. Gaharu is used for making insence, perfume and traditional medicines; these make the price of gaharu very expensive (Kakino *et al*., 2010). The high demands especially from consumers in countries in Asia, Europe and the Middle East have caused all species of *Aquilaria* to be listed in the Appendix of the Convention International Trade Species of Wild Fauna and Flora (CITES, 2013). *Aquilaria* is a tropical tree that is not well-studied despite its valuable gaharu. Gaharu is produced as a result of defense mechanism from wounding, pathogen and insect attack. To understand the mechanism of gaharu production, we studied sesquiterpene biosynthesis and its regulation via the terpenoid pathway using molecular methods. The main fragrant compounds of gaharu are sesquiterpene and phenylethyl chromones, but more sesquiterpenes are found in high quality gaharu (Yagura *et al.*, 2003). Recently, the first enzyme that catalyzes the conversion of farnesyl diphosphate (FPP) into the 15-carbon sesquiterpene has been cloned and characterized from *A. crassna* (Kumeta and Ito, 2010). The responsible enzyme is known as **δ**-guaiene synthase.

Materials and Methods

Total RNA was isolated from 0.5g *A. malaccensis* callus tissue (Jayaraman *et al.*, 2014) using the RNeasy Plant Mini Kit (Qiagen, Germany) and the first-strand cDNA synthesized (SuperScript ™ First –Strand Synthesis System, Invitrogen). Primers were designed from partial transcriptomic sequence using the Beacon Designer 7 software (Premier Biosoft, USA). The forward and reverse primers were 5' ACACCGCACCGCCGAAACG 3' and 5' AGCCGCCGAGATGCCAATTACC 3', respectively. PCR products were cloned into the pGEM T-Easy vector (Promega) and sequenced. The 5'- and 3'- cDNA ends were cloned using the RACE approach (FirstChoice® RLM-RACE, Ambion, USA). Based on the partial sequences, RACE primers were derived as follow: AmGS 5' RACE g.s outer primer (5'-GAAGCAGTTGAGAGGTGGGACATTG-3'), AmGS 5' RACE g.s inner primer (5'-ATGTTGATTGGAAGGGCAGAGTTTG -3') and AmGS 3'RACE g.s outer primer (5'-TCCCACGCCTAGACGAATGATTTTG -3'). The final full length sequences were cloned into the pGEM T-Easy vector (Promega).

Results and Discussion

We isolated the δ-guaiene synthase genes from *A. malaccensis* using reverse transcriptase-PCR amplification and specific primers derived from our in-house transcriptome. One partial cDNA sequence of 1350bp was cloned. Using RACE, the full length cDNAs including the start codon and poly A tail, were successfully cloned. Sequence analysis revealed that there were two clones, designated as *AmGS2* and *AmGS3*. They both contained an open reading frame of 1644 bp, flanked by 51bp 5'UTR and 73 bp 3'UTR, encoding a peptide of 547 amino acids.

Sequence alignment (Figure 1) revealed that both genes are similar to known δ-guaiene synthases from other species. Blastx analysis predicted high similarity between *AmGS2* and *AmGS3* with δ-guaiene synthases cloned from related species, *Aquilaria microcarpa, Aquilaria crassna* and *Aquilaria sinensis* (96% to 97% identity and E-value at 0.0 with 99% query cover). Although sharing the same length, their predicted amino acid sequences had about 5% differences at acid amino level. Our findings suggest that the two genes are probably involved in terpenoid biosynthesis.

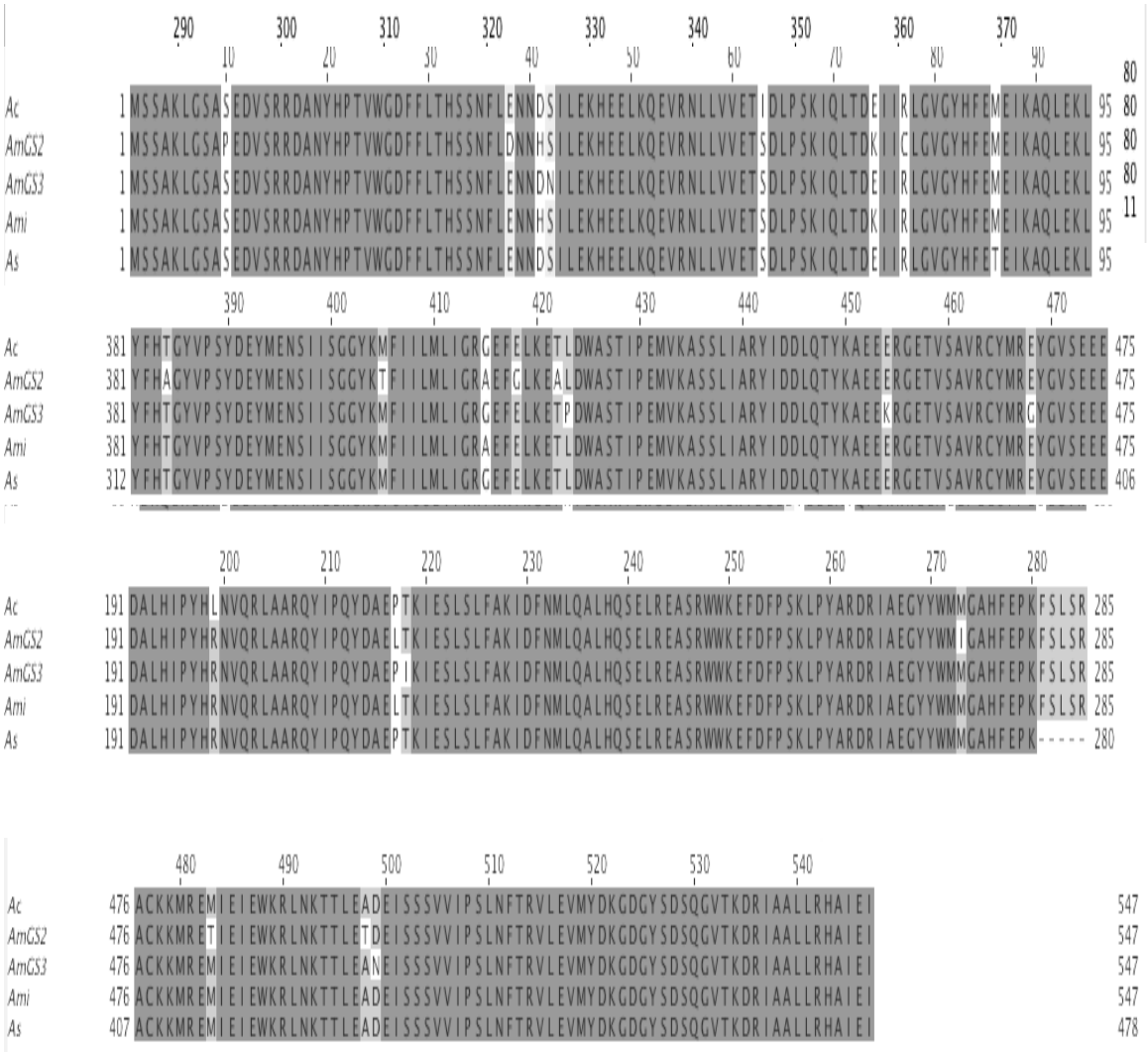

Figure 1: Multiple sequence alignment of δ-guaiene synthases.

Amino acid sequences from *Aquilaria malaccensis* (AmGS2 and AmGS3) were aligned with those from *Aquilaria microcarpa* (Ami, GenBank accession no: AHH25146.1), *Aquilaria crassna* (Ac, GenBank accession no. AEG77018.1), and *Aquilaria sinensis* (As, GenBank accession no. AFV99466.1). Alignment of the amino acid sequences was obtained using CLUSTALW with BOXSHADE (http://bioweb.pasteur.fr), in which gaps are marked as dashes and the conserved residues are highlighted in black, identical residues in dark gray and similar residues in light gray.

Conclusion

In this work, we report cloning of two genes encoding for δ-guaiene synthase from *A.malaccensis*, the major gaharu producer in Malaysia. This is the first report of sesquiterpene synthase genes from *A. malaccensis*. δ -guaiene is a sesquiterpene compound often found in gaharu. Terpenes have an active part in plant secondary metabolite research because of their diverse functions. These compounds have been extensively utilized in pharmaceutical, fragrance, cosmetic, and other related industries. Our findings provide new genes for the purpose of generating specific compounds of interest via in vitro methods such as through metabolic engineering.

Acknowledgements

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Proteomics and bamboo research

*Khalid Rehman Hakeem, Faridah Hanum Ibrahim, Rozi Mohamed

Faculty of Forestry, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
**Corresponding author.Tel.:+603 89467211; email address: kur.hakeem@gmail.com*

Bamboo, being one of the fastest growing plants needs only between 3 and 4 years to mature before they are ready for harvesting and utilization. This makes bamboo the best possible alternative to replace timber in the future. As a result, bamboo has been the focus of research in recent years. Currently, there is a growing demand for thicker walled and rapidly growing bamboo from the industry due to its mechanical strength, high durability and uninterrupted as well as sustainable supply. However, the information on the physical, mechanical and chemical properties for different bamboo species and age-groups is rather limited. In the current *Omics era*, 'Proteomic Analysis' has now become one of the basic technologies to obtain essential information of the biological systems. Proteomics is a powerful tool to study the global changes in protein synthesis in response to environmental stimuli as well as during development. At present, we are performing the proteomics studies on various commercially important known species of bamboo to understand the molecular mechanisms of rapid growth as well as thick wall lumen. This will help us to better understand the growth characteristics and physical properties of bamboo at molecular level by identifying the novel proteins associated with the production of thick wall and rapidly growing culms of bamboo. We believe that the present proteomics study shall provide a new dataset and the gene screening list, which will be a useful resource for future genetic as well as genomic studies for the development of high quality bamboo cultivars.

Keywords: Proteomics, bamboo, thick wall lumen

Identification and expression analysis of putative SDP1 homologue in rice *(Oryza sativa)*

*Xin Yi Ng, Hue Seow Mun and Sadequr Rahman.

School of Science, Monash University Malaysia Campus, 47500 Bandar Sunway, Selangor.
**Corresponding author.Tel.:+6016 5055295; email address: zoeyng512@gmail.com*

Oryza sativa is an important staple food for half of the world population. Although the main product from paddy is polished rice, oil can also be produced from the bran. However, genes involved in the production and breakdown of lipids in rice bran are not well characterized. A *SUGAR-DEPENDENT 1* gene (*SDP1*) was identified in *Arabidopsis thaliana* and was found to encode a patatin-like phospholipase. Its expression was detected in non-oil storage tissues. This gene encoded protein has triacylglycerol lipase activity during seed germination and the mutant plant was found to have retarded growth, impaired oil metabolism and oil accumulation in different tissues. Due to its potential to accumulate plant oil, homologue of this gene in rice was studied. To study the expression pattern of this gene, rice RNA samples were collected from different *Indica* and *Japonica* rice in different development stages. The putative *SDP1* was found expressing in non-oil storage tissues. Transcriptomics study of selected Bangladesh BD 192 white rice and Indonesia black rice showed no differences in term of this gene expression, but expressed significantly higher in seedling tissues than in leaf. For gene characterization study, a full length *SDP1* cDNA clone was used as template for transformation. Amplified *SDP1* gene was then cloned into an expression vector and transformed into *Escherichia coli* as host for protein expression. Two amino acid bands were observed on 12% SDS-PAGE that were about 40kDa, but their identity remains unknown. Further protein analysis will be done in the near future.

Keywords: *SUGAR-DEPENDENT 1* gene, *Oryza sativa*, gene expression, triacylglycerol lipase, transcriptomics

Identification of novel rice genes influencing starch digestibility

Ishrat Jabeen[1], Wickneswari Ratnam[2], Tilakavati Karupaiah[3], Ton So Ha[1], Sharifah Dipti[4], Khandaker Aminul Kabir[4] and Sadequr Rahman[1]

[1]*School of Science, Monash university, Malaysia*
[2]*Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[3]*Faculty of Allied Health Sciences, National University of Malaysia, Kuala Lumpur, Malaysia*
[4] *Grain quality and Nutrient Division of Bangladesh Rice Research Institute (BRRI)*
**Corresponding author.Tel.: 4608303 551; email address: sadequr.rahman@monash.edu*

Rice is one of the most versatile crops cultivated worldwide. It serves as the primary dietary source of carbohydrate for most Asians. Eating and cooking qualities of rice are influenced by the physiochemical properties of starch in endosperm, primarily by the amylose. Rice with improved carbohydrate qualities could offer potential as dietary strategy for preventing and managing several chronic diseases, thus promoting population health. Glycemic Index (GI) is a concept used to compare the blood glucose raising responses to carbohydrates of different foods in response to consumption of equal quantities of glucose as a reference. Consumption of low GI food was found to have a beneficial effect in lowering postprandial glucose responses. Thus is it important to study commonly consumed rice in Malaysia with low GI. In this study, twelve healthy individuals were subjected to consume five different rice lines (and their blood samples were collected to determine glucose and insulin responses). Data obtained from the *in vivo* results of our study revealed two of the rices as low GI whilst the remaining three as intermediate. Sequence differences between the three rices in the Waxy gene have been uncovered. Further work will also involve transcriptome sequencing to identify differences between rices. Research is still in progress to establish an *in vitro digestibility* test which will facilitate the identification of genes associated with low GI trait by analysing suitable crosses. Development of markers and the *in vitro* test should enable the rapid development of low GI varieties optimised to Malaysian conditions.

Keywords: Endosperm, glycemic index (gi), incremental area under the curve (iauc), postprandial glucose response

Isolation, subcellular localization and phylogenetic of Transaldolase genes from *Zea mays* cv sweet corn bi-color

*Nahid Kalhori, Rosimah Nulit and Ang Poh Nee

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

**Corresponding author.Tel.:+603 89466615; email address: rosimahn@upm.edu.my*

The Pentose Phosphate Pathway (PPP) also is known as 6-phosphogluconate pathway which occurs in the cytosol of the plant cell. There are two main arms in PPP; the oxidative and non-oxidative arms. The main functions of this pathway is to generate reducing equivalents in the form of NADPH, for reductive biosynthesis reactions within cells in the biosynthesis of fatty acids and steroid. This pathway also provide the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids. Transaldolase (TAL) is an enzyme which plays an important role in the non-oxidative portion of the pentose phosphate pathway. The actual reaction is between glyceraldehydes 3-phosphate and sedoheptulose 7-phosphate, resulting the formation of C_4 product erythrose 4-phosphate and fructose 6-phosphate. In this study, transaldolase (TAL) has been successfully isolated and identified from Zea mays cv Sweet corn bi-color. The objectives of the study were achieved where the specific primer designed has function effectively in isolated TAL with 773 bp of nucleotide sequences. Analysis of homology through multiple sequence alignment revealed that TAL from Z. mays cv Sweet corn bi-color is highly similar with the plant species compared to animal and bacteria. ClustalW analysis found that TAL from Z. mays hit the score of 85.0 as they are close related in terms of taxonomy level which they are from the family of Poaceae. Other plants such as Solanum lycopersicum and Solanum tuberosum are from family Solanaceae, Dimorcarpus longan is from family Sapindaceae whereas Hyacinthus orientalis is from Hyacinthus family. However, it is discovered that TAL from Z. mays cv Sweet corn bi-color with TAL in Oryza sativa subsp. Indica only possess score of 64.0.

Keywords: Transaldolase, pentose phosphate pathway, subcellular localisation. phylogenetic.

Strain improvement of *Aspergillus brasiliensis* through mutagenesis for overproduction of xylanase in submerged fermentation

*Hooi Ling Ho and Kuan Fai Ho

Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, Cheras, 56000 Kuala Lumpur, Malaysia.
**Corresponding author.Tel.:+603 91018880; email address: hohl@ucsiuniversity.edu.my ; hohooiling@gmail.com*

Introduction

Mutagenesis is defined as the changes of genetic information of an organism in a stable, heritable manner, which could either occur naturally or performed experimentally through chemicals or radiation. There were two types of mutations involved in this study, ultraviolet (UV) irradiation and chemical mutagenesis using ethyl methyl sulfonate (EMS) and N-methyl-N-nitro-N-nitrosoguanidine (MNNG). Increase of the enzyme production rate was achieved by exposing fungi to UV light with different exposure time (Irfan *et al.*, 2011). As a result, mutations improve enzyme yield by several folds than the parental strains. It has been proven that with mutation, certain genes may urge to be more efficient. Like UV mutation, chemical mutagen may also enhance the efficiency of some genes through deletion and duplication mechanism. Chemical mutagens such as EMS and MNNG may cause the alkylation of guanidine residues to produce resident lesions within the DNA molecules. Apart from that, such effects may increase the expression of genes responsible for encoding enzymes production in fungi (Haq *et al.*, 2008). Therefore, in this study, the objectives of the study are to improve the production of xylanase by *Aspergillus brasiliensis* using UV and chemical mutagenesis and to determine the most effective mutagenesis method in the strain improvement of *A. brasiliensis* for the overproduction of xylanase.

Materials and Methods

A. brasiliensis ATCC 16404 was subcultured on PDA and incubated at 30°C for 3 days. Then**,** *A. brasiliensis* on PDA were treated with the UV light of the wavelength 2537 Angstroms from a distance of 10 cm for 10, 20, 30 min, respectively. The control experiment was conducted with non-treated *A. brasiliensis* without the exposure of UV. The mutant and control strains were then incubated for 2 days at 30°C. For EMS mutagenesis, *A. brasiliensis* on PDA plate were harvested and resuspended into sterile distilled water. 9 mL of spore suspension was then mixed with 1 mL of Sodium Phosphate Buffer (SPB) at pH 7 used as the non-treated control. Another 9 mL of spore suspension were mixed with 1 mL of 150 µg/mL EMS in SPB at pH 7 used as the sample for EMS mutagenesis. Then, the mutagenesis sample was incubated at 30°C for 30, 60 and 90 min intervals. For MNNG treatment, 5 mL of spore suspension were then mixed with 5 mL of SPB at pH 7 used as the non-treated control. Another 5 mL of spore suspension were harvested and mixed with 5 mL of 150 µg/mL MNNG in SPB at pH 7 used as the sample for MNNG mutagenesis. Then, the mutagenesis sample was incubated at 30°C for 30, 60 and 90 min intervals. After incubation, all spores from UV and chemical mutagenesis

were harvested and 1 × 10^6 spores were transferred to 150 mL PDA broth at pH 6.5 with three replications. The inoculums were incubated at 30°C at 150 rpm for 24 h. Xylanase activity was carried out every 24 h for 6 days. Xylanase activity was measured according to Bailey *et al*. (1992).

Results and Discussion

Figure 1 shows xylanase overproduction by *A. brasiliensis* after exposed to UV. The *Aspergillus* mutant which exposed to UV for 10 min possessed xylanase activity of 3.49 ± 0.034 U/mL with total protein of 0.175 ± 0.0034 g/mL by total of 138.67 ± 1.53 × 10^6 spores/mL at medium pH 4.63 ± 0.03 at 48 h. There was about 12.69% increment of xylanase production as compared to 144.67 ± 0.58 x 10^6 spores/mL of wild type that produced 3.097 ± 0.089 U/mL of xylanase with total protein of 0.205 ± 0.0037 g/mL at 48 h at pH 4.75 ± 0.05. As for *Aspergillus* that exposed to UV for 20 min, it produced higher xylanase activity of 4.86 ± 0.095 U/mL with total protein of 0.214 ± 0.007 g/mL by 177.67 ± 2.08 × 10^6 spores/ mL at pH 4.47 ± 0.02 at 48 h. In fact, there was about 56.93% increment of xylanase production as compared to the wild type. The UV light was capable of modifying the structure of pyrimidine which led to the formation of thymine dimer which distorted the structure of DNA helix. In common cases, UV mutation is not beneficial but sometimes, it may lead to the improved adaptation of the fungi to its environment with better biocatalytic performance (Irfan *et al.*, 2011). UV exposed *A. brasiliensis* mutant experienced the highest percentage of 56.93% in the overproduction of xylanase as compared to EMS and MNNG treated mutants with the increment of only 1.34% and 17.14% (data not shown), respectively.

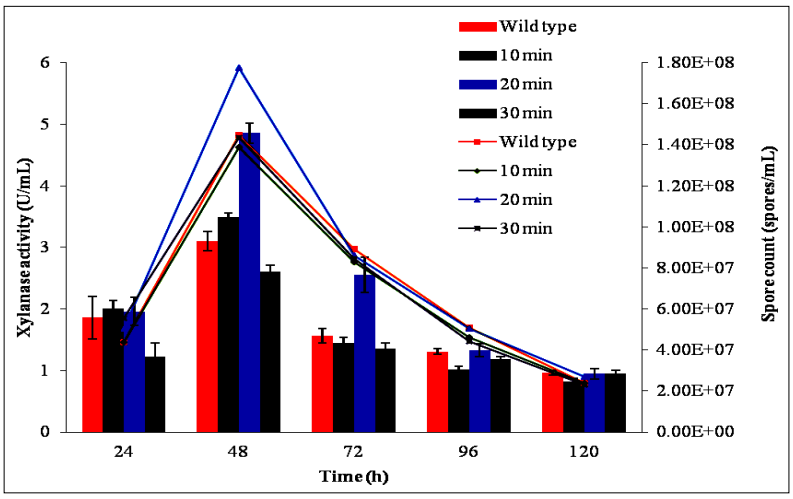

Figure 1: Comparison of xylanase production between wild type and mutants of *A. brasiliensis* after being exposed to UV from 10 cm distance at time interval of 10, 20 and 30 min. Xylanase activity is presented as column chart while spore count is presented as line graph.

Conclusion

In conclusion, UV mutagenesis was among the most effective mutagenic approach in inducing the overproduction of xylanase by *A. brasiliensis* compared to EMS and MNNG.

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Preparation, characterization and reduction of burst release of bovine serum albumin (BSA) from biodegradable PLGA microspheres

*M.M. Rahman[1], R.H. Ansary[1], H. Katas[2] and M. B. Awang[3]

[1]*Kulliyyah of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Malaysia*
[2]*Faculty of Pharmacy, University Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia*
[3]*Faculty of Pharmacy, Cyberjaya University College of Medical Sciences, 63000 Cyberjaya, Malaysia*

** Corresponding author.Tel.:+609 5716400 (ext. 4939); email address: mdrahman@iium.edu.my*

Introduction

In conventional drug administration method, macromolecular drugs such as protein and peptide drugs are administered by daily, sometimes multiple injections via a parenteral route due to their short half life *in vivo*. To improve patient compliance and convenience, poly(lactide-co-glycolide) (PLGA) based micro/nanoparticles have been developed for controlled release formulations of protein and peptide drugs [1]. In general, the major drawbacks in the development of PLGA based micro/nanoparticles formulations in protein and peptide drugs delivery are the high initial burst, incomplete release and instability of the encapsulated proteins [2]. Initial burst release means the rapid release of a large amount of encapsulated drug. In this study, bovine serum albumin (BSA) has been encapsulated in hydroxyl-terminated PLGA microspheres with a view to reduce the high initial burst release of BSA from PLGA microspheres.

Materials and Methods

The glucose star-type hydroxyl-terminated PLGA (LA:GA 1:1) with a number averaged molecular weight 50000 and acid-terminated PLGA 50:50 (molecular weight 24000-38000) were purchased from Sigma-Aldrich (USA), bovine albumin fraction V was purchased from R & M chemicals (U.K). Micro bicinchoninic acid (micro BCA) protein assay kit was obtained from Sigma-Aldrich (USA). All other chemicals used were of analytical grade.

BSA loaded hydroxyl-terminated PLGA microspheres have been prepared by a conventional water-in-oil-in-water (w/o/w) and a modified water-in-oil-in-oil-in-water (w/o/o/w) emulsion solvent evaporation method [3]. For comparison, acid terminated PLGA microspheres were also prepared by same method. In modified w/o/o/w emulsion technique, 100 µl of BSA solution (30 mg/ml) in 1% aqueous PVA (w/v) was emulsified with 50 mg of acid-terminated PLGA in ethyl acetate (EA). This water/oil emulsion was then emulsified with 50 mg of hydroxyl-terminated PLGA in dichloromethane (DCM). The obtained w/o/o emulsion was further emulsified with 10 ml of 1% PVA (w/v). The resulting w/o/o/w emulsion was transferred into 50 ml of 0.5% PVA (w/v) aqueous solution and stirred for three hours to evaporate EA/DCM solvent mixture by vacuum evaporation at room temperature. After that the microspheres were separated by centrifugation and washed 3 times with 60 ml distilled water and freeze dried overnight. In conventional w/o/w method, BSA loaded

hydroxyl-terminated PLGA and acid-terminated PLGA microspheres have been prepared separately as well. Particle size, and size distributions of microspheres were measured by a laser particle size analyzer. The particle size distribution was expressed as the volume median diameter (D 50%). BSA encapsulation efficiency and *in vitro* release were determined by a micro BCA method [4]. The shape and surface morphology of the microspheres were observed by field-emission scanning electron microscope.

Results and Discussion

Microspheres prepared with w/o/w and w/o/o/w emulsion technique exhibited high encapsulation efficiency except formulation F3 (Table 2). High initial burst release of BSA was observed in all formulations prepared by w/o/w emulsion technique. A significant reduction of initial burst release of BSA was observed when microspheres were prepared by modified w/o/o/w emulsion method (Figure 1). The *in vitro* cumulative release profiles of the optimized formulation F4 of BSA loaded microspheres showed reduced initial burst and prolonged sustained release of BSA over 70 days (Figure 2). During this time period more than 80% BSA was released. The optimized formulation F4 was nonporous, smooth-surfaced, and spherical shape (Figure 4) under field-emission scanning electron microscope (FE-SEM) with a mean particle size of 3.95 µm and encapsulation efficiency of 98.46%. In contrast, microspheres prepared with w/o/w double emulsion technique were porous (Figure 3) which might be a reason of high initial burst release of BSA [5].

Table 1: Formulation conditions of BSA loaded PLGA microspheres.

Formulation code	**Method**	**Hydroxyl terminated PLGA (mg)**	**Carboxyl terminated PLGA (mg)**	**Dichloromethane (ml)**	**Ethyl acetate (ml)**
F1	w/o/w	50	-	1	-
F2	w/o/w	-	50	1	-
F3	w/o/w	-	50	-	1
F4	w/o/o/w	50	50	1	1

Table 2: Results of particle size, encapsulation efficiency, and yield of BSA loaded PLGA microspheres.

Formulation code	**Mean particle size (µm ± SD)**	**Encapsulation efficiency (% ± SD)**	**Microsphere yield (% ± SD)**
F1	8.16 ± 0.68	83.63 ± 3.95	65.05 ± 2.75
F2	8.38 ± 0.52	98.25 ± 2.83	68.17 ± 3.42
F3	17.35 ± 0.76	21.43 ± 1.95	75.29 ± 3.28
F4	3.95 ± 0.45	98.46 ± 2.62	53.60 ± 3.04

SD = Standard deviation, n=3

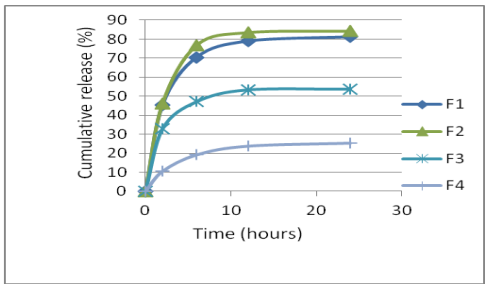

Figure 1: *In vitro* release profiles (24 hours) of BSA loaded PLGA microspheres in different formulations.

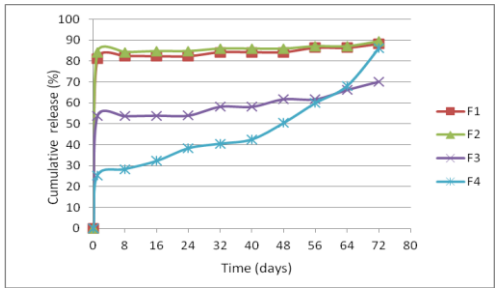

Figure 2: *In vitro* release profiles (72 days) of BSA loaded PLGA microspheres in different formulations.

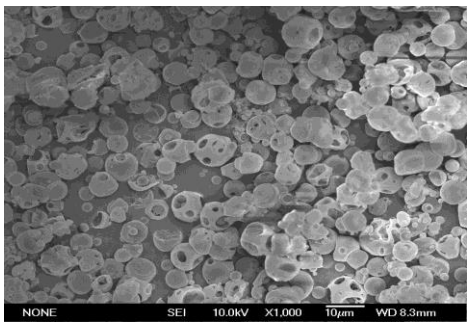

Figure 3: SEM picture of F1.

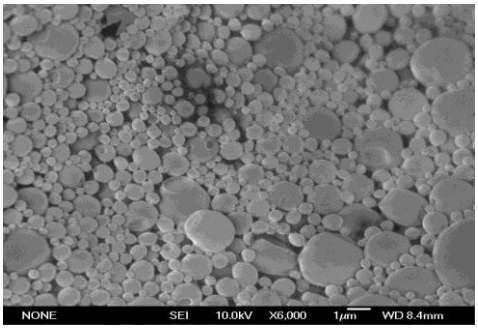

Figure 4: SEM picture of F4.

Conclusion

In this work, BSA loaded PLGA microspheres were prepared successfully using w/o/w and w/o/o/w emulsion solvent evaporation technique with respect to particle size, encapsulation efficiency and yield. Formulation F4 prepared by w/o/o/w consisting of hydroxyl-terminated PLGA and carboxyl-terminated PLGA in a binary solvent mixture exhibited a reduced burst release followed by sustained release over 70 days. This formulation could be proposed as a potential delivery system of therapeutic proteins.

Acknowledgements

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Physiology

Extraction and purification of astaxanthin by *Aeromonas hydrophila*

Jeeyin Cheong[1], *Muskhazli Mustafa[1], Nor Azwady Abd Aziz[1], Siti Aqlima Ahmad[2] and Azleen Ahmad Adli[1]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Biochemistry, Faculty of Biotechonlogy and Biomolecular Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
**Corresponding Author. email address: muskhazli@upm.edu.my*

Introduction

Shrimp processing generates wastes up to 60% of its initial weight. These wastes were previously managed using strong acid and alkali to recover certain valuable substances of which in turn leads to pollution. Therefore, recent environmental friendly approaches using microbes and LAF has been widely adopted to extract valuable substances from crustacean wastes (Khanafari *et al*., 2008) as it mimics chemical effects. Carotenoids were extracted out from shrimp shell wastes using lactic acid fermentation due to the oxidative properties of carotenoids. However, the process of anaerobic fermentation is not feasible to be conducted elsewhere as special anaerobic monitoring is required and can be costly. Hence in this study, aerobic fermentation with *Aeromonas hydrophila* was much interested to determine carotenoids extracted mainly astaxanthin and purified. Various cell disruptions efficiencies were also being studied.

Materials and Methods

Preparations

Shrimp shell wastes obtained from the wet market were cleaned thoroughly and lyophilized before grinding into powder form and kept in -20°C away from light until use. *Aeromonas hydrophila* was cultured in its optimum media of 0.1% (w/v) K_2HPO_4, 0.05% (w/v) $MgSO_4.7H_2O$, 3% (w/v) monosodium glutamate, 1% (w/v) glucose, and 9% (w/v) shrimp shells, pH 7.0 for 48 hours, 150 rpm at 30 ± 0.5°C. Carotenoproteins were retained after microbial fermentation by centrifuging the culture at 8000 x g for 20 minutes before lyophilized and kept in -80°C until extraction. Astaxanthin estimations were done according to Babu *et al*. (2008).

Cell disruptions

Cell disruptions were conducted on shrimp shells before subjecting it to bacterial fermentation. Shells were subjected to autoclaving (dry and wet), heating (75°C, 30 mins), autolysis and sodium carbonate treatment (5M, soaked 2 hours). Methods were done following Xiao *et al*. (2008). All filtrates were lyophilized before subjected to microbial fermentation.

Extraction of the carotenoids

Carotenoprotein extraction was extracted by soxhlet extraction according to (Thana *et al.,* 2008). The crude extract was subjected to rotary evaporation until the amount remains 1-2 ml.

Purifications and identification of astaxanthin

Thin Layer Chromatography using Merck 60 Silica Aluminum Sheet was used to purify the carotenoids obtained from the shrimp shells after microbial fermentation. Mobile phases used were suggested by Khanafari *et al*. (2008). The entire purification process was conducted under low light conditions. Purified carotenoids (visible bands) were scraped out and dissolved in acetone (HPLC grade) before subjected to HPLC analysis. Purified carotenoids were subjected to HPLC analysis equipped with C18 reverse phase column, 5µm pore size for astaxanthin determination. Mobile phase and conditions used were according to Dong *et al*. (2014).

Results and Discussion

Cell disruptions were found to expose more astaxanthin in yeasts cells and algae cells as it has been successfully reported in Xiao *et al*. (2008). However when conducted on shrimp shells, no significant increase in astaxanthin amount was detected. This maybe due to the differences in cells structures between yeast and algae with shrimp shells as shrimp shells generally have calcification. Table 1 shows the amount of astaxanthin available comparing various cell disruptions after bacterial fermentation. Heat treated treatments showed lesser gain of astaxanthin perhaps due to heat degradation while sodium carbonate and control showed no significant differences though having higher amount of astaxanthin. Therefore, cell disruption was not opted as control treatment is able to produce more or less same amount as cell disruption.

Table 1: Amount of astaxanthin estimated through various cell disruptions.

Cell Disruption	Estimated astaxanthin (µg)
Autolysis	2.11 ± 0.096^{a}
Dry autoclave	1.76 ± 0.172^{b}
Wet autoclave	1.30 ± 0.121^{c}
Heating	1.67 ± 0.188^{b}
Sodium carbonate treatment	2.41 ± 0.182^{d}
Control	2.30 ± 0.118^{d}

Note: The different superscript in each column are significantly different ($p<0.05$)

The crude extract was bright red-orange in color which suggests the presence of carotenoids such as astaxanthin and β-carotenes. Thin Layer Chromatography developed with n-hexane: acetone has yielded clear bands and is in agreement with Khanafari *et al*. (2008) and Sindhu and Sherief (2011) as the best mobile phase solvent system to separate carotenoids especially in the concern of astaxanthin extraction. The R_f value of approximately 0.33 ± 0.02 (internationally accepted) was found to be astaxanthin and the analysis of HPLC has confirmed this.

Aerobic fermentation has proven to be able to extract carotenoids (astaxanthin) from shrimp shells while studies done on lactic acid fermentation may give a higher recovery of astaxanthin. Nevertheless, it is suggested in future studies to compare the amount extracted by both of the system.

Conclusion

Aeromonas hydrophila has proven to be able to extract carotenoids (astaxanthin) from shrimp shell wastes through aerobic fermentation and purification of the carotenoids has found the presence of astaxanthin at R_f value of 0.33 and has been confirmed by HPLC.

Acknowledgements

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Comparative study on protein expression of chicken meat between Malaysian broilers and indigenous chicken

*****Muhamad Shafiq Abu Hassan and Noraslinda Muhamad Bunnori

Kulliyyah of Science, International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200 Kuantan, Pahang

**Corresponding author.Tel.:+6013 2747325; email address: shafiq.iium@gmail.com*

Introduction

Broilers are the most notable chicken meat in the market. The increment of demand is due to well acceptance of chickens as food by multi-religious and multi-cultural citizen and also inexpensive. Farmers breed the chickens in the modern integrated poultry raising facilities with antibiotics to keep them healthy. Due to rapid juvenile growth, broilers are experiencing many metabolic problems such as ascites, lameness and sudden death (Fanatico *et al.*, 2009). Apart from broilers, there is indigenous chicken (*ayam kampung*) that been grown by traditional means. The farmers feeding them with organic feeds and avoids chemicals, fertilizers, and also antibiotics. This chickens are freely scavenging around, utilizing spaces. This type of chicken has less fat and weight less compared to broilers. The stress from rapid juvenile growth in broilers may give result on different protein expression in meat. In order to understand this issue, this study has been carried out to characterize the protein profile between broilers and indigenous chicken muscle.

Materials and Methods

Revised method from Wu *et al*. (2009). One hundred milligrams of muscle tissues from broilers and indigenous chicken were homogenized separately under liquid nitrogen with pre-chilled mortar and pestle. The grounded tissue was dissolved into the 1.0 ml of extraction buffer (7 M Urea, 2 M thiourea, 2% CHAPS, 65 mM DTT, 0.2% Bio-Lyte 3/10, 1 mM protease inhibitor). Then the mixture was vortexed vigorously for 2 minutes and rest on the ice for another 2 minutes, repeated five times. The sample then was centrifuge at 14,000 x g at 15˚C for 1 hour. The supernatant was taken and further quantify by using Bradford (1976) method. 400 µg of sample was added to the rehydration buffer (7M Urea, 2M thiourea, 2% CHAPS, 65mM DTT, 0.2% Bio-Lyte 3/10) resulting in a total volume of 125 µl. The immobilized pH gradient (IPG) strips (pH 3-10, 7cm) was rehydrated overnight with premixed protein sample mentioned above. The first dimensional electrophoresis, isoelectric focusing (IEF), was performed in PROTEAN IEF Cell (Bio-Rad) at 20°C, using the mode 200V, 30 min, then 1000V, 1 h, 3000V, 5 h, 10000 to 60000V•h and hold at 500V according to the Gorg *et al.* (1999) protocols with slight modifications. The strips were equilibrated with a buffer containing 6M Urea, 20% glycerol, 2% SDS, 1.5M Tris pH 8.8 and 1% (w/v) DTT for 15 minutes and subsequently equilibrated for additional 15 minutes with

replacement of DTT with 2.5% (w/v) iodoacetamide. The equilibrated strips were moved onto 12% polyacrylamide gels. The second dimensional separation was performed using the mode 150 V, for 1 hour. The gels were stained by using Coomasie Brilliant Blue solution for 1 hour and destained for overnight. The resolved protein spots were then obtained by GS-8000 Densitometer using Quantity One software and further analyzed with PDQuest application. The selected spots were excised and analyzed with MALDI-TOF.

Results and Discussion

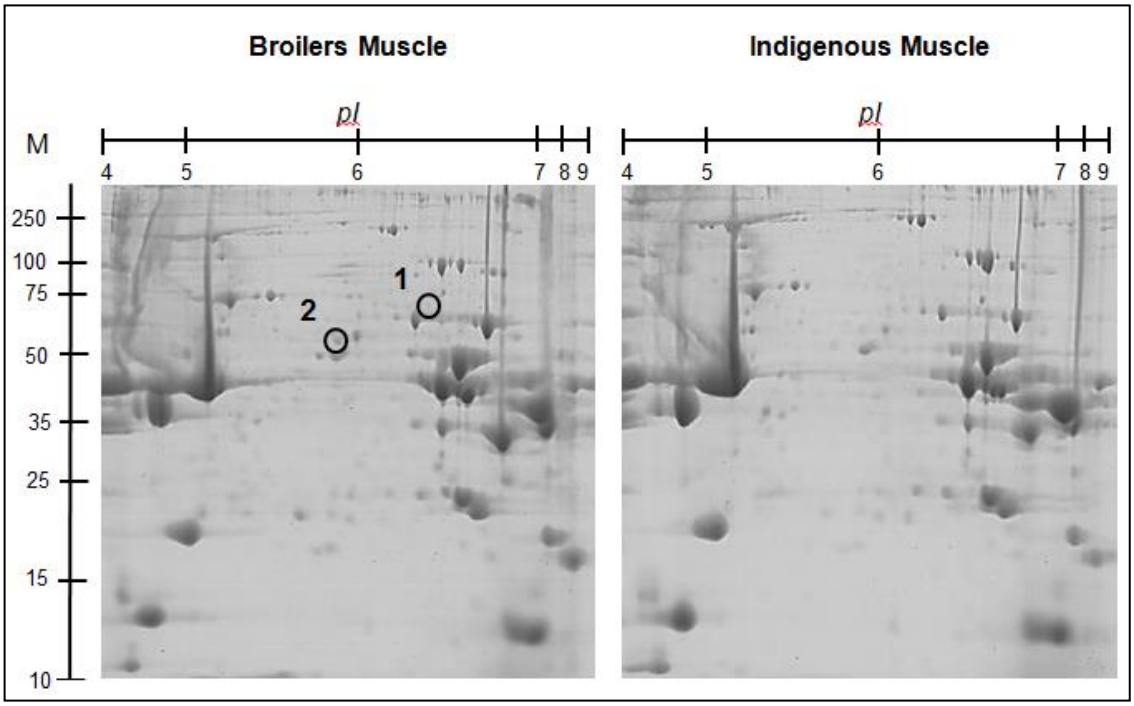

Figure 1: Differential protein expression in chicken's muscle sample by using 2D-PAGE. The circles indicate the unmatched spots when compared between both gels. M, protein marker (kDa); pI, isoelectric point; 1, Molybdopterin synthase catalytic subunit; 2, Proteasome regulatory particle base subunit RPT1.

The result from Bradford assay showed the protein concentration for broiler's and indigenous chicken muscle are 7.935 ± 0.635 mg/ml and 8.288 ± 1.245 mg/ml respectively. The result from PDQuest detected 640 spots by applying all spots intersection with conformation of 90% student t-test statistical analysis in comparison for both replicate gels. Among those spots, two spots have been selected for further analysis with MALDI-TOF. The data from MALDI-TOF revealed the several peaks which then were searched through non-redundant NCBI database by using Mascot Peptide Mass Fingerprint application (http://www.matrixscience. com/cgi/search_form.pl?FORMVER= 2&SEARCH=PMF). The first protein is belongs to Molybdopterin synthase catalytic subunit with score of 84% and coverage sequence of 63% while the

second protein is belongs to Proteasome regulatory particle base subunit RPT1 which having the score of 77% and coverage sequence of 47%. Molybdopterin synthase catalytic subunit (MOCS2) is a subunit of enzyme that catalyzes the conversion of precursor Z into molybdopterin by incorporation of two sulfur atoms to generate a dithiolene group. Molybdopterin acts as a cofactor to several enzymes includes sulfite-oxidase which is required in catalyzing the oxidation of sulfite to sulfate and also metabolizing sulfur-containing amino acids methionine and cysteine (Kisker *et al*., 1997). The expression of MOCS2 in muscle may act as sulfur carrier to generate molybdopterin which then to be used as a cofactor with sulfite-oxidase to metabolize amino acids methionine and cysteine efficiently. This may resulted to a better meat quality in broilers. Proteasome regulatory particle base subunit RPT1 is a small subunit protein that attached to proteasome which generally involved in many essential cellular functions includes protein catabolism, stress signaling, inflammatory responses and apoptosis (http://www.genome.jp/dbget-bin/www_bget? pathway+sce03050). The expression of RPT1 protein with associated with proteasome may show the increment of high protein catabolic activity in broilers muscle. This conforms with the ability of broilers to convert feed into meat in efficient way to produce greater mass of meat compared to indigenous chicken.

Conclusion

In conclusion, the two proteins have been identified with specific functions contribute insight at molecular level which may arises from the effect of rapid juvenile growth in broilers meat.

Acknowledgments

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Identification of attractant-derived bioactive compounds in the haemolymph of pest male carambola fruit fly, *Bactrocera carambolae* and melon fly, *B. cucurbitae*

*Alvin Kah-Wei Hee

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor

**Corresponding author. email address: alvinhee@upm.edu.my*

Carambola fruit fly (CFF), *Bactrocera carambolae* and melon fly, *B. cucurbitae* are important fruit pests of economic importance. These flies are known to be strongly attracted to two different groups of male attractants- CFF to methyl eugenol (ME), and melon fly to raspberry ketone (RK) that have been successfully used in male annihilation and quarantine detection. Pharmacophagy of those attractants resulted in the biotransformation of ME to *(E)*-coniferyl alcohol (CF) that is a booster component of male sex pheromone in CFF whilst RK was sequestered unchanged as one of the male sex pheromone components in the melon fly. The presence of either CF or RK that sequestered in the rectal gland prior to emission during courtship period at dusk was investigated in the male circulatory system. These results and their implications in relation to that known about the oriental fruit fly, *B. dorsalis* will be discussed.

Keywords: Methyl eugenol, raspberry ketone, *(E)*-coniferyl alcohol, carambola fruit fly, melon fly, haemolymph

Developmental exposure to mercury (II) chloride alter behaviour of zebrafish (*Danio rerio*) larvae

Noraini Abu Bakar and *Wan Norhamidah Wan Ibrahim

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

**Corresponding author.Tel.:+603 89466625 ; email addres: wnwi@upm.edu.my*

Introduction

A growing body of evidence from epidemiological and experimental studies has revealed an association of exposure to environmental pollutants during early life predispose the organism to higher susceptibility for pathological condition later in life. Since developing brain is inherently more susceptible to toxicant as compared to adult brain, currently Developmental Neurotoxicity (DNT) testing has become one requirement in toxicity testing of chemicals (U.S. EPA, 1998). Only 5 industrial chemicals are classified as toxic to human neurodevelopment namely arsenic, lead, toluene, polychlorinated biphenyls and methylmercury (MeHg) (Grandjean and Landrigan, 2006). Considering there are still a vast number of chemicals in commerce without available DNT data, there is a high push for the scientific community to develop testing strategies that can quicken up the process of DNT testing.

Materials and Methods

The zebrafish embryos were exposed to 6 different nanomolar concentrations of $HgCl_2$ (7.5, 15, 30, 100, 125 and 250) in triplicates from 5 hours post fertilization (hpf) until hatching under semi-static exposure regime with the daily renewal of the test solution. To avoid bias in the behavioural testing, we excluded embryos with morphological abnormalities. Behavioral alterations in normal treated embryos or larvae were compared against respective control. Five embryos and larvae from each replicates were randomly tested. Number of spontaneous tail coiling in 24 hpf embryos were quantified under dissecting microscope within 1 minute. Meanwhile, spontaneous swimming activity in 6 dpf larvae were quantified using a modified protocol by Samson and Shenker (2001). The larvae was individually placed in a petri dish with a diameter of 60 mm containing 15 mL of embryo medium and placed over a grid of 1.0 cm^2 squares. After 1 min acclimatization period, the number of squares larvae swam through 1 minute was recorded by using SONY JVC E505B Full HD Camcorder. The number of lines crossed by the larvae was counted. The *one-way* analysis of variance (*ANOVA*) was *used to* determine whether there are any significant differences of the behavioural alterations between the $HgCl_2$ exposed larvae and the control.

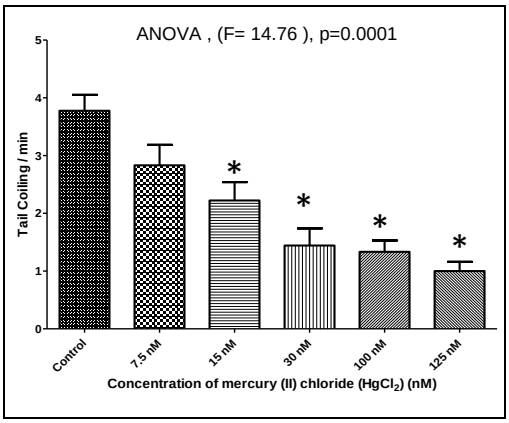

Figure 1: Exposure to $HgCl_2$ toward zebrafish embryos decreased the tail coiling of zebrafish (*Danio rerio*) embryo at 24 hpf in a dose dependent manner. *Significantly different from control (P ≤ 0.05).

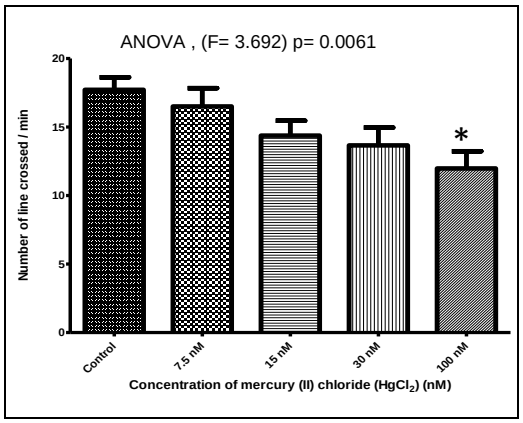

Figure 2 : Exposure to $HgCl_2$ decreased the swimming activity of 6 dpf larvae in a dose dependent manner. *Significantly different from control (P ≤ 0.05).

Results and Discussion

Tail coiling is the first motor movement in zebrafish (Saint-Amant & Drapeau, 1998). In our study we found that embryonic exposure to $HgCl_2$ induced a significant decrease in spontaneous tail coiling at 24 hpf in a dose dependent manner (Figure 1). Reduction in the number of spontaneous tail coiling could be due to delays in the normal development of the neuromuscular system or the muscular system per se. Cole and Ross (2001) demonstrated that induction of apoptosis due to oxidative stress in the hindbrain may result in perturbation of the on-going morphogenesis of the cerebellum, a part of the brain that plays an important role in muscle contraction around the tail area. Moreover, embryonic exposure to $HgCl_2$ may also disrupt the normal development and function of the neuromuscular system by affecting the cholinergic neuromuscular transmission (Candura *et al.,* 1997). In addition, Shi *et al*., (2008) suggested that the rate of proliferation and apoptosis that occurs in the tail area will determine the fundamental barrier between normal development and susceptibility toward toxicant exposure. Exposure to concentrations below 30 nM at 5 hpf did not produce significant alterations in the larvae spontaneous swimming activity whereas exposure to 100 nM caused a decrease in swimming activity (Figure 2). In contrast, the embryo treated with 5 µg/l methylmercury exposed at 24 hpf did not cause any significant behavioural effects in the larvae (Samson and Shenker, 2001). This is interesting because it is proved by a molecular study which revealed that organic methylmercury chloride is more toxic than inorganic $HgCl_2$ (Debes *et al*., 2006; McElwee *et al.*, 2013). However, reduction in swimming activity could be due to distruption of sensitivity in the larval lateral line system that contains mechanosensory receptors along the body surface (neuromasts) which can detect water motion as well as exhibit other types of behaviours. In fact, this ability could associate with swimming motor neuron activity in larval zebrafish (*Danio rerio)* since inhibition of a single neuromast (receptor) could deficit a swimming response (Haehnel-Taguchi *et al.*, 2014). Yet, further research should be done in order to validate whether the deficit behavioural changes displayed in zebrafish in this study

actually represent in motor activity or are secondary to other functional impairment such as cognitive, sensory, or activity changes.

Conclusion

This study emphasizes the idea that embryonic exposure to $HgCl_2$ affected the normal behaviour at sublethal concentrations as compared to the control even when no morphological abnormalities observed. This finding revealed that behavioural parameter is a profound parameter that can be used as the endpoint for DNT testing.

Acknowledgements

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The comparative study on the collagen and gelatin derived from earthworms *Eudrilus euginiae* and *Lumbricus rubellus*

Sujithra Devi, A.[1], *Nor Azwady, A. A.[1], Muskhazli, M.[1], Shuhaimi, M.[2] and Teng, S. K.[1]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Halal Product Research Institute, Universiti Putra Malaysia, Putra Infoport, Universiti Putra Malaysia, 43400Serdang Selangor, Malaysia*
**Corresponding author. email address: azwady@upm.edu.my*

Introduction

The rising interest in the economical value of industrial by-products is the main reasons why exploring different species of collagen and gelatin has attracted the attention of researchers over time. The abundant sources of gelatin comes from the pig skin, bovine hide and, pork and cattle bones, however, the industrial use of collagen or gelatin obtained from non-mammalian species is growing in importance (Gomez *et al*., 2011). The classical food, photographic, cosmetic and pharmaceutical application of gelatin is based mainly on its gel-forming properties. Recently, in the food and medicinal industry, an increasing number of new applications have been found for gelatin such as emulsifiers, foaming agents, colloid stabilizers, biodegradable film-forming materials and micro-encapsulating agents, in par with the growing trend to replace synthetic agents with natural ones. Gelatin is a soluble protein compound obtained by partial hydrolysis of collagen, the main fibrous protein constituent in bones, cartilages and skins; therefore, type of collagen is an intrinsic factor influencing the properties of the gelatins (Johnston-Banks, 1990). Although up to 27 different types of collagen have been identified, type I collagen is the most widely occurring collagen in connective tissue. Gelatin quality for a particular application depends largely on its rheological properties (Stainsby, 1987). Apart from basic physico-chemical properties, such as composition parameters, solubility, transparency, colour, odour and taste, the main attributes that best define the overall commercial quality of gelatin are gel strength and thermal stability (gelling and melting temperatures). Physical properties of gelatin influence its quality and potential application as they are related to gelatin structure (Yang and Wang, 2009).

It was found that earthworm body contains 60-70 % proteins (high content of hydroxyproline, glycine, and alanine) and X-ray diffraction pattern showed the presence of collagen in the cuticle and epidermis of earthworms which gives an indication that gelatin 'should be' available in earthworms as well. In this study, *Eudrilus euginiae* was used as a comparative species as most of the current research was focusing on *Lumbricus rubellus* and furthermore *Eudrilus euginiae* are commonly cultured locally and has an advantage of size. This study aims to compare the properties of gelatine obtained from earthworms for the commercial use of industries.

Materials and Methods

Two species of earthworm, *Eudrilus euginiae* (African Nightcrawler) and *Lumbricus rubellus* was cultured in-situ in small scale for 12 weeks. The feeding diet of earthworms was a mixture of Empty Fruit Bunch(EFB), tofu waste and spinach.The optimum condition such as temperature (~26^0C) and moisture (70-80%) was maintained at all times. Each treatment was prepared with five replicates. Each container measured 16 cm in diameter and 16cm in height. Ten worms of almost the same length and weight were inoculated into each replicates. The incubation period of substrate prepared was kept for 10 days before introducing worms. Gelatine was extracted from earthworm collagen extracted from acid-alkaline method using the standard Protein Method protocols (International patent PCT/-S01/00275) of acid swelling steps. The parameters measured were the protein content, viscosity, thermostability and Bloom strength value.

Results

From the results obtained, it can be seen that there is no significant difference between the gelatine obtained from *Eudrilus euginiae* and *Lumbricus rubellus*.

Table 1: Comparison of gelatine properties obtained from *Eudrilus euginiae* and *Lumbricus rubellus.*

Properties	***Eudrilus euginiae***	***Lumbricus rubellus***
Bloom strength	101.25 ± 1.84^a	90.6 ± 1.72^a
Protein content (%)	47.63 ± 1.22^a	45.66 ± 0.98^a
Viscosity (Cp)	3.03 ± 0.13^a	2.99 ± 0.56^a
Thermostability	moderate	moderate

(Same alphabets indicate no significant differences)

The protein content obtained from the gel extracted was found to be high in both species with the percentage of 47.63 ± 1.22% in *Eudrilus euginiae* and 45.66 ± 0.98% in *Lumbricus rubellus*.

Discussion

The gel obtained was very soft with low Bloom strength value and low viscosity property. This may be due to the fact of using high extraction temperature which may cause reduced ability of alpha chains to anneal correctly during the formation of gel. Although there was no significant difference in the Bloom strength value, the gel obtained from *Eudrilus euginiae* was found to be in stronger category gel compared to *Lumbricus rubellus*. However, the values obtained were not comparable to the common sources of gelatine from mammals such as bovine and porcine which are commonly used which range from 200 to 300 (Raja *et al*., 2011). The low strength of gelatine was found to be a good source for cosmeceutical use particularly in skin creams as it can be easily absorbed.

The high protein content found in gelatine accounts for much beneficial gelatine being produced by earthworms as earthworm protein are known for many good bioactive compounds such as enzymes which may aid the medicinal, pharmaceutical, food and agriculture industries.

The gel obtained from both earthworm species were also identified to be in the range of low viscosity range. Such gels are found to be with low cohesive force and low deformation ability, thus having high moisture content which is an important character for the use in cosmetic creams as it is water based with high absorption rate (Lee *et al*., 2004). Low gelling temperatures (shown as moderate thermostability in Table 1) of obtaining earthworm gelatine (8-10^{0}C) also offers new potential applications as biomaterials. Gelatines with low melting points could also be used in dry products (micro-encapsulation), as one of the major applications of gelatin is in the microencapsulation of vitamins and pharmaceutical additives.

Conclusion

Although the properties found in the gel obtained from earthworms were not significantly comparable to the commercially obtained gelatine. The present study suggested that the earthworm gelatine may be of great use in the medicinal and pharmaceutical industry with its highly beneficial enzyme and its properties being a good source of biomaterials. However, further studies have to be done to determine the properties of gelatine chemically and its expanded usage not only in medicinal field but also in the much demanding industries such as in biotechnology industry and agriculture.

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Ethanol induces alteration in swimming activity in zebrafish larvae

Nurul Farhana Ramlan, Siti Norhidayah Hassan and *Wan Norhamidah Wan Ibrahim

Department of Biology, Faculty Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

**Corresponding author.Tel.:+603 89466625; email address: wnwi@upm.edu.my*

Introduction

By definition, neurotoxicity is "any adverse effect on the chemistry, structure or function of the nervous system, during development or at maturity, induced by chemical or physical influences (LG, 1998). The developing central nervous system is often more vulnerable to injury than the adult one. Exposure to ethanol during pregnancy can cause birth defects and neurodevelopmental disorders in mammals, including human. Zebrafish has been chosen as model organism due to the transparency of the embryos and larvae allows detailed non-invasive observation of organ-system development.

Materials and Methods

Fish husbandry

Adult male and female of zebrafish was rear in an aquarium with 30L of dechlorinated aged tap water at temperature 26^0c-30^0c and 14 h light : 10 h dark controlled photoperiod. The fish were fed three times a day with brine shrimp (*Artemia salina*, San Francisco Bay Brand, San Francisco,CA) and commercial dry flake food (Sera Vipan). The fish were maintained healthy and free from any sign of disease.

Breeding and egg collection

The breeding tanks was prepared with a spawning tray, consisting a fine net with a small hole equip with a fake grass in order to provide natural environment for zebrafish to breed. In order to enhance the rate of spawning, 200 ml of breeding solution was added into the tank. The breeding tank was left overnight inside the main tank and the egg was collected on the next day. Pasteur pipette was used to collect the eggs and rinse it with distilled water before transferred it into petri dish containing embryo media.

Ethanol exposure

Each petri dish containing 30 fertilized eggs and was exposed to several concentration of ethanol (0.25%, 0.50%, 0.75%, 1.50% and 2.00%) at 5 hpf. The duration of the exposure is until hatching with daily renewal of the medium. The embryo was observed daily for the neurotoxic effect of ethanol such as mortality rate and tail coiling/min at 6 dpf, swimming behavior was tested.

Behavioral analysis

Spontaneous tail coiling were evaluated in embryos aged 24 to 26 hpf in duration one minute period. At 6 dpf, swimming behavior was tested by average number of line crossed in one minute period.

Results and Discussion

Figure 1 shows the result of spontaneous tail coiling of larvae zebrafish for one minute period. Tail coiling is the first motor behavior consists of spontaneous movement shown by embryos during 17 hours post fertilization (Saint Aman and Drapeau,1998). This spontaneous movement is independent of sensory stimulation and is driven by activity in the spinal cord. Exposure to ethanol had showed significantly decreased the spontaneous tail coiling as the concentration increase compare to control. Though embryos first react to touch at 21 hpf, it is suggested that use of embryos at 26 hpf or later makes it easier to distinguish the touch response since much of the spontaneous coiling has stopped (McKeown *et al*., 2009)

Figure 2 showed the behavioral test assessed on zebrafish larvae as they reached 6 days post fertilization (dpf) after being exposed to different concentrations of ethanol. Swimming behavior was observed on 6 dpf because the larvae are already becoming mature swimmers with functioning sensory and motor systems allowing studies of locomotor, escape, goal-oriented and optomotor responses (Drapeau *et al*., 2002). Exposure to 0.75% ethanol showed significantly increase swimming activity in the zebrafish larvae. But exposure to high dose of ethanol concentration which is 1.50% showed decreasing in swimming activity behavior. Alcohol exposure affects zebrafish locomotion which is at low concentrations, fish tend to swim faster and as the dose increases, swimming typically slows. This result was similarly as previous study done by Nicole (2010) with the solid evidence that larval expose to ethanol at lower dose (0.50%) induced swimming activity which is also known as hyperactivity. In contrast, exposure of larvae to higher concentration of ethanol will induce hypoactivity, which is referring to the act of decreasing the swimming activity of larvae. Ethanol has been shown to decrease inhibitory behavior in zebrafish.

Conclusion

Exposure to several concentration of ethanol induced significant behavioral changes on zebrafish larvaein term of tail coiling and swimming behavior. Ethanol also induced hyperactivity and hypoactivity in larvae at concentration of 0.75% and 1.50% respectively.

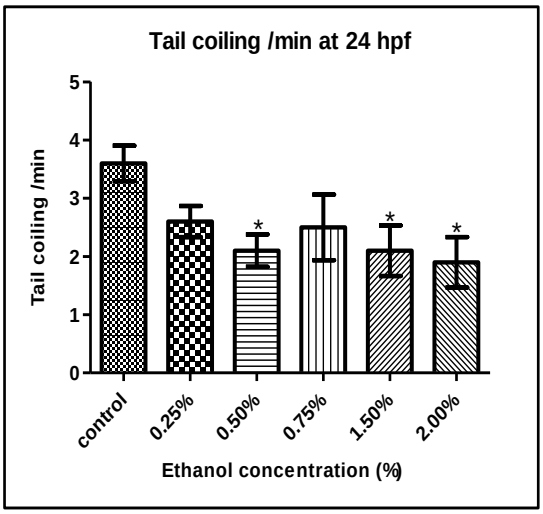

Figure 1: Effect of ethanol on tail coiling of larvae zebrafish exposed to different concentration of ethanol.

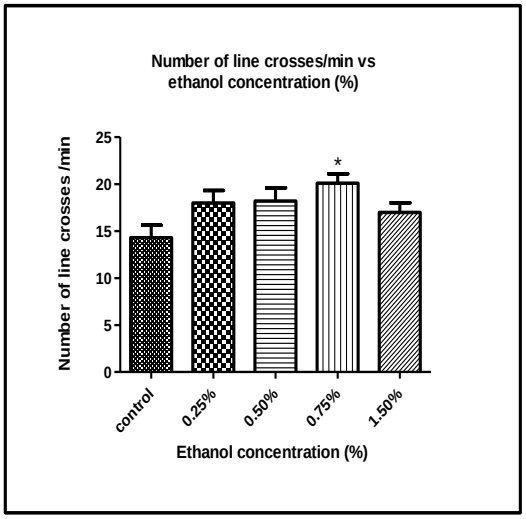

Figure 2: Effect of different ethanol concentrations on average number of line crosses by zebrafish larvae per minute.

Acknowledgements

I would like to take this opportunity to express my profound gratitude and deep regards to my supervisor Dr Wan Norhamidah Binti Wan Ibrahim for his exemplary guidance, monitoring and constant encouragement throughout the course of this writing. I also take this opportunity to thank Siti Norhidayah Binti Hassan for giving me a permission to present her work.

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Mycoremediation of phenanthrene by a fungal isolate from coastal sediment

Safiya Yakubu[1], Nor Azwady Abd. Aziz[1], Mohamad Pauzi Zakaria[2], Noormashela Ulul Azmi[1], Nurul Shaziera Abdul Ghafar[1], Cheong Jee Yin[1], Azleen Ahmad Adli[1] and *Muskhazli Mustafa[1]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Faculty of Environmental Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
** Corresponding author. email address: muskhazli@upm.edu.my*

Introduction

The rapid rise in industries over the lpast decades has led to the indescriminate disposal of persistent organic contaminants into our environment. Polycyclic aromatic hydrocarbons (PAHs) as organic pollutants have prompted concerns among researchers due to their harmful effect on the ecosystem, having the ability to be toxic, mutagenic and carcinogenic therefore are targets for statutory monitoring and control in most environmental samples(Matsubara *et al*., 2006). Response approaches to the elimination of these compounds has been through the utilization of physico-chemical techniques many of which serve for transfer and containment without actual elimination or detoxification of these aromatic compounds (Semple, 2007). Mycoremediation through the use of fungi species may have a significant advantage via extracellular enzyme production with low substrate specificities coupled with the ability to penetrate recalcitrant substrates due to hyphal growth and extention (Casas *et al*., 2009). This study aims to isolate, screen and evaluate the degradative potentials of fungi species from the environment (soils and sediments) for PAHs biodegradation using phenanthrene as a model substrate.

Materials and Methods

Soil and sediment samples were collected within Banting, Selangor Malaysia. Soil serial dilution technique on streptomycin supplemented Potato Dectrose Agar (PDA) was utilized for fungi isolation. Screening of fungi species was according to Argumedo-Delira *et al*. (2012). Screening for potent isolates in liquid medium was done using Mineral Salt Broth (MSB) supplemented with Trace Element Solution (TES) (Hadibarata *et al*., 2007; Arora and Gill, 2001). Extracts obtained from fungal culture by liquid-to-liquid extraction was analysed for quantitative phenanthrene degradation by GC-FID (Bhattacharya *et al*., 2012; Hadibarata *et al*., 2007). Best isolate was identified both morphologically and molecularly. Qualitative phenanthrene degradation by the best isolate (*Trichoderma* sp.) after screening was determined both in short-term (day 1 to day 10) and long-term (day 15, 20 and 30) incubations and metabolites were detected and identifed by GC-MS by comparism with a mass spectra data base (NIST, 08).

Results

Forty four fungal isolates designated SY1-SY44 were successfully obtained. Nine isolates were found to be tolerant to 250mg.l^{-1} phenanthene, exhibiting growth percentages above 50% relative to their controls (Table 1). Isolate SY1 and SY11 exibited a better tolerance to phenanthrene with percentage growth efficiencies of 76.9% and 85.3% respectively and showing less significant difference between control and treated samples.

Table 1: Mean differences of isolates at 72hrs for control and treated samples with growth percentages >50%.

	% Growth at 72hrs		
Isolate	**Control †**	**Treated ‡**	**%Growth***
SY1	**80.0 ± 0^{f}**	**61.5 ± 3.8^{e}**	**76.9**
SY2	80.0 ± 0^{f}	54.8 ± 4.6$^{c, d}$	68.5
SY3	80.0 ± 0^{f}	57.0 ± 1.8^{d}	71.3
SY4	80.0 ± 0^{f}	57.5 ± 2.2^{d}	71.9
SY5	80.0 ± 0^{f}	52.3 ± 4.5^{c}	65.4
SY6	80.0 ± 0^{f}	57.0 ± 1.8^{d}	71.3
SY7	80.0 ± 0^{f}	55.8 ± 0.3^{d}	69.8
SY8	80.0 ± 0^{f}	57.1 ± 0.9^{d}	71.4
SY11	**23.2 ± 0.3^{b}**	**19.8 ± 0.8l^{a}**	**85.3**

Superscript ($^{a-f}$): Combined variations in means for control and treatment samples at p<0.05

Phenanthrene biodegradation in liquid MSB showed that the isolate SY1 utilized 60.4% of the initial phenanthrene added within 10 days of incubation, with isolate SY11 achieving only 49.8% phenanthrene degradation and showing significant difference (P<0.05) within same incubation period (Figure 1).

Evaluation into the phenanthrene biotransformation capabilities SY1 showed that this fungal strain could degrade phenanthrene to metabolites as shown below in Table 2.

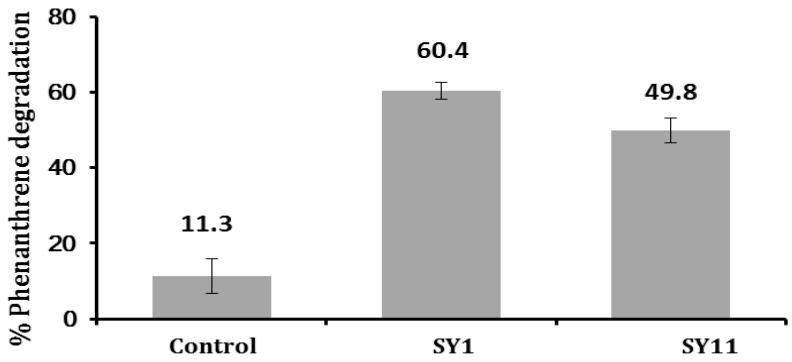

Figure 1: Phenanthrene degradation by isolates SY1 and SY11 after 10days incubation in MSB. Data points represent mean ± SD, p<0.05.

Table 2: Metabolites from fungal phenanthrene degradation.

Class of compound	**Degradation product**
Phenol	9-Phenanthrol
Hydrated phenanthrene	9,10-dihydrophenanthrene
	1,2,3,4-tetrahydrophenanthrene
Dihydrodiol	9,10-dihyro-9,10-dihydroxtphenanthrne
Ring-cleavage product	Phthalates

Discussion

The potent isolate, SY1 was identified to belong to the genus *Trichoderma* and its efficiency in tolerating and quantitatively reducing phenanthrene can be attributed to the environment from which it was isolated alongside its fasts growth habit. Biotransformation of phenanthrene by this isolate to other metabolites (Table 2) suggests a ligninolytic and non-ligninolytic fungal PAH degradation mechanism.

Conclusion

Evidence provided in this study shows a successful isolation of a sediment fungus *Trichoderma* sp., capable of tolerating, reducing and bio-converting phenanthrene and thus serves as a potential isolate that could be utilized in the production of bio-products for possible application in PAHs remediation purposes.

Acknowledgements

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Alpha-tocopherol reduce endosulfan-induced toxicity of goats semen

Baharin, A., Ahyar, A. I., Adanan, N. F., Aminudin, M. T. and *Shikh Maidin, M.

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

**Corresponding author.Tel.:+603 89466767; email address: mashitah@upm.edu.my*

Introduction

Endosulfan is introduced in 1954 as one of the first registered organochlorine insecticide's product at United States and it used widely on crops plantation. It hve been reported the exposure of endosulfan could harm animal and human, where later it would bioaccumulated in the body. One of the common organ where it bioaccumulate is the reproductive organ and for male is testes.

In male reproduction, at molecular level of cells, this organochlorine insecticide have ability to produced reactive oxygen species (ROS) that could generates oxygen free radicals which then bind itself to polyunsaturated lipid membrane of cells/sperms thus leading to production of lipid peroxidation. With respects to sperm production, an increase in lipid peroxidation would reduce sperm motility and progressive score or sperms as well as increases the number of sperm abnormalities (Khan and Sinha, 1996). However, semen quality could improve by nourished with antioxidant supplementation (Shikh Maidin *et al.*, 2014). Antioxidants such as, α-tocopherol functions as a chain-breaking chemical that prevents the propagation of free radical reactions and therefore reduces the amount of harmful free radicals. For that reason, we observed the effect of α-tocopherol against endosulfan on sperm parameters; motility, progressive score and abnormalities of *Capra hircus* (*C. hircus*) *in vitro*. Consequently, the motility and progressive score of spermatozoa in *C. hircus* will be increased and sperm abnormalities will be reduced after adding α-tocopherol into endosulfan treatment.

Materials and Methods

From ten mature goats (*C. hircus*), only two were continuously used for semen collection. The live body weight range of these two bucks is from 21.50 ± 0.72 kg to 28.90 ± 0.72 kg. The goat's semen was collected using prepared artificial vagina (AV) and collection was done in three consecutive weeks. All semen that has been collected were measured the motility and semen with motility more than 80% were pooled and divided into five different treatment groups: Control, T1 (50 nmol/mL of endosulfan), T2 (100 nmol/mL of endosulfan), T3 (50 nmol/mL of endosulfan and 1000 µmol/L of α-tocopherol) and T4 (100 nmol/mL of endosulfan and 1000 µmol/L of α-tocopherol). The pooled semen was diluted with TCAYE extender in ratio of 1:9. In this study, sperm motility and progressive score were observed and the sperm viability was determined through smear staining method. All the semen parameters was analysed using statistic software SPSS for Windows version 22.0; Linear Mixed Model and the mean's value of significance is $p \leq 0.05$.

Results and Discussion

The mean of sperm motility and progressive score in T3 and T4 that had been supplemented with vitamin E was significantly higher ($p \leq 0.05$) than the other treatment groups (Table 1). This showed that vitamin E reversed the effect of endosulfan on the sperm's parameters. Endosulfan induced the production of ROS and resulted in imbalanced concentration of ROS and antioxidant in the sperms. Free radicals of ROS bind to the polyunsaturated lipid membrane, leading to production of lipid peroxidation. This leads to cell apoptosis or necrosis that directly impact motility and progressive score of sperms.

Table 1: Mean ± SE of percentage of sperm motility, progressive score and percentage of sperm abnormalities score between Treatments and Time (h).

		Time (h)		
	Group	**0**	**1**	**2**
Motility,%	Control	85.75 ± 1.11[a]	80.00 ± 2.20[a]	72.50 ± 4.11[b]
	T1	86.75 ± 0.75[a]	79.25 ± 3.07[a]	65.00 ± 1.22[c]
	T2	85.00 ± 1.47[a]	78.25 ± 3.15[a]	61.75 ± 1.03[c]
	T3	83.72 ± 2.52[a]	83.05 ± 1.86[a]	76.05 ± 1.45[a,b]
	T4	85.39 ± 2.19[a]	80.72 ± 2.89[a]	76.05 ± 1.45[a,b]
Progressive score	Control	0.54 ± 0.00[b]	0.46 ± 0.04[b,c]	0.41 ± 0.04[b,c]
	T1	0.57 ± 0.01[a]	0.48 ± 0.04[b,c]	0.35 ± 0.03[c,d]
	T2	0.55 ± 0.01[a,b]	0.47 ± 0.04[b,c]	0.37 ± 0.05[c,d]
	T3	0.55 ± 0.02[a,b]	0.54 ± 0.01[b]	0.50 ± 0.03[b]
	T4	0.53 ± 0.03[b]	0.55 ± 0.01[b]	0.52 ± 0.01[b]
Abnormalities %	Control	0.63 ± 0.21[a]	0.59 ± 0.12[a]	1.07 ± 0.13[a,b]
	T1	0.82 ± 0.12[a]	1.26 ± 0.28[a,b]	1.10 ± 0.27[a,b]
	T2	0.83 ± 0.32[a]	1.37 ± 0.20[b]	1.17 ± 0.12[a,b]
	T3	0.88 ± 0.12[a]	0.93 ± 0.15[a,b]	0.75 ± 0.17[a]
	T4	0.84 ± 0.11[a]	1.02 ± 0.32[a,b]	1.06 ± 0.31[a,b]

Note. Means sharing a letter in their superscript are not significantly different at the 0.05 according to a Duncan test.

Treatment 1 (50 nmol/ml of endosulfan)
Treatment 2 (100 nmol/ml of endosulfan)
Treatment 3 (50 nmol/ml of endosulfan and 1000 µmol/L of α-tocopherol)
Treatment 4 (100 nmol/ml of endosulfan and 1000 µmol/L of α-tocopherol

Kumar *et al*. (1995) proved that degeneration of axoneme due to endosulfan binding caused slow progression and non motile sperms. Abnormalities such as crooked or coiled-like structure and detached-tail were caused by depolymerisation of microtubules in sperms by endosulfan. A reduction in sperm abnormalities was shown after 2 hours of observation caused by administration of vitamin E into endosulfan treatment in T3 and T4 while T1 and T2 shown opposed result. Vitamin E binds to free radicals from ROS forming α-tocopheroxyl radicals which is inert thus prevented lipid peroxidation from occurring. The results of this study were closely

related to Takhshid *et al*. (2012) where vitamins E and C reduced the production of lipid peroxidation thereby improved the motility and viability of sperms. We conclude that administration of vitamin E (α-tocopherol) enhances the sperm parameters; motility, progressive score and reduces sperm abnormalities by acting as counter-reactant to ROS produced by endosulfan.

Acknowledgements

We thank to Mr Razali Jonit (UM) for their assistance in this study. This project was financially supported by RUGS-UPM (9374600).

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Preliminary study on antinociceptove effect of aqueous extract of *Boesenbergia pandurata* in formalin-induced nociception test in mice

Ai Chin Ee[1], Ahmad Akira[1], Enoch Kumar Perimal[1], Daud Ahmad Israf[1], Lee Ming-Tatt[2] and *Mohd Roslan Sulaiman[1]

[1]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Faculty of Pharmaceutical Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia*
**Corresponding author.Tel.:+603 89472346; email address: mrs@upm.edu.my*

Pain is an unpleasant sensation associated with body state dysfunction that negatively affects the productivity of patients. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used as over-the-counter pain reliever medication due to its cost effectiveness. However, prolonged usage of NSAIDs usually accompanied with adverse side effects such as ulcer, nausea and even kidney failure. Hence, researchers are now focused on traditional herbal research to search for potential analgesic substances that are with minimal or no adverse effects. *Boesenbergia pandurata*, it is also known as *temu kunci* in Malaysia is a perennial herb that belongs to Zingiberaceae family. *Boesenbergia pandurata* is widely distributed in Southeast Asia and its rhizomes are commonly used as food ingredients or as traditional medicine to treat diseases conditions such as inflammation, cancer, and fungal infection. The aim of this study is to evaluate the inhibitory effect of aqueous extract of *Boesenbergia pandurata* (AEBP) on formalin-induced nociception test in mice. Mice were pre-treated with AEBP via intraperitoneal injection 30 min before challenged with intraplantar injection of formalin. It was demonstrated that intraperitoneal administration of AEBP at doses (0.3, 1, 3 and 10 mg/kg) produced significant antinociceptive response in both neurogenic and inflammatory phases of pain response induced by formalin. The findings indicated preliminary study on antinociceptive effect of AEBP, but further study should be conducted to explore the exact mechanism of pain inhibition by AEBP.

Keywords: *Boesenbergia pandurata*, antinociceptive, neurogenic, inflammatory pain

Activity of cardamonin on chemical model of nociception in mice

Chung Pui Ping, Lee Ming-Tatt, Muhammad Nadeem Akhtar, Enoch Kumar Perimal, Ahmad Akira, Daud Ahmad Israf and *Mohd Roslan Sulaiman

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, 43400 UPM Serdang, Malaysia.
**Corresponding author.Tel.:+603 89472346; email address: mrs@upm.edu.my*

Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are among the most widely used medication in reducing pain. Prolonged usage of these drugs leads to undesirable side effects such as gastrointestinal bleeding, respiratory depression and tolerance. Thus, there is a demand to search for new pharmacologically potent analgesic compounds with fewer or no adverse effects. Cardamonin is a naturally occurring chalcone, which are commonly found in plant kingdom. Previous reports showed that cardamonin has anti-inflammatory effects and inhibit generation of nitric oxide and prostaglandin E_2 via interruption of NF-κB pathway. In the present study, we evaluated the antinociceptive property of cardamonin using acetic acid-induced abdominal writhing test in mice. Cardamonin (0.3, 1, 3 and 10 mg/kg), vehicle (10 ml/kg) or indomethacin (10 mg/kg) was administered either intraperitoneally or orally, 30 minutes or 60 minutes respectively before injection of 0.8% acetic acid. The number of abdominal writhes was recorded for 30 minutes, starting from 5 minutes after acetic acid injection. Cardamonin showed significant reduction in abdominal writhes. These findings suggested that cardamonin exerted pronounced antinociceptive activity when assessed in chemical model of nociception in mice.

Keywords: Cardamonin, nociception, acetic acid

Kenaf Water Use Efficiency Under stress

*Elroda Abdelhalim Ibrahim[1], Ahmed Ainuddin Nuruddin[1,2], Hazandy Abdul Hamid[1,2] and Mohd Shahwahid Haji Othman[3]

[1]*Institute of Tropical Forestry and Forest Products, INTROP Universiti Putra Malaysia, UPM,*
[2]*Faculty of Forestry Universiti Putra Malaysia, UPM*
[3]*Faculty of Economics and Management Universiti Putra Malaysia, UPM*
43400 UPM Serdang, Selangor

**Corresponding author.Tel.:010285690; email address: rodahala@gmail.com*

Research on kenaf water use efficiency received more attention these days regarding to different wates supply problems in countries growing kenaf (*Hibiscus cannabinus* L.) traditionally for fiber production as a source of raw material for rope, canvas and sacking and recently as a multi-purpose crop for energy and paper pulp production. Some recent studies used Deficit irrigation (DI) methods of high crop water use efficiency (WUE) which can maintain high crop yields if it is properly used. Deficit irrigation is a kind of irrigation to maximizing WUE for higher yields per unit of irrigation water applied. This current study exposed kenaf to certain levels of water stress throughout growing season based on crop evapotranspiration (ETc) using Tensiometers for soil moisture measurement and Irrigation Scheduling. The expectation is that any yield reduction will be economically not significant compared with the benefits due to saving water, increase planted area and irrigates more crops around the year. Through this research we can explain the critical period of water deficit for kenaf life cycle and try to avoid it and re-arrange irrigation schedules. Kenaf has a great potential in terms of dry biomass production, achieving a maximum of 21–24 t ha as total biomass and 18–19 t ha as stems, under no water limitations (100% ETc restoration), also (50% ETc restoration) may be advantageous, since a 44% irrigation water saving, when compared to the fully irrigation treatment, against no significant yield reduction.

Keywords: Kenaf, water use efficiency, deficit irrigation (DI)

Macroscopic evaluation of wounds healing progress treated with collagen-calcium alginate film dressing with therapeutic ultrasound

*Khaled, M. A.[1], Jalila, A.[1], Kalthum, H.[2], Noordin, M.[1] and Asma. Saleh, W.[3]

[1]*Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan*
[3]*Faculty of Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru, 71800 Nilai, Negeri Sembilan, Malaysia*
**Corresponding authors. email address: kelhmri@yahoo.com*

Introduction

A wound is a physical bodily injury resulting in the disruption of the normal continuity of structures and wound healing is the restoration of the continuity. The treatment of wounds has improved considerably in the past 30 years, and will continue to progress rapidly with the advancing technology and a greater understanding of chronic wounds (Ballard and Baxter, 2000; Arul Jothi *et al.,* 2006). The objectives of any wound management are relief of pain and distress to the animal, functional and cosmetic repair, economic and time efficient procedures and prompt decision making in the event of signs of delayed healing (Cockbill, 2002; Khaled *et al.,* 2014). In chronic wounds, the major focus of wound healing has been on the relationship between tissue destruction by excess inflammation and tissue synthesis stimulated by a pro-healing environment. Natural polymers have been increasingly studied for applications in health care due to their biocompatibility, biodegradability, and nontoxity (Mali *et al.,* 2006). The present paper discusses the macroscopic evaluation wounds healing treated with collagen-calcium alginate film dressing with therapeutic ultrasound in a rat.

Materials and Methods

Twenty four healthy female Sprague-Dawley rats weighing between 300 to 350g were used in this study over a 20-day period. They were allocated randomly into 4 groups of 6 animals each. After the creation of 2cm x 2cm open wound, Group I was control treated with Gentamycin ointment. Groups II, II and IV were treated with Therapeutic ultrasound massage, collagen-calcium alginate film and Collagen-calcium alginate film with therapeutic ultrasound

Results and Discussion

On application, the Collagen-calcium alginate film with therapeutic ultrasound was well accepted by the animals without any adverse reaction. On bacteriological examination, *Staphylococcus aureus, Pseudomonas, Escherichia coli, Proteus* and *Klebsiella species* were isolated from all the groups. Mean percentage of epithelialistion, wound contraction and total healing were significantly better in Group IV ($P<0.05$) (Figures 1, 2 and 3).

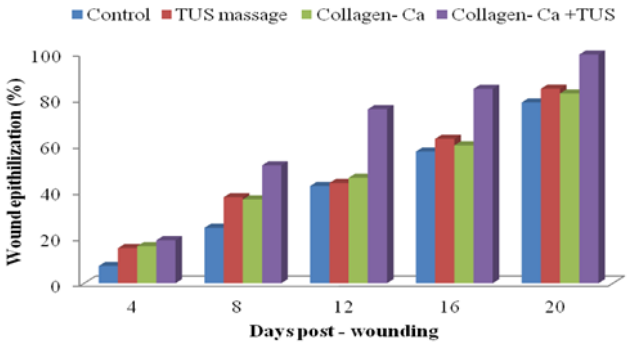

Figure 1: Percentage of epithelization of the wound.

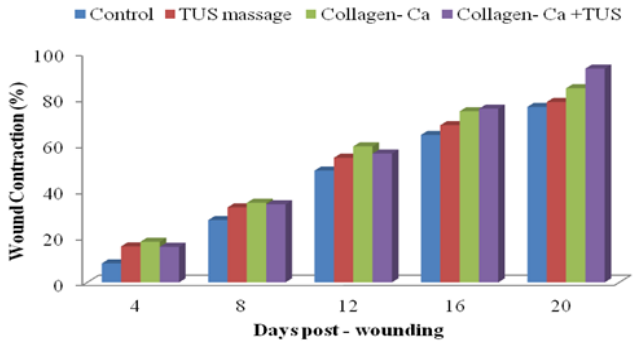

Figure 2: Percentage of contraction of the wound.

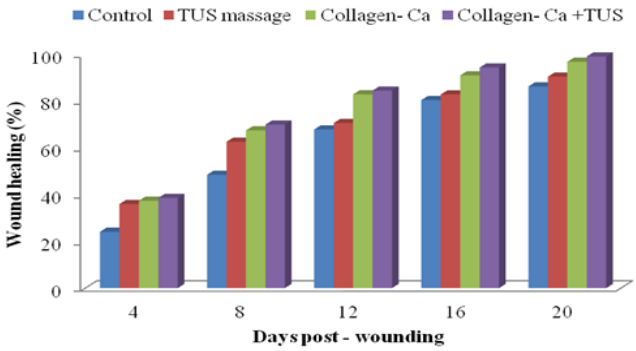

Figure 3: Percentage of total wound healing of the wound.

Conclusion

Collagen is a biocompatible protein that does not interfere with the body's normal immunologic response and can be used in non-healing chronic wounds, which require a trigger to stimulate the normal healing process. In extensive wounds when there is lack of autologous tissue, biomaterials like Collagen-Calcium alginate may be beneficial and can be used.

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Anti-inflammatory activity of *Jatropha curcas* extracts

Ahmad Razi Othman[1], *Norhani Abdullah[2,3], Syahida Ahmad[2] and Intan Safinar Ismail[1]

[1]*Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Biochemistry, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[3]*Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
**Corresponding author.Tel.:+603 89471147; email address: norhani@upm.edu.my*

The *Jatropha curcas* plant or locally known as “pokok jarak” has been widely used as remedies for various conditions including arthritis, gout, jaundice, wound and inflammation. In this study, the seed, leaves, stem and root of *J. curcas* plant were screened for anti-inflammatory (nitric oxide inhibition) and cytotoxic activities (MTT assay) by using RAW 264.7 murine macrophage cells. The highest anti-inflammatory activity was observed in the methanolic extract of root. However, root extract showed high inhibition towards RAW 264.7 cells growth due to it cytotoxicity. Further extraction procedure by using four solvents (hexane, chloroform, ethyl acetate and water) with different polarities was conducted on the root sample. The hexane partition showed high anti-inflammatory activity, at the same time high cytotoxicity towards RAW 264.7 cells at 1 mg/mL. Analysis of this extract by GCMS showed the presence of high levels of terpenes and diterpenes which are known to possess cytotoxic activity. Fractionation process of the hexane partition using column chromatography gave five spots, where two spots (H-4 and H-5) showed anti-inflammatory activity and low cytotoxicity. The two spots showed the presence of hexadecanoic acid and octadecanoic acid by GCMS analysis. This finding suggests that these two compounds are responsible for producing the anti-inflammatory activity of the *J. curcas* root.

Keywords: Plant extracts, RAW 264.7 cells, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, gas chromatography mass spectrophotometer analysis

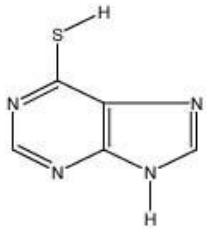
Cytotoxicity effect of 6-mercaptopurine and 6-mercaptopurine riboside on RAW 264.7 and HIG-82 cell lines

*Che Ku Dahlan Che Ku Daud[1], Muhammad Zulhilmi Zailani[1], Nor Erma Suryana Sukiman[1], Tham Chau Ling[1], Zuraini Ahmad[1], Yong Yoke Keong[2], Zetty Nadia Md Zain[3] and Muhammad Nazrul Hakim Abdullah[1,4]

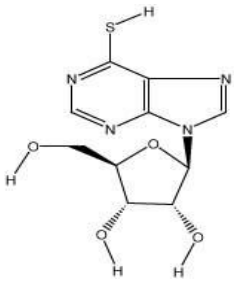
[1]*Department of Biomedical Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia*
[3]*Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia,*
[4]*Halal Products Research Institute, Universiti Putra Malaysia*
**Coresponding author.Tel.:+013 9533183; email address: dahlanckd@gmail.com*

Introduction

Purinethiol or 6-mercaptopurine (6-MP) (Figure 1) is a purine sulphur derivative approved as an antitumor drug by Food and Drug Administration (FDA) in 1953 (Pilar *et al.,* 1999). 6-MP was one of the first effective treatments for childhood leukaemia and dramatically improved the previously dismal prognosis of this disease then further developed as an effective anticancer and immunosuppressant drug (Elion, 1989). 80% of children's leukaemia diseases are treated with 6-MP concurrently with other anti-tumour drugs.



6-Mercaptopurine
FM: $C_5H_4N_4S.H_2O$



6-Mercaptopurine riboside
FM: $C_{10}H_{12}N_4O_4S$

Figure 1: Chemicals Structures of 6-Mercaptopurine (6-MP), 6-Mercaptopurine riboside (6-MPR).

Recently, 6-MP is the most commonly used as immunomodulatory drugs in the treatment of inflammatory bowel disease such as ulcerative colitis, Crohn's disease and arthritis, for more than 30 years since 1970's (Nielsen *et al.,* 2001). More recently, the combination of 6-MP and gold has showed cytotoxicity activity against cancer cells with a more potent cytotoxic action than free 6-MP alone and even than cisplatin (Cuin *et al*., 2011; Pilar *et al.,* 1999). The combination of chemotherapy is very useful to enhance the efficacy of treatment to all most cancers such as liver, breast and lungs (Kaur *et al.,* 2013). Besides the anti-tumoral activity, this complex also showed an excellent MIC value against *M. Tuberculosis* (Pilar *et al.,* 1999). In this study, the effect of 6-Mercaptopurine and its derivative (6-Mercaptopurine riboside) on murine macrophages RAW 264.7 cell and rabbit fibroblast-like

synoviocytes (HIG-82) cell line were investigated. The findings perhaps might be useful as a preliminary reference in developing a new rheumatoid arthritis drugs.

Materials and Methods

Chemical and Plating of cells

6-mercaptopurine and 6-mercaptopurine (Acros Organic BVBA, Belgium) and diclofenac (Sigma-Aldrich, USA) were serially dissolved in 0.2% dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) at 0.05 M stock solution. A serial dilution with Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St Loius, USA) and nutrient mixture Hams F12 growth medium (Sigma Chemicals, St. Louis, MO, USA) to give a final concentration of 200 µM. Macrophage Abelson murine leukaemia virus transformed; RAW 264.7 cell line derived from mouse and synoviocytes fibroblast HIG-82 cell line (ATCC CRL-1832) derived from rabbit were purchased from American Type Culture Collection (ATCC Rockville, MD USA). Both cell lines were cultured in following growth medium as recommended by ATCC. All media were supplemented with 10 % fetal bovine serum (Biowest, South America) and flasks with 90 to 100% confluence are harvested, detached from the flask surface by trypsinization. The concentration of cells was done by Trypan blue (Sigma Chemicals, USA) exclusion. 1 $x10^6$ cells/mL and 1 x 10^4 cells/mL for both respectively cells were pipette into each well of the 96-well microtiter plate, incubated 3 hours for RAW 264.7 cell and 24 hours for HIG-82 cells at 37 °C with 95 % O_2 / 5% CO_2 prior to treatment.

Dosing and 3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

Methods of Pozzolini *et al.* (2003) were employed with slight modifications. Approximately after incubation hours completed, serial dilution with final concentration of 100, 50, 25, 12.5, 6.25 and 3.125 µM of compounds were added to the working wells. The plates were then further incubated for 24 hours. Cell viability was determined using common MTT assay.

Statistical analysis

Data are expressed as mean ± SEM of duplicates separate experiments using GraphPad Prism 5 software. Statistical significance was defined at $p \leq 0.05$ using analysis of student's T-test. Significant treatment means were further subjected to Bonferroni posttests.

Results and Discussion

Table 1 shows the percentage viability of two cells used in this study assayed by MTT assay for 24 hours incubation with 6-MP doses. After 24 hours incubation, RAW 264.7 cell demonstrated a doses dependent reduction in cell viability. However, but not doses dependent reduction on HIG-82 cell line. The viability of RAW 264.7 cells at 50 and 100 µM were 100.73 and 93.90%, respectively. Eventually, 6-MP compound was more potent to HIG-82 cells where viability calculated at 90.45 and 88.53% at the same concentration doses respectively.

Table 1: Viability of synoviocytes, HIG-82 cell line treated with various concentration of 6-mercaptopurine after 24 hours treatment.

6-mercaptopurine (µM)	Percentage viability of cells	
	RAW 264.7	HIG-82
Normal	154.65±52.72 [a]*	127.87±29.34[b#]
3.124	159.85±65.82 [a]*	92.79±7.02[b#]
6.25	158.25±61.40[a]*	99.20±6.77[b#]
12.5	149.42±55.27[a]*	94.41±9.24[b#]
25	127.39±46.38[a]*	90.62±10.00[b#]
50	105.73±37.15[a]*	90.45±10.57[b#]
100	93.90±34.16[a]*	88.53±7.13[b#]

Note: n=4 (RAW 264.7 cell)/group and n=3 (HIG-82 cell)/group for duplicates separate experiments. [a-b] Mean with different superscripts significantly no different ($p < 0.05$) in same column; [*#] means with different superscripts significantly different ($p < 0.05$) in same row.

Table 2 demonstrated the percentage of viability of the cells treated with 6-MPR after 24 hours incubation. The results showed that, 6-MPR incubation induced a dose dependent reduction in cell viability for RAW 264.7 cells similar trend to 6-MP but slightly dose-dependent reduction effect on HIG-82 cell. Similar to 6-MP, RAW 264.7 cells were less sensitive to 6-MPR when compared to HIG-82 cells. Viability of RAW 264.7 cell were at 145.70 and 140.96 % at the 2 highest concentrations of 6-MPR. However, HIG-82 cells were statistically more susceptible to 6-MPR compared to RAW 264.7 cells. HIG-82 cells showed better viability at 91.19 and 92.31% at the same doses 50 and 100 µM of 6-MPR respectively.

Table 2: Viability of synoviocytes, HIG-82 cell line treated with various concentration of 6-mercaptopurine riboside after 24 hours treatment.

6-mercaptopurine riboside (µM)	Percentage viability of cells	
	RAW 264.7	HIG-82
Normal	154.65±52.72[a]*	127.87±29.34[b#]
3.124	177.49±69.86[a]*	100.99±17.29[b#]
6.25	169.07±63.34[a]*	97.59±15.28[b#]
12.5	167.52±58.32[a]*	98.76±9.52[b#]
25	146.61±65.93[a]*	94.12±6.14[b#]
50	145.70±67.37[a]*	91.19±3.73[b#]
100	140.96±63.67[a]*	92.31±10.60[b#]

Note: n=4 (RAW 264.7 cell)/group and n=3 (HIG-82 cell)/group for duplicates separate experiments. [a-b] Mean with different superscripts significantly no different ($p < 0.05$) in same column; [*#] means with different superscripts significantly different ($p < 0.05$) in same row.

Table 3 shows the viability of the cells incubated with Diclofenac as a common standard NSAID used in treating rheumatoid arthritis disease. The drug was shown a dose-dependent reduction on both cells. However, the drug was more cytotoxic to HIG-82 cells than RAW 264.7 cells almost similar to 6-MP pattern. The viability of RAW 264.7 cells were at 101.38 and 91.84% at 50 and 100 µM, respectively. Meanwhile, the viability of HIG-82 cells were at 89.42 and 69.02 % at the same concentrations respectively.

Table 3: Viability of synoviocytes, HIG-82 cell line treated with various concentration of Diclofenac after 24 hours treatment.

Diclofenac (µM)	Percentage viability of cells	
	RAW 264.7	HIG-82
0	154.65±52.72[a]*	127.81±29.34[b#]
3.124	167.21±61.01[a]*	92.91±1.51[b#]
6.25	151.54±55.58[a]*	90.31±5.84[b#]
12.5	147.98±72.35[a]*	86.58±2.03[b#]
25	136.74±67.60[a]*	83.58±1.52[b#]
50	101.38±26.44[a]*	89.42±1.47[b#]
100	91.84±35.22[a]*	69.02±19.59[b#]

Note: n=4 (RAW 264.7 cell)/group and n=3 (HIG-82 cell)/group for duplicates separate experiments. [a-b] Mean with different superscripts significantly no different ($p < 0.05$) in same column; [*#] means with different superscripts significantly different ($p < 0.05$) in same row.

Figures 2 and 3, demonstrated statistically pattern for all the drug compounds used in this cytotoxicity study on both cells RAW 264.7 and HIG-82 cells respectively at 2 highest concentrations; 50 and 100 µM. The percentage of viability of RAW 264.7 cell was statistically higher than others at same concentrations of 6-MPR (Figure 2). However, the viability of HIG-82 cells at same concentrations was modestly lower than RAW 264.7 cells (Figure 3). 6-MP and Diclofenac showed the strong cytotoxicity effect for both cells but not for 6-MPR.

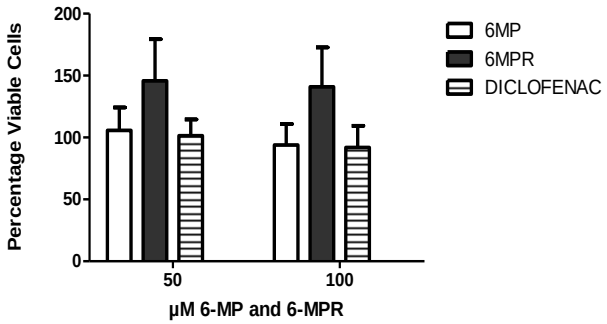


Figure 2: Percentage of cell viability on RAW 264.7 cell line after 24 hours incubated.

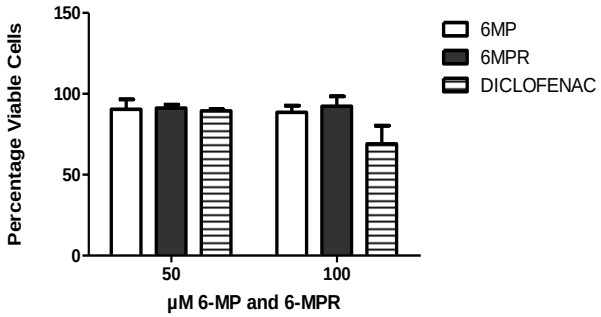


Figure 3: Percentage of cell viability on HIG-82 cell line after 24 hours incubated.

Interestingly, all the three compounds demonstrated the same capability to reduce the HIG-82 cells induced by a stimulant to create an inflammation state to the cells at the concentrations. HIG-82 cell was less resistant compared to RAW 264.7 cell for all drug used especially 6-MP (Figure 3). Meanwhile 6-MPR was the lowest potency compound compared to others on both cell types. There are no significant different among the 2 doses of same drugs and between same dose of differences drugs.

This present study demonstrated that the two purine compounds namely 6-mercaptopurine (6-MP) and 6-mercaptopurine riboside (6-MPR), with diclofenac, a common non-steroidal anti-rheumatic drug (NSAID) were used to investigate the cytotoxicity effects on RAW 264.7 and HIG-82 cell lines. All the compounds induced dose-dependent cytotoxicity effect on RAW 264.7 cell line after incubation 24 hours. However, the cytotoxicity effect on HIG-82 cell statistically induced low dose-dependent manner after 24 hours incubation. Interestingly, 6-MP showed almost the same potent effect like diclofenac effect, but stronger toxicity effect compared to 6-MPR. All three compounds were moderately effective towards HIG-82 cell line but low toxic to RAW 264.7 cell line after 24 hours treatment.

In this current study found that, 6-MP a common drug used in acute leukemia among children in USA and European region in chemotheraphy was showed the most potent cytotoxic agent again both cell RAW 264.7 and HIG-82 cell lines (a normal synoviocytes cell originated from rabbit knee) however 6-MPR showed the less potent cytotoxic agent. However, the long used of potent drug such as 6-MP might be induced the critical adverse drug reactions (ADR's) in human body, such as fever, rash, nausea, hypertension, hepatitis, pancreatitis, bacterial liver abscesses, cytomegalovirus infections, life-threatening myelosuppression, bone marrow suppression, gastrointestinal symptoms, hypersensitivity reactions and tumorigenicity (Gaya *et al.,* 1995). Regardings to those side effects, there are a few strategies in reducing the ADR's induced by 6-MP treatment.

A strategy to reduce the ADRs is using the glucocorticoids. Glucocorticoids are still the most effective remission-inducing medication but unfortunately for patients who fail to respond and for those who develop side-effects or require long-term glucocorticoid treatment expose to serious side-effects, so immunomodulatory drugs are important supplements or act as alternatives (Nielsen *et al.,* 2001). Immunomodulatory drug such as prodrug of 6-MP, azathioprine is able to minimize and reducing ADR's induced by 6-MP. However, endless, the toxic accumulation of 6-thioguanine in cells were activates the apoptosis or cell death programme further induce tissues and organs tumoregeneticity (Yatscoff and Aspeslet, 1998). The intracellular accumulation of 6-thioguanine nucleotides in infants is also believed to be responsible for the cytotoxic effects of these drugs through the inhibition of purine synthesis involving DNA synthesis and RNA replication to new infant tissues and organs (Lennard, 1992).

Another strategy besides using glucocorticoids and pro-drug replacement is using nano-encapsulated drug delivery system. Recently reported that, the cellular proliferation rates in the presence of the anti-cancer drugs delivered by the gold nanoparticle conjugated were found to be statistically lower than those of cells exposed to the cytostatic drugs alone, indicating that nano-participle delivery facilitated an increased susceptibility of cancer cells to drugs used plus ribavirin

(Tomuleasa *et al*., 2012). The nano-particle of the drug will be produced the better efficacy in lower dosage of the drug concentration to the target tissues, subsequently reduced the adverse drug reactions to the patients.

Conclusion

As a conclusion, results from this preliminary study demonstrated the potential use of 6-MP in treating rheumatoid arthritis disease on HIG-82 and RAW264.7 cell culture in-vitro model. The both drugs candidates are promising usefulness to be listed as new drugs compound to be used in treating the rheumatoid arthritis in the future with further studies need to be conducted with intent into the mechanism of action for reducing the adverse drug reactions to the patients.

Acknowledgements

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Effect of pomegranate on metabolic indexes and inflammatory biomarkers in STZ-NA induced diabetic rats

Seyedeh Zeinab Taheri Rouhi, *Asmah Rahmat and Fauziah Othman

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
**Corresponding author. email address: asmah@upm.edu.my*

Type 2 Diabetes is associated not only with hyperinsulinaemia and hyperglycemia but also with other disorders such as atherosclerosis, hypertension, inflammatory disorders and abnormal lipid profiles in dyslipidemia. Fruit extracts have been used universally in treatment of chronic diseases, because they are natural, safe, and readily accessible. Pomegranate (*Punica granatum Linn*) has been used, as pomegranate fractions have therapeutic potential stated by multitudinous scientists using various *in vitro* assay systems which is due to the presence of unique bioactive compounds and polyphenolic constituents. This study was performed to evaluate the pomegranate juice and seed consumption and their potential effect on treatment of diabetes and its associated complications such as abnormalities in plasma glucose, insulin, inflammatory factors, lipid profile level and pancreatic tissue in STZ-NA induced diabetic rats. Forty adult healthy male Sprague Dawley rats were dedicated in 5 groups of 8 rats each. Diabetes mellitus was induced in 4 groups by administration of streptozotocin-nicotinamide. Diabetic animals were further treated differently for 21 days, by using oral gavage by force-feed with needle. Rats in all groups were sacrificed on day 22. The results of the study suggests that the antioxidant flavonoids and Polyphenolic active constituents present in pomegranate are possibly responsible for hypolipidemic and anti-inflammatory effect in diabetic rats which can help the cure and management of diabetes. Although the biochemical mechanisms underlying Pomegranate seed and juice activities are not yet clear, our results demonstrated that *Punica granatum* has a suppressor effect versus lipid abnormalities and overexpression of inflammatory cytokines which are side effects of diabetes.

Keywords: Diabetes, pomegranate polyphenolic constituents, lipid abnormalities, inflammatory cytokines

Evaluation on the effectiveness of bacteriophage cocktail to control the growth of *Escherichia coli* on food contact surface during simulation study

*Nur ilida Mohamad, Mohd Effendi Mohamed Noor and Nur Baizura Sa'dom

Food Technology Research Centre, MARDI HQ, P.O Box 12301, 50774, Kuala Lumpur.
**Corresponding author.Tel.:+603 89437077; email address: ilida@mardi.gov.my*

Introduction

Food safety is a public health priority worldwide as millions of people fall ill every year due to consumption of unsafe food. In certain countries, rates of illnesses are increasing significantly every year. Most of the food-borne illness cases are resulting from the consumption of food, contaminated by the pathogenic microorganism.

Food contamination is a major concern in the food industry and may be considered as the occurrence of any objectionable matter in or on the food (Wan Zairi, 2006). Furthermore, contamination of buildings, equipments, food processing facilities and other facilities of strategic importance by pathogenic bacteria remains a serious problem. Decontamination of such facilities and surfaces also presents considerable challenges because of the increased resistance of many potentially pathogenic bacteria to traditional sanitizers (Tamar *et al*., 2008).

Escherichia coli has been known as one of the most common bacteria found in the intestinal tract of human and warm blooded animals. Their ability to survive outside the body for longer period of time makes them an ideal indicator organism to test food and environmental samples for fecal contamination. *E.coli* species are generally understand as harmless intestinal flora but they are somehow an opportunistic outside their normal habitant and some of the strains have been identified as the serious causal agents of various illness. Pathogenic *E.coli* strains cause several type of human diarrhea and their infection is a major cause of public health problems in developing countries (Ke Xin and Kwai Lin 2008). The health hazards associated with *E.coli* have become complicated by the fact that some of the causal agents have over the years, developed resistance against commonly used antibiotics.

Previous studies done by other researchers on the ability of bactericidal bacteriophages to control several human pathogens have shown promising results to an alternative way, which is believed as natural, nontoxic, safer and environmentally friendly. Bacteriophage is bacterial viruses which in its virulent state infects the bacterial cell, multiplies within it, eventually causing the cell to burst (lysis). Their activities ceases once the bacterial cell are killed. They do not attack bacteria indiscriminately; instead they usually attack a specific type and importantly, it does not infect humans or animals.

This study was conducted to evaluate the effectiveness of a cocktail containing several *Escherichia coli* bacteriophages in controlling the growth of *E.coli* strain on food contact surface (stainless steel) during simulation study.

Materials and Methods

A sterile 25 x 25 cm stainless steel plate surfaces were pretreated with agar as artificial contaminant and let to dry at room temperature for 20-30 minutes. After drying, the surfaces were contaminated with *E. coli* strain. The experiment was carried out using a high density *E. coli* cell culture (10^8 cfu/ml) and also a low density *E.coli* cell culture (10^4 cfu/ml) respectively. The cultures respectively, were introduced to a previously prepared 25 x 25 cm stainless steel plate surfaces and later, bacteriophage cocktail containing approximately 10^{10} pfu/ml cell count was applied to treat them. The control surfaces were not treated with bacteriophages. The experiment was also conducted using two different concentrations ratio of bacteria to bacteriophage as below: (i) Bacteria:Bacteriophage (1:2) (ii) Bacteria:Bacteriophage (1:4). The effectiveness of bacteriophage cocktail was evaluated by comparing the number of viable *E.coli* organisms (cfu/ml) recovered from the test and control surfaces at every hour intervals until 3 hours of bacteriophage treatment to the surfaces.

Results and Discussion

The results showed that for a low density *E. coli* cell culture (10^4 cfu/ml) with bacteria to bacteriophage ratio of 1:2, the *E. coli* count was totally reduce after 3 hours of bacteriophage treatment and same pattern was evaluated for 1:4 ratio (Figures 1 and 2). As for a high density *E. coli* cell culture (10^8 cfu/ml), the bacteria count was reduced from 10^8 cfu/ml to approximately 10^4 cfu/ml after 3 hours of bacteriophage treatment for both 1:2 and 1:3 ratio respectively (Figures 3 and 4).

Most of the studies regarding the effectiveness of bacteriophages to control the growth of pathogens have shown positive results in the application within the foods and only some has been cited for the application on food contact surfaces. Both Tamar *et al*. (2008) and Wan Zairi (2006), have shown the possibility of using bacteriophages to reduce contamination by *E.coli* on food contact surfaces and their finding is in line with this study's finding. Wan Zairi (2006) indicated that the use of 10% bacteriophage suspension appeared to be effective in reducing the *E.coli* number on stainless steel contact surface while Tamar *et al*. (2008) reported that treatment with two most concentrated bacteriophages preparations have reduced the *E. coli* organism on gypsum board surfaces.

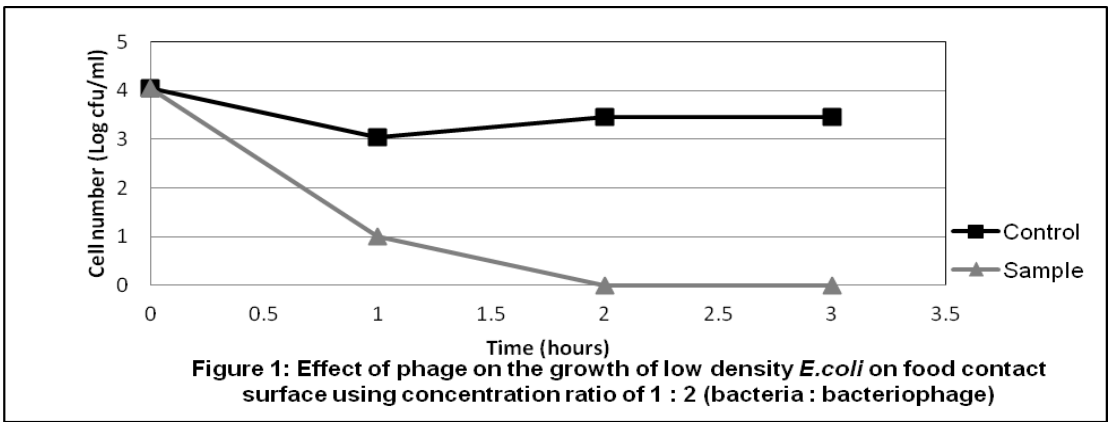

Figure 1: Effect of phage on the growth of low density *E.coli* on food contact surface using concentration ratio of 1 : 2 (bacteria : bacteriophage)

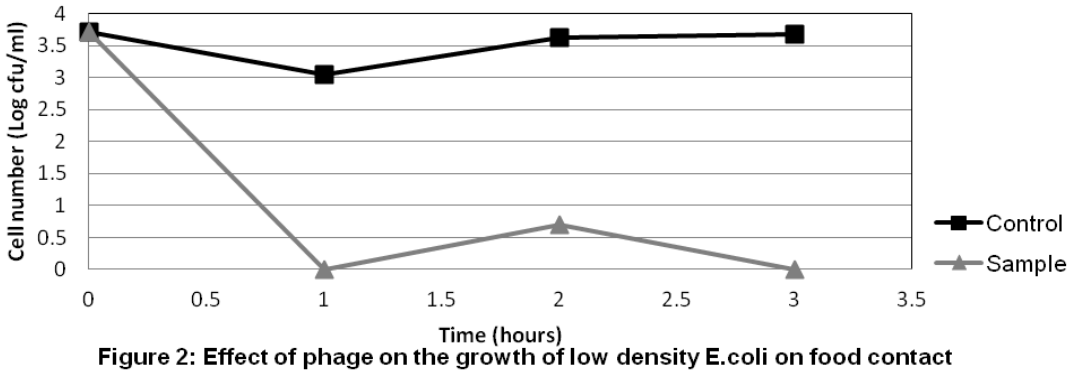

Figure 2: Effect of phage on the growth of low density E.coli on food contact surface using concentration ratio of 1 : 4 (bacteria : bacteriophage)

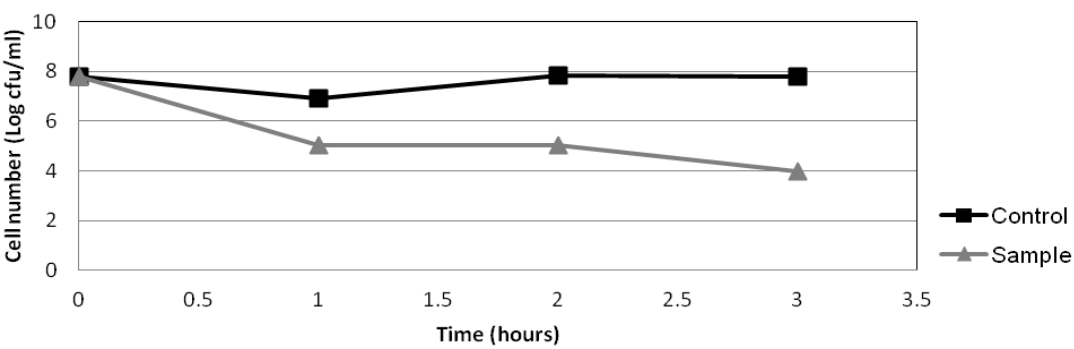

Figure 3: Effect of phage on the growth of high density *E.coli* on food contact surface using concentration ratio of 1 : 2 (bacteria : bacteriophage)

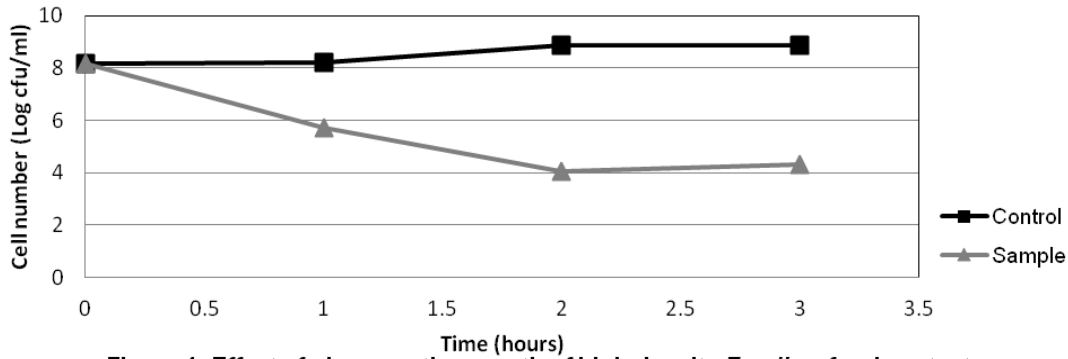

Figure 4: Effect of phage on the growth of high density *E.coli* on food contact surface using concentration ratio of 1 : 4 (bacteria : bacteriophage)

Conclusion

The results suggest that the bacteriophage coctail used in this study may have potential for reducing contamination of *E.coli* on food contact surface. These bacteriophages may be used in the future as biological agent for decontamination of food processing plants and facilities.

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Feed intake and kidding rate of goats under intensive and semi-intensive management systems in Peninsular Malaysia

Norhazirah Abdul Halim, Shohaimi, S., Nulit, R. and *Shikh Maidin, M.

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

** Corresponding author.Tel.:+603 89466767; email address: mashitah@upm.edu.my*

Reproductive efficiency of goat production in Malaysia is low, despite an increase in production of kidding rate from year to year. It has been reported that, feed intake most probably affect the reproductive performance of goat by redusing the kidding rate and this has commonly been shown in sheep. With this unease, therefore, we assess the effect of dietary intake and kidding rate of three common breeds in Peninsular Malaysia (Boer, Jamnapari and Katjang) in two different animal husbandries; semi-intensive and intensive management system. A survey on type of feed intakes and kidding rate of goats were conducted from intensive and semi-intensive management farms in every states of Peninsular Malaysia from January 2014 until July 2014. Results show that the breed of goats (Boer, Jamnapari and Katjang) and the feed intake (Napier grass, soya hulls, oil palm fronds and pellet) do not have any significant effect on the kidding rate of goats in Peninsular Malaysia under intensive and semi-intensive system ($p>0.05$). The mean kidding rate ± SD of goats for intensive management system was 1.22 ± 0.64 while for semi-intensive management system the kidding rate was 1.08 ± 0.53 ($p>0.05$). Although the feeding systems for intensive farms seems to be more controlled and generally well-managed compared to the semi-intensive system, the kidding rate between each breed at both farm management systems were not significantly ($p>0.05$). A further study should be done in details on amount and frequency of feeds given in a day.

Keywords: Kidding rate, goat, breed, survey

Histopathologic toxicity evaluation of liposome-encapsulated diclofenac sodium

*Jun Zheng Goh[1], Hoe Siong Chiong[1], Yoke Keong Yong[2], Ahmad Zuraini[1] and Muhammad Nazrul Hakim[1]

[1]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
**Corresponding author. email address: junzhenggoh@hotmail.com*

Introduction

Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAIDs) which is commonly prescribes as worldwide used in the treatment of acute and chronic inflammatory conditions. Non-steroidal anti-inflammatory drug is related to gastrointestinal, hepatic and renal adverse events are commonly reported. Liposomal drug delivery systems have been shown to improved toxicity profiles of encapsulated drugs. Present study was conducted to compare the toxicity profiles between nanoencapsulated and free form diclofenac in Sprague Dawley rat.

Materials and Methods

Commercial Pro-lipo™ Duo from Lucas Meyer Cosmetics (France), were used. Diclofenac sodium salt was purchased from Sigma® (USA), dimethyl sulfoxide (DMSO) 99.5 % from Sigma®Life Science (USA). Healthy adult female Sprague Dawley rats, weighing 180–220 g were used in present study.

Liposomes formulation was prepared in accordance with the manufacturer's instruction with some modifications. Animals were subjected to 14 days repeated dosing procedure, toxicity signs were observed and recorded. Organs collection of stomach, liver and kidney were performed on day 15 and macro and microscopic histopathological assessment (gastric lesion assessment, histological damage score) were carried out.

Results and Discussion

Both macro and microscopic examination of organs shown significant improvement ($p<0.05$) in toxicity parameters for liposome encapsulated diclofenac when compared to baseline, control groups and free form drug at equivalent dosage. Data of gastric lesion assessment showed that free form diclofenac resulted in a significantly higher ulcer index score and longer ulcer length when compared to baseline and control groups, whereas none of the liposomes encapsulated diclofenac cause significant gastric lesions when compared to control. Data of stomach, liver and kidney histopathological scores showed similar trend with lower scoring obtained in liposomes encapsulated diclofenac treatment groups. A positive correlation was observed between the pathological changes of organs with the increases of treatment dosage.

Present study demonstrated liposomes as an effective protective agent against NSAID's adverse effects which were likely due to the promotion of lymphatic transport system, maintenance of stomach protective mucosa barrier and gastric cells prostaglandin synthesis effect of liposomal drug delivery system.

Table 1: Effect of different treatment on macroscopic stomach lesion.

Treatment group	Drug dosage (mg/kg)	Ulcer index score	Ulcer length (mm)
Baseline	0	0	0
Free Form Diclofenac	0(control)	0.33±0.21	0.05±0.03
	0.2	0.83±0.31	1.33±0.57
	1	1.33±0.21°	2.17±0.65°*
	1.8	2.33±0.21°*	3.43±0.19°*
Liposomes encapsulated diclofenac	0	0.33±0.21	0.05±0.03
	0.2	0.67±0.42	1.0±0.68
	1	0.83±0.17	0.92±0.26
	1.8	1.33±0.33°	1.23±0.11#

Values were mean ± SEM (n = 6/group)
° sig (P<0.05) when compared to baseline
* sig (P<0.05) when compared to control
# sig (P<0.05) when compared to group with equivalent dosage of diclofenac

Table 2: Effect of different treatment on microscopic stomach, liver and kidney damage score.

Treatment group	Drug dosage (mg/kg)	Stomach	Liver	Kidney
Baseline	0	0.33±0.21	0.33±0.21	0.33±0.21
Free Form Diclofenac	0(control)	0.55±0.22	0.67±0.21	0.33±0.21
	0.2	1.50±0.22	1.67±0.67	1.83±0.31°*
	1	1.67±0.33°	4.67±0.42°*	5.67±0.21°*
	1.8	3.33±0.42°*	6.50±0.22°*#	6.83±0.40°*
Liposomes encapsulated diclofenac	0	0.33±0.21	0.50±0.22	0.33±0.21
	0.2	0.67±0.21	0.67±0.33	1.33±0.21
	1	1.17±0.31	3.83±0.31°*	4.33±0.21°*#
	1.8	1.83±0.31°*#	4.83±0.31°*	5.33±0.33°*#

Values were mean ± SEM (n = 6/group)
° sig (P<0.05) when compared to baseline
* sig (P<0.05) when compared to control
# sig (P<0.05) when compared to group with equivalent dosage of diclofenac

Conclusion

Results of present study indicated that nanoencapsulated diclofenac were promising in reducing NSAIDs associated gastrointestinal, liver and renal toxicity thus led to improved drug toxicity profiles.

Acknowledgments

The authors thank Fundamental Research Grant Scheme from the Ministry of Higher Education, Malaysia (04-10-07-277FR) for providing financial support.

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Morphology study of *Chaetoceros anastomosans* and *Chaetoceros baculites* isolated from coastal water of Pahang, Malaysia

*Mohamad Fuad Mohamad Anuar[1], Normawaty Mohamad-Noor[2], Ahmed Jalal Khan Chowdury[2] and Kamaruzzaman Yunus[2]

[1]*Institut Kesihatan Umum, Kementerian Kesihatan Malaysia, Jalan Bangsar, 50590 Kuala Lumpur, W.P Kuala Lumpur*
[2]*Kulliyyah of Science, International Islamic University Malaysia, Jalan Istana, Bandar Indera* Mahkota, 25200 Kuantan, Pahang Darul Makmur
**Corresponding author.Tel.:+603 22829421; email address: crystal_fuad@yahoo.com.sg*

Introduction

Genus *Chaetoceros* is one of the largest cosmopolitan marine phytoplankton genera (Be´rard-Therriault *et al*., 1999) and most important genus in marine planktonic diatoms. The genus includes both neritic and oceanic species. Additional, there are no true freshwater species but some species occur at very low estuarine salinities and in inland saline lakes (Jensen and Moestrup, 1998).

Genus *Chaetoceros* live in coastal areas, producing high biomass which comprises very long chains of cells and magnificent resting spores (Jensen and Moestrup, 1998). Due to that, this genus had been listed as major contributor to primary production in near-shore upwelling regions and coastal areas (Rines and Theriot, 2003), where it contributes about 20–25% of the total marine primary production (Jensen and Moestrup, 1998).

Identification at the species level within the genus *Chaetoceros* is not an easy task and mainly based on gross morphology investigated by light microscopy (LM). Some morphological characters of taxonomical value can only be detected in detail using Scanning electron microscopy (SEM). Due to that, this study will focus on morphology study on one of the *Chaetoceros* species, *Chaetoceros baculites* within the laboratory by using LM and SEM.

Materials and Methods

Sample Collection: Samples of phytoplankton were collected by using 20 µm plankton net meshes in size, along the coastal water. The samples were then kept in polyethylene bottle and transported to the laboratory for isolation.

Establishment of Pure Culture: In the Laboratory, single cell of the phytoplankton was isolated using one cell isolation technique (Mohammad-Noor, 2012) under Compound Light microscope, Model: Leica DME. The isolated cell was put into 24 well plates containing 1 mL of F/2 media (Harrison and Berges, 2004). The pH of the medium was adjusted from 7.2 to 8.2, salinity of 28 $\pm$ 1 ppt, pressure was within 35 PSV (Normal Atmosphere) and temperature of 24$\pm$ 1 ^{0}C. For light intensity, cold lights were used with the intensity of 4000-5000 Lux (Rika Partiwi *et al*., 2009). During cultivation, the air flow was given for 24 hours by using air aerator and the light: dark cycle was 12:12 hours.

Identification: Both Light Microscope (LM) and Scanning Electron Microscope (SEM) were used for identification to species level. For LM, identification was done

under Compound Light Microscope, Model: Leica DME. For details view of the morphology, SEM was used. The method for fixation and dehydration of the samples were followed Mohammad-Noor (2012). After the dehydration process, the samples were critical point dried using Critical Point Dryer (CPD) machine; model CPD 030 BAL-TEC. Next, the samples were coated with gold using coating machine; model LEICA EM SCD 005. The samples were then examined using Zeiss Evo 50 at magnification of 300x to 15000x. Pictures were captured using SmartSEM Version V05 program.

Results and Discussion

Chaetoceros baculites Meunier

Morphological Characteristics: The cell form straight, narrow and fragile chain with 2-3 cells. The cells are delicate and fragile due to thinness of the cells. The setae originate inside the valve edge with short basal parts and were thin and fragile. The terminals setae are long and thin with the spine sparsely arranged. One chloroplasts was observed inside the cells.

Observation: Brown color culture detected. No mucous appeared.

C. baculites has so far been identified from Danish Coastal water (Jensen and Moestrup, 1998). Morphological description for this species is still limited. In this study, new data obtained on the size of aperture, the terminal setae and spine arrangement which will add on the available knowledge. This species is fragile and very delicate compared to *C. affinis* var*. willei* with the size of aperture is 3.5 $\pm$ 0.5 µm. The terminal setae are long and thin with the thickness similar to intercalary setae. The seta is very thin with spine arranged sparsely and only two to three cells can be seen in one chain. This is a new record of this species in tropical water.

Conclusion

This is the second report of *C. baculites* after first recorded in the Danish Coastal Water. Details data on the size of aperture, the terminal setae and spine arrangement of *C. baculites* will be add as a new data on the descriptions of this species.

Acknowledgements

The authors would like to express greatest appreciation and gratitude to the Research Management Centre, International Islamic University Malaysia for funding this study (EDW-B11-004-0482) and staff of Institute of Oceanography And Maritime Studies for continuous support throughout the project period.

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Morphology study of two varieties *Chaetoceros affinis isolated from coastal water of Pahang, Malaysia*

*Mohamad Fuad Mohamad Anuar[1], Normawaty Mohamad-Noor[2], Ahmed Jalal Khan Chowdury[2] and Kamaruzzaman Yunus[2]

[1]*Institut Kesihatan Umum, Kementerian Kesihatan Malaysia, Jalan Bangsar, 50590 Kuala Lumpur, W.P Kuala Lumpur*
[2]*Kulliyyah of Science, International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200 Kuantan, Pahang Darul Makmur*
**Corresponding author.Tel.:+603 22829421; email address: crystal_fuad@yahoo.com.sg*

Introduction

Genus *Chaetoceros* is one of the largest cosmopolitan marine phytoplankton genera (Be´rard-Therriault *et al*., 1999) and most important genus in marine planktonic diatoms. The genus includes both neritic and oceanic species. Additional, there are no true freshwater species but some species occur at very low estuarine salinities and in inland saline lakes (Jensen and Moestrup, 1998).

Genus *Chaetoceros* live in coastal areas, producing high biomass which comprises very long chains of cells and magnificent resting spores (Jensen and Moestrup, 1998). Due to that, this genus had been listed as major contributor to primary production in near-shore upwelling regions and coastal areas (Rines and Theriot, 2003), where it contributes about 20–25% of the total marine primary production (Jensen and Moestrup, 1998).

Identification at the species level within the genus *Chaetoceros* is not an easy task and mainly based on gross morphology investigated by light microscopy (LM). Some morphological characters of taxonomical value can only be detected in detail using Scanning electron microscopy (SEM). Due to that, this study will focus on morphology study on one of the *Chaetoceros* species, *Chaetoceros affinis* var. *affinis* within the laboratory by using LM and SEM.

Materials and Methods

Sample Collection: Samples of phytoplankton were collected by using 20 µm plankton net meshes in size, along the coastal water. The samples were then kept in polyethylene bottle and transported to the laboratory for isolation.

Establishment of Pure Culture: In the Laboratory, single cell of the phytoplankton was isolated using one cell isolation technique (Mohammad-Noor, 2012) under Compound Light microscope, Model: Leica DME. The isolated cell was put into 24 well plates containing 1 mL of F/2 media (Harrison and Berges, 2004). The pH of the medium was adjusted from 7.2 to 8.2, salinity of 28 $\pm$ 1 ppt, pressure was within 35 PSV (Normal Atmosphere) and temperature of 24$\pm$ 1 ^{0}C. For light intensity, cold lights were used with the intensity of 4000-5000 Lux (Rika Partiwi *et al*., 2009). During cultivation, the air flow was given for 24 hours by using air aerator and the light: dark cycle was 12:12 hours.

Identification: Both Light Microscope (LM) and Scanning Electron Microscope (SEM) were used for identification to species level. For LM, identification was done

under Compound Light Microscope, Model: Leica DME. For details view of the morphology, SEM was used. The method for fixation and dehydration of the samples were followed Mohammad-Noor (2012). After the dehydration process, the samples were critical point dried using Critical Point Dryer (CPD) machine; model CPD 030 BAL-TEC. Next, the samples were coated with gold using coating machine; model LEICA EM SCD 005. The samples were then examined using Zeiss Evo 50 at magnification of 300x to 15000x. Pictures were captured using SmartSEM Version V05 program.

Results and Discussion

Chaetoceros affinis var. *affinis* Lauder 1864

Morphological Characteristics: The cells form into medium to long chains and have very thick cell walls. Inside the cell, one large chloroplast can be seen. The labiate or rimoportula appear at the center of end cells and the cells in rectangular shape with girdle. The setae are long and basal part can be seen. The setae originated from valve margin. Terminal setae are thicker compare to intercalary setae with U or V-shaped. However, the intercalary setae are moderately thick. At setae, the spines are visible with spiral arrangement.

Observation: Brown color appeared during cultivation. Not producing mucous.

Based on LM and SEM, *C. affinis* var. *affinis* is the strain where major features of *C. affinis* still appeared. These major features consist of thick terminal setae where the shape in U-shaped or V-shaped, thick cell wall with visible setae, the appearance of rimoportula and visible spines. According on Jensen and Moestrup (1998), both strains could be identified as distinct taxon whereby both strains can be distinguished clearly based on morphological characteristics. In addition, both species are cosmopolitan and can be found almost in any marine waters in the world (Shevchenko *et al*., 2006).

C. affinis var. *affinis* has very distinct features that can be easily identified under SEM and light microscopy. Based on the available references, the morphological characteristic such as rimoportula, chloroplast, setae, spine arrangement, cell length and aperture opening are in agreement with other studies. However, for the apical axis, the length is shorter compared to other references. The average length in this study is about 8 µm with the longest length is 10 µm, whereas other references recorded between 9 to 30 µm. The differences in the length of at apical axis may due to culture condition.

Conclusion

As a conclusion, *C. affinis* var. *affinis* has shorter apical axis compared to other studies. In addition, *C. affinis* var. *affinis* has thick and rigid cell wall compared to *C. affinis* var. *affinis*. All the information regarding the morphology of this species will be added to description data for the algae species ecology and identification from Coastal Water of Pahang, Malaysia.

Acknowledgements

The authors would like to express greatest appreciation and gratitude to the Research Management Centre, International Islamic University Malaysia for funding

this study (EDW-B11-004-0482) and staff of Institute of Oceanography And Maritime Studies for continuous support throughout the project period.

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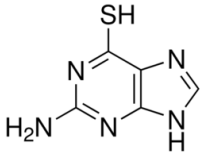
Nitric Oxide (NO) secretion inhibition and cytotoxicity effects of LPS-induced RAW 264.7 macrophage cells and PMA-induced HIG-82 cells by 6-Hydroxy-2-mercaptopurine and 6-Thioguanine

*Nurul Syuhada Nordin[1], Nor Erma Suryana Sukiman[1],Muhamad Zulhilmi Zailani[1], Che Ku Dahlan Che Ku Daud[1], Yong Yoke Keong[2], Zuraini Ahmad[1], Zetty Nadia Md Zain[3] and Muhammad Nazrul Hakim[1,4]

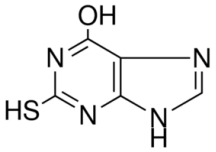
[1]Department of Biomedical Sciences, [2]Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.
[3]Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia,
[4]Halal Products Research Institute, Universiti Putra Malaysia.
**Corresponding author.Tel.:017 2230787; email address: lav_vender@yahoo.com*

Introduction

Activation of immune cells leads to the mediator secretion of pro-inflammatory cytokines thus amplifying the inflammation. Nitric oxide (NO) produced by activated cell plays critical role in the inflammatory process. Thiopurines, are purine antimetabolites have gained a reputation as effective anti-cancer compounds, treatment for organ transplant recipients and immunosuppressive drugs (Fotoohi *et al*., 2010) Cancer is often linked to inflammation or assigned as chronic inflammatory disease and allows the overlap of cancer and inflammation in drug delivery illustration context (Crielaard *et al*., 2012) Being categorized as inactive pro-drugs, their cytotoxicity is exerting after metabolized intracellularly by either inhibit de novo purine synthesis (DNPS) or are incorporated into DNA to products (Karim *et al*., 2011).



6-Thioguanine (6TG)



6-Hydroxy-2-Mercaptopurine (6H2MP)

In this study, the cytotoxicity effects and anti-inflammatory role of 6-hydroxy-2-mercaptopurine and 6-thioguanine is being investigated towards their inhibition of NO secretions evaluated in HIG-82 cell and RAW 264.7 cells, with PMA and LPS as the inducer towards inflammation respectively.

Materials and Methods

The rabbit synoviocytes cell line HIG-82 (CRL-1832) is purchased from the American Type Culture Collection (ATCC). Phorbol myristate acetate (PMA) is used to activate the HIG-82 synovial cell while Lypopolysaccharide (LPS) is used to activates the RAW 264.7 cell and induce the inflammation. Cell viability was determined and observed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The synoviocytes cell HIG-82 and RAW 264.7 (*ATCC* TIB-71) cell line (1×104 cells/ml) were plated in each well of a 96-well plate and allowed to adhere and spread for 24 hours. The culture mediums were changed on the next day and cells

were exposed to the serial dilutions of compound 6TG and 6H2MP. After 24 hours, the cell cytotoxicity were observed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Gosslau *et al*., 2014) MTT solution (5 mg/ml) is added to each of 96 well microplate and the cultures were incubated for an additional 4 hours to allow the formazan crystals solubilised in SDS buffer. The absorbance at 570 nm was determined in each by using Infinite Tecan M200 Bioelisa Reader. The inhibition of these two compounds upon nitric oxide is evaluated in the cellular models of inflammation which assessed the inhibitory effects of 6-Thioguanine (6TG) and 6-hydroxy-2-mercaptopurine (6H2MP) from inflammatory induced HIG-82 cells with PMA and LPS-induced RAW 264.7 macrophage cells. NO was predicted as nitric oxide metabolites (NOx) by Griess reaction after transformation of nitrate to nitrite by nitrate reductase. Tecan M200 Bioelisa Reader read the ensuing color changes at 550 nm (Sosroseno *et al*., 2011).

Results and Discussion

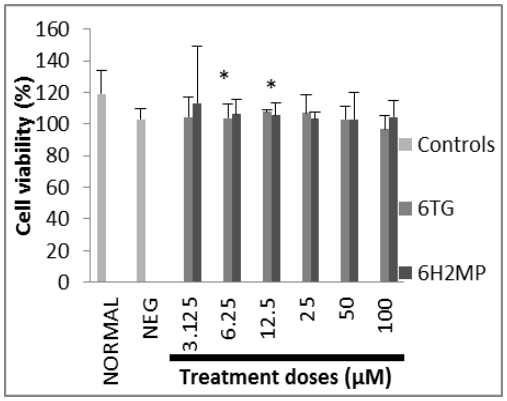


Figure (a)

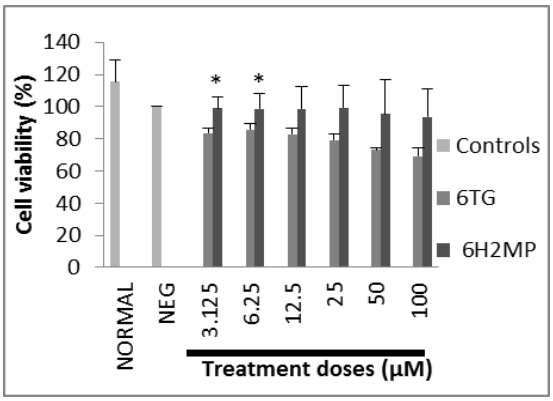


Figure (b)

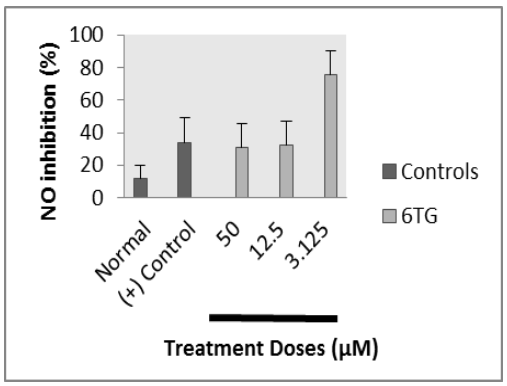


Figure (c)

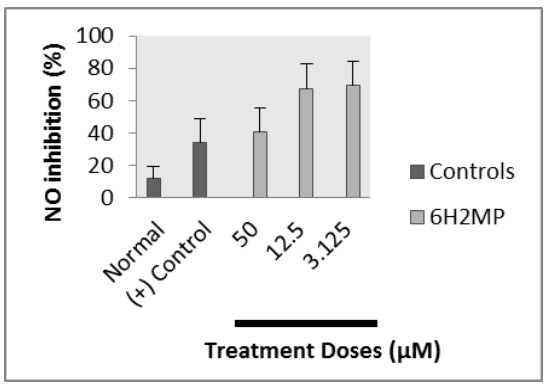


Figure (d)

Figure (a) shows the MTT result of PMA-induced HIG-82 cells treated with 6TG and 6H2MP. The cell viability percentage increase starting at dose 12.5 µM to 25 µM of 6TG and is high at dose 6.25 µM to 25 µM of 6H2MP. Cell viability percentage for all the dose treatment that treated with 6TG upon exposure to PMA is relatively higher compared to untreated group (Negative control). There is undetectable NO synthesized by HIG-82 cell line under PMA-induce culture condition. Figure (b) shows the result of LPS-induced RAW 264.7 macrophage cells viability percentage

treated with 6TG and 6H2MP. The cell viability percentage is high at dose 3.125 µM to 12.5 µM of 6TG and is high starting at dose 3.125 µM to 25 µM of 6H2MP. The percentage of viability of cells that treated with 6TG is lower than the Normal and Negative control groups. This might because of the proliferative effect of the cells shown in the control groups. The percentage of NO inhibition of LPS-induced RAW 264.7 macrophage cells by 6TG is shown in Figure (c). The inhibition percentage is increasing from the dose 50µM to 3.125µM. Figure (d) shows the percentage of NO inhibition on LPS-induced RAW 264.7 macrophage cells by 6H2MP. The inhibition percentage is also increasing from the dose 50µM to 3.125µM. Data are represented as mean (± S.E.M) of three independent experiments. *P≤0.05 compared with normal. 6H2MP was found to reduce NO production in a dose-dependent manner compared to 6TG. The increase of NO secretions in LPS-induced RAW 264.7 cell is regulated by reactions such as superoxides and nitrogen oxide species. Purine analogues drugs cytotoxicity in medium culture might be due to apoptosis and correlated with intracellular drug accumulation.

Conclusion

There is undetectable NO synthesized by HIG-82 cell line under PMA-induce culture condition. 6-Thioguanine (6TG) and 6-hydroxy-2-mercaptopurine (6H2MP) demonstrate inhibitory potential of Nitric Oxide (NO) production in inflammatory process in LPS-induced RAW 264.7 cells. The findings are crucial in making arrangement for further development of both compounds into anti-inflammatory drug. However further studies need to be perform to study the mechanism of action in depth.

Acknowledgements

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Possible participation of protein kinase c and cyclic guanosine monophosphate pathway in antinociception of ethanol extract of *Ficus deltoidea* var *angustifolia* leaves in mice

*Mohd Khairi Hussain, Hamizah Abu Bakar, Zainul Amiruddin Zakaria, Mohd Roslan Sulaiman and Muhammad Nazrul Hakim

Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang
**Corresponding author. email address: khairi@upm.edu.my*

This study was conducted to evaluate the involvement of protein kinase C (PKC) and cyclic guanosine monophosphate (cGMP) pathway in the antinociception of ethanol extract of *F. deltoidea* (EEFD) leaves. To evaluate the involvement of PKC, the mice were treated with EEFD or acetylsalicylic acid (ASA, 100 mg/kg) 30 minutes before receiving phorbol 12-myristate 13-acetate (PMA, 0.03µg/paw) intraplantarly at the right hind paw. The mice were observed to record the licking and biting the injected paw. To estimate the involvement of cGMP, the mice were divided into four groups (n = 7). Two groups were pre-treated with methylene blue (20 mg/kg) and after 20 minutes, they were given EEFD or vehicle. Another two groups were pre-treated with vehicle 20 minutes before EEFD or vehicle administration. After 30 minutes, induced with 0.6% acetic acid and the number of abdominal writhing was recorded. EEFD produced significant effect of inhibition of abdominal writhing in dose dependent manner. Similarly, administration of EEFD at the similar doses resulted in significantly reduced in nociception induced by intraplantar injection of PMA as it showed reduction in paw licking time. The pre-treatment with methylene blue significantly enhanced the anticoceptive activity of EEFD.The present results suggested that PKC and cGMP pathway possibly inactivated in the antinociceptive action exerted by EEFD.

Keywords: *Ficus deltoidea*, antinociception, PKC, cGMP

Potential of fresh POME as a growth medium in mass production of *Arthrospira platensis*

Puganeswary Sukumaran[1], Rosimah Nulit[1], Shahrizim Zulkifly[1], Normala Halimoon[2], Hishamuddin Omar[1] and *Ahmad Ismail[1]

[1]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor*
[2]*Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor*
**Corresponding author.Tel.:+603 89466617; email address: aismail@upm.edu.my*

Introduction

Fresh Palm Oil Mill Effluent (POME), non-toxic in nature (Lam and Lee, 2011) is suggested to be suitable supplementary nutrient in *A. platensis* cultivation proposed for beneficial application in pharmaceutical, cosmetics and food industries. Fertilizing properties of POME which contains appreciable amounts of macro- and micronutrients (Singh *et al*., 2010) is suitable to support good *A. platensis* growth. Besides that, fresh POME is readily available in Malaysia and it is also cost effective feed for *A. platensis* growth in commercial cultivation. Previously, small scale studies have been done on Spirulina cultivation using digested or treated POME in laboratory condition (Parkavi *et al*., 2011). The novelty of this study is to optimize productivity of *A. platensis* cultured in outdoor tanks (10 L) in the climate of Malaysia using fresh POME which were supplemented with commercial fertilizers as nitrogen, phosphorus source because fresh POME is lack of these macronutrients (Mohd Nasir *et al*., 2013) and sodium bicarbonate as carbon source and to maintain the alkalinity of culture medium. The aim of this study is to find the optimum fresh POME concentration (0%, 1%, 2% 3%, and 4% v/v) to culture *A. platensis* without affecting its productivity and pigments content as in control medium.

Materials and Methods

Control medium was prepared based on Kosaric medium (Tompkins *et al*., 1995) with modification using commercial fertilizers as followed (g/L): 4.5 $NaHCO_3$, 0.25 NaCl, 0.1 $CaCl_2$, 0.2 $MgSO_4.7H_2O$, 0.221 Urea, 0.07 H_3PO_4, 0.242 KOH, 0.02 $FeSO_4.7H_2O$ and 0.5 mL/L of trace metals solution composed of following elements (g/L): 2.86 H_3BO_4, 1.81 $MnCl_2.4H_2O$, 0.22 $ZnSO_4.7H_2O$, 0.08 $CuSO_4.5H_2O$, 0.01 MoO_3, and 0.01 $COCl_2.6H_2O$. Treatments, T1 to T5 were supplemented with different concentration of fresh POME (0%, 1%, 2%, 3% and 4% v/v) and commercial fertilizers as followed (g/L): 4.5 $NaHCO_3$, 0.25 NaCl, 0.221 Urea, 0.07 H_3PO_4.

Productivity and specific growth rate was calculated according to Kong *et al*. (2011). Total chlorophyll and carotenoid was extracted with 95 % ethanol in a water bath at 70°C for 5 min evaluated at 664, 649, 470 nm according to Lichtenthaler (1987). Phycocyanin was extracted using 0.1M phosphate buffer (pH 7) and evaluated at 615 and 652 nm according to Bennett and Bogorad (1973). Productivity, specific growth rate and pigments production of *A. platensis* cultured in different POME concentrations were analysed using SPSS software version 21

through one-way independent analysis of variance (ANOVA) and followed by Tukey HSD (Honestly Significant Difference) multiple comparison test.

Results and Discussion

Table 1 shows productivity and specific growth rate of *A. platensis* cultured in T2 (1% v/v fresh POME) were significantly higher ($p < 0.05$) compared to control and other treatments. Based on Table 2, *A. platensis* grown in control and T2 had significantly higher ($p < 0.05$) chlorophyll content. Furthermore, carotenoid content was significantly higher ($p < 0.05$) in T2 while phycocyanin content was significantly ($p < 0.05$) higher in control.

Table 1: Results of productivity (g L^{-1} d^{-1}) and specific growth rate (μ) of *A. platensis* grown in different POME concentrations.

Treatments	Productivity (g L^{-1} d^{-1})	Specific growth rate (μ d^{-1})
Control	0.181 ± 0.0034^{b}	0.227 ± 0.0031^{b}
T1	0.057 ± 0.0019^{e}	0.130 ± 0.0037^{d}
T2	0.211 ± 0.0034^{a}	0.250 ± 0.0026^{a}
T3	0.168 ± 0.0034^{b}	0.218 ± 0.0066^{b}
T4	0.130 ± 0.0031^{c}	0.181 ± 0.0042^{c}
T5	0.082 ± 0.0022^{d}	0.142 ± 0.0016^{d}

Each value is presented as mean ± SE (n = 5). Means within each column with different letters (a-e) differ significantly ($p < 0.05$).

Table 2: Pigments content (% dry weight) in *A. platensis* cultured in control and treatments with different POME concentrations.

Treatments	Chlorophyll (% Dry weight)	Carotenoid (% Dry weight)	Phycocyanin (% Dry weight)
Control	1.026 ± 0.023^{a}	0.533 ± 0.004^{b}	13.585 ± 0.192^{a}
T1	0.235 ± 0.014^{e}	0.175 ± 0.004^{f}	4.636 ± 0.229^{e}
T2	1.045 ± 0.024^{a}	0.573 ± 0.005^{a}	12.013 ± 0.110^{b}
T3	0.793 ± 0.016^{b}	0.484 ± 0.008^{c}	9.770 ± 0.104^{c}
T4	0.623 ± 0.022^{c}	0.384 ± 0.011^{d}	6.108 ± 0.098^{d}
T5	0.509 ± 0.011^{d}	0.330 ± 0.005^{e}	5.011 ± 0.103^{e}

Each value is presented as mean ± SE (n = 5). Means within each column with different letters (a-f) differ significantly ($p < 0.05$).

Besides that, productivity, specific growth rate and high value pigments of *A. platensis* cultured in medium with increasing POME concentration from T2 (1 % v/v fresh POME) to T5 (4 % v/v fresh POME) were decreased. This phenomenon can be evidently related with increasing turbidity and darker color of growth medium due to the presence of tannic, humic and fulvic acids (Yaser *et al*., 2013) which limit light penetration and photosynthesis reaction through shading effect in the cultivation system (Habib *et al*., 2003). This phenomenon altered the POME based treatments (T2 to T5) to mixotrophic and heterotrophic states. Consequently, rate of photosynthesis metabolism decreased while rate of oxidative glucose metabolism increased in the culture medium with increasing POME concentration. As a result, photosynthetic pigments reduced in darker medium (Madhyastha and Vatsala, 2007).

Hence, microalgae have greater growth rate under mixotrophic condition than heterotrophic and autotrophic condition as proved by Kong *et al.* (2011).

Moreover, *A. platensis* grown in T1 (0 % v/v fresh POME) had significantly lower productivity, specific growth rate and pigments content compared to other treatments. This suggests *A. platensis* cultured in T2 to T5 benefited the organic nutrients available in fresh POME for its growth and productivity.

Conclusion

1 % v/v fresh POME is optimum for *A. platensis* growth and pigments production in the climate of Malaysia. Besides, fresh POME contains appreciable amount of vital nutrients for algal growth thereby, addition of macro and micro minerals except nitrogen and phosphorus can be omitted which could reduce the cost of production. This study also gives a good view on fresh POME as a cheaper and easily available organic fertilizer source in Malaysia to culture *A. platensis* in larger scale.

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Study on growth and digestion of climbing perch (*Anabas testudineus*)

Intan Faraha A. Ghani[1] and Mazlan Abd Ghaffar[2]

[1]Fakulti Sains dan Bioteknologi, Jabatan Akuakultur– Universiti Selangor Kampus Bestari Jaya, 45600 Bestari Jaya, Selangor
[2]Pusat Pengajian Sains Perikanan dan Akuakultur, Universiti Malaysia Terengganu 21030 Kuala Terengganu, Terengganu
**Corresponding author. email address: intanfaraha@unisel.edu.my*

Introduction

Anabas testudineus, also known as Climbing perch, is hardy, small brown fish originated from the Southern Asia countries such as India, Indochina peninsular, southern China, Taiwan, Philipines and Indonesia (Hitchcock, 2008). It belongs to the family of Anabantidae (Marimuthu *et al.*, 2009), breathe through complicated air-respiratory organ by gasping atmospheric oxygen (Jalilah *et al.*, 2011). To promote growth in culture operations, fish should be fed a nutritionally adequate diet (Singh and Srivastava, 1985). Feed management is critical in order to get maximum growth with lowest feed input during culture operations (Soares *et al*., 2005). On the other hand, excessive food supply leads to fouling and wastage. The study was carried out to determine the growth, to trace the movement of food items by X-radiography techniques and to locate the site of nutrient absorption which passing through the alimentary tract of *A. testudineus.*

Materials and Methods

Growth Experiment

All samples, juvenile stages of *Anabas testudineus* were obtained from a local aquaculture dealers. Five pairs of *A. testudineus* with a total range between 9-11 cm were kept in an aquarium (30W x 61L x 56H cm) complete with an aeration and filtration system. Samples were fed with commercial diet twice daily (0900 and 1600h) at 3% BW. During the 4 week experiments, the fish were manually fed until apparent satiation was reached. It was determined as the point when the fish stop actively feeding and uneaten pellets remained at the bottom of the tanks for more than 2 min. Uneaten pellets were then siphoned out of the tanks and immediately dried to constant mass in an oven (80^{O}c for 24 h). The amount of food consumed was calculated as the difference between the mass food of the food offered and that of the uneaten food after drying. Food consumption rate (FCR) was calculated for each tank as :

FCR : (Final body weight – Initial Body Weight) / Total food intake
SGR : (ln Final mass – ln Initial mass/t) * 100

Gastric Emptying and Digestion Experiment

Total no of 78 adult stage samples were used for both gastric emptying and digestion experiment. For X-radiography techniques, fish were deprived of food for 72 hours to empty the stomach. Thereafter, they were fed to apparent satiation with commercial diets (mix with $BaSO_4$) and sampled (every 2 hours, 8 fish in total) started from 0 to 14 hours after feeding. The remaining fish were sampled for every 2 hours (2 per tank, 6 fish in total per treatment) at 0-12 h post feeding. At each time, all samples were killed and their alimentary tract were cut into 3 sections (stomach, anterior intestine,posterior intestine). All contents from every section were removed and freeze-dried prior to proxymate analysis.

Statistical Analysis

A square root 'non-linear' regression model (Mazlan *et al*., 2002) was used to analyze the movement of stomach content along the alimentary tract for every specified time after feeding.

Results and Discussion

Growth Rate

The rate of growth and development of the *A. testudineus* were conducted for four weeks with the temperature and density, feeding equivalent for each Aquarium. Results showed that the increasing of the body length in line with the increase in weight. However, the increasing rate for both is different for every set Aquarium although all three sets of the Aquarium had the same control of the density, temperature and water quality.

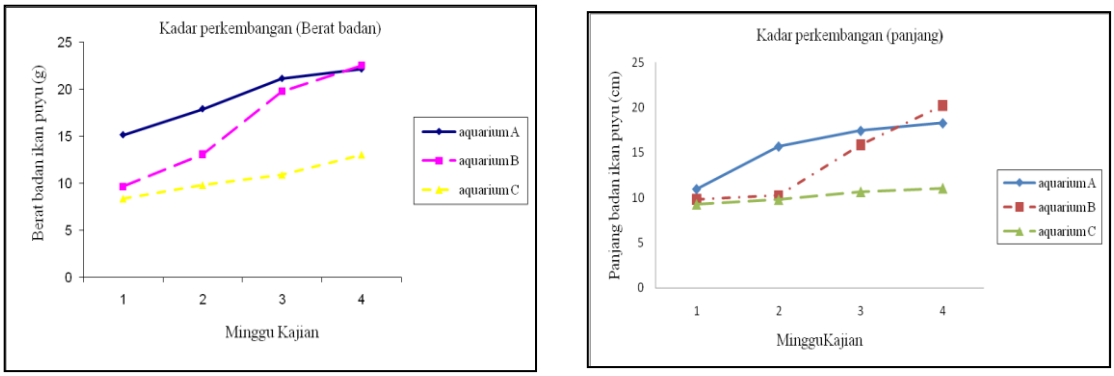

Figure 1: Growth rate *A. testudineus* (weight and length) during 4 weeks experiment.

Gastric Emptying

Result shows the condition of food items 0,2,4,6,8,10,12,14 h post feeding in adult size *A.testudineus*. Within 2-4 h after feeding, the stomach is full and food has already entered the intestine loop but still has not reached the rectum; 6-8 h post feeding, approximately 30%-40% of the food items still remain in the stomach Within 14 h post feeding, the food items have completely left the stomach and have concentrated in the posterior part of the intestine and rectum, ready to be defecated. The stomach content for every specified time after feeding, shown by the plotted graph using square root 'non-linear' regression model (Mazlan *et al*., 2002).

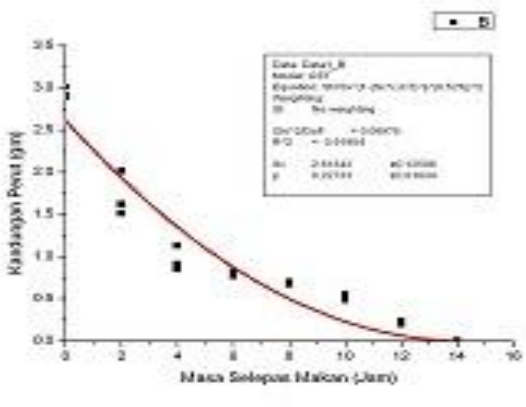

Figure 2: Concentration of stomach content at various time after feeding with commercial pellet.

Proxymate Analysis

The graphs shows the concentration of nutrients, ash and water content at variant time after feeding. Water content decreased 73% to 48% along the gut. In contrast, ash concentration increased steadily towards posterior regions of the gut to reach 56%. The concentration of protein and lipid decreased along the gut, approximately 43% of protein 11% of lipid appear to have been absorbed in passage between the stomach and the interior intestine. In the passage of the remaining nutrient from mid intestine to rectum, approximately 37% protein and 6% lipid were absorbed. Carbohydrate concentrations increase from 0.64% to 0.81% towards the anterior intestine before declining to 0.78% towards posterior region.

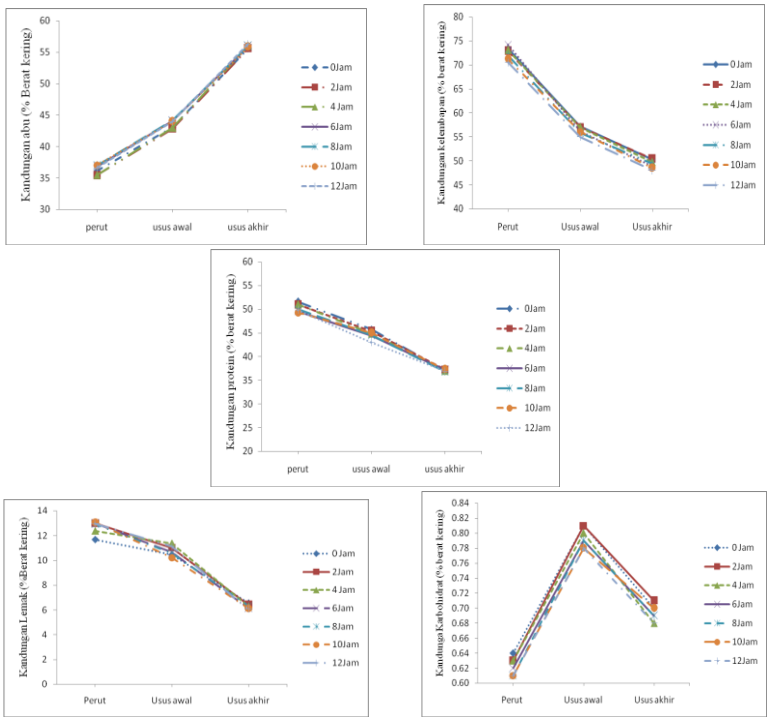

Figure 3: Nutrient concentrations in digesta from different gut sections at different times after feeding with commercial diet.

Conclusion

The study shows the differences between growth rate for each aquarium. Growth rate was influenced by external factors (aquaculture practice, temperature, density, feeding rate and water quality) and internal factors (physiology and fish genetic). Result from this experiment shows that the growth rate was influenced by the internal factors of the fish. The movement of stomach content were observed by X-radiography completely evacuated the stomach within 14 h post-feeding. The decrease in the water level content and increase of ash during the process showed, despite use as an internal marker, play the important role as compacting factors in digestion process (Mazlan and Grove, 2003). Concentrations of protein and lipid decreased in similar trend as transit the food along the gut. Lipid digestion and absorption takes place mainly in the anterior intestine where the pancreatic lipase are secreted. Absorption of carbohydrate was poor in comparison with protein and lipid. Carbohydrate absorption are difference in every species. Knowledge in growth rate, gastric emptying and digestion of *A.testudineus* will contribute to high productivity in Aquaculture.

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The morphology, physiology and biochemical changes in *Oryza sativa* planted under cyclic water stress with different potassium input

Nurul Amalina Mohd Zain[1,2], *Mohd Razi Ismail[2], Maziah Mahmood[3], Adam Puteh[4]

[1]*Institute of Biological Science, Faculty of Science, University of Malaya,* 50603 *Kuala Lumpur, Malaysia*
[2]*Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
[3]*Department of Biochemistry, Faculty of Bioctechnology and Biomolecular Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*
[4]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia*
**Corresponding author.Tel.:+603 66471045; email address: razi@agri.upm.edu.my*

Four levels of potassium rates (Control, 80 kg K_2O/ha, 120 kg K_2O/ha and 160 kg K_2O/ha) and two types of potassium (KCl and K_2SO_4) were exposed to rice to investigate the influence of potassium fertilization in minimizing the impact of cyclic water stress in rice production. It was observed that panicle dry weight/hill, root dry weight, rice yield, Catalase activity, proline, maliondialdehyde and harvest index were influenced by potassium rates. The leaf numbers, total tillers and 1000-grain weight, was influenced by potassium types. Interaction effects (potassium rate x potassium type) was observed in shoot dry weight, leaf area, total spikelet/ panicle, net assimilation rate, transpiration rate and instantaneous water use efficiency. It was found as fertilization rates increased from 80<120<160 kg K_2O/ha, the production of proline was increased. The increases of proline production was simultaneously enhanced the production of Catalase and malondehyde (MDA). Proline have a significant positive correlationship with Catalase (r^2 =0.891; p≤0.05) and maliondialdehyde (r^2 = 0.912; p≤0.05). As the potassium rate increased from 80>120>160 kg K_2O /ha the transpiration rate was observed to be increased in both MOP and K_2SO_4. The result suggested that high potassium rates would reduce water stress effects by having high transpiration rate. The study has showed that application of potassium fertilizer would minimize the effects on rice growth and physiology under cyclic water stress condition.

Keywords: Cyclic water stress, potassium, physiology, biochemical changes

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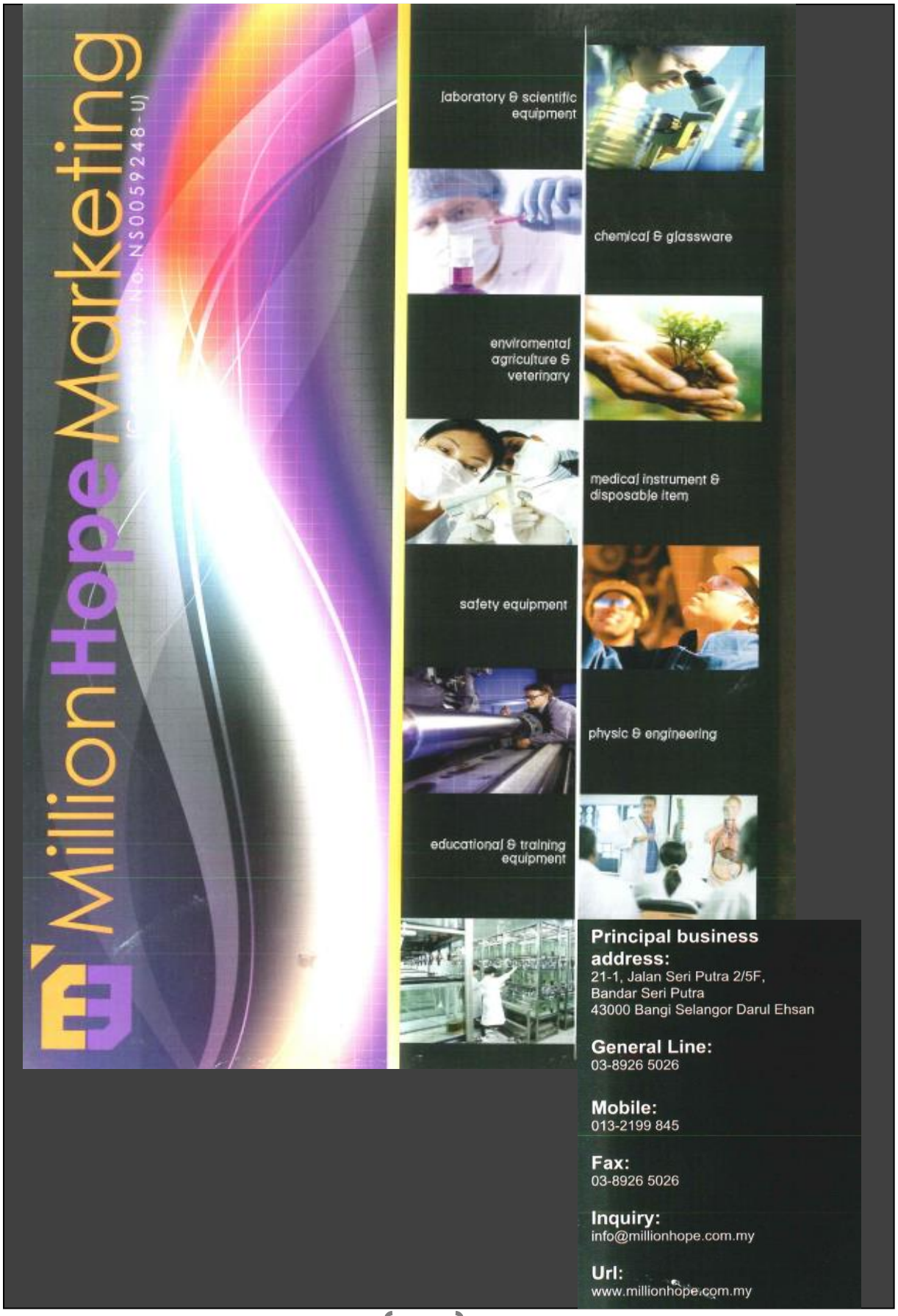
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Fax:
03-8926 5026
Inquiry:
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