ACLS 2014

Global Trends in the Life Sciences

The Asian Conference on the Life Sciences and Sustainability

Official Proceedings

ISSN 2188-3971

The 2014 Asian Conference on the Life Sciences and Sustainability

KKR Hotel Hiroshima, Japan

27 – 29 August 2014

http://www.esdfocus.org/life-sciences-conference/

The Asian Conference on the Life Sciences and Sustainability
Official Proceedings: ISSN 2188-3971

Index of Proceedings

Official ACLS 2014 Program
Pages: 4 to 52

ACLS 2014 Full Papers
Pages: 53 to 116

ACLS 2014 / WASH 2014

The Inaugural Asian Conference on the Life Sciences & Sustainability (ACLS)
&
The Inaugural Asian Symposium on Water, Sanitation and Hygiene (WASH)

Schedule & Agenda

August 27-29, 2014 at the KKR Hotel Hiroshima, Japan

Sponsored by:
The PRESDA Foundation of Japan
www.presdafoundation.org

Welcome Letter from the Organizing Committee Chairperson

Dear Friends,

It is my sincere honor and privilege to welcome all of you to the Inaugural Asian Conference on the Life Sciences (ACLS) and its sister event the Inaugural Asian Symposium on Water, Sanitation and Hygiene (WASH).

Since the founding of PRESDA in 2010, we have endeavored to offer unique events every year in Hiroshima during the memorial period of the atomic bombing. Our first event in 2012, examined the future of sustainable energy following the nuclear accident in Fukushima.

Thus, we are quite pleased that more than 130 presenters, audience and invited guests from 30 countries have accepted our invitation to come to Hiroshima for these special events.

As always, the overarching goal is to provide an opportunity for academics, scholars, non-profit workers, students and others concerned with human rights and ESD to exchange views in a setting encouraging respectful dialogue.

The range of topics and caliber of presenters is impressive. We are confident that everyone will find reason to be inspired by the research presented during these combined events.

It is in this spirit of friendship, peace and international cooperation that we express our warmest welcome to every participant.

We hope you will have a memorable and fruitful experience here in Japan's City of Peace: Hiroshima!

Warmest regards,

Takayuki Yamada
Chairman, Board of Governors
PRESDA Foundation
Co-Founder, Chubu Mirai Rotary
Polio-Plus Committee, Rotary Japan

Welcome Letter from the Editorial Committee

Dear ACLS/WASH Participants,

On behalf of the PRESDA Foundation and everyone else involved in the journey leading to this special event, it is with open arms and minds that we welcome you to the inaugural ACLS/WASH 2014 Conference on the Life Sciences and Sustainability, and Symposium on Water, Sanitation and Hygiene in Asia.

We have come here today united by our drive and shared vision of a better world in which scientific advances and water take center stage. And it is through this intersection of interests that meaningful discourse and change arise. Your commitment to these objectives and gathering at the KKR Hotel Hiroshima bring renewed hope and ideas to a continent.

We sincerely thank you for your participation in ACLS/WASH 2014 and for broadening the intellectual scope and understanding of the life sciences, water, and sustainability in Asia and elsewhere. We hope you enjoy your stay in Hiroshima as well as the city's rich cultural heritage while advancing our shared goals.

On a personal note, I would also like to thank the PRESDA Foundation for permitting me to take part in its development and this conference as well as for its greater mission of education for sustainable development.

Sincerely,

John Latzo
Editorial Committee
ACLS/WASH 2014
Hiroshima, Japan

Welcome Letter from the ACLS/WASH 2014 Conference Program Director

Dear Participants of ACLS/WASH 2014,

It is with great pleasure that I welcome each and every one of you to our jointly held Asian Conference on the Life Sciences and Sustainability and the Asian Symposium on Water, Sanitation and Hygiene here at the KKR Hotel in beautiful Hiroshima, Japan.

I'm very excited about the quality of the presentations that we have lined up as well as the fact that so many of our participants have traveled quite the distance to be here with us for this important joint event. My hope is that we not only come away with new knowledge and ideas from our time together, but also to create new networks and friendships that will last a lifetime.

I would like to give thanks to the PRESDA Foundation as well as both the ACLS and WASH organizing committees for all their hard work and commitment in planning these two very important events.

Finally, I would like to thank all of the presenters and audience members for your contributions to this conference. As members of the organizing committee, our role was to produce the program, but the truth is, this is your conference and without your participation none of this would be possible.

I hope you have a wonderful time during your stay in Hiroshima.

Yours sincerely,

Michael Sasaoka
Conference Program Director
ACLS/WASH 2014
Hiroshima, Japan

Letter of Welcome from the Organizing Committee Vice Chairperson

Dear Colleagues,

I would like to thank everyone for traveling to Hiroshima to present and share your ideas with others from across Asia and around the world. I am sure that we will all learn a great deal from each other, but one thing I have known from the start is that our presenters are truly the heart and soul of this conference. We appreciate the great deal of time and effort that you have put in to make this a fantastic conference.

This conference brings together two very important subjects, which are pressing for many parts of today's world. As climate change continues to change weather patterns at an ever-increasing rate for many people access to clean and safe water supplies is reaching critical levels. Together with Life Sciences we can forge new avenues to help improve the lives of people everywhere. The ideas being presented here can help create solutions to many problems both in the future as well as in the present.

Before I close, I'd like to thank each of your for attending this conference and bringing your expertise here. You have the vision, the knowledge, the wherewithal and the experience to help pave the way into the future. You are truly our greatest asset today and tomorrow, and we could not accomplish what we do without your support and leadership. Throughout this conference, I ask you to stay engaged, keep us proactive and help us shape the future. My personal respect and thanks goes out to all of you.

Sincerely,

Gary Smith Hara

ACLS / WASH 2014 ACKNOWLEDGEMENTS

We extend our deepest appreciation to the following institutions and people:

ACLS / WASH 2014 Sponsor
The PRESDA Foundation (Japan)

ACLS / WASH 2014 Institutional Affiliates
University of Guadalajara (Mexico)
Sonoma State University (USA)
The Institute for Environmental Science & Policy, University of Illinois at Chicago (USA)
Norwegian University of Life Sciences (Norway)
Dominican University of California (USA)
The Faculty of Agricultural Production at Maejo University (Thailand)
Cervelet Management and Strategy Consultants (USA)
The International Management Institute (India)

ACLS / WASH 2014 Program Committee
Takayuki Yamada, Chairperson, The PRESDA Foundation (Japan)
Gary Smith-Hara, Environmental Filmmaker, Seven Summits
Prof John Latzo, Pohang University of Science and Technology (Rep. of Korea)
Salvador Peniche, Ph.D., Professor, Economics Dept, University of Guadalajara (Mexico)
Martin G. Romero-Morett, Ph.D, Chair, Economics Dept, University of Guadalajara (Mexico)
William P. Kittredge, Ph.D., President of Cervelet Management and Strategy Consultants
Prawit Puddhanon, Ph.D., Maejo University (Thailand)
Luis Maria Bo-ot, Ph.D., University of the Philippines – Dillman
Bhimaraya Metri, Ph.D., The International Management Institute (India)
Michael Sasaoka, Academic Director, Tierra Corp. (Japan)
Claudio Delang, Ph.D., Hong Kong Baptist University (Hong Kong, SAR)
Gabriel Barhaim, Ph.D., Bar Il-lan University (Israel)
John Stayton, Executive Director, Graduate Programs, Sonoma State University (USA)
Sayantan Sinha, PRESDA Foundation Board of Advisers (Japan)
Oliver Rabara, Ph.D., University of the Northern Philippines

About the Sponsor

The Pacific Rim Education for Sustainable Development Alliance (PRESDA) was established in 2010. We are an alliance of grassroots non-profit organizations, teachers, business people, and concerned citizens from around Asia and beyond who wish to make a difference. Incorporated under the laws of Japan, the PRESDA Foundation is an independent, not-for-profit foundation helping to fulfill the Millennium Development Goals by the year 2015.

For more information about the PRESDA Foundation and our projects, please visit our homepage at www.presdafoundation.org

Finally, on behalf of the ACLS / WASH 2014 team, we would like to extend our deepest thanks to the people of Hiroshima City for their support and gracious hospitality.

ACLS / WASH 2014 GENERAL INFORMATION

DESIGNATED HOTEL – KKR Hotel Hiroshima

All ACLS / WASH sessions will be held in the KKR Hotel Hiroshima, which is located a short distance from Hiroshima Castle and the government offices of Hiroshima Prefecture.

Three public transportation rail systems pass within a short distance of the KKR Hotel Hiroshima:

1. JOHOKU Station on the ASTRAM Rail Line is only 3 minutes west on foot.
2. HAKUSHIMA Station on the Dentetsu Streetcar Line (also known as the 'Hiroden') is also 3 minutes west on foot. Please transfer at "Hacchobori" to Hakushima-line and get off at the termination.
3. JR HIROSHIMA Station or JR YOKOGAWA Station is only 5 minutes by taxi

VENUE

As one of the largest cities in western Japan, Hiroshima has been a bustling, affluent and prosperous city with a rich history dating back to 1589, when it was established as the capital city of a powerful samurai warlord. Today, of course, Hiroshima is most well-known as the first city in the world to have suffered an atomic bombing, which occurred towards the end of World War II on August 6, 1945. Around the city of Hiroshima, you will find many well-used green spaces, none of which are more important than the poignant Peace Memorial Park, where various statues and monuments reside, as well as the A-Dome building, which has become a symbol of peace and has been registered as a UNESCO World Heritage Site. Apart from war memorials, Hiroshima boasts some of the best traditional Japanese landscaping, including the beautiful Shukkeien Garden complete with tea houses, ponds and bridges.

Finally, there is the iconic Miyajima Shrine Island, which is revered as on of the "three views of Japan" with its towering red torii gate standing in the midst of the inland sea. Built in 593, it was renovated to its current splendor in 1168. The main hall of the shrine is furnished with vermillion-lacquered columns and Japanese cypress bark roofing. The shrine pavilion is a unique example of Heian Period architecture and has been designated as a national treasure of Japan. The Miyajima Shrine is also a UNESCO World Heritage Site and its close proximity to Hiroshima makes for a wonderful afternoon visit.

DRESS

During conference sessions and receptions, the expected dress code is business casual.

CLIMATE

In general, expect Hiroshima to be hot and humid. August temperatures in Hiroshima can range from an average high of 37.9 C (100 F) to an average low of 24.8 C (76 F). The average daily temperature is 32.5 C (90 F).

When moving around outside we suggest wearing the following:

- light, casual clothes
- a hat (preferably with a wide brim)
- sunglasses
- comfortable walking shoes
- sun screen

In addition, you should drink plenty of fluids to remain hydrated and also consider carrying a hand towel or face towel.

EQUIPMENT

Presentation rooms are equipped with PC notebook computers, screens, projectors, laser pointers and microphones. We also provide onsite technical support, if needed.

If possible, please send us your presentation in advance, which we can upload to the computers, which will save time and avoid glitches.

POSTER SESSION STRUCTURE

We will have two poster sessions during the conference. The first will be during the welcome dinner reception on August 27th. The second poster session will be during the networking reception on August 28th.

If you are giving a poster presentation, here are the instructions for poster presenters:

1. Poster boards mounted on stands will be provided with tape.
2. Posters must be set up 15 minutes before the start of the session and taken down after the session ends.
3. Poster materials should include the title of the presentation and the list of authors. Bring all illustrations needed — figures, tables, color photographs, charts.
4. Dimensions for posters should be A0 size portrait style 47 x 33 inches (118cm x 84cm). Portrait style is standard but if you need to use landscape that is fine.
5. Poster material should be well labeled and easy to read from a distance of one meter. Lettering should be bold. If you will be posting typed material, use a large font size (24 to 30 pt) on white, pale yellow or cream-colored non-glossy paper. Avoid use of non-standard fonts. Use 1-inch margins. Use upper and lower case letters.
6. No audiovisual equipment is permitted for poster presentations.
7. Please stay near your poster so that you are available to answer questions.

ORAL SESSION STRUCTURE

Sessions A, B and C on August 28th will be 75 minutes in length with a 15-minute break before the next session begins. Presenters have been allotted 25 minutes, which includes time for questions and answers. If you need additional time for discussion, please do so during the recess periods or coffee breaks.

Session D on August 29th is a special featured session, which will last 2 hours and have 4 presenters. Each presenter has been allotted 30 minutes, which includes time for questions and answers.

** Please give your presentation within the allotted time on the schedule. If a presenter does not arrive at the scheduled time, please proceed with the next presenter.*

PROCEEDINGS

Presenters will receive the proceedings in electronic format by September 5, 2014. As we are attempting to be a carbon-neutral conference as much as possible, we kindly request participants to understand the proceedings will be in electronic format only:

- **ACLS ISSN 2188-3971**
- **WASH ISSN 2188-3440**

INTERNET ACCESS
WIFI access is available in the KKR lobby area. Please see the front desk for login and password details. If you are staying in the KKR, then you can access the Internet free-of-charge in your room using the LAN cable provided.

Having difficulty connecting to the WIFI inside the KKR? Please note that the PRESCO organizers have no control over the WIFI access, so please inquire at the hotel front desk.

PRINTING
Photocopies can be made at the Lawson convenience store across from the KKR. Alternatively, for larger printing needs, there is a Kinko's located on Hon-Dori Avenue. See the KKR front desk for directions.

STORES NEAR THE KKR
There are two convenience stores within easy walk of the KKR: the nearest is a Lawson across from the KKR. Also, there is a 7-11 approximately 5 minutes on foot. Ask the front desk for a map and directions.

Banks / ATM / Currency Exchange
There is a bank that offers currency exchange within 5 minutes walk from the KKR. Please ask the hotel front desk for a map and directions.

All banks in Hiroshima are open until 3:00 p.m. Monday to Friday and closed on Saturday and Sunday. Please remember to bring your passport when exchanging money. International ATMs with English menus are also available at **7-Eleven** convenience stores, which are open 24 hours with locations throughout the city.

Tour Options: UNESCO World Heritage Site of Miyajima

Half-Day Tour to Miyajima: 'The Shrine Island'

Constructed in 593 A.D., Itsukushima (popularly known as Miyajima Shrine Island) was undamaged by the atomic bombing. The island consists of a series of temple buildings, teahouses and quaint shops. The long pier from which the torii in the sea can be seen offers majestic views of the inland sea and is considered one of Japan's three most iconic views. As a UNESCO World Heritage site, it is considered a 'must see' for travelers to Japan. The three-hour tour includes roundtrip transportation, temple fees and guidance in English. Due to the popularity of this tour, the number of participants is limited.

Full-Day Tour to Miyajima: 'The Shrine Island' + Mt. Misen Hiking Trail

The tour begins with a visit to Miyajima: The Shrine Island. After the tour of Miyajima, you will go to Mt Misen: at 500 meters above sea level, Mount Misen is the highest peak on Miyajima. It is also considered a holy mountain with a number of Buddhist structures near the peak, some of which are more than 1200 years old. It affords spectacular views of the Seto Inland Sea and as far as Hiroshima City. A ropeway leads up the mountain from town, but it is also possible to climb up on foot. There are three hiking trails leading up Misen. It takes 1.5 to 2 hours to reach the summit from town. The six-hour tour includes roundtrip transportation, ferry fees, lift fees and guidance in English. The hiking portion of the tour is led by an experienced guide, hiker and photographer.

** Details regarding tour dates, departure times and costs are available at the registration table.*

Table of Contents

Page 1:	Main Schedule Overview (Day 1, 2, 3)
Pages 2-4:	Section I: Featured Presentations
Pages 5- 9:	Section II Oral Session A
Pages 10-14:	Section III Oral Session B
Pages 15-18:	Section IV Oral Session C
Pages 18-21:	Section V Oral Session D
Pages 21-27:	Section VI Poster Session A & Welcome Reception
Pages 27-32:	Section VII Poster Session B & Networking Reception
Pages 32-34:	Section VIII Virtual Presentations
Page 35:	Section IX Luncheon and Closing Remarks

The Asian Conferences on the Life Sciences & Sustainability
The Asian Symposium on Water, Sanitation and Hygiene
August 27-29, 2014 at the KKR Hotel Hiroshima, Japan

MAIN SCHEDULE OVERVIEW

DAY 1: Wednesday, August 27, 2014

13:00 to 16:45	Participant Check-in (Name Badge, Certificate, Receipt, Schedule, Tour information, etc.) Location: KKR Hotel, Main floor lobby
17:00 to 19:00	Commencement (attendance is open to registrants only)*
17:00 to 17:15	Address from Host Organizer
17:15 to 17:20	Welcome Toast from Chairman
17:20 to 19:30	Dinner Reception
17:20 to 19:30	Poster Session A

DAY 2: Thursday, August 28, 2014

08:00 to 09:00	Participant Check-in (Name Badge, Certificate, Receipt, Schedule, Tour information, etc.)
08:00 to 09:15	Virtual Presentations
09:00 to 09:15	Introduction
09:15 to 09:45	Featured Presentation I
09:45 to 10:00	Question and Answer
10:00 to 10:30	Featured Presentation II
10:30 to 10:45	Question and Answer
10:45 to 11:15	Featured Presentation III
11:15 to 11:30	Question and Answer
11:30 to 12:30	Welcome Lunch
12:30 to 13:00	Oral Presenter Set-up Period
13:00 to 14:15	Oral Session A
14:30 to 15:45	Oral Session B
16:00 to 17:15	Oral Session C
16:45 to 17:15	Poster Presenter Set-up
17:15 to 18:30	Networking Reception
17:15 to 18:30	Poster Session B

DAY 3: Friday, August 29, 2014

09:00 to 11:00	Oral Session D
11:00 to 12:00	Closing Remarks
12:00 to 16:00	Optional Tour

Section I
Featured Presentations

Thursday, August 28, 2014
09:15 to 11:30

FEATURED PRESENTATIONS

Thursday, August 28, 2014
09:15 to 11:30

Featured Presenters

Dr Erizal Mukhtar of Andalas University, Indonesia
Dr David O'Brien of the University of Melbourne, Australia
Dr Emmanuel Akpabio of Kyoto University, Japan

08:00 to 09:00 Participant Check-in
09:00 to 09:15 Introduction

Featured Presentation I by Dr Erizal Mukhtar
09:15 to 09:45
09:45 to 10:00 Question and Answer

Ecology
0143 *The change of tree diversity and carbon stock during three decades in Ulu Gadut, West Sumatra, Indonesia*
Erizal Mukhtar, Faculty of Mathematics and Natural Science, Andalas University, Indonesia[1]
Syamsuardi, Faculty of Mathematics and Natural Science, Andalas University, Indonesia[2]
Hermansah, Department of Soil Science, Faculty of Agriculture, Andalas University, Indonesia[3]

Featured Presentation II by Dr David O'Brien
10:00 to 10:30
10:30 to 10:45 Question and Answer

Household Treatment and Safe Storage
0215 *Climate change, health and composting toilets in coastal communities: Sipaia, Papua New Guinea*
David O'Brien, Faculty of Architecture, Building and Planning, The University of Melbourne, Australia[1]
Matt Breen, Faculty of Architecture, Building and Planning, The University of Melbourne, Australia[2]
Ilari Lehtonen, Faculty of Architecture, Building and Planning, The University of Melbourne, Australia[3]

Featured Presentation III by Dr Emmanuel Akpabio
10:45 to 11:15
11:15 to 11:30 Question and Answer

Hygiene and Behavioral Change
0210 *Science or Local Knowledge? Exploring the Worlds of WASH and the Dilemma of Intervention in sub-Saharan Africa*
Emmanuel Akpabio, Department of Geography and Natural Resource Management, University of Uyo, Akwa Ibom State, Nigeria; (Visiting Scholar) Kyoto University, Japan[1]

Featured Presenter Biographies

Featured Presentation I: Erizal Mukhtar has been teaching at Andalas University since 1986, where he specializes in forest ecology with an emphasis on carbon stock and regeneration processes. Much of his field research has been conducted through the study of a permanent plot in the Ulu Gadut Forest in West Sumatra, which was established by Andalas University. In addition, he has conducted research collaboration with several scientists from some of Japan's most prestigious national universities: Kyoto University, Osaka University, Kagoshima University and Kyushu University. Erizal Mukhtar earned his masters at Ehime University (JP) and his doctorate at Yokohama National University. He is a member of the Japanese Association of Tropical Ecology (JASTE).

…

Featured Presentation II: David O'Brien has 25 years experience in the construction field designing and building for both commercial and domestic clients. He has project managed the construction of community development projects in Western Australia, the Northern Territory, Queensland, Thailand and Papua New Guinea. His teams include trainees, students, architects, engineers, building contractors, government agencies and aid workers. His academic research investigates the links between construction technologies and sustainable housing cultures. Since 2008, David has coordinated the Bower Studio and has more recently developed the HomesPLUS housing initiative. Research fellowships, academic prizes, institute awards and the Australian Government through the Direct Aid Program and AusAID have supported David's programs in Thailand and Papua New Guinea. He has worked closely with eleven community groups as well as six partner universities. David's work with indigenous communities within Australia has significant and widespread support from the University of Melbourne, Federal and Territory Governments, industry partners, philanthropic agencies and various Aboriginal corporations.

…

Featured Presentation III: Emmanuel Akpabio is a currently a visiting scholar at Kyoto University, Japan. He is also a lecturer in the Department of Geography and Regional Planning, University of Uyo, Nigeria. Emmanuel Akpabio was a Commonwealth scholar (2004-2006) at Lancaster University, UK. He was a British Academy visiting Fellow to Newcastle University, UK (2008). He was Alexander von Humboldt Fellow and Senior Researcher at Center for Development Research, University of Bonn, Germany (2011-2012). He is currently serving as a JSPS Fellow, Disaster Prevention Research Institute, Kyoto University. He is one of the Directors in the Board of the International Water Resources Association (IWRA) and a member of the International Scientific Steering Committee for the XVth World Water Congress to be held at Edinburgh (May 25-29, 2015). He has several publications to his credit. In 2012, he was awarded a prize from The Water Institute at North Carolina University (USA) for the publication of his paper entitled *Water meanings, sanitation practices and hygiene behaviors in the cultural mirror: a perspective from Nigeria*.

Section II
Oral Session A

Thursday, August 28, 2014
13:00 to 14:15

ORAL SESSION A

Thursday, August 28, 2014

Oral Session A
13:00 to 14:15

Topics in Agriculture I
Chidori Room
Presenters: 0132, 0133, 204

13:00 to 13:25
Agriculture
0132 *Profil Fiber Digestibility in Vitro of Oil Palm Trunk Fermented by Phanerochaete chrysosporium*
Yetti Marlida, Animal Nutrition and Feed Technology, Andalas University, Padang, Indonesia[1]
Mirnawati Mirnawati, Animal Nutrition and Feed Technology, Andalas University, Padang, Indonesia[2]
Syukri Arief, Chemisty Department, Andalas University, Padang, Indonesia[3]

13:25 to 13:50
Agriculture
0133 *Supplementation of Sacharomyces cerevisiae and Sapindus rarak on feed based of Oil Palm Frond (OPF) in Goat Diet*
Mardiati Zain, Animal Nutrition and Feed Technology, Andalas University, Indonesia[1]
Jurnida Rahman, Animal Nutrition and Feed Technology, Andalas University, Indonesia[2]
Khasrad Khasrad, Animal Production, Andalas University, Indonesia[3]

13:50 to 14:15
Agriculture
0204 *The supplementation of macro mineral on protein microbial synthesis of forages in West Sumatra, Indonesia*
Evita Yani, Faculty of Animal Husbandry, Andalas University, Indonesia[1]
Maramis, Andalas University, Indonesia[2]
Armina Fariani, Sriwijaya University, Indonesia[3]
Lili Warly, Faculty of Animal Science, Andalas University, Indonesia[4]

ORAL SESSION A

Thursday, August 28, 2014

Oral Session A
13:00 to 14:15

WASH I: Sanitation and Health
Suehiro Room
Presenters: 0203, 0206, 0209

13:00 to 13:25
Sanitation and Health
0203 *Economic Impact of Inadequate Sanitation on Women's Health: A Study in Andhra Pradesh, India*
Suresh Lal, Kakatiya University, Warangal, India[1]

13:25 to 13:50
Ecological Sanitation
0206 *Analysis of Physicochemical and Biological parameters of Vembanad back waters, near Alleppy, Kerala*
Prameela Devi Yalavarthy, Kakatiya University, Warangal, Telangana, India[1]

13:50 to 14:15
Sanitation and Health
0209 *Reframing the role WASH in containing antimicrobial resistance*
Stephanie Fletcher, NPS MedicineWise, Australia[1]

ORAL SESSION A

Thursday, August 28, 2014

Oral Session A
13:00 to 14:15

Microbiology
Aki Room
Presenters: 0124, 0148, 0157

13:00 to 13:25
Microbiology
0124 *Improving The Quality of Rice Bran and Blood Mixture as Poultry Feed Through Fermentation by Bacillus amyloliquefaciens*
Wizna Wizna, Animal Nutrition and Feed Technology, Andalas University, Indonesia[1]
Helmi Muis, Animal Nutrition and Feed Technology, Andalas University, Indonesia[2]
Jafrinur Jafrinur, Animal Nutrition and Feed Technology, Andalas University, Indonesia[3]

13:25 to 13:50
Microbiology
0148 *Isolation and Screening of Thermostable Alkaline Protease Producing Bacillus spp. from Kerinci Hot Spring, Indonesia*
Anthoni Agustien, Department of Biology, Andalas University, Indonesia[1]
Yetria Rilda, Department of Chemistry, Andalas University, Indonesia[2]
R.R. Megahayati, Faculty of Education STKIP PGRI, Indonesia[3]
Nasrazudhy, Polytechnic of Medicine, Indonesia[4]
Rozana Zuhry, Faculty of Education STKIP YPM Bangko, Jambi, Indonesia[5]
Akmal Djamaan, Laboratory of Biotechnology, Biota Sumatra, University of Andalas, Indonesia[6]

13:50 to 14:15
Microbiology
0157 *Hydrolysation of Palm Oil Empty Fruit Bunch by Using Crude Extract Enzymes of Aspergillus niger and Analysis of Its Hydrolizates for Bioethanol Production*
Kahar Muzakhar, Biology Department, Faculty of Math and Natural Sciences, The University of Jember, Jember, Indonesia[1]
Sutoyo, Biology Department, Faculty of Math and Natural Sciences, The University of Jember, Jember, Indonesia[2]
Siswoyo, Biology Department, Faculty of Math and Natural Sciences, The University of Jember, Jember, Indonesia[3]
Rosita Fitrah Dewi, Biology Department, Faculty of Math and Natural Sciences, The University of Jember, Jember, Indonesia[4]
Widya Yuniar, Biology Department, Faculty of Math and Natural Sciences, The University of Jember, Jember, Indonesia[5]

ORAL SESSION A

Thursday, August 28, 2014

Oral Session A
13:00 to 14:15

Biotechnology I
Takasago Room
Presenters: 0138, 0141, 0144

13:00 to 13:25
Biotechnology
0138 *Effect of Impeller Tip Speed on Biosurfactant Production by Streptomyces sp. R1*
Nor Syafirah Zambry, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[1]
Latiffah Zakaria, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[2]
Ahmad Ramli Mohd Yahya, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[3]

13:25 to 13:50
Biotechnology
0141 *Isolation and Identification of Actinobacterial-Biosurfactant Producers*
Intan Sakinah Mohd Anuar, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[1]
Mor Kar Mun, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[2]
Ahmad Ramli Mohd Yahya, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[3]

13:50 to 14:15
Biotechnology
0144 *Rhamnolipid production in Pseudomonas aeruginosa USM-AR2 fed-batch fermentation*
Nur Asshifa Md Noh, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[1]
Ahmad Ramli Mohd Yahya, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia[1]

Section III
Oral Session B

Thursday, August 28, 2014
14:30 to 15:45

ORAL SESSION B

Thursday, August 28, 2014

Oral Session B
14:30 to 15:45

Topics in Agriculture II
Chidori Room
Presenters: 0107, 0203, 0205

14:30 to 14:55
Agriculture
0107 *Influence of Dietary Buffer and Rice Straw in Lactating Cows Fed Pineapple Peel as Main Roughage*
Suntorn Wittayakun, Rajamangala University of Technology Lanna, Lampang Campus,Thailand[1]
Weera Innaree, Rajamangala University of Technology Lanna, Lampang Campus,Thailand[2]
Worawut Chainetr, Rajamangala University of Technology Lanna, Lampang Campus,Thailand[3]
Sukunya Innaree, Rajamangala University of Technology Lanna, Lampang Campus,Thailand[4]
Charoen Saengdee, Rajamangala University of Technology Lanna, Lampang Campus,Thailand[5]

14:55 to 15:20
Agriculture
0203 *Chromium Status of Lactating Cows Supplemented with Organic Chromium and Ganoderma lucidum*
Fauziah Agustin, Faculty of Animal Husbandry, Andalas University, Indonesia[1]
Toto Toharmat, Bogor Agriculture University, Indonesia[2]
Dwi Erra Evvyerni, Bogor Agriculture University, Indonesia[3]
Darmono Taniwiryono, Research Institute of Indonesia Plantation Biotechnology, Bogor, Indonesia[4]
S. Tarigan, Research Institute of Veterinary, Bogor, Indonesia[5]

15:20 to 15:45
Agriculture
0205 *Improving the Quality of Palm Kernel Cake Content as Poultry Feed Through Fermentation by Combination of Various Microbe and Substrate Composition*
Mirnawati Mirnawati, Faculty of Animal Nutrition and Feed Technology, Andalas University, Indonesia[1]
Ade Djulardi, Faculty of Animal Science, Andalas University, Indonesia[2]
Yetti Marlida, Faculty of Animal Science, Andalas University, Indonesia[3]

ORAL SESSION B

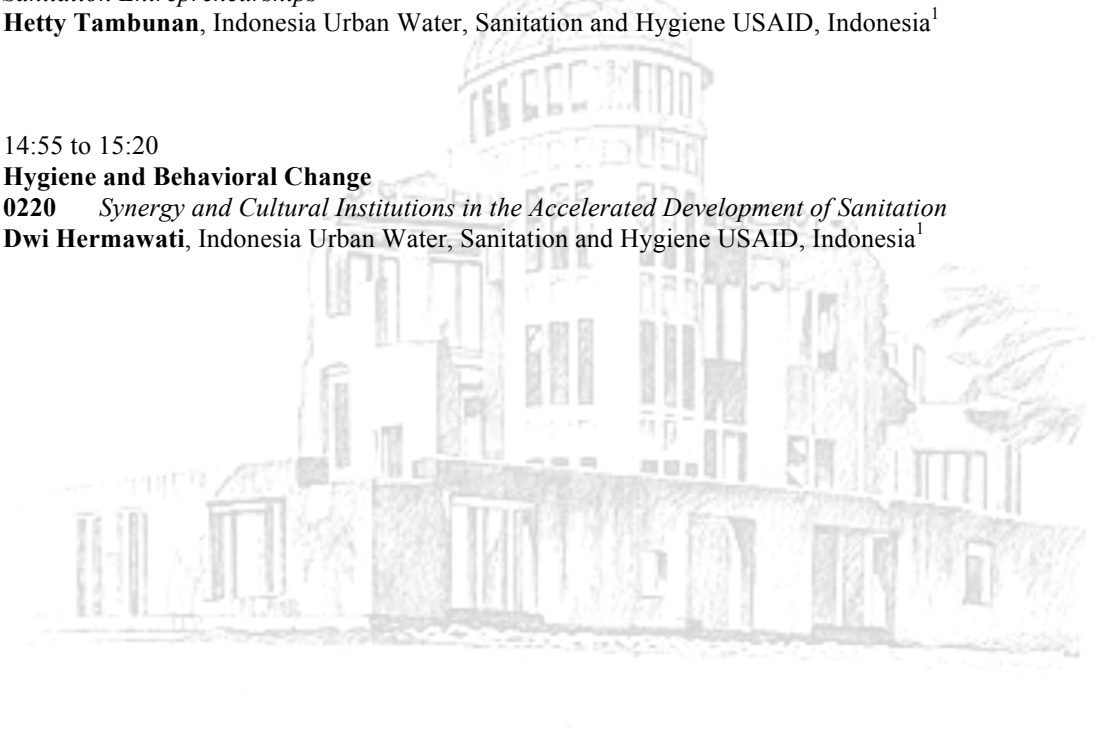
Thursday, August 28, 2014

Oral Session B
14:30 to 15:45

WASH II: Sanitation & Hygiene
Suehiro Room
Presenters: 0204, 0220

14:30 to 14:55
Sanitation: Urban and Rural
0204 *Driving Improvement Access of Individual Sanitation towards Awakening the Model of Sanitation Entrepreneurships*
Hetty Tambunan, Indonesia Urban Water, Sanitation and Hygiene USAID, Indonesia[1]

14:55 to 15:20
Hygiene and Behavioral Change
0220 *Synergy and Cultural Institutions in the Accelerated Development of Sanitation*
Dwi Hermawati, Indonesia Urban Water, Sanitation and Hygiene USAID, Indonesia[1]



ORAL SESSION B

Thursday, August 28, 2014

Oral Session B
14:30 to 15:45

Biodiversity and Conservation Biology
Aki Room
Presenters: 0119, 0122, 0154

14:30 to 14:55
Biodiversity and Conservation Biology
0119 *Managing bamboo resources for rural sustenance in Arunachal Himalaya, Northeast India*
Sumpam Tangjang, Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh, India[1]
Tapi Taka, Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh, India[2]
Tonlong Wangpan, Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh, India[3]

14:55 to 15:20
Biodiversity and Conservation Biology
0122 *Diversity of Wild Orchid species in West Sumatera, Indonesia*
Marlis Rahman, Graduate Study Program, Andalas University, Indonesia[1]
Mairawita Marlis, Department of Biology, Andalas University, Indonesia[2]

15:20 to 15:45
Biodiversity and Conservation Biology
0154 *Geographic Variation of Morphological Characters in Puntius lateristriga (Valenciennes, 1842) from Sumatra and the Adjacent Island*
Dewi Imelda Roesma, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Indonesia[1]
Ada Chornelia, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Indonesia[2]

ORAL SESSION B

Thursday, August 28, 2014

Oral Session B
14:30 to 15:45

Biotechnology II
Takasago Room
Presenters: 0125, 0190, 0191

14:30 to 14:55
Biocontrol and Biopesticide
0125 *Biopesticides of Elettariopsis slahmong and Cimbopogon nardus against Trigona minangkabau the vector of Blood Disease Bacterium on banana in Indonesia*
Nasril Nasir, Faculty of Mathematics and Natural Science, Andalas University, Indonesia[1]
Abdi Dharma, Faculty of Mathematics and Natural Science, Andalas University, Indonesia[2]
Fumio Hashimoto, Faculty of Agriculture, Kagoshima University, Japan[3]
Nurmansyah, Spice and Estate Crops Research Institute, Bogor, Indonesia[4]
Mairawita, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia[5]
Fredrika Eliesti, Faculty of Agriculture, The University of Muhammad Yamin, Indonesia[6]

14:55 to 15:20
Biotechnology
0190 *Electro-osmotic parthenogenetic activation of porcine oocyte*
Candrani Khoirinaya, College of Veterinary Medicine, Seoul National University, Korea[1]
Islam M. Saadeldin, Seoul National University, South Korea, College of Veterinary Medicine, Seoul National University, Korea[2]
Su Jin Kim, Seoul National University, South Korea, College of Veterinary Medicine, Seoul National University, Korea[3]
Sanghoon Lee, Seoul National University, South Korea, College of Veterinary Medicine, Seoul National University, Korea[4]
Hyun Ju Oh, Seoul National University, South Korea, College of Veterinary Medicine, Seoul National University, Korea[5]
Byeong Chun Lee, Seoul National University, South Korea, College of Veterinary Medicine, Seoul National University, Korea[6]

15:20 to 15:45
Biotechnology
0191 *Improving Canine Semen Preservation via Modifying the Osmolyte Content*
Erif Maha Nugraha Setyawan, College of Veterinary Medicine, Seoul National University, Korea[1]
Hyun Ju Oh, College of Veterinary Medicine, Seoul National University, Korea[2]
Min Jung Kim, College of Veterinary Medicine, Seoul National University, Korea[3]
Geon A Kim, College of Veterinary Medicine, Seoul National University, Korea[4]
Young Kwang Jo, College of Veterinary Medicine, Seoul National University, Korea[5]
Seok Hee Lee, College of Veterinary Medicine, Seoul National University, Korea[6]
Yoo Bin Choi, College of Veterinary Medicine, Seoul National University, Korea[7]
Se Chang Park, College of Veterinary Medicine, Seoul National University, Korea[8]
Byeong Chun Lee, College of Veterinary Medicine, Seoul National University, Korea[9]

Section IV
Oral Session C

Thursday, August 28, 2014
16:00 to 17:15

ORAL SESSION C

Thursday, August 28, 2014

Oral Session C
16:00 to 17:15

Conservation and Ecology
Chidori Room
Presenters: 0123, 0126, 0158

16:00 to 16:25
Agriculture
0123 *Transmission Mechanism of Ralstonia solanacearum Phylotype IV by Insects on Banana Plant in West Sumatera*
Mairawita Marlis, Department of Biology, Andalas University, Indonesia[1]
Trimurti Habazar, Faculty of Agriculture, Andalas University, Indonesia[2]
Nasril Nasir, Faculty of Mathematics and Natural Science, Andalas University, Indonesia[3]
Suswati, Department of Agriculture, Medan Area University, Indonesia[4]
Ahsol Hasyim, Fruit Plants Research Institute, Bogor, Indonesia[5]

16:25 to 16:50
Agriculture
0126 *The Long-term Effect of Cascade Irrigation System on the Paddy soil Properties and Rice Productivity in West Sumatra, Indonesia*
Darmawan Darmawan, Faculty of Agriculture, Andalas University, Indonesia[1]

16:50 to 17:15
Insect Ecology and Diversity
0158 *Ant Species (Hymenoptera: Formicidae) in Dragon fruit plantations (Hylocereus spp.) of West Sumatera, Indonesia*
Henny Herwina, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia[1]
Rijal Satria, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia[1]
Diyona Putri, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia[1]
Ranny, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia[1]
Yaherwandi, Department of Pests and Plant Deseases, Faculty of Agriculture, Andalas University, Indonesia[2]
Jumjunidang, Indonesian Tropical Fruit Research Institute, Indonesia[3]

ORAL SESSION C

Thursday, August 28, 2014

Oral Session C
16:00 to 17:15

Water Policy, Governance and Sustainability
Suehiro Room
Presenters: 0163, 0207, 0214

16:00 to 16:25
Data Management
0163 *FDI and Governance Sustainability*
Chun-Ping Chang, Shih Chien University, Taiwan[1]

16:25 to 16:50
Water Policy and Governance
0207 *Drivers of water use in China from 2002 to 2010*
Yuan Zhi, School of Environment, Beijing Normal University, Beijing, P.R. China[1]
Zhifeng Yang, School of Environment, Beijing Normal University, Beijing, P.R. China[2]
Xin-an Yin, School of Environment, Beijing Normal University, Beijing, P.R. China[3]

16:50 to 17:15
Water Policy and Governance
0214 *Long-term characteristics of dry season water allocation in the Chao Phraya River basin, Thailand: Implications for comparative institutional analysis of water governance*
Mari Takeda, The University of Tokyo, Japan[1]
Athit Laphimsing, Chulalongkorn University, Thailand and The University of Tokyo, Japan[2]
Aksara Putthividhya, Chulalongkorn University, Thailand and The University of Tokyo, Japan[3]

ORAL SESSION C

Thursday, August 28, 2014

Oral Session C
16:00 to 17:15

Agriculture and Aquaculture
Takasago Room
Presenters: 0208, 0209, 0210

16:00 to 16:25
Nutrition Science
208 *Fermented Product by Phanerochaete chrysosporium and Neurospora crassa in the Diet of Broiler*
Nuraini Nuraini, Faculty of Animal Science, Andalas University, Indonesia[1]
Ade Djulardi, Faculty of Animal Science, Andalas University, Indonesia[2]
Maria Endo Mahata, Faculty of Animal Science, Andalas University, Indonesia[3]

16:25 to 16:50
Agriculture
209 *The Application of the LEISA System (Low External Input and Sustainable Agriculture) on the Growth and Production of King Grass (Pennisetum Purpupoides) on the second Harvesting*
Lili Warly, Faculty of Animal Science, Andalas University, Indonesia[1]
Suyitman, Faculty of Animal Science, Andalas University, Indonesia[2]
Armina Fariani, Faculty of Animal Science, Andalas University, Indonesia[3]
Evita Yani, Faculty of Animal Science, Andalas University, Indonesia[4]
Mega Delta Riska, Faculty of Animal Science, Andalas University, Indonesia[5]

16:50 to 17:15
Agriculture
210 *Factors Affecting the Animal Food Consumption by Household Consumers In Padang City, West Sumatra, Indonesia*
Jafrinur Jafrinur, Faculty of Animal Science, Andalas University, Indonesia[1]
Noni Novaista, Faculty of Animal Science, Andalas University, Indonesia[2]

Section V
Oral Session D

Friday, August 29, 2014
09:00 to 11:00

ORAL SESSION D

Friday, August 29, 2014

Oral Session D
09:00 to 11:00

WASH Perspectives and the Global Impact
Aki Room
Presenters: 0201, 0208, 0213, 0228

09:00 to 09:30
Prevention and Treatment of Waterborne Illnesses
0201 *Typhoid outbreak – Pune district, Maharashtra, India, 2014*
Kapil Goel, National Center for Disease Control, New Delhi, India[1]

09:30 to 10:00
Water and Sanitation Capacity Building
0208 *WASH Capacity Building through Empowering Disadvantaged Youth: The Engineering Orphanage Approach*
J. Scott Remer, System and Information Engineering, University of Virginia, USA[1]
Garrick Louis, System and Information Engineering, University of Virginia, USA[2]

10:00 to 10:30
Water Quality
0213 *Determination of Trimethylamine at Ultra-Trace–Level in Ultra-Pure Water Samples by Gas Chromatography–Mass Spectrometry Coupled with Purge and Trap*
Feng GU, GE Singapore Water Technology Centre at NUS, Singapore, GE Water & Process Technologies, Singapore[1]
Qiantao Cai, GE Singapore Water Technology Centre at NUS, Singapore, GE Water & Process Technologies, Singapore[2]
George Delong, GE Water & Process Technologies, USA[3]

10:30 to 11:00
Sanitation in Schools
0228 *Promoting Sanitation and Hygiene through Schools - Some Strategic Interventions*
Bidhu Samanta, Multi Applied System, India[1]

ORAL SESSION D

Friday, August 29, 2014

Oral Session D
09:00 to 11:00

Perspectives in the Life Sciences
Takasago Room
Presenters: 0150, 0187, 0189, 0196

09:00 to 09:30
Sport Science
0150 *The effects of stress management intervention on physiological arousal before competition among track and field Malaysian young athletes*
Norsilawati binti Abdul Razak, Sultan Idris University of Education, Tanjung Malim, Perak, Malaysia[1]
Balbir Singh Gill, University of Malaya, Malaysia[2]

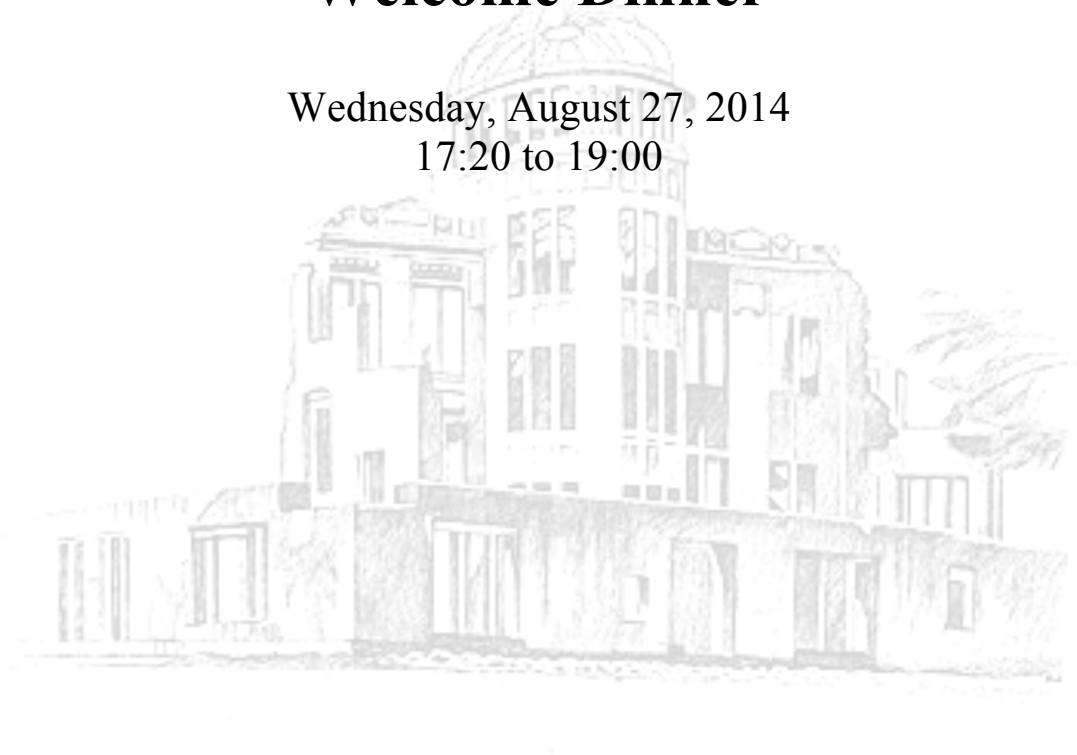
09:30 to 10:00
Neuroscience
0187 *Human Behavior Related with Cut Locus in Daily Life*
Nathaphon Boonnam, School of Science and Technology, Tokai University, Japan[1]
Pakkinee Chitsakul, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Thailand[2]

10:00 to 10:30
Pharmacology and Pharmaceutical Science
0189 *Antibiotic utilization in dental teaching hospitals: The importance of guidelines*
Mayu Winnie Rachmawati, Universitas Gadjah Mada, Indonesia; Kanazawa University, Japan[1]
Naoko Yoshida, Kanazawa University, Japan[2]
Hirohito Tsuboi, Kanazawa University, Japan[3]
Kazuko Kimura, Kanazawa University, Japan[4]
Andrea Orozco Lara, Kanazawa University, Japan[5]

10:30 to 11:00
Nutrition Science
0196 *Emotion Estimation from Facial Expressions Using Kinect*
Chaiyapruk Thongkam, Graduate School of Engineering, Tokai University, Japan[1]
Osamu Uchida, Department of Human and Information Science, Tokai University, Japan[2]
Natthapon Boonnam, School of Science and Technology, Tokai University, Japan[3]

Section VI
Poster Session A
&
Welcome Dinner

Wednesday, August 27, 2014
17:20 to 19:00



POSTER SESSION A

Wednesday, August 27, 2014

16:45 to 17:15
Poster Set-up Period

17:15 to 18:30
Poster Presenters

0103, 0104, 0106, 0108, 0109, 0115, 0117, 0118, 0120,
0121, 0128, 0135, 0140, 0145, 0146, 0151, 0152, 0153

LIST OF POSTER PRESENTERS IN SESSION A

Biopolymers
0103 *Bone morphogenetic protein 2 (BMP-2) gene delivery via polyethylene imines grafted chondroitin sulfate non-viral gene carrier*
Po-Liang Lai, Chang Gung University, Taoyuan, Taiwan[1]
Yu-Shiang Peng, Chang Gung University, Taoyuan, Taiwan[2]
I-Ming Chu, National Tsing Hua University, Hsinchu, Taiwan[3]

Biopolymers
0104 *Glial cell line-derived neurotrophic factor (GDNF) gene delivery via a polyethylene imine grafted chitosan carrier*
Yu-Shiang Peng, Chang Gung Memorial Hospital, Linkou, Taiwan[1]
I-Ming Chu, National Tsing Hua University, Hsinchu, Taiwan[2]
Po-Liang Lai, Chang Gung University, Taoyuan, Taiwan[3]

Microbiology
0106 *In vitro antimicrobial properties of propolis against caries-associated microorganisms*
Boonyanit Thaweboon, Mahidol University, Bangkok, Thailand[1]
Sroisiri Thaweboon, Mahidol University, Bangkok, Thailand[2]
Rattiporn Kaypetch, Mahidol University, Bangkok, Thailand[3]

Biotechnology
0108 *Immobilization and Stability Studies of Organic Solvent-tolerant Lipase Production by Bacillus sp. SC3*
Chotmanee Soisuwan, Chiang Mai University, Thailand[1]
Warunya Kaewthip, Chiang Mai University, Thailand[2]
Angkana Wipatanawin, Chiang Mai University, Thailand[3]

Environmental education
0109 *The effect of elementary children's place-based environmental learning though butterfly ecological curriculum module instruction*
Chiung Fen Yen, Providence University, Taichung, Taiwan[1]
W.J. Shen, Providence University, Taichung, Taiwan[2]
Tsung-Wei Yao, Providence University, Taichung, Taiwan[3]

POSTER SESSION A

Wednesday, August 27, 2014
(continued)

Biomechanics
0115 *A LabVIEW Based Personal Authentication System Using Electrostatic Induction Technique*
Koichi Kurita, Kinki University, Higashi-Hiroshima, Hiroshima, Japan[1]
Tomohiro Fukuda, Kinki University, Higashi-Hiroshima, Hiroshima, Japan[2]

Ecology
0117 *Life history traits explain the body size patterns of frogs in different altitudes*
Yeong-Choy Kam, Tunghai University, Taichung, Taiwan[1]
K.T. Feng, Tunghai University, Taichung, Taiwan[2]
M.F. Chuang, Tunghai University, Taichung, Taiwan[3]

Biomechanics
0118 *Noncontact and nonattached human physical activity measurement method by using variation in electric potential of human body*
Koichi Kurita, Kinki University, Higashi-Hiroshima, Hiroshima, Japan[1]
Tomohiro Fukuda, Kinki University, Higashi-Hiroshima, Hiroshima, Japan[2]

Biotechnology
0120 *Isolation and Characterization of Polylactic Acid Degrading Bacteria*
Nuttagarn Gaewyana, Chiang Mai University, Chiang Mai, Thailand[1]
Angkana Wipatanawin, Chiang Mai University, Chiang Mai, Thailand[2]

Physiology
0121 *Enhanced salinity tolerance of the euryhaline tadpoles depends on increased Na+, K+-ATPase expression after salinity acclimation*
Chi-Shiun Wu, Chinese Culture University, Taipei, Taiwan[1]
Jou-Chieh Lai, Tunghai University, Taichung, Taiwan[2]
Yeong-Choy Kam, Tunghai University, Taichung, Taiwan[2]

POSTER SESSION A

Wednesday, August 27, 2014
(continued)

Neuroscience
0128 *Effects and after-effects of voluntary intermittent light finger touch on body sway*
Leif Johannsen, Technical University Munich, Germany[1]
Shu-Zon Lou, Chung Shan Medical University, Taichung, Taiwan[2]
Hui-Ya Chen, Chung Shan Medical University, Taichung, Taiwan[3]

Marine Biology
0135 *Phylogeny and phylogeography study of the Dichotomaria marginata complex in Taiwan*
Pei-Husan Lai, National Changhua University of Education, Changhua, Taiwan[1]
Shao-Lun Liao, National Changhua University of Education, Changhua, Taiwan[2]
Wei-Lung Wang, National Changhua University of Education, Taiwan[3]

Biodiversity and Conservation Biology
0140 *Isolation of microsatellites in the native frog, Polypedates braueri and cross amplification in the alien species P. megacephalus in Taiwan*
Yi-Huey Chen, Chinese Culture University, Taiwan[1]
Yuan Cheng Cheng, Chinese Culture University, Taiwan[2]

Biotechnology
0145 *Enhancement of Stress Tolerance in Transgenic Cyanobacteria to Increase Culture Efficiency in Outdoor*
Hsiang-Yen Su, National Sun Yat-sen University and Academia Sinica, Kaohsiung, Taiwan[1]
Hsiang-Hui Chou, National Sun Yat-sen University, Kaohsiung, Taiwan[2]
Hsien-Jung Chen, National Sun Yat-sen University, Kaohsiung, Taiwan[3]
Te-Jin Chow**,** Fooyin University, Kaohsiung, Taiwan[4]

POSTER SESSION A

Wednesday, August 27, 2014
(continued)

Medical and Veterinary Sciences
0146 *Hepatoprotective and Antioxidant Effects of Wu-Zi-Yuan-Chung-Wan against CCl4-Induced Oxidative Damage in Rats*
Hao Yuan Cheng, Chung-Jen Junior College of Nursing, Health Sciences and Management, Taiwan[1]
Jung Chao, National Yang-Ming University, Taipei, Taiwan[2]
Chung-Jen, National Yang-Ming University, Taipei, Taiwan[3]
Chiu Sung Chuan, Hsin Sheng College of Medical Care and Management, Taoyuan, Taiwan[4]
I-Chien Hsieh, School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung, Taiwan[5]
Lung-Yuan Wu, School of Chinese Medicine for Post Baccalaureate, Kaohsiung City, Taiwan[6]
Wen-Huang Peng, School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung, Taiwan[7]

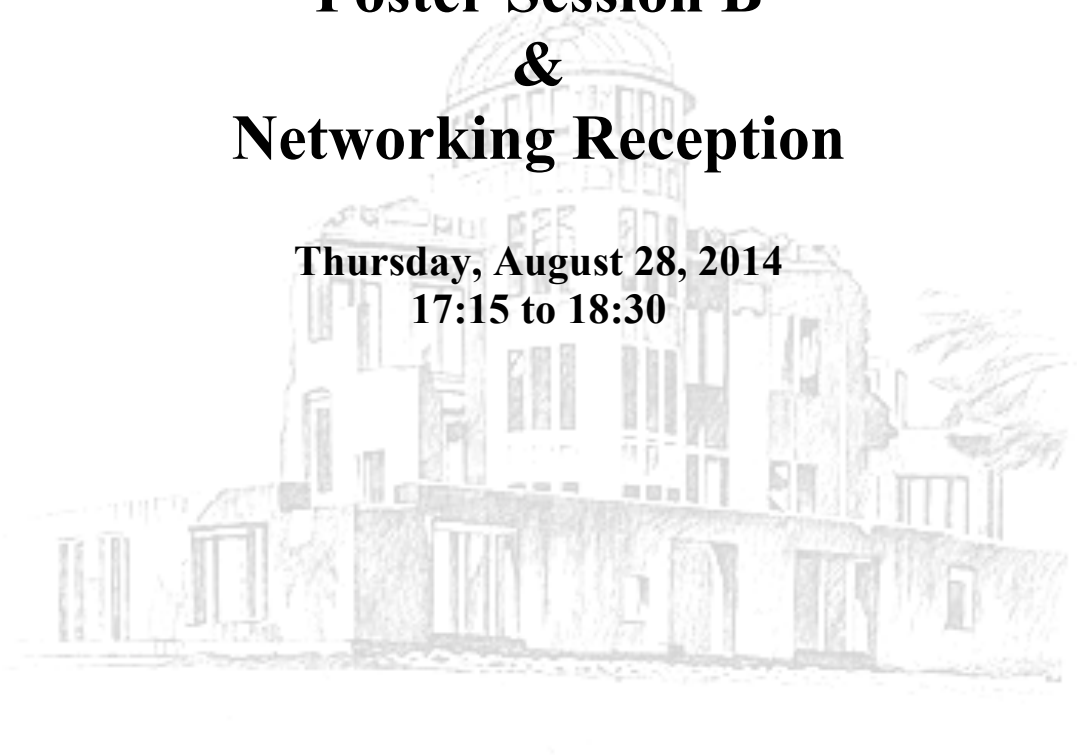
Environmental Science
0151 *Influences of Different Air Layer Thickness of Green Wall on Building Cooling Effect*
Chenyu Chen, National Cheng Kung University,Tainan, Taiwan[1]
Shang-Shuang Huang, National Cheng Kung University, Tainan, Taiwan[2]

Biomedical Sciences
0152 *Cytotoxic and antioxidant activities of group of Thai medicinal ingredient for cancer treatment*
Bhanuz Dechayont, Faculty of Medicine, Thammasat University, Thailand[1]
Chayaporn Limpichai, Faculty of Medicine, Thammasat University, Thailand[2]
Kornrawee Kornwisitwathin, Faculty of Medicine, Thammasat University, Thailand[3]
Arunporn Itharat, Faculty of Medicine, Thammasat University, Thailand[4]

Biomedical Sciences
0153 *Anti-allergic, Anti-inflammatory and Antioxidant activities and Total phenolic content of the bark extracts from Jatropha multifida Linn*
Pathompong Phuaklee, Faculty of Medicine, Thammasat University, Thailand[1]
Chayaporn Limpichai, Faculty of Medicine, Thammasat University, Thailand[2]
Kornrawee Kornwisitwathin, Faculty of Medicine, Thammasat University, Thailand[3]
Arunporn Itharat, Faculty of Medicine, Thammasat University, Thailand[4]

Section VII
Poster Session B
&
Networking Reception

Thursday, August 28, 2014
17:15 to 18:30



SCHEDULE OVERVIEW OF POSTER SESSION B

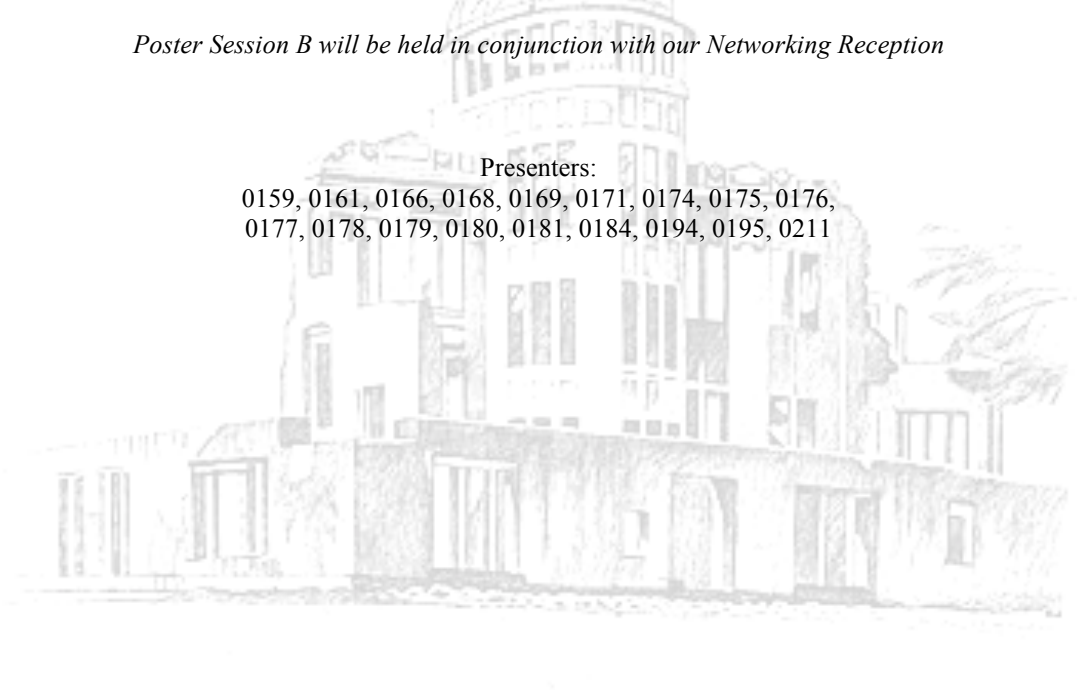
Thursday, August 28, 2014

16:45 to 17:15 Poster Set-up Period

17:15 to 18:30 Poster Session B*

Poster Session B will be held in conjunction with our Networking Reception

Presenters:
0159, 0161, 0166, 0168, 0169, 0171, 0174, 0175, 0176,
0177, 0178, 0179, 0180, 0181, 0184, 0194, 0195, 0211



LIST OF POSTER PRESENTERS IN SESSION B

Sports Science
0159 *Durations of Hyperbaric Oxygen (HBO2) Exposure on Post-Intensive Exercise Recovery in Elite Volleyball Athletes*
Chartlada Pawong, Sports Authority of Thailand, Bangkok,Thailand[1]

Environmental Science
0161 *Study of indoor air quality in public area by mobile learning of students*
Chihmei Chou, JinWen University of Science and Technology, Taiwan[1]

Health Sciences
0166 *Effect of yoga on self-esteem, body imagery, and subject well-being in menopause women*
Hui-ling Lai, National Taiwan University of Physical Education and Sport, Taiwan[1]
Chiao-lin Nien, National Taiwan University of Physical Education and Sport, Taiwan[2]
Tzu-chieh Jian, National Taiwan University of Physical Education and Sport, Taiwan[3]

Sports Science
0168 *I can't help but love doing it: Examination passion under a social cognitive approach*
Chiao-Lin Nien, National Taiwan University of Physical Education and Sport, Taiwan[1]
Sheng-chun Shen, National Taiwan University of Physical Education and Sport, Taiwan[2]
Wan-shiun Chen, National Taiwan University of Physical Education and Sport, Taiwan[3]

Biomedical Sciences
0169 *Protective Effects of Liposomal Astaxanthin Against Lipopolysaccharide-Induced Acute Hepatotoxicity*
Charng-Cherng Chyau, Hungkuang University, Taiwan[1]
Pin-Der Duh, Department of Food Science and Technology, Chia Nan University of Pharmacy and Science, Taiwan[2]
Hsiang-Ting Lin, Research Institute of Biotechnology, Hungkuang University, Taiwan[3]
Chi-Huang Chang, Research Institute of Biotechnology, Hungkuang University, Taiwan[4]
Chun-Hung Chiu, Research Institute of Biotechnology, Hungkuang University, Taiwan[5]

Biomechanics
0171 *Develop an instrumented pedal for three-dimensional kinetic analysis of lower limbs during cycling*
Kai-Han Liang, National Chung-Hsing University, Taichung, Taiwan[1]
Yi-Hung Ho, National Chung-Hsing University, Taichung, Taiwan[2]
Li-Cheng Hsieh, National Chung-Hsing University, Taichung, Taiwan[3]
Hong-Wen Wu, National Taiwan University of Physical Education and Sport, Taiwan[4]

POSTER SESSION B

Thursday, August 28, 2014
(continued)

Food Chemistry, Engineering and Processing
0174 *Chemical Compositions, Amylose and Resistant Starch Content, Functional and Pasting Properties of Starches and Flours Prepared from Mung Bean (Vigna Radiata (L.) R. Wilcz.) and Cowpea (Vigna Unguiculata (L.) Walp.)*
Ratchaneeporn Photinam, Mahasararakham University, Thailand[1]
Anuchita Moongngarm, Mahasarakham University, Thailand[2]
Tatdao Paseephol, Mahasarakham University, Thailand[3]

Food Chemistry, Engineering and Processing
0175 *Effects of Varieties and Harvesting Stages on Chemical Compositions and Bioactive Compounds in Maize (Zea may L.)*
Amaraphon Homduang, Mahasarakham University, Thailand[1]
Anuchita Moongngarm, Mahasarakham University, Thailand[2]
Tatdao Paseephol, Mahasarakham University, Thailand[3]

Biomedical Sciences
0176 *Non uniform deposition of pMDI aerosol in a large volume spacer*
Nicholas Ogrodnik, Carleton University, Canada[1]
Elizabeth Sprigge, Carleton University, Canada[2]

Cell Biology
0177 *Roles of RapGAP5 in developmental process of Dictyostelium*
Taeck Jeon, Chosun University, Republic of Korea[1]
Mi-Rae Lee, Chosun University, Republic of Korea[2]
Ara Lee, Chosun University, Republic of Korea[3]

Cell Biology
0178 *Induction of Apoptosis by Fucoidan in Osteoblasts*
Mi Rae Lee, Chosun University, Republic of Korea[1]
Hyeseon Kim, Chosun University, Republic of Korea[2]
Ara Lee, Chosun University, Republic of Korea[3]
Taeck J. Jeon, Chosun University, Republic of Korea[4]

POSTER SESSION B

Thursday, August 28, 2014
(continued)

Cell Biology
0179 *Subcellular Localization of RapGAP9 in Dictyostelium*
Ara Lee, Chosun University, Republic of Korea[1]
Mi-Rae Lee, Chosun University, Republic of Korea[2]
Madina Hur, Chosun University, Republic of Korea[3]
Taeck J. Jeon, Chosun University, Republic of Korea[4]

Cell Biology
0180 *The effects of fucoidan on cell adhesion and migration in osteoblasts*
Taeck Jeon, Chosun University, Republic of Korea[1]
Hyeseon Kim, Chosun University, Republic of Korea[2]
Mi-Rae Lee, Chosun University, Republic of Korea[3]
Ara Lee, Chosun University, Republic of Korea[4]
Madina Hur, Chosun University, Republic of Korea[5]

Biophysics
0181 *Graphene oxide-based biosensor film for immunoassay applications*
Nan-Fu Chiu, National Taiwan Normal University, Taiwan[1]
Teng-Yi Huang, National Taiwan Normal University, Taiwan[2]

Sports Science
0184 *Social Support as a Norm Predictor for Catharsis in the Life of Student Athletes*
A.Rahim M.Shariff, Sultan Idris Education University, Malaysia
Nik Mohd.Zaki Nik Mohamed, Sultan Idris Education University, Malaysia

Food Chemistry, Engineering and Processing
0194 *Application of Thermosyphon Heat Exchanger for Cooling Oil of a Hydraulic System in Sugarcane Harvester*
Tanongsak Moontree, Mahasarakham University, Thailand[1]
Sampan Rittidech, Mahasarakham University, Thailand[2]
Sanhawat Thongdaeng, Rajamangala University of Technology Isan Sakonnakhon Campus, Thailand[3]

Food Chemistry, Engineering and Processing
0195 *Stability of Gamma-oryzanol encapsulated using different wall materials*
Jeerasupa Amornpan, Mahasarakham University, Thailand[1]
Anuchita Moongngarm, Mahasarakham University, Thailand[2]
Orya Porneimmongkol, Mahasarakham University, Thailand[3]

Marine Biology
0211 *Comparison of propylene glycol and dimethyl sulfoxide based vitrification solutions for the cryopreservation of gorgonian (Junceella juncea) sperm*
Chiahsin Lin, National Museum of Marine Biology and Aquarium, Taiwan[1]
Sujune Tsai, Mingdao University, Taiwan[2]
Weihsu Yen, National Museum of Marine Biology and Aquarium, Taiwan[3]

Section VIII
Virtual Presentations

Thursday, August 28, 2014
08:00 to 09:15

Virtual Presentations

Thursday, August 28, 2014

08:00 to 09:15

Takasago Room
Presenters: 0112, 0142, 0170, 0199, 0222

(Presentations will be shown in order below and repeated. We will be happy to forward questions and / or comments directly to the presenters. Please send all inquiries to secretariat@presdafoundation.org)

Nutrition Science
0112 *Prevalence of metabolic syndrome and its association with oxidative stress markers in rural Thai adults*
Pornpimon Chupanit, Khon Kaen University, Khon Kaen Province, Thailand[1]
Benja Muktabhant, Khon Kaen University, Khon Kaen Province, Thailand[2]
Ratthaphol Kraiklangi, Khon Kaen University, Khon Kaen Province, Thailand[3]

Nutrition Science
0142 *Biochemical Characterization and Sensory Attributes of Traditional Indian Food Products Formulated from Quality Protein Maize*
Vishakha Singh, Maharana Pratap University of Agriculture & Technology, Udaipur, India[1]

Cell Culture
0170 *The Effects of Carica papaya Leaves Extract Against Dengue Virus Infected Vero Cells*
Siti Latifah Abd Kadir, Universiti Teknologi Malaysia, Malaysia[1]
Harisun Yaakob, Institute of Bioproduct Development, Universiti Teknologi Malaysia, Malaysia[2]
Razauden Mohamed Zulkifli, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, Malaysia[3]
Ehsan Moghaddam, Faculty of Medicine, Universiti Malaya, Malaysia[4]

Urban Ecology
0199 *The model of ecological rehabilitation of Russian towns*
Elkhana Riabova, The International University of Nature, Society and Man "Dubna", Russia[1]

Sanitation and Health
0222 *Improved sanitation can make children taller and smarter in rural Tanzania*
Maria Quattri, The World Bank, UK[1]
Emily Christensen Rand, The World Bank, USA[2]
C. Ajith Kumar, The World Bank, Tanzania[3]
Yolande Coombes, The World Bank, Kenya[4]
Craig Kullmann, The World Bank, USA[5]

Section IX

Closing Remarks*
Suehiro Room

** Coffee and light snacks will be served*

Friday, August 29, 2014
11:00 to 12:00

Tour Option 1: (Half Day)
Miyajima: 'The Shrine Island'

Friday, August 29, 2014
12:00 to 16:00

Tour Option 2: (Full Day)
Hiking Mt. Misen + Miyajima: 'The Shrine Island'

Saturday, August 30, 2014
09:00 to 16:00

This year we will be offering two tours to the world famous Shrine Island of Miyajima.
Miyajima is one of the crown jewels of Japan, and certainly one of its finest views. It is considered to be one of the three most beautiful and famous scenic spots in all of Japan. As a result, the entire island and surrounding vicinity was declared a UNESCO World Heritage Site in 1996.

We will be offering two types of tours:

Half-day Tour to Miyajima: constructed in 593 A.D., Itsukushima (popularly known as Miyajima Shrine Island) was undamaged by the atomic bombing. The island consists of a series of temple buildings, teahouses and quaint shops. The long pier from which the torii in the sea offers majestic views of the inland sea and is considered one of Japan's three most iconic views. As a UNESCO World Heritage Site, it is considered a 'must see' for travelers to Japan. The tour includes roundtrip transportation and guidance in English. Due to the popularity of this tour, the number of participants is limited. The tour fee is JPY 3,500.

Full-day Tour of Miyajima + Hiking Mt Misen: the tour begins with a visit to Miyajima Shrine Island, a UNESCO World Heritage Site. After the tour of Miyajima, you will go to Mt Misen: at 530 meters above sea level, Mount Misen is the highest peak on Miyajima. The hiking trail is safe and consists of earthen steps. Hikers will pass by waterfalls, temples and hundreds of maple trees. It is the perfect trip for the nature lover! On clear days, it affords spectacular views of the Seto Inland Sea and as far away as Hiroshima City. There are also a number of Buddhist structures near the peak and one of the oldest eternal flames in the world. A ropeway leads up the mountain from town, but it is also possible to climb up on foot. It takes 1.5 to 2 hours to reach the summit from town. The tour fee is JPY 8,000.

The tours will be conducted in English with a bilingual guide. All transportation, park entrance fees, temple entrance fees and cable car tickets are included. We recommend wearing loose clothing, comfortable shoes and a hat.

Information regarding the tour dates, departure times and tour payment is available at the registration desk. Please note that the tour fee must be paid in advance to confirm your place. All payments must be in JPY cash. As these tours are very popular, please understand that the tour group size is limited.

The Asian Conference on the Life Sciences and Sustainability
Official Proceedings: ISSN 2188-3971

Index of Full Paper

The change of tree diversity and carbon stock during three decades in Ulu Gadut, West Sumatra, Indonesia
Erizal Mukhtar, Faculty of Science, Andalas University, Indonesia
Pages: 54 to 59

Isolation and Screening of Thermostable Alkaline Protease Producing Bacillus spp. From Kerinci Hot Spring, Indonesia
Anthoni Agustien, Department of Biology, Andalas University, Indonesia
Pages: 60 to 67

Geographic Variation of Puntius lateristriga (C.V) From Sumatra and Adjacent Island
Dewi Roesma, Department of Biology, University of Andalas, Indonesia
Pages: 68 to 74

Hydrolysation of Palm Oil Empty Fruit Bunch by Using Crude Extract Enzymes of Aspergillus niger and Analysis of Its Hydrolizates for Bioethanol Production
Kahar Muzakhar, Biology Department, Faculty of Math and Natural Sciences, The University of Jember, Jember, Indonesia
Pages: 75 to 80

Ant Species (Hymenoptera: Formicidae) in Dragon fruit plantations (Hylocereus spp.) of West Sumatera, Indonesia
Henny Herwina, Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia
Pages: 81 to 86

Human Behavior Related with Cut Locus in Daily Life
Nathaphon Boonnam, Tokai University, Japan
Pages: 87 to 91

Emotion Estimation from Facial Expressions Using Kinect
Chaiyapruk Thongkam, Tokai University, Japan
Pages 92 to 97

Diversity of Wild Orchid species in West Sumatera, Indonesia
Mairawita Marlis, Department of Biology, Andalas University, Indonesia
Pages: 98 to 109

The model of ecological rehabilitation of Russian towns
Elkhana Riabova, The International University of Nature, Society and Man "Dubna", Russia
Pages: 110 to 116

THE CHANGE OF TREE DIVERSITY AND CARBON STOCK DURING THREE DECADES IN ULU GADUT, WEST SUMATRA

Erizal Mukhtar
Department of Biology,
Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia

Syamsuardi
Department of Biology,
Faculty of Mathematics and Natural Sciences, Andalas University, Indonesia

Hermansah
Department of Soil Science,
Faculty of Agriculture, Andalas University, Indonesia

ABSTRACT

This study aimed to clarify changes of tree diversity and its carbon stock on the stand dynamics of a tropical rainforest in Sumatra based on long-term observations of a 1-ha plot from 1981 to 2102. Tree biomass was calculated by using the non destructive sampling method. All trees with stem diameter the breast height ≥ 8 cm were measured. Our result showed that diversity index were varied during three decades, the rate increased at year 1981 until 1987 but after that decreased to year 2012. Carbon stocks were varied and it was increased 43.21% during three decades. We found that decreased of diversity index about 22.7 % during three decades were not affected to the carbon stock.

Key word: Diversity index, carbon stock, tropical forest, West Sumatra

Correspondence author: erizal@fmipa.unand.ac.id

INTRODUCTION

Tropical forests are globally significant ecosystems; accounting for ~50% of global forest area (Malhi and Grace, 2000), storing ~45% of all carbon in terrestrial vegetation (Baccini *et al.,* 2012; Saatchi *et al.,* 2011), maintaining high biodiversity (Myers *et al.,* 2000), and providing ecosystem services, such as timber, non-timber forest products [Timko *et al*., 2011), and climate change mitigation (Phillips *et al*., 1998). The role of biodiversity in ecosystem functioning has become a central issue in ecology (Malhi and Grace, 2000; Wang *et al*., 2011). Forest ecosystems play a crucial role in regional and global terrestrial carbon (C) cycles because they store large quantities of C in vegetation, detritus, and soil, and exchange large amounts of C with the atmosphere through photosynthesis and respiration (Dixon *et al.,* 1994).

Numerous studies have investigated the relationship between species diversity and productivity in forest ecosystems (Caspersen and Pacala, 2001, Bunker *et al.,* 2005, Creed *et al.,* 2009). The literature suggests that the relationship between productivity and species diversity is often positive (Whittaker and Heegaard, 2003; Wang *et al*., 2010). Although studies on species diversity effects on productivity and C stocks are numerous, little information exists concerning relationships between tree diversity and carbon storage at the stand scale during three decades.

Several ecological studies in a foothill rain forest in Ulu Gadut, West Sumatra have been carried out since 1980. The research about growth and death of trees (Kohyama *et al.* 1989), spatial distribution pattern of representative tree species (Kohyama *et al.,*1994), impact of dry weather on the stand dynamics (Yoneda *et al*., 2006), and soil quality characterization in relation to tree species diversity (Kubota *et al.,* 2000), dispersal and survival several tree species (Mukhtar *et al.* 1992,

1998; Mukhtar and Koike, 2007, 2009). However, evaluation of changes in forest conditions that have an impact on changes of forest community and carbon stock had never been reported. Knowledge of changes in community diversity and carbon stock in a forest ecosystem can provide a snapshot of the impact of disturbances on forests in the past, predict the effects of global climate change on tropical forests and know the contribution of forest ecosystems to the global carbon balance. Therefore, the objective of this study was to determine changes of carbon stock and tree diversity of long-term permanent plot in Ulu Gadut forest, West Sumatra.

METHODS

This study was carried out from June 2012 to October 2012 at a 1- ha permanent plot, named Pinang-pinang plot (590 - 620 m above sea level) in a foothill forest of Mt. Gadut (Lat. 0° 55' S, Long. 100° 30' E), 17 km east from Padang, West Sumatra, Indonesia. The Pinang-pinang plot was set up in 1981 consisted of 115 sub plots of approximately equivalent area.
Above ground biomass was calculated by using the non destructive sampling method. All trees with stem diameter at breast height (dbh) ≥ 8 cm were re-measured, number of individuals and species were recorded. Data series from 1981, 1983, 1985, 1987, 1996, 1999, 2007 and 2012 were used to quantify the amount of tree biomass using allometric equations based on stem diameter.

Data Analysis

a. *Shannon Diversity Index*
Diversity index was calculated using the following equation (Shannon and Weaver, 1949):

$$H = - \sum\{(ni/N) \log (ni/N) \quad (1)$$

Where:
H = Shannon Diversity Index
ni = Important value of each species
N = Total of Important value

b. *Above-ground biomass*
Above-ground biomass was estimated by using allometric equation Kettering (Hairiah *et al*., 2001)

$$\mathbf{(AGB)_{est} = 0{,}11 \times \rho \times D^{2{,}62}} \quad (2)$$

Where : $(AGB)_{est}$ = Above ground biomass (Kg)
D = Stem diameter (cm)
ρ = wood density (g/cm^3)
Wood density refers to the ICRAF website: www.*worldagroforestry*.org

c. *Carbon stock*
The amount of carbon stock was estimated by using equation (Hairiah *et al*., 2001):

$$\mathbf{C = 0.5 \times W} \quad (3)$$

Where:
C = Carbon stock (kg)
W = Trees biomass (kg)

RESULTS AND DISCUSSION

The change of trees composition

Number of trees were various during three decades of measurements as shown in Figure 1. The number of tree was decreased at year 1996 and 1999 but it was increased again after year 2007. In general the number tree was increased 20.8 % during three decades. Furthermore, number of species and families were not so differences but tree diversity index was decreased 22.8 % during three decades. We suggested that impact of the climate change where during year 1995 to year 2001 rainfall decreased cause of the El-Nino affected to these phenomena which the big tree of *Swintonia schwenkii* was fallen down as shown in Figure 2 and Figure 3. Eventhough the diversity index was decreased but it was still in high category (H=3.5). Comparison to several researchers in Sumatra, found diversity index in Batang Gadis National Park, North Sumatra were ranged between 2.5 to 3.5 (Kuswanda and Bambang, 2008). In East Borneo, found the diversity index was 3.68 (Hairiah *et al*., 2001).

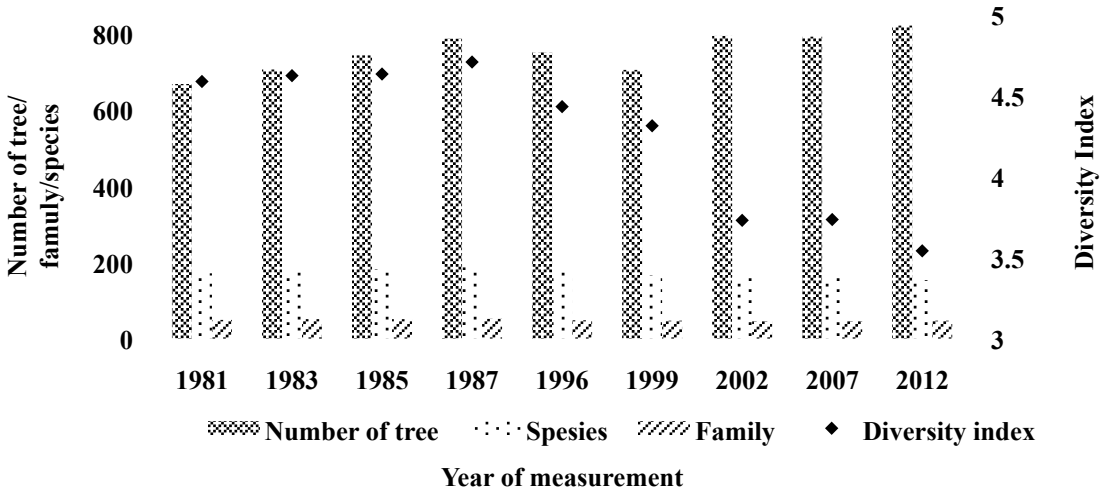


Figure 1. Change of tree composition and diversity index in Ulu Gadut forest during three decades

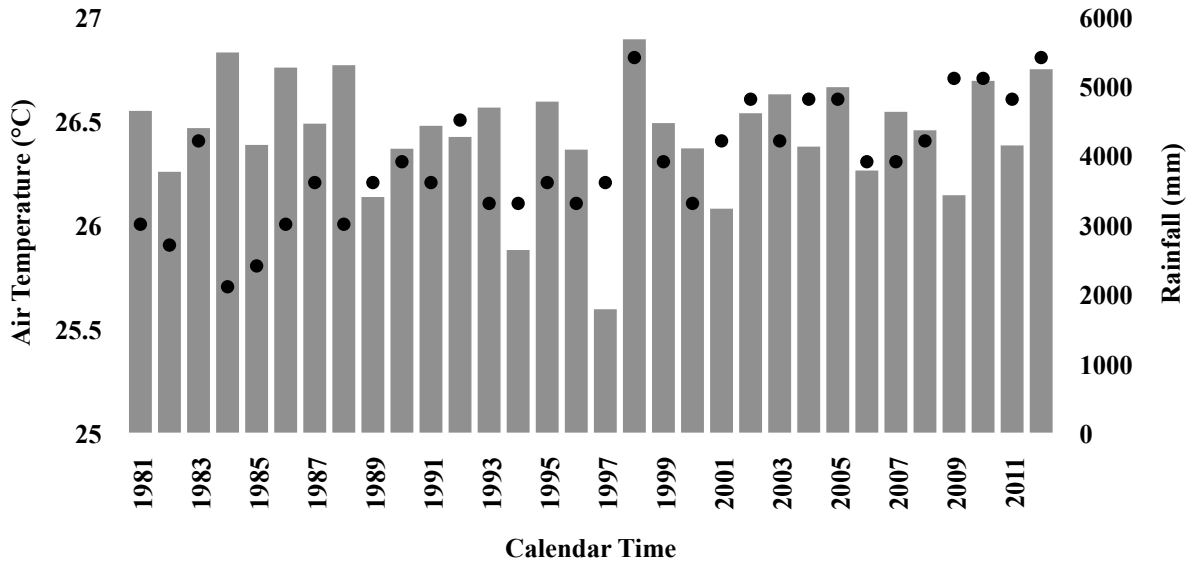


Figure 2. Air temperature and rainfall during three decades in Padang city, West Sumatra

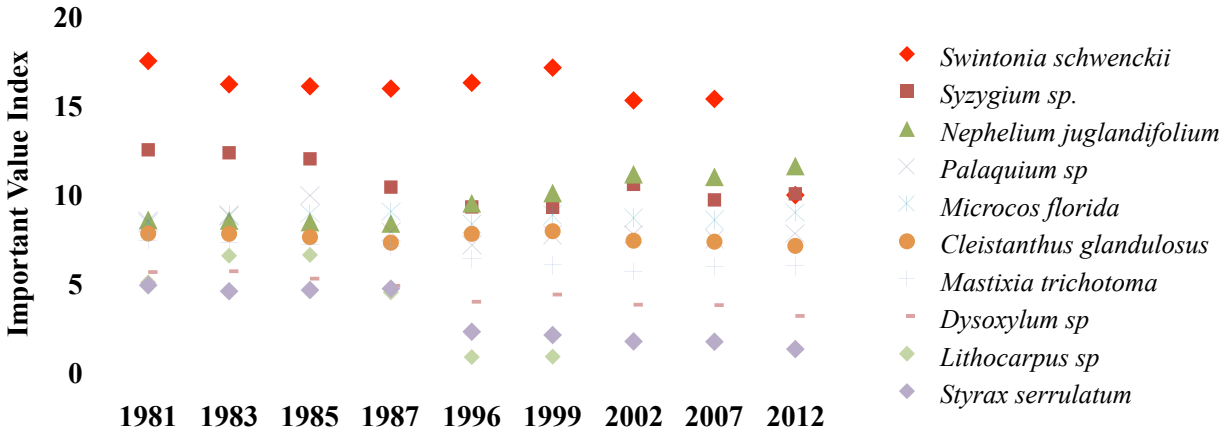


Figure 3. Important Value Index pattern from main tree species

The change of carbon stock

The carbon stocks were varied between year measurements. During three decades the rate was increased about 33.3 % from 197.4 t C ha^{-1} to 249.6 t C ha^{-1} as shown in Figure 4. The carbon stock rate per year was 2.07 t C $ha^{-1}year^{-1}$. These values are higher than those reported by Yang and Guan (2008) in China (21.81 t C $ha^{-1}year^{-1}$. If we focused to carbon stock per species we found various characteristics between tree species (Figure 5). *Swintonia schwenkii* was decreased after year 2007 but almost species showed increased on their carbon stock.

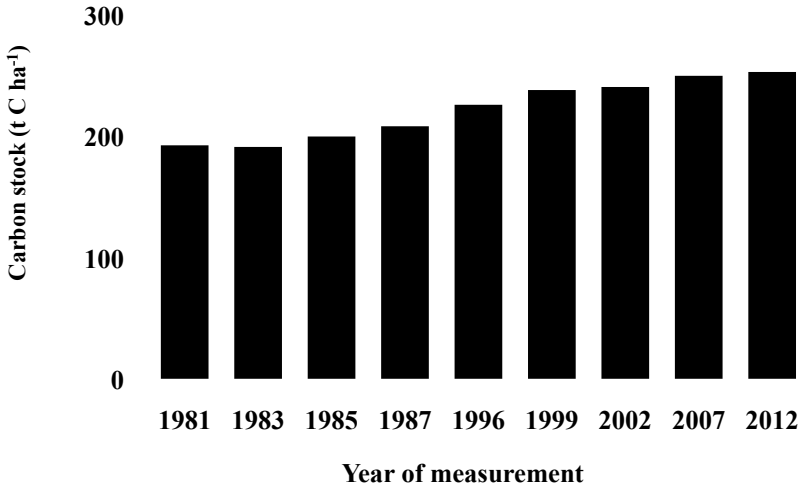


Figure 4. Changes of carbon stock in Ulu Gadut forest during three decades

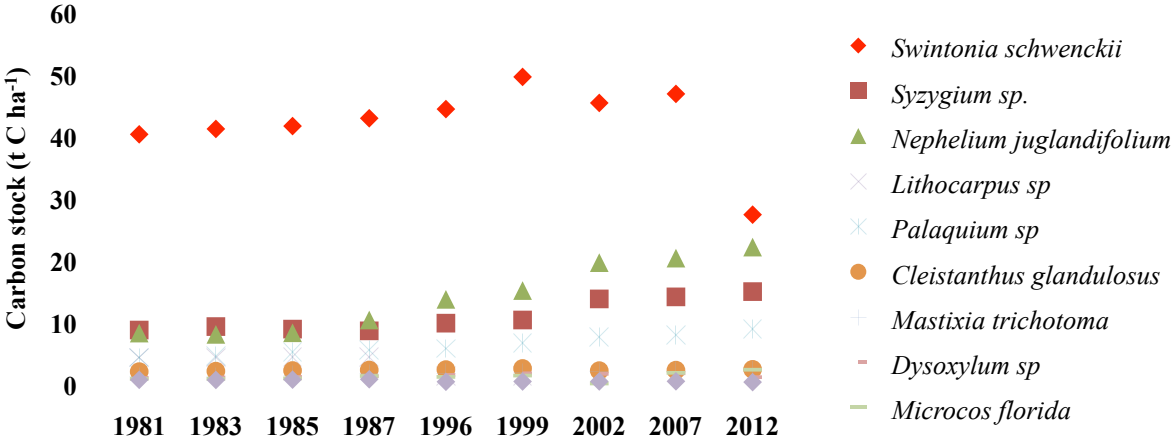


Figure 5. Carbon stock changes from main tree species during three decades

In diameter class distribution we found that change of carbon stock was varied as shown in Figure 6. The tree that have bigger diameter size (dbh>60 cm) was contributed more than half of the carbon stock. Therefore, we could be concluded that the big tree was important play role to increased carbon stock in this forest. However, the rate of changes was also depended on their forest condition. In China, carbon stock was increased about 14.31% of 19.08 t C ha^{-1} became 21.81 t C ha^{-1} between 1989-2003 (0.19 t C $ha^{-1}year^{-1}$) (Yang and Guan, 2008). Furthermore, in Indonesia, Rahaju (2008) reported that carbon stock was decreased 43,250 t C ha^{-1} between 1990-2000.

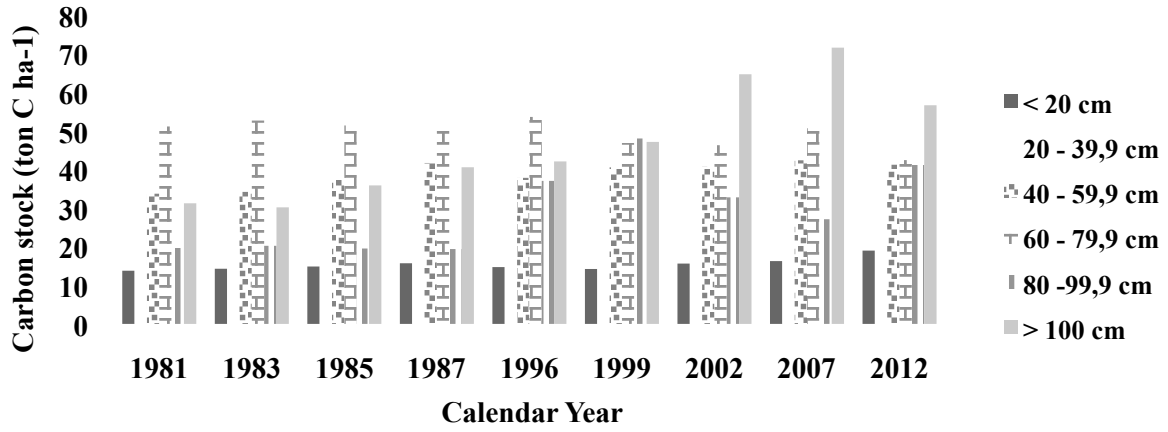


Figure 6. Changes of carbon stock based on diameter class during three decades

The relationship between Diversity Index and carbon stock is shown in Figure 7. Simple regression analysis indicates that carbon stock was not so affected by Diversity Index. One reason is the rate not so differences and second reason is the rate still in high range of diversity index. Our found here indicated that the carbon stock was not decreased cause of change of diversity index when the changes were not so big and still in high category. These result concluded that the condition Ulu Gadut forest still in good condition during three decades.

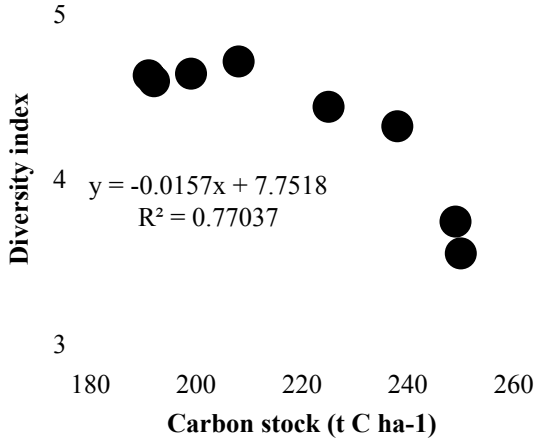


Figure 7. Correlation between carbon stock and diversity index during three decades

CONCLUSION

It can be concluded that diversity index were varied during three decades, the rate increased at year 1981 until 1987 but after that decreased to year 2012. Carbon stocks were varied and it was increased 43.21% during three decades. Number of species and families were not so differences but tree diversity index was decreased 22.8 % during three decades but it was not affected to the carbon stock. The tree that have bigger diameter size (dbh>60 cm) was contributed more than half of the carbon stock. Therefore, we could be concluded that the big tree was important play role to increased carbon stock in this forest.

ACKNOWLEDGEMENTS

We would like to express our appreciation to Prof. Tsuyoshi Yoneda, Prof. Eizi Suzuki and Dr. Aiba of Kagoshima University for their initiation of this study and valuable advices for this manuscript. This study is based on long-term study observation over 30 years. This study was supported by Directorate General of Higher Education, Ministry of National Education Republic of Indonesia FY 2013 contract Number 01/UN.16/PL/KLNPI/2013 and FY 2014 contract Number No. 03/UN.16/PL/KLNPI/2014.

REFERENCES

Baccini A, Goetz SJ, Walker WS, Laporte NT, Sun M, Sulla-Menashe D, Hackler J, Beck PSA, Dubayah R, Friedl MA, Samanta S and R.A. Houghton. 2012. Estimated carbon dioxide emissions from tropical deforestation improved by carbon-density maps. *Nat Clim Change* 2:182–185.

Caspersen, J.P and S.W. Pacala. 2001. Successional diversity and forest ecosystem function. *Ecological Research* 16, 895–903.

Dixon, R. K., R. A. Brown, R. A. Houghton, A. M. Solomon, M. C.Trexler, J. Wisniewski. 1994. Carbon pools and flux of global forest ecosystems. *Science* 263: 185-190.

Hairiah, K., S.M. Sitompul., M. van Noordwijk and C. Palm. 2001. Methods for sampling carbon stocks above and below ground. *International Centre for Research in Agroforestry*. Bogor, Indonesia

Kohyama, T., Hotta, M., Ogino, K., Syahbuddin and E. Mukhtar. 1989. Structure and dynamics of forest stands in G. Gadut, West Sumatra. In: Diversity and plant animal interaction in equatorial rain forests (ed. Hotta, M.), pp. 33−47. Occasional papers of the Kagoshima University Research Center for South Pacific 16.

Kohyama, T., Suzuki, E and M. Hotta, M. 1994. Spatial distribution pattern of representative tree species in a foothill rain forest in West Sumatra. *Tropics* 4: 1-15

Kubota, D., Masunaga, T., Hermansyah., Hotta, M. And T. Wakatsuki. 2000. Soil quality characterization in relation to tree species diversity in tropical rain forest, West Sumatra, Indonesia. I. Comparison of two 1−ha plots. *Tropics* 9: 133−145.

Malhi, Y., Grace, J. 2000. Tropical forests and atmospheric carbon dioxide. *Trends Ecol. Evol* 15 (8): 332–337

Mukhtar, E., Suzuki, E., Kohyama, T. and M. Rahman. 1992. Regeneration process of a climax species *Calophyllum soulattri* in tropical rain forest of West Sumatra. *Tropics* 2: 1−12.

Mukhtar, E., Yoneda, T., Zalfiati and M. Rahman. 1998. Regeneration process of a climax *species Calophyllum soulattri* in tropical rain forest of West Sumatra: population dynamics of a cohort from mast fruiting in 1981. *Tropics* 7: 183−194.

Mukhtar, E and F. Koike. 2007. Dispersal and survival of juveniles of several tree species in a tropical rain forest of West Sumatra. *Tropics* Vol 16(3); 205-219

Mukhtar, E and F. Koike. 2009. Juvenile growth rate of seven major tree species in a tropical rain forest of West Sumatra. Tropics Vol. 18(1); 1-6.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 2000, 403:853–858.

Phillips OL, Malhi Y, Higuchi N, Laurance WF, Nunez PV, Vasquez RM, Laurance SG, Ferreira LV, Stern M, Brown S and J. Grace.1998. Changes in the carbon balance of tropical forests: evidence from long-term plots. Science 282:439–442.

Saatchi SS, Harris NL, Brown S, Lefsky M, Mitchard ETA, Salas W, Zutta BR, Buermann W, Lewis SL, Hagen S, Petrova S, White L, Silman M and A. Morel.2011. Benchmark map of forest carbon stocks in tropical regions across three continents. *Proc Natl Acad Sci* 2011, 108:9899–9904.

Shannon CE, Weaver W. 1949. The mathematical theory of communication. University of Illinois Press, Champaign

Timko JA, Waeber PO and R.A. Kozak. 2010. The socio-economic contribution of non-timber forest products to rural livelihoods in Sub-Saharan Africa: knowledge gaps and new directions. Int For Rev 12:284–294.

Wang, S., J. M. Chen, W. M. Ju, X. Feng, M. Chen, P, Chen and G. Yu. 2007. Carbon Sink and Source in China's Forest During 1901-2001. *Journal of Environmental Management*. 85: 524-537

Wang, W, X. Lei, Z. Ma, D. Kneeshaw and C. Peng. 2011. Positive relationship between aboveground carbon stocks and structural diversity in Spruce-Dominated forest stands in new brunswick, canada. *For.Sci* 57(6); 506-515

Whittaker , R.J and E. Heegaard . 2003. What is the observed relationship between species richness and productivity? Comment. *Ecology* 84(12):3384 –3390.

Yang, K and Guan D. 2008. Change in forest biomass carbon stock in the Pearl River Delta between 1989 and 2003. *Journal of Environmental Science*. 20: 1439-1444

Yoneda, T., Mizunaga, H., Nishimura, S., Fujii, S., Mukhtar, E., Hotta, M and K. Ogino. 2006. Impacts of recent dry weather on a tropical rain forest in Sumatra with special reference to stand dynamics during the last two decades. *Tropics*, 15: 177-187.

ISOLATION AND SCREENING OF THERMOSTABLE ALKALINE PROTEASE PRODUCING *Bacillus* spp. FROM KERINCI HOT SPRING, INDONESIA

Anthoni Agustien
Department of Biology, Faculty of Mathematics and Natural Sciences
University of Andalas, Padang, Indonesia

Yetria Rilda
Department of Chemistry, Faculty of Mathematics and Natural Sciences
University of Andalas, Padang, Indonesia

R.R. Megahayati
Faculty of Education STKIP PGRI, Padang, Indonesia

Nasrazuhdy
Polytechnic of Medicine, Jambi, Indonesia

Rozana Zuhri
Faculty of Education STKIP YPM Bangko, Jambi, Indonesia

Akmal Djamaan
Laboratory of Biotechnology, Biota Sumatra, University of Andalas, Padang, Indonesia

ABSTRACT
This research aimed to isolate and screening the potential isolate which produce thermostable alkaline protease. Thermophilic *Bacillus* spp. isolated using selective medium (TB medium). Screening of bacteria producing alkaline protease using TB medium containing 2% casein.One hundred dan sixty five thermophilic*Bacillus* spp.isolates were collected from Kerinci hot spring, out of which 34 were alkaline protease producers, which include 14 isolate potent. SR-09 isolates had a proteolyticindex , and the highest enzyme specific activity and enzyme is heat stable up to 20 hours at 60 ^{0}C respectively.

Key words : isolation, screening, thermostable, *Bacillus* spp., hot spring

INTRODUCTION

Intensive research needs to be done to isolate thermophilic bacteria from several habitats with the intended use of microorganisms and enzymes they produce to be applied in the field of biotechnology (Souza and Martins, 2001). The use of thermophilic bacteria in industrial areas is likely to increase, due to the application of enzymes in biotechnology that require heat-resistant enzymes (Suhartono, 1991). One source is a thermostable

enzyme producing thermophilic bacteria, mostly from thermophilic bacteria studied were of the genus *Bacillus* isolated from thermal environments (Schallmey *et al.,* 2003).

Bacillus a major concern in biotechnology because it is relatively easy for bacteria isolated from various environments and are able to grow in a synthetic medium (Johnvesly and Naik 2001). Various different types of *Bacillus* fementation ability to perform the process at pH acidic, neutral, alkaline, and the combination with the thermophilic nature in this genus, has indicated to be developed to produce enzymes that are stable at high temperatures (Schallmey*et al.,* 2003).

Bacillus bacteria can produce a thermostable proteases are used in various fields, especially for the detergent industry. Protease is an enzyme which is most important, because 60% of industrial production in the world is the enzyme protease and 25% of them are thermostable. *Bacillus* spp. can produce alkaline proteases are widely used as detergent additives which have economic value (Rao *et al.,* 1998). Alkaline protease contained in the detergent serves to hydrolyze materials consisting of proteins during washing (Banik and Prakash, 2004).

Jambi province, especially in Kerinci has several active volcanoes, one of which is Mount Kerinci, which is the highest mountain on the island of Sumatra and also has hot springs which are at two different locations, namely the Sungai Medang and Semerup. Conditions hot springs alkaline Kerinci is different from the hot springs located in West Sumatra. Alkaline hot springs, rich in minerals while the acid, poor in minerals, so it is predicted that there are hot springs in Kerinci Jambi, have a diversity of different microorganisms. Present this research reports the isolation and screening of thermostable alkaline protease producing *Bacillus* spp. from Kerinci hot spring.

METHODS

This research was conducted at the laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Science, Andalas University, Padang, Indonesia.

Isolation of thermophilic*Bacillus* spp.

Thermostable producing *Bacillus* spp. was isolated from samples collected from Kerinci hot springs at Sungai Penuh, Jambi province. Ten water samples were obtained from nine points of hot spring. These samples were collected from 10 cm below the water surface where the temperature was between 45 until 80 ^{0}C, and the pH around 8. One mL of sample was inoculated to Petridish containing TB medium (trypton 0.2%; yeast extract 0.1%; NaCl 0.05%; $CaCl_2$ 1.0%; minerals 1.0%; vitamins 1.0%, agar 1.5%; gelrite gellan gum 0.5%. pH 9.0) The plates incubated at 60 C for 24 h. After incubation white and circular colonies was obtained. Purification of each colony was carried out using the streaking plate method, and single colony to transferred on slant media miring and labeled.The organisms were maintained on TB agar plates and stored at 4°C.

Screening for alkaline protease production

One hundred and sixty five *Bacillus* spp. thermophilic isolated Kerinci hot spring were screened for their ability of alkaline production at 60 ^{0}C, 24 h on TB medium pH 9.0 incorporated with casein 2%. The incubation was carried out at 60 ^{0}C during 24 h.

The clear zone formed around colony was indicated the ability of the isolate to produce extracellular alkaline protease and the diameter of these clear zones as an indication of protease activity was noted. The Proteolytics Index (PI) was determined by measuring diameter of clear zone compare to diameter of the colony.

Protease production

The inoculums were prepared by inoculating colony from the slant medium into 25 ml basal medium contain casein (g/l: NaCl 5,0 g, K_2HPO_4 0,3 g; KH_2PO_4 0,3 g; $MgCl_2$. 6 H_2O 0.1 g; kasein 10 g and pH 9.0) in Erlenmeyer 250 ml, incubated at 150 rpm in a shaker for 24 hours at 50°C. 2.5 ml of inoculum (10^7 sel/ml) was inoculated into 100 ml growth medium and incubated at 50 °C for 24 h. Culture was harvested by centrifuged at 5000 rpm for 15 min. at room temperature. The supernatant as crude enzyme was used for the assay of protease activity.

Protease Assay

Protease activity was determined by applying a modified method by Takami *et al.* (1989). The mixture containing of 0.25 ml of 1% casein in 0.025 Tris-Cl buffer pH 8 was pre- incubated for 5 min and 0.25 ml of enzyme added, incubated for 10 min. The enzyme reaction was stopped by adding 0.5 ml of 0.4 M TCA. The mixture was centrifuged at 12000 rpm for 10 min. Supernatant (0.5 ml) was mixed with 2.5 ml of 0.4 M Na_2CO_3 and 0.25 ml of 1 N Folin Ciocalteu's and incubated for 30 min at room temperature. The absorbance of the solutions were read against the blank sample by spectrophotometer at 600 nm . Tyrosin standard solution, in the range of 0-200 mg/L was used a standard curve. One unit (U) of alkaline protease was defined as the amount of enzyme that could produce 1 μg of tyrosine in one minute under the defined assay conditions.

Thermal Stability

The stability of enzyme against thermal was determined by incubating the crude enzyme for each isolate at 60 ^{0}C up to 28 h. The enzyme activity was measured every 1 h and expressed as relative activity against the enzyme without thermal treatment as 100% enzyme activity.

RESULTS

Conditions of temperature and pH of the water in a Kerinci hot spring. The water temperature in the hot springs of the Sungai Medang (5 pools) which ranges from 50-78 ^{0}C with a pH of alkaline water with a pH that ranges from 8.45 to 8.71. While the hot springs Semurup, has 4 pools temperature conditions ranging from 65-92 ^{0}C with a pH between 8.7 to 8.9. Vegetation around the hot springs in the form of shrubs and grasses. Isolation of *Bacillus* spp. by using selection medium (TB medium) and incubated at 60 ^{0}C for 24 hours, the medium contained 165 colonies of bacteria that indicate the type isolates of *Bacillus* spp. are characterized by rounded and colored bacterial colonies white (Figure 1).

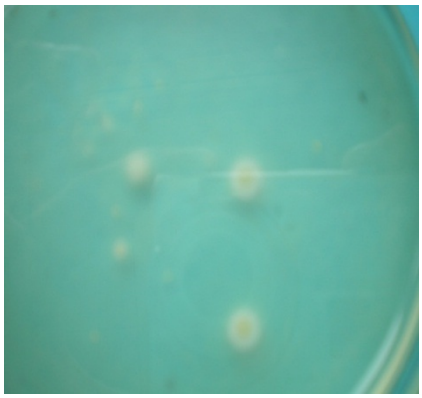


Figure 1. Colonies of *Bacillus s*pp. on TB medium

Screening results of 165 isolates of thermophilic bacteria that have the ability to produce alkaline protease was obtained 34 isolates of bacteria. Thirty-four isolates showed proteolytic properties, which have the ability to produce alkaline protease.

Proteolytic index (IP) of all isolates ranged from 0.25 to 3.20 A total of 14 isolates, IP of eight isolates Semerup origin of the hot springs and the origin of six isolates Sungai Medang hot springs have more than 2. It showed that 14 isolates indicated potential as a producer of alkaline protease.

Table 1.Index and proteolytic enzyme activity of isolates of *Bacillus* spp.

Bacillus spp.	Proteolytic Index (PI)	Aktivitas spesifik (Unit/mg)
SR-01	2,33	0,280
SR-02	2,25	0,242
SR-04	2,36	0,285
SR-09	3,20	0,712
SR-10	2,20	0,220
SR-14	2,57	0,482
SR-15	2,25	0,230
SR-16	2,20	0,185
SM-02	2,64	0,520
SM-06	3,00	0,644
SM-08	2,25	0,212
SM-10	2,11	0,165
SM-14	2,57	0,510
SM-15	2,00	0,125

Measurement of alkaline protease activity of each thermo-proteolytic bacterial isolates showed that all isolates had an alkaline protease enzyme activity Alkaline protease

activity of 14 isolates potential, enzyme activity ranged from 0.125 to 0.712 U / mg . Isolate SR-09 had the highest enzyme activity (0.712 U / mg) (Table 1).

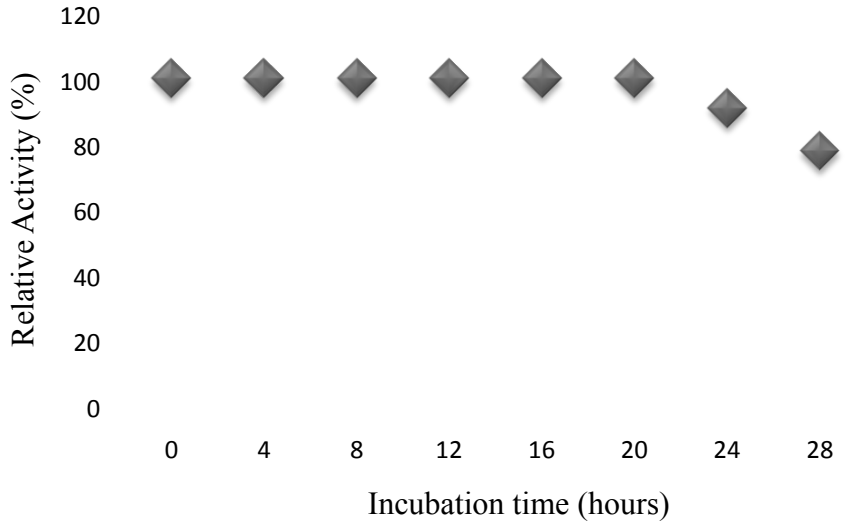


Figure 2. Profile of alkaline protease stability at 60^0C.

Alkaline protease stability of SR-09 isolates of thermophilic bacteria to temperature, suggesting that the enzyme is stable 100% up to 20 hours of incubation at 60 ^{0}C, then decreased stability at 24 hours of incubation approximately 91% and the activity of the enzyme can still be maintained at about 78% to 28 hours incubation (Figure 2).

DISCUSSION

Colonies of bacteria *Bacillus* sp. were grown in TB medium, at high temperatures will have a characteristic round colonies colored white, do not form pigment (Atlas, 1997).Thermophilic microorganisms containing protein denaturation heat resistant and resistant to be able to adapt to conditions of extreme temperature environment to live and survive (Kumar and Nussinov, 2001).Thermophilic microorganism is a microorganism that grows at an optimum temperature above 45 ^{0}C, the optimum temperature thermophilic microorganisms mostly below 80 ^{0}C (Madigan *et al.,* 2000). Alkaline hot springs rich in minerals, these circumstances lead to different diversity biota (Cowan, 1992). The minerals contained in water source heat allows thermophilic microorganisms can live and survive (Edwards, 1990). Trace elements" such as calcium, magnesium and iron are required elements in the metabolism of bacteria, nitrate is used as a source of nitrogen, iron acts as a cofactor in enzymatic reactions and sulfite needed cells as a source of sulfur (Madigan *et al.,* 2000). At endospore forming bacteria, high concentrations of calcium in spores and diphicolynate acid binds to calcium, which protects DNA (Singleton, 2004). Conditions of a biotic factors such as temperature and pH of the water and biotic factors such as vegetation of hot springs, survived and life-sustaining heat resistant microorganisms. Dirnawan *et al*. (2000), reported that the falling leaves, branches twigs, grass seeds, pollen, and insect carcasses were found in the hot springs is an organic material that can be utilized by microorganisms that live in the hot springs.

Specific activity of different enzymes from isolates of this is probably due the amount of enzyme and amino acid sequence of the enzyme protein produced each isolate different from each other. Alkaline protease specific activity of 52 isolates of *Bacillus* spp. the origin of the hot springs of West Sumatra ranged from 0.080 to 0.512 U / mg (Agustien, 2010). Dirnawan *et al*., (2000), reported sixteen isolates of *Bacillus* spp. of thermophilic hot springs Gunung Pancar,West Java, have colonies with diameters between 1-7 mm, with four isolates were proteolytic index > 3, however, the specific activity of the protease is very small between 0.01 to 0.08 U / mg. Protease activity of microorganisms is influenced by a number of enzymes and amino acid sequence of the enzyme (Suhartono, 1991).

Thermo-proteolytic bacterial isolates SR-09 has the highest proteolytic index (3.20), so it is a potential bacteria producing alkaline protease for use as a detergent additive. In general *Bacillus* can secrete proteases on environment, it can be detected by a clear zone around bacterial colonies on solid media containing skim milk (Priest, 1992). Formation of a clear zone around bacterial colonies on solid medium containing casein, this is due to the bacteria produce proteases and secreted in the medium, then the proteases would hydrolyze casein substrate causing visible translucent medium (Fuad *et al.,*2004). Agustien (2010) reported that the *Brevibacillus agri* A-03 isolated thermophilic bacteria from Ambayan hot spring with proteolytic index 3.50 and has proven to have the ability as a producer of alkaline protease for detergent additives.

This indicates that the alkaline protease produced SR-09 isolates, showed high stability, so that it can be said that the category of enzymes and thermostable enzymes potentially applied in industry. Stability to heat an enzyme it can still be retained due to the intrinsic structure of the protein or protein conformational stability preservation and maintenance of the effect of the hydrophobicity of the enzyme that causes protein "folding" of the enzyme (Steel and Walker 1991; Vielle and Zaikus, 2001). Stability of the temperature profile of the alkaline protease from *Bacillus licheniformis* RP1 showed the enzyme is stable at a temperature of 55 ^{0}C, after incubated for 1 h at pH 10.5 (Kamoun *et al.,* 2008). Guangrong *et al.* (2006) reported that *Bacillus* sp. HS08 thermophilic alkaline protease produced with optimum temperature 65 ^{0}C, and the stability of the enzyme at a temperature of 650 ^{0}C for 60 min incubation showed protease activity of 75%. *Brevibacillus agri* thermophilic from West Sumatra hot springs produce thermostable alkaline protease activity of the enzyme that can sustain up to 30 hours of incubation (Agustien, 2010).

CONCLUSION

Tirty four thermophilic *Bacillus* spp. isolated Kerinci hot spring were alkaline protease producers, which include 14 isolate potent. SR-09 isolates had a proteolytic index (IP = 3.20) and the highest enzyme specific activity (0.712 U/mg) and enzyme is heat stable up to 20 hours at 60 ^{0}C respectively.

ACKNOWLEDGEMENTS

This study was funded through a grant scheme funded by the Graduate Team Funds Directorate General of Higher Education, Ministry of Education and Cultural Affairs of Republic Indonesia, through the DIPA (Budget Implementation List) Andalas University Number: Dipa-023.04.2.415061/2013, 05 December 2012.

REFERENCES

Agustien, A. 2010. Protease bakteri termofilik.Unpad Press, Bandung, ISBN: 978-602-8743-08-2.

Atlas, R.M. 1997. Handbook of Microbiological Media, CRC. Press.Inc. New York.

Banerjee, U.C., R.K. Sani, W. Azmidan R. Soni. 1999. Thermostable alkaline proteases from Bacillus brevis and its characterization as a loundry detergent additive. J.Pro. Biochem., 35. 213-219.

Banik, R.M. dan M. Prakash. 2004. Laundry detergent compatibility of the alkaline protease from *Bacillus cereus*. Microbiol. Research. 159, 135-140.

Cowan, D. A. 1992. Biochemistry and Molecular Biology of the Extremely Thermophilic Archaeabacteria, in : Molecular Biology and Biotechnology Extremophiles. Ed. R.A. Herbert and R.J. Sharp, Blackie & Sons. New York.

Edwards, C. 1990. Thermophiles, in: Microbiology of Extreme Environments, Graw-Hill Publ. Company. New York.

Gupta, R., Q.K. Beg and P. Lorenz. 2002. Bacterial alkaline proteases : molecular approaches and industrial applications. Appl. Microbiol. Biotech., 59, 13-32.

Haki, G.D. and S.K. Rakshit. 2003. Developments in industrially important thermostable enzyme : a review. J. Biores. Technol., 89.17-34.

Johnvesly, B and G.R. Naik. 2001. Study on production of thermostable alkaline protease from thermophilic and alkaliphilic Bacillus sp. JB99 in a chemically defined medium. J. Proc. Biochem., 37, 139-144.

Kumar, S. and R.Nussinov. 2001. How do thermophilic proteins deal with heat? A review. Cell Mol. Life Scien., 58, 1216-1233.

Madigan, M.T., J.M. Martinko and J. Parker. 2000. Biology of Microorganisms. 9th Ed. Prentice Hall International, Inc., New Jersey.

Rao, M.B., A.M. Tanksale, M.S. Gahtge and V.V. Deshpande.1998.Moleculer and biotechnological aspects of microbial proteases.Microbiol.Rev., 62.597-635.

Schallmey, M., A. Singh and O.P. Ward. 2003. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol., 50, 1-17.

Singleton, P. 2004. *Bacteria : In Biology, Biotechnology and Medicine*. The Quarterly Review of Biology. 6th Ed..Willey & Sons. New York.

Souza, A.N. and M.L.L. Martins. 2001. Isolation, properties and kinetics of growth of a thermophilic *Bacillus*, Brazil. J. Microbiol, 32, 4, 2001.

Suhartono, M.T. 1991. Protease, PAU Bioteknologi IPB. Bogor.

Takami, H., T. Akiba and K. Horikoshi, 1992. Substrate specificity of thermostable alkaline protease from *Bacillus* sp. No.AH-101.Biosci.Biotechnol.Biochem., 56, 333-3341.

Todar, K. 2009. The Genus Bacillus.Todar's online textbook of bacteriology. University of Wisconsin-Madison. Madison.

Vieille, C. and G.J. Zeikus. 2001. Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. *Microbiol.and Mol. Biol. Rev.*, 65, 1-43.

Geographic Variation of Morphological Characters in *Puntius lateristriga* (Valenciennes, 1842) From Sumatra and the Adjacent Island

Dewi Imelda Roesma
Department of Biology, Faculty of Mathematics and Natural Sciences
University of Andalas, West Sumatra, Indonesia

Ada Chornelia
Department of Biology, Faculty of Mathematics and Natural Sciences
University of Andalas, West Sumatra, Indonesia

ABSTRACT

Puntius lateristriga (Valenciennes, 1842) is a tropical freshwater fish belonging to the Cyprinidae. Intraspecific variation among *P. lateristriga* from Sumatra Island and Singkep as adjacent island was studied based on 23 morphological characters by using UPGMA (*Unweighted Pair Group Method Arithmetic Average*) analysis. The data consist of new data from west Sumatra subpopulations (valley of mount Tujuh) and the old data from Haryono's report (Aceh, Sekundur, Bahorok, Lampung and Singkep). The study aimed to analysis the morphological characters variation of *P. lateristriga* in Sumatra and the adjacent island populations. The present results show that there are morphological variation on characters of *P. lateristriga*. *P. lateristriga* from west Sumatra which represent the middle part of Sumatra population closely related to *P. lateristriga* from Singkep island with 0.10 Euclidean distance and hold in one cluster with Lampung population which represent the south part of Sumatra population, while the Aceh, Sekundur and Bahorok populations which represent the north part of Sumatra populations were existed in another one cluster. Geologically active regions create many geographically isolated areas which can reflect in morphological characters of *P. lateristriga*.

Keywords: Geographic; morphology; Cyprinidae

1 INTRODUCTION

Puntius is a genus of cyprinids, widespread in Sumatra, Bangka Belitung, Singkep, Java and Malaysia (Weber and Beaufort, 1916; Roberts, 1989), while Taki *et al*. (1978) wrote that Puntius occur throughout the region from Pakistan to southern China, inhabiting various types of fresh waters. Puntius consist of some species with interesting colour pattern. One of the species from this genus is *Puntius lateristriga* (Valenciennes,1842). Fish of this species has colour pattern with a broad longitudinal stripe in addition to two cross band. *P. lateristriga* is a synonym to *Barbus lateristriga* (Valenciennes, 1842) and *Systomus lateristriga* (Valenciennes, 1842). According to Weber & Beaufort (1916) and Kottelat *et al.* (1993), this species distributed in Sundaland and their colour pattern showed geographically and ontogenetically variation. Tweedie (1961) found the variation of color pattern of six populations in Malaysia and found black reduction of colour pattern on the populations at the north of Kedah and Perlis. Other than high variation of colour pattern, morphological variation also exists in this species. Haryono (2001) found the existence of morphological and pattern of the color variations of *P. lateristriga* population in five places in Sumatra that is Aceh, Sekundur, Bahorok, Lampung and Singkep. Aceh, Sekundur and Bahorok were located at the north of Sumatra; Lampung was at the south of Sumatra while Singkep was an Island close to east Sumatra Island at the middle part (Figure 1.). There is no data from west or the middle part of Sumatra included in that report.

Sumatra is a unique island, at least there are two main barrier in Sumatra, first is Bukit Barisan mountain range that predicted formed around 2.5 million years ago (Miosen) and second is the eruption of Toba mount, as the biggest eruption in world history around 71.000 years ago and caused disruption of Bukit Barisan corridor (Hamilton , 1979: Whitten *et al*., 1987). Bukit Barisan

mountain range divided the island becomes west and east Sumatra as unequally parts, and the north and the south parts of Sumatra could be influence by Toba eruption. It was predicted that so many variation of fresh water fish in Sumatra according to its geography history. In other to complete the information and to analyze the variation of morphology characters of *P. lateristriga* in Sumatra and the adjacent island we used the Haryono's data (2001) instead of our new data from west Sumatra subpopulations. The study aimed to analyze the morphological variation of *P. lateristriga* in Sumatra and the adjacent island populations.

MATERIAL AND METHODS

Fish collection was made along 600-meters on each of six rivers in the valley of Mount of Tujuh in West Sumatra using standard procedures according to Cailliet *et al*. (1996) and specimens handling followed to procedures of Kottelat *et al*. (1993). Our new data were measured from 48 individuals while Haryono (2001) data as comparison initially from Sekundur (6 individuals), Bohorok (6 individuals), Aceh (2 individuals),Lampung (12 individuals) and Singkep Island (8 individuals). The distribution of samples is enclosed in Figure 1.

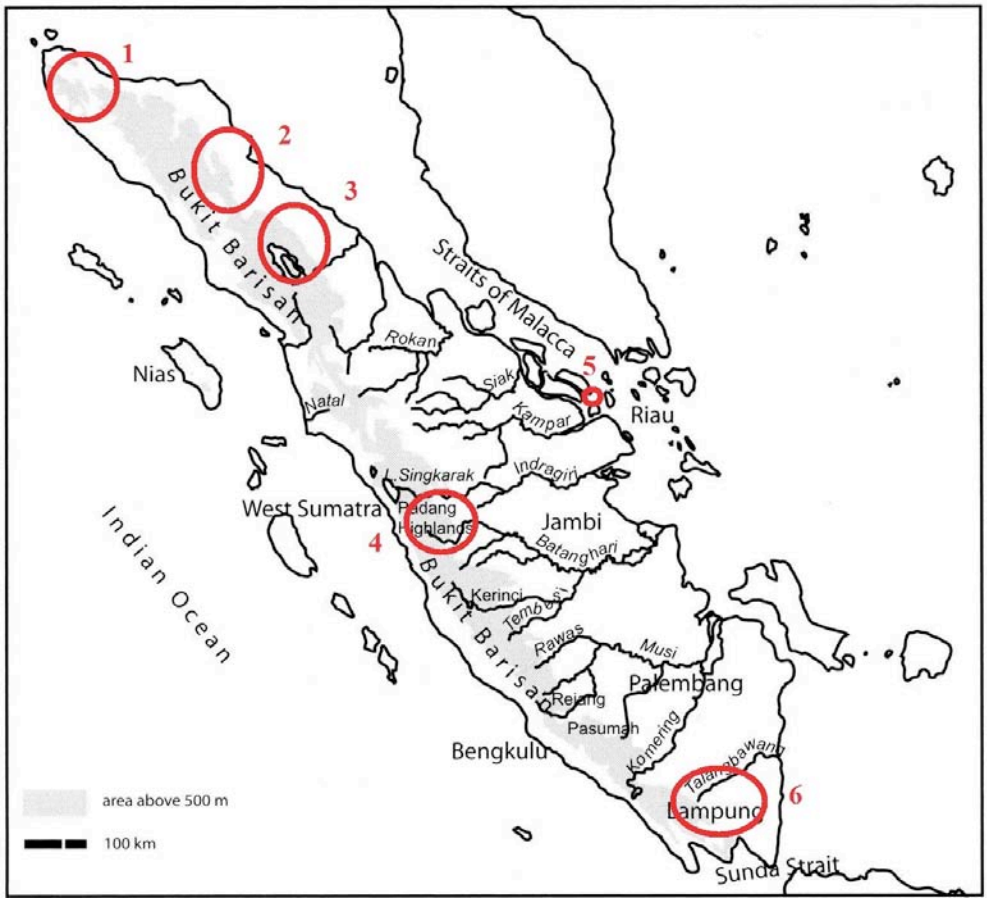


Figure 1. The location of samples of data (Note: 1. Aceh; 2. Sekundur; 3. Bohorok; 4. West Sumatra; 5. Singkep Island; dan 6. Lampung)

Each individual was measured based on Strauss and Bookstein (1982) with modification to nearest 0.1 mm using digital calipers on 23 morphometric characters. Those characters were also followed as Haryono (2001) did (Figure 2.). There were Total Length (TL), Standard Length (SL), Head Length (HL), Pre Dorsal Length (PDL), Pre Pelvic Length (PPL), Pre Anal Length (PAL), Head Depth(HD), Body Height (BD), Depth of Caudal Peduncle (DCP), Length of Caudal Peduncle (LCP), Snout Length (SNL), Body Width (BW), Eye Diameter (ED), Inter Orbital Width (IW), Length of Dorsal Base (LDB), Length of Anal Base (LAB), Length of Pelvic (LPVF), Length of Pectoral (LPCF), Length of Upper Caudal (LUCL), Length of Middle Caudal (LMCR), Length of Lower Caudal (LLCL), Maxillary Barbell Length (MXB), and Snout Barbell Length (SNB).

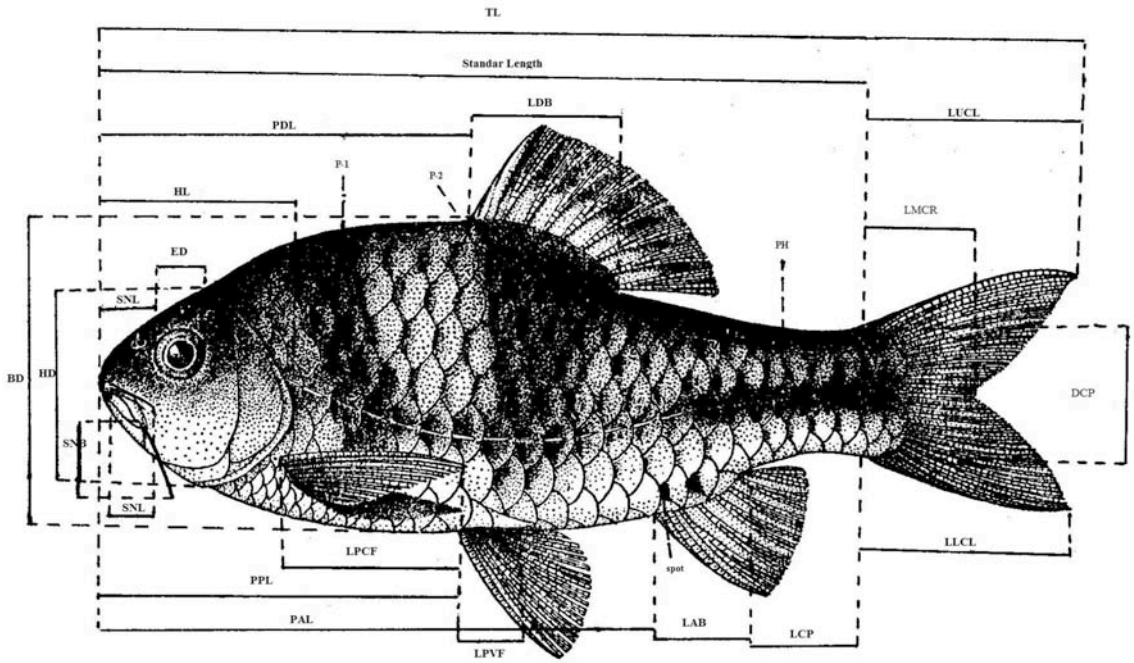


Figure 2. Morphometric characters measurement (Haryono, 2001)

The measured characters were transformed to percent of standard length (SL) and to common logarithms. In order to analyze the relationship of *P. lateristriga* population in Sumatra and the adjacent island based on new and Haryono (2001) data, cluster analysis was done by using NTSyst Ver. 2.0.2i program (Rohlf, 1998).

.

RESULT

The result of the measurement of 23 characters morphometric included in Table 1. The data consist of Haryono's (2001) data from Aceh, Sekundur, Bahorok, Lampung, Singkep and from west Sumatra as a new data.

		ACEH	SEKUNDUR	BAHOROK	LAMPUNG	SINGKEP	WEST SUMATRA
		n=2	n=6	n=6	n=12	n=8	n=48
	TL	134.02 ± 0.83	130.43 ± 1..99	134.89 ± 1.78	132.38 ± 1.37	131.52 ± 2..81	133.25 ± 0..22
2	SL	80.51 ± 10.45	80.02 ± 7.48	63.67 ± 6.75	64.62 ± 4.59	78.60 ± 10.59	66.81 ± 15.47
3	HL	29.78 ± 2.39	28.02 ± 1.46	29.18 ± 1.44	29.74 ± 2.01	25.17 ± 4.91	27.01 ± 0.02
4	PDL	51.71 ± 0.37	54.50 ± 0.96	55.33 ± 1.22	54.22 ± 1.16	53.49 ± 1.25	51.14 ± 0.04
5	PPL	55.36 ± 0.19	50.82 ± 1.65	49.61 ± 6.55	53.35 ± 1.55	51.73 ± 1.51	50.72 ± 0.02
6	PAL	76.91 ± 1.28	74.93 ± 1.15	74.62 ± 1.35	76.26 ± 1.61	75.89 ± 1.77	74.15 ± 0.02
7	HD	27.24 ± 1.60	24.90 ± 1.20	24.31 ± 0.75	24.31 ± 1.55	25.02 ± 1.27	23.44 ± 0.05
8	BD	41.80 ± 0.25	39.65 ± 1.61	40.89 ± 2.36	40.13 ± 1.19	40.77 ± 0.87	39.41 ± 0.03
9	DCP	14.60 ± 0.34	14.85 ± 0.33	15.74 ± 0.70	15.79 ± 0.39	15.24 ± 0.44	16.24 ± 0.01
10	LCP	19.30 ± 1.88	19.47 ± 1.65	18.06 ± 0.55	17.62 ± 1.04	17.93 ± 1.24	19.48 ± 0.02
11	SNL	9.46 ± 0.89	9.17 ± 0.64	9.41 ± 0.27	9.63 ± 0.91	9.78 ± 0.97	7.35 ± 0.01
12	BW	19.38 ± 0.92	19.85 ± 1.37	19.44 ± 1.07	19.69 ± 1.54	20.37 ± 1.63	14.91 ± 0.03
13	ED	7.24 ± 0.24	7.39 ± 0.56	8.13 ± 0.57	7.99 ± 0.36	6.91 ± 0.55	8.06 ± 0.01
14	IW	11.13 ± 0.86	11.09 ± 0.49	11.01 ± 0.41	10.79 ± 0.30	10.92 ± 0.52	10.90 ± 0.01
15	LDB	20.43 ± 0.29	19.82 ± 1.93	21.38 ± 1.62	21.68 ± 0.85	21.74 ± 0.53	19.48 ± 0.02
16	LAB	10.57 ± 0.13	10.08 ± 0.62	10.97 ± 1.07	11.84 ± 0.70	11.07 ± 0.60	9.85 ± 0.01
17	LPVF	22.41 ± 0.49	21.48 ± 1.02	22.99 ± 0.91	21.54 ± 0.71	20.17 ± 0.63	6.23 ± 0.01
18	LPCF	24.63 ± 0.28	23.68 ± 1.15	24.92 ± 0.38	22.26 ± 0.51	21.60 ± 1.61	23.35 ± 0.02
19	LUCL	34.07 ± 0.18	30.70 ± 1.46	33.57 ± 1.05	34.21 ± 1.53	31.74 ± 1.85	30.83 ± 0.03
20	LMCR	15.59 ± 1.48	15.04 ± 0.62	16.61 ± 1.32	16.32 ± 1.37	17.20 ± 1.93	13.85 ± 0.02

Table 1. Morphometric characters measurement of *P. lateristriga* new and Haryono (2001) data

The Total Length of *P. lateristriga* ranges from 130.43-134.89 mm and Standard Length from 63.67-80.51 mm. Based on the characters that have been measured, there was character divergence between populations. Those characters were: Standard Length (SL), Pre Pelvic Length (PPL), Body Width (BW), Length of Pelvic (LPVF), Length of Lower Caudal (LLCL) and Maxillary Barbell Length (MXB). To ascertain the existence of this character divergence, the analysis statistic such as Krusskal-Wallis and Mann-Whitney test to be needed. Because of acquirement data from Haryono (2001) was incomplete, and then prediction was conducted based on the result of analysis at level subpopulation of *P. lateristriga* in west Sumatra (Roesma, Chornelia & Putra, 2014 (unpublished). Analysis Kruskall-Wallis at those six subpopulations showed the character divergence between them. There were six characters found that experience of divergence that is Head Length (HL), Pre Anal Length (PAL), Depth of Caudal Peduncle (DCP), Body Width (BW), Eye Diameter (ED) and Length of Middle Caudal (LMCR). Analysis with Mann-Whitney test at subpopulations showed 1-8 characters differ between rivers in west Sumatra (subpopulations from six rivers).

Based on the value of Euclidean distance between six population of *P. lateristriga* in Sumatra and the adjacent island (Table 2.), it can be concluded that based on morphological characters, the most closely related populations was between *P. lateristriga* from West Sumatra and Singkep (0.10), followed by Bahorok-Sekundur (0.118) and Sekundur-Aceh (0.158). The smaller Euclidean distance value, the closer their relationship.

Tabel 2. Euclidean distance between six populations of *P. lateristriga* in Sumatra and the adjacent island.

Population	Aceh	Sekundur	Bahorok	Lampung	Singkep	WestSumatra
Aceh	-					
Sekundur	0.158	-				
Bahorok	0.58	0.118	-			
Lampung	0.21	0.21	0.21	-		
Singkep	0.21	0.21	0.21	0.14	-	
WestSum	0.21	0.21	0.21	0.14	0.10	-

Figure 3. show the result of UPGMA analysis on 23 morphological characters. There were two main cluster, first consist of *P.lateristriga* from West Sumatra, Singkep Island and Lampung, second consist of population from Aceh, Sekundur, and Bahorok. The Euclidean distance between those two groups was 0.21 (Table 2). All populations on each cluster also tend to subdividing, the first cluster was West Sumatra and Singkep populations held-up with Lampung population, and second cluster was Bahorok and Sekundur populations held-up with Aceh population.

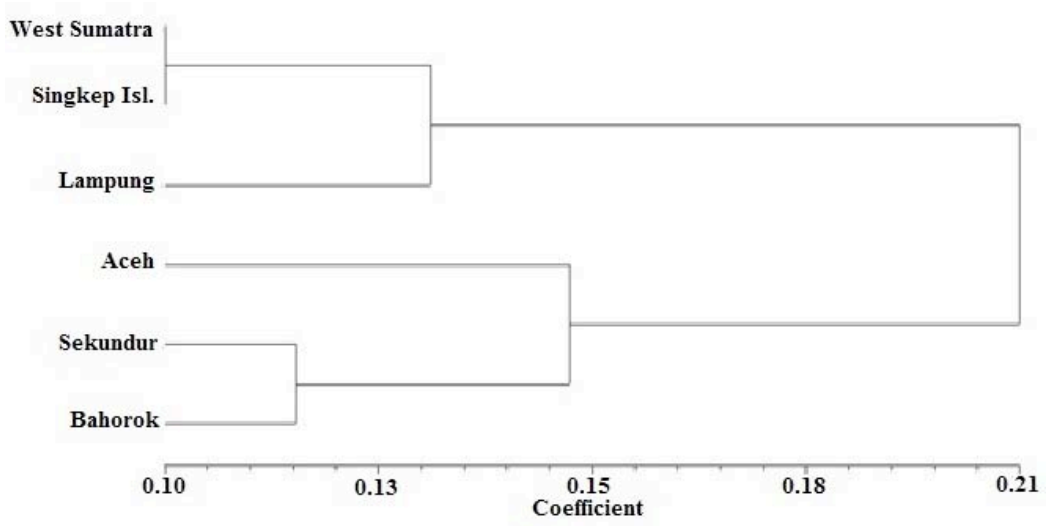


Figure 3. Dendogram of *P. lateristriga* between populations in Sumatra and adjacent island.

DISCUSSION

The existence of characters variation of morphology that found between *P.lateristriga* subpopulation in six rivers in West Sumatra (Roesma *et al*. 2014 unpublished) suggested that character divergence between populations were happened in Sumatera. Base on characters comparison that experience of divergence at subpopulation in west Sumatra and populations in the north and south Sumatra and Singkep Island, the Body Width (BW) was a character that constantly showed divergence between populations. It was predicted that those variation of character morphology caused of environmental pressure which vary geographically. It has been reported that the differences at body size character can be triggered by predator (Bronmark and Miner, 1992), territorial water condition (lotic or bentic) (Day *et al*., 1994), temperature difference between rivers (Beacham, 1990) and food type and way to eat (Day, *et al*., 1994; Robinson and Wilson, 1996).

According to Kondrashov and Kondrashov (1951) the characters variation of morphology that happened inter populations could be triggered by the process of speciation that caused by reproduction insulation. Losos and Glor (2003) stated that besides reproduction insulation, geographical insulation also supports the variation that tends to make subspecies or new species. Haryono (2001) concluded that pattern of widespread and morphology variation that emerges were caused by the different interregional of physical environment. There for it could be assumed that the primary factors which result in character divergence between populations are geographical factor, reproduction insulation and physical pressure from environment. According to Krukk (1999), the pressure of physical environment factors can result in divergence at individual in interpopulation. Those factors can be in form of ecological selection, sexual selection and natural selection. As a consequence, adaptation to the environment will be done and adaptation or non adaptation process could be happen. According to Haryono (2001) the environmentally pressure which produce changes and adjustments is physical factor like water temperature, pH, salinities, water brightness, water current, altitude and others. Thompson (1991) state that the factors of physical environment influence the phenotype as a consequence of genotype response to the different of environmental pressure. De Silva and Liyanage (2006) state that the environmental factor that affect on Puntius variation of morphology character is altitude either between different rivers or same river and that condition called as morphological plasticity.

Variations of morphology character made a cluster between populations. Analysis cluster is conducted to know the value of distance Euclidean between populations (Tables 2, Figure 3). There is a tendency that geographical distance has positive correlation by distance Euclidean. The bigger geographical distance, the bigger value of distance Euclidean and vise versa. The smaller value of

the distance Euclidean indicates that the variation of characters morphology between population getting smaller. Bernatchez *et al*.(1992) state that subdividing a population in to cluster can be caused by life-history and dissociation process that happened because of process geologic millions year ago. According to Taki (1978), that existence of equality in one cluster can be assumed that they have common ancestors. There for, there is a possibility that subdividing between population in one species resulted from sharing common ancestor, nevertheless disjointed by geological process and history biogeography of Sumatra millions year ago.

Sumatera is an unstable land that disjointed at a period of Pleistocene, formed because of continuously interaction between physical factor, land/ground and climate (Hamilton, 1979 and Whitten *et al*., 1987). In the beginning of Miocene, formed of Bukit Barisan mountain range divide the island into two unequal part and condition. This unsymmetrical part is also affected by activities of volcanic in mountain range; as a consequence the cycle of hydrology in Sumatra is varying. The western rivers relative short and flows to the West coast of Sumatra while the eastern rivers relative sloping, wide, length and flows to Sea of South Chinese (Figure 1) (Whitten *et al*., 1987 and Colombijn, 2005). Geologically active regions create many geographically isolated areas and followed by various ecosystem conditions.

It is interesting to study more detail about this spesies, why the population in west Sumatra and Singkep Island has a smaller Euclidean distance than other part of Sumatra. Do Bukit Barisan mountain range and Toba eruption could be affect in morphology and genetic of others fresh water fish in the north and the south parts of Sumatra.

CONCLUSION

There are geographic variation of morphological characters in *P. lateristriga* in Sumatra and the adjacent island. The results of analysis show that *P. lateristriga* from west Sumatra which represent the middle part of Sumatra population closely related to *P. lateristriga* from Singkep island with 0.10 Euclidean distance and hold in one cluster with Lampung population which represent the south part of Sumatra population, while the Aceh, Sekundur and Bahorok populations which represent the north part of Sumatra populations were existed in another one cluster

ACKNOWLEDGEMENTS :

We wish to express our thanks to Manager and staff of Tidar Kerinci Agung Company for the grant and facilities, to Mr. Haryono for the data on paper, and to Dr. Wilson Novarino and team for their collaboration.

REFERENCES

Beacham TD. 1990. A genetic analysis of meristic and morphometric variation in chum salmon (*Oncorhynchus keta*) at three different temperatures. Canadian. *Journal of Zoology.* 68: 225-229

Bernatchez. L, R. Guyomard‘and F. Bonhomme.1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *SaZmo tvutta* populations. *Molecular biology.* 1: 161-173

Cailliet, G.M, M.S. Love and A.W. Ebeling. 1986. Fishes. A Field and Laboratory Manual on Their Structure, Identification and Natural History. Waveland Press, Inc.

Colombijn, F.2005. A Moving History of Middle Sumatra 1600-1870. *Modern Asia Studies.* 39: 1-38

Day T. Pritchard J. and Schluter D. 1994. A comparison of two sticklebacks. *Evolution*.48:1723-1734

De Silva, M.P.K.S.K., and N.P.P Liyanage. 2006. Intra specific morphological plasticity in three *Puntiu*s species in Srilanka. *Ruhuna Journal of science.*1: 82-95

Hamilton. 1979. Tectonic of the Indonesian Region. GEOLOGICAL Survey Professional Paper. United States Government Printing Office, Washington

Haryono. 2001. Variasi Morfologi dan Morfometri Ikan Dokun (*Puntius* lateristriga) di Sumatera. *Biota*. 6: 109-116

Kondrashov and Kondrashov. 1999. Interaction among quantitative traits in the course of sympatric speciation. *Nature.* 40: 351-354.

Kottelat, M A. J. Whitten, S. N. Kartikasari and S. Wirjoatmodjo (1993*). Freshwater Fishes of Western Indonesia and Sulawesi*. Periplus Editions (HK) Ltd. and EMDI : Indonesia, Singapore

Kruuk, L. 1999. Sticklers for Sympatry. *Tree.*14 : 465-466

Losos, J.B and R.E Glor. 2003. Phylogenetic Comparative Methods and the Geographic of Speciation. *Trends in Ecologi and Evolution.* 18: 220-226.

Roberts, T.R. 2989. The Freshwater fishes of western Borneo (Kalimantan Barat, Indonesia). *Memoirs of the California Academy of Science*. 14: 1-120

Robinson B.W. and D.S Wilson. 1996. Genetic variation and phenotypic plasticity ina trophically polymorphic population of pumpkinseed sunfish (Lepomis gibbus). *Evolutionary Ecology* 10:631-652

Roesma, D.I, A. Chornelia, A.A. Putra, 2014. Variasi Morfometri Ikan *Puntius lateristriga* Valenciennes, 1842 (Pisces: Cyprinidae) Dari Beberapa Sungai di Kawasan PT. Tidar Kerinci Agung (TKA) Sumatera Barat. Unpublihed.

Rohlf, F.J. 2001. NT Syst. *Numerical Taxonomy and Multivariate Analysis System Version 2.0.2.* New York: Applied Biostatistic Inc.

Strauss, R. E. and F. L. Bookstein. 1982. The Truss: Body Form Reconstruction in Morphometrics. *Syt. Zool*. 31: 113-135

Taki,Y. A. Katsuyama, T. Urushido, 1978. Comparative Morphology and Interspecific Relationships of the Cyprinid Genus *Puntius*. *Jap. J. Icht*.Vol. 25. No. 1

Thompson J.D. 1991. Phenotypic plasticity as a component of evolutionary change. *Trends in Ecology and Evolution.* 6:246-249

Tweedie, M.W.F. 1961. Notes on Malayan Fresh Water Fishes.9. Regional differentiation in the colour pattern of *Puntius lateristriga. Bulletin of the Raffles Museum.* 26: 178-182

Voris, H.K. 2000. Maps of Pleistocene sea level in southeast Asia : shorelines, River systems and Time Duration. *Journal of Biogeography.* 27: 1153-1167

Weber, M. and .L.F. de Beaufort. 1916. *The Fi*shes of Indo-Australian Archipelago (ostariophysi II: Cyprinoidea, Apodes, synbranchi). E.J. Brill leiden.Ltd

Whitten, A.J., J. Anwar, and N. Nisyam. 1987. *The Ecology of Sumatra*. Yokyakarta : Gadjah Mada University Press

Hydrolysation of Palm Oil Empty Fruit Bunch by Using Crude Extract Enzymes of *Aspergillus niger* and Analysis of Its Hydrolizates for Bioethanol Production

Kahar Muzakhar
Biology Dept., Faculty of Math. and Nat. Sci., The University of Jember, Indonesia

Sutoyo
Biology Dept., Faculty of Math. and Nat. Sci., The University of Jember, Indonesia

Siswoyo
Chemistry Dept., Faculty of Math. and Nat. Sci., The University of Jember, Indonesia

Rosita Fitrah Dewi
Graduate Student at Biology Dept., Faculty of Math. and Nat. Sci., The University of Jember, Indonesia

Widya Yuniar
Graduate Student at Biology Dept., Faculty of Math. and Nat. Sci., The University of Jember, Indonesia

ABSTRACT

A huge amount of lignocellulosic-rich palm oil empty fruit bunch (POEFB) biomass was released during palm oil harvested. Observation showed that *Aspergillus niger* could grow well in POEFB without any nutrion added. And after 5 days cultivated at 30^{o}C the extracellular enzyme of *A. niger* was extracted using 1% NaCl. Of about 500 ml crude enzyme was concentrated by ammonium sulphate precipitation at 70% saturation. The precipitates was then dissolved to 50 ml and dialysed against 20mM acetate buffer at pH 5 to remove remaining ammonium sulphate. Four hundred milligrams of powdered POEFB was suspended in 20 ml dialysed crude enzyme for 36 hours hydrolysation at 37^{o}C and 100 rpm shaken. The hydrolyzates was then fermented using *Saccharomycess cerrevisae* at 30^{o}C for 24 hours. Colorimetric analysis at OD 580nm using QuantiChrom Kit DIET-500 showed that fermentation produced alcohol 0.88%, respectively. Suggested POEFB is an alternative biomass potential than can be used for bioethanol production efficiently.

Keywords: hydrolysation, POEFB, fermentation, bioethanol

INTRODUCTION

Indonesia as agriculture countries is well known for its potential in agriculture biomass wastes such as palm waste, sugarcane bagasse and paddy rice. At present and from forcasted production of palm oil for the year 2000–2020 [26], Indonesia is the largest producer and exporter of palm oil in the international market. In the process production of palm oil from the fruit, a huge mount of lignocellulosic material oil palm empty fruit bunch (POEFB) is generated as a waste product [6, 33], accumulated in the field, and considered to be difficult materials to digest [20] so that the utilisation of this biomass waste product is still less attention. In practice this biomass is burned in incinerators [5]. It was reported that 14.5 million tons of POEFB biomass waste is generated annually in the world and half of POEFB was produced in Indonesia [10, 30].

A bioconversion of biomass including organic waste materials to fuel is receiving interest as they are low cost, renewable and widespread in nature [28]. Researchs related to POEFB utilisation has been demonstrated for biohydrogen [2, 9], activated carbon [3], biochar [12], xylooligosaccharides [13], sugars [21], latic acid [23], biogas [29], cellulase [31], biodiesel [32] and bioethanol production [14, 19, 27]. However to convert ethanol from POEFB still limited gave low efficiency so that some researchs effort to meet POEFB conversion with high yield be done. In this paper will be explained POEFB saccharification to produce sugars by using exctracellular enzymes from *A. niger*, followed by fermentation to produce ethanol.

2 MATERIALS AND METHODS

2.1 Extracellular Enzyme Production of *A. niger* for POEFB Hydrolysation

Source extracellular enzyme was produced from one kilogram of sterilized POEFB in a 5 liter flask which inoculated with *A. niger* and incubated at 30°C for 5 days. To harvest the enzyme was done by adding 500 ml water containing 1% NaCl, shaken at room temperature for 9 hours and filtered using paper filter on funnel Buchner. Remaining debris from the filtrate was removed by centrifugation at 12000 rpm for 20 minutes. The supernatant as crude enzyme was then concentrated by ammonium sulfate at 70% saturation, dissolved and dialyzed against water for 3 days to remove the remaining ammonium sulphate, bring to 50 ml total volume with the same buffer and stored at 4°C until needed for POEFB hydrolysis.

2.2 POEFB Enzymatic Hydrolysis

One percent of powdered POEFB substrate (400 milligrams/20ml) was suspended into concentrated crude enzyme, incubated at 37°C, shaken 100 rpm, 36 hours for hydrolysis. The resulting supernatant as hydrolysate was harvested from the remaining POEFB substrate by centrifugation at 4000 rpm for 10 minutes. Hydrolysate was stored at 4°C for next sugars component analysis. This procedure was done in double.

2.3 Analysis of Sugar Component of POEFB

The reducing sugar of hydrolysate was quantified using Somogy [24] and Nelson [16] methods. For total sugars of POEFB was estimated colorimetric using phenol-sulfuric method [11]. Further analysis for sugar composition of hydrolysate was done by using Thermo Scientific Trace 1310 Gas Chromatograph (GC) using TG-225MS 15m x 0.25mm x 0.25µm column. One milliliter hydrloisate was prepared as alditol acetates [5, 25] and 0.5µl injected to GC for analysis. During analysis, the GC was setup at 190°C (5 minute hold) to 250°C at 8°C/min (5 minute hold) and carrier gas hydrogen ajusted at flow rate 45cm/sec. The hydrolysate was also analyzed by using TLC in silica gel plate (Merck, silica gel 60 F254). A Mixture (v/v) of butanol : ethanol : chloroform : amonia (4 : 7.5 : 4 : 8) was used as solvent system and 0.1% Sulfuric acid containing o.1% vanilin was employed for detection.

2.4 Anaerobic Fermentation

The yeast cells *S. cerreviseae* for anaerobic fermentation was preculture aerobically in 100 ml medium pH 6 in 500 ml shaker flasks, containing yeast-extract (0.3%), malt extract (0.3%), pepton (0.5%) and glucose (1%). The culture was incubated at 25°C under shaker set at 120 rpm for 24 hours, and active yeast cells were harvested by centrifugation at 4000 rpm for 5 minutes. The pellet (≈20 mg) was suspended in 1 ml sterilized water and used for source inoculum in anaerobic fermentation POEFB hydrolysate. Fermentation was done in 20 ml medium using mini fermenter at 30°C. The medium was inoculated with 2 mg cells (≈0.1ml) of source inoculum prepared above.

2.5 Analysis of Ethanol Concentration

The ethanol concentration was analysis using QuantiChrom Kit DIET-500 colorimetric method at OD 580nm. For data comparison, analysis was also analysed using GC above equipped with Trace GOLD TG-1301MS GC column. The machine was setup at 250°C with carrier gas helium at flow rate 35cm/sec.

3 RESULTS AND DISCUSSION

3.1 Enzyme Production of *A. niger* on POEFB

Production of exctracellular enzyme of *A. niger* in POEFB medium without any nutrient added was successfully done. Evidence showed that after 3 days cultivation, *A. niger* grow well in POEFB. Much mycelium with their black spores covered whole of medium. Mean, *A. niger* certainly utilized carbon and nitrogen from POEFB for the growth. Further, we observed that after 5 days cultivation, small amount liquid was produced indicated that POEFB hydrolysed by extracellular enzyme which secreted by *A. niger* during cultivation. The exctracellular enzyme was then harvesting by 1% NaCl extraction.

3.2 Enzymatic Hydrolysis of POEFB

Hydrolysis was done without any adjusting of buffer usage as well as optimum pH and temperature. We found that this enzyme readily hydrolysed alkali extract of POEFB and maximum degree of hydrolysis was 62% after 36 hours incubation at 37°C. The longer incubation time, the more higher the degree of hydrolysis. However, thereafter 72 hours the hydrolysis is still in progress and could presumably attain the same hydrolysis at very much later time.

3.3 Determination of Sugars Component in Hydrolysate

POEFB hydrolysate was rich in monosaccharide but poor in oligosaccharides. The oligosaccharide concentration constituted less than 1% of the total sugar content. By the TLC analysis, it was clear that the hydrolysis product was predominantly monosaccharide glucose respectively as shown in the Figure 1 as follow.

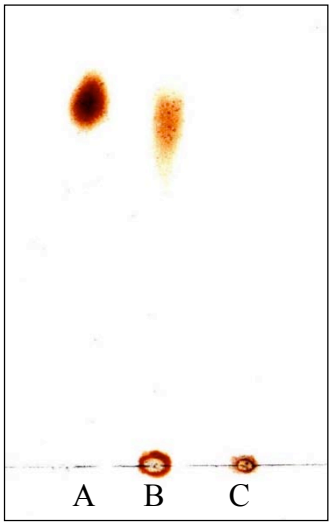


Figure 1: TLC analysis of hydrolysate ; standard glucose (A), POEFB hydrolysate (B) and POEFB before hydrolysis (C)

GC analysis revealed that POEFB-hydrolysate consisted of glucose (86.1%) while the minor are xylose (4.1%), and other monosaccharides less than 1%. GC analysis of total sugars as monosaccharides in hydrolysate 12.4 mg/ml. And GC calculation of glucose and xylose are 10.7 mg/ml and 0.51 mg/ml respectively. The capability of crude enzyme to hydrolyze POEFB and produce glucose and xylose as monosaccharide could be due to the fact that some genus *Aspergillus* secrets of various cellusases [4, 15], glucoamylase [1] and xylanase [7]. POEFB-hydrolyzate was also contain crude protein nearly 0.3% on dry-basis which was needed as a nitrogen source for fermentation.

3.4 Ethanol Production Analysis

Anaerobic fermentation to produce ethanol using POEFB hydrolysate was done directly by innoculating cells yeast of *S. cerrevisae* and without any adjusting of both pH or sugar concentration of hydrolysate at 30^{o}C for 24 hours. The maximum concentration of ethanol produced was 0.88% when analysed using colorimetric analysis. This concentration precentage also similar result when GC analysis used, that was 0.81%. Calculation ethanol production efficiency 72%.

3.5 Ethanol Production Analysis

After fermentation, the remaining sugar in hydrolysate also analysed by GC and we found the glucose concentration was very low with value 0.2 mg/ml. However, the xylose almost nearly same comparing with initial concetration before fermentation, that was 0.49 mg/ml respectively. This evidence proved that *S. cerrevisae* could not ferments xylose from POEFB hydrolysate, but in contrast *S. cerrevisae* definitely utilized and converted glucose to ethanol as well as for their cells growth. By these results, improvement to increase the yield of is needed. Optimizing condition both enzymatic hydrolysis [22, 30, 17] and environtment factors as well as parameters in fermentation such as feed sugar concentration [8, 18], pH of medium and temperature [8].

4 CONCLUSION

Bionconversion of POEFB biomass to bioethanol in laboratory scale was successfully done and resulting in production efficiency was 72%, respectively. Suggested POEFB is an alternative biomass potential than can be used for bioethanol production efficiently so that improvement in industrial scale must be planed. However, effort in optimizing of the process as well as progressively research incuding economically calculation must be highly considerated.

ACKNOWLEDGEMENT

The author wishes to thank the Directorate General Higher Education, Ministry of Culture and Education, Government of Indonesia who financially supported for research and conference.

REFERENCES

[1] Abe J., Takeda Y., Hizukuri S., 1982, "Action of glucoamylase from *Aspergillus niger* on phosphorylated substrate", Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, Vol. 703, Issue 1, Pages 26-33.

[2] Abdul P. M., Jahim J. M., Harun S., Markom M., Hassan O., Mohammad A. W., Asis A. J., 2013, "Biohydrogen production from pentose-rich oil palm empty fruit bunch molasses: A first trial", International Journal of Hydrogen Energy, Vol. 38, Issue 35, Pages 15693-15699.

[3] Alam Md. Z., Muyibi S. A., Mansor M. F., Wahid R., 2007, "Activated carbons derived from oil palm empty-fruit bunches: Application to environmental problems", Journal of Environmental Sciences, Vol. 19, Issue 1, Pages 103-108.

[4] Anthony T., Raj C. K., Rajendran A., Gunasekaran P., 2003, "High molecular weight cellulase-free xylanase from alkali-tolerant *Aspergillus fumigatus* AR1", Enzyme and Microbial Technology, Vol. 32, Issue 6, Pages 647-654.

[5] Arai, M. and Murao. S., 1978, “Characterization of oligosaccharides from an enzymatic hydrolyzate of red yeast cell walls by lytic enzyme”, Agric. Biol. Chem., Vol. 42, Pages 1651-1659.

[6] Baharuddin A. S., Sulaiman A., Kim D. H., Mokhtar M. N., Hassan M. A., Wakisaka M., Shirai Y., Nishida H., 2013, "Selective component degradation of oil palm empty fruit bunches (OPEFB) using high-pressure steam", Biomass and Bioenergy, Vol. 55, Pages 268-275.

[7] Botella C., Diaz A., Ory I., Webb C., Blandino A., 2007, "Xylanase and pectinase production by *Aspergillus awamori* on grape pomace in solid state fermentation, Process Biochemistry, Vol. 42, Issue 1, Pages 98-101.

[8] Cazetta M.L., Celligoi M.A.P.C., Buzato J.B., Scarmino I.S., 2007, "Fermentation of molasses by *Zymomonas mobilis*: Effects of temperature and sugar concentration on ethanol production", Bioresource Technology, Vol. 98, Issue 15, Pages 2824-2828.

[9] Chong P. S., J. Jahim M., Harun S., Lim S. S., Mutalib S. A., Hassan O., Nor M. T. M., 2013, "Enhancement of batch biohydrogen production from prehydrolysate of acid treated oil palm empty fruit bunch", International Journal of Hydrogen Energy, Vol. 38, Issue 22, Pages 9592-9599.

[10] DOA (Departement of Agriculture), 2006. "Pedoman Pengelolaan Limbah Industri Kelapa Sawit", Departement of Agriculture Report Republic of Indonesia.

[11] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956, "Colorimetric method for determination of sugars and related substances", Anal. Chem., Vol. 28, Pages 350-356.

[12] Harsono S. S., Grundman P., Lau L. H., Hansen A., Salleh M. A. M., Meyer A. A., Idris A., Ghazi T. I. M., 2013, "Energy balances, greenhouse gas emissions and economics of biochar production from palm oil empty fruit bunches", Resources, Conservation and Recycling, Vol. 77, Pages 108-115.

[13] Ho A. L., Carvalheiro F., Duarte L. C., Roseiro L. B., Charalampopoulos D., Rastall R. A., 2014, "Production and purification of xylooligosaccharides from oil palm empty fruit bunch fibre by a non-isothermal process", Bioresource Technology, Vol. 152, Pages 526-529.

[14] Jung Y. H., Kim I. J., Han J., Choi I., Kim K. H. , 2011, "Aqueous ammonia pretreatment of oil palm empty fruit bunches for ethanol production", Bioresource Technology, Vol. 102, Issue 20, Pages 9806-9809.

[15] Liu D., Zhang R., Yang X., Wu H., Xu D., Tang Z., Shen Q., 2011, "Thermostable cellulase production of *Aspergillus fumigatus* Z5 under solid-state fermentation and its application in degradation of agricultural wastes", International Biodeterioration & Biodegradation, Vol. 65, Issue 5, Pages 717-725.

[16] Nelson, N. 1994, "A photometric adaptation of the Somogy method for the determination of glucose". J. Biol. Chem., Vol. 153, Pages 375-380.

[17] Newman R. H., Vaidya A. A., Sohel M. I., Jack M. W., 2013, "Optimizing the enzyme loading and incubation time in enzymatic hydrolysis of lignocellulosic substrates", Bioresource Technology, Vol. 129, Pages 33-38.

[18] Ozmihci S., Kargi F., 2007, "Effects of feed sugar concentration on continuous ethanol fermentation of cheese whey powder solution (CWP)", Enzyme and Microbial Technology, Vol. 41, Issues 6–7, Pages 876-880.

[19] Piarpuzán D., Quintero J. A., Cardona C. A., 2011, "Empty fruit bunches from oil palm as a potential raw material for fuel ethanol production", Biomass and Bioenergy, Vol. 35, Issue 3, Pages 1130-1137.

[20] Purwandari F. A., Sanjaya A. P., Millati R., Cahyanto M. N., Horváth I. S., Niklasson C., Taherzadeh M. J., 2013, "Pretreatment of oil palm empty fruit bunch (OPEFB) by N-methylmorpholine-N-oxide (NMMO) for biogas production: Structural changes and digestion improvement", Bioresource Technology, vol. 128, Pages 461-466.

[21] Saleha S., Shah U. K. M., Zainudin H., Aziz S. A., Kamal S. M. M., Shirai Y., Hassan M. A., 2012, "Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars", Biomass and Bioenergy, Vol. 36, Pages 280-288.

[22] Shill K., Miller K., Clark D. S., Blanch H. W., 2012, "A model for optimizing the enzymatic hydrolysis of ionic liquid-pretreated lignocellulose", Bioresource Technology, Vol. 126, Pages 290-297.

[23] Sitompul J. P., Simangunsong R. F., Asrizal A. A., Alisyahbana H., Lee H. W., Rasrendra C. B., 2014, "Catalytic Conversion of Empty Fruit Bunch of Palm Oil for Producing Lactic Acid", Procedia Chemistry, Vol. 9, Pages 88-93.

[24] Somogy M., 1952, "Notes on sugar determination", J. Biol. Chem. Vol.195, Pages 19-23.

[25] Spiro, G., 1972, "Study of carbohydrates of glycoproteins, Methods in Enzymology". Academic Press, New York and London Vol.XXVIII. Ginsburg I.V., (ed.), Part B. p. 1-11.

[26] Sumathi S., Chai S.P., Mohamed A.R., 2008, "Utilization of oil palm as a source of renewable energy in Malaysia", Renewable and Sustainable Energy Reviews, vol. 12, Pages 2404–2421.

[27] Tan L., Yu Y., Li X., Zhao J., Qu Y., Choo Y. M., Loh S. K., 2013, "Pretreatment of empty fruit bunch from oil palm for fuel ethanol production and proposed biorefinery process", Bioresource Technology, Vol. 135, Pages 275-282.

[28] Thambirajah J. J., Zulkali M. D. and Hashim M. A., 1995, "Microbiological and biochemical changes during the composting of oil palm empty-fruit bunches effect of nitrogen supplementation on the substrate", Bioresource Technology, Pages 133-144.

[29] Thong S. O, Boe K., Angelidaki I., 2012, " Thermophilic anaerobic co-digestion of oil palm empty fruit bunches with palm oil mill effluent for efficient biogas production", Applied Energy, Volume 93, Pages 648-654.

[30] USDA, 2012. “Palm Oil: World Supply and Distribution”, Foreign Agricultural Services.

[31] Wang Z., Ong H., Geng A., 2012, "Cellulase production and oil palm empty fruit bunch saccharification by a new isolate of *Trichoderma koningii* D-64", Process Biochemistry, Vol. 47, Issue 11, Pages 1564-1571.

[32] Yaakob Z., Sukarman I. S. B., Narayanan B., Abdullah S. R. S., Ismail M., 2012, "Utilization of palm empty fruit bunch for the production of biodiesel from *Jatropha curcas* oil", Bioresource Technology, Vol. 104, Pages 695-700.

[33] Zainudin M. H. M., Hassan M. A., Tokura M., Shirai Y., 2013, "Indigenous cellulolytic and hemicellulolytic bacteria enhanced rapid co-composting of lignocellulose oil palm empty fruit bunch with palm oil mill effluent anaerobic sludge", Bioresource Technology, Vol. 147, Pages 632-635.

Ant Species (Hymenoptera: Formicidae) in Dragon Fruit Plantation (*Hylocereus* spp.) of West Sumatra, Indonesia

Henny Herwina
Rijal Satria
Diyona Putri
Ranny
Department of Biology, Faculty of Mathematics and Natural Sciences
Andalas University, Indonesia

Yaherwandi
Department of Pests and Plant Deseases, Faculty of Agriculture
Andalas University, Indonesia

Jumjunidang
Indonesian Tropical Fruit Research Institute, West Sumatra, Indonesia

ABSTRACT

A study about ant species was conducted in five Dragon fruit (*Hylocereus* spp.) plantations in West Sumatra, Indonesia. Bite traps, soil core sampling and hand collection methods were used to collect ants from July 2013 to July 2014. A total of 34 species of ants that belonging to five subfamilies, 13 tribes, 22 genera and 8524 individual was collected. Myrmicinae was the highest in the number of species (15 species) followed by Formicinae (11 species), Dolichoderinae and Ponerinae (four species each). Aenictinae was the lowest with only one species. *Pheidole* and *Tetramorium* were the genera with the highest in the number of species (four species each). *Tapinoma melanochepalum* was recorded more frequently followed by *Selenopsis geminata* and *Monomorium floricola.* The effect of invasive ant *Solenopsis geminata* on dragon fruit health and production were also discussed.

Keywords: Dragon fruit; plantations; invasive; ants

1 INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is a tropical fruit native to Mexico and United States. This fruit has been cultivated commercially in Indonesia since 2000 [10]. Because of its good markets, dragon fruit plants grow very fast in some area of Indonesia [9]. Currently the dragon fruit was facing serious problems due to pests and diseases attacks. The diseases caused reducing of dragon fruit production up to 80% in several dragon fruit producing area of Indonesia [10].

The dragon fruit plantation in United States is relatively free from harmful pests and diseases [12] meanwhile yellow stem rot, stem freckle and stem spots/antrachnose, those were three major diseases attacking of dragon fruit plants in all locations observed in West Sumatra and Riau Islands Province. Pests found attacking dragon fruit plants in West Sumatra are scales fleas, lice shells and red ants. This pests and diseases caused the plant failed to produce, even most of them destroyed plantation [10].

Cardiocondyla wroughtoni, *Paratrechina longicornis, Pheidole megachepala,* and *Solenopsis geminata* were reported as pests to dragon fruit in Thailand [3]. During our observation to five traditional dragon fruit plantations in West Sumatra we saw several species of ant on plant stems, flowers and fruits as well as in ground. Some ants caused a serious problem for farmers during maintenance and harvesting. The information about ant in dragon fruit is still scanty so we are considering the importance of study about ant species in dragon fruit plants of West Sumatra.

2 MATERIALS AND METHODS

Ants were collected from five dragon fruit traditional plantations (Lubuk Minturun plantation in Padang City, Ketaping and Gasang plantation in Pariaman Regency, Aripan in Solok Regency, and Ujung Gading in West Pasaman Regency) of West Sumatra Province (1°00'S, 100°30'E, Figure 1). The altitudes of the locations range from 2 to 450 m above sea level, temperature-range during the study was about 28 to 32°C.

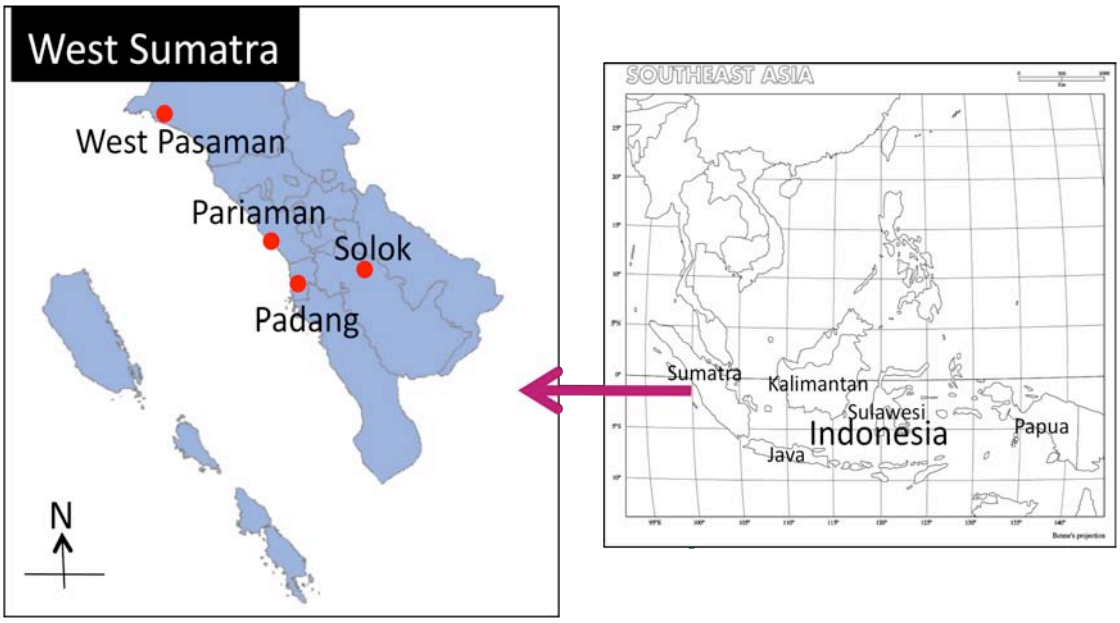


Figure 1: The location of one city and three regencies where ants were collected from dragon fruit plantations in West Sumatra.

In each plantation, one transect was set, 180 m long and subdivided into three 60 m sampling sections. Each sampling section was sampled by the three different methods, hand collecting (HC), soil core sampling (SC), and honey bait trap (BT). These three ant collection methods were adapted from four methods in Quadra Protocol Methods [4] except litter shifter. The methods involved the following procedures:

HC: Ants were picked up using forceps or an aspirator. This procedure was carried out for 30 minutes per sampling section.

SC: Five soil cores, each 20 x 20 x 15 cm deep, were taken at equal intervals along the transect in each sampling section. These soil cores were sifted using a hand sieve and white pan, and the ants collected.

BT: Fifteen baits with honey solution were set on the plantation floor at four-meter intervals along the transect in each sampling section. The ants attraced to the baits were collected with forceps.

Ants were sorted to genus and morphospecies level at the Animal Taxonomy Laboratory of the Department of Biology of Andalas University, following Bolton (1994). The ant specimens are housed in the Laboratory of Animal Taxonomy, Department of Andalas University, Indonesia.

3 RESULTS AND DISCUSSION

A total of 34 species of ants that belonging to five subfamilies, 13 tribes, 22 genera and 8524 individual was collected from five dragon fruit plantation in West Sumatra (Table 1). About 15

species were collected by hand collection on dragon fruit plant stem, flower or fruit and the remain were collected from ground. The total number of species collected quite high considering dragon fruit plantation is kind of monoculture plantation. Ant study in banana plantation with BBTV symptom only collected 24 species of ants [6].

Table 1. List of subfamily, tribe, genera and species of ants collected from five dragon fruit plantation in West Sumatra. N: Total number of individual, F: Frequency of ants among 45 unit sampling (nine units per location base on three methods per subsection).

No	Subfamily	Tribe	Genera	Species	N	F
1	Aenictinae	Aenictini	*Aenictus*	*Aenictus* sp.	52	2
2	Dolichoderinae	Dolichoderini	*Bothriomyrmex*	*Bothriomyrmex* sp.	4	4
3			*Dolichoderus*	*Dolichoderus thoracicus* (Smith, 1860)	37	8
4			*Iridomyrmex*	*Iridomyrmex anceps* (Roger, 1863)	70	5
5			*Tapinoma*	*Tapinoma melanochepalum* (Fabricius, 1793)	3961	29
6	Formicinae	Camponitini	*Camponotus*	*Camponotus (Colobopsis)* cf. *saundersi* Emery 1889	2	2
7				*Camponotus (Colobopsis) leonardi* Emery (1889)	3	1
8			*Polyrachys*	*Polyrhacys (Myrma) hosei*Doristhrorpe 1942	3	2
9				*Polyrhacys (Myrma) proxima*Roger	3	1
10				*Polyrhacys (Myrma) villipes*Smith 1857	1	1
11		Lasiini	*Nylanderia*	*Nylanderia* sp. 1	282	16
12				*Nylanderia* sp. 2	4	2
13				*Nylanderia* sp. 7	1	1
14			*Paratrechina*	*Paratrechina longicornis* (Latreile, 1802)	196	7
15		Oechophylini	*Oechophylla*	*Oechophyla smaragdina* (Fabricius, 1775)	3	2
16		Plagiolepidini	*Anoplolepis*	*Anoplolepis gracilipes* (Smith, 1857)	2	1
17	Myrmicinae	Dacetonini	*Strumigenys*	*Strumigenys koningsbergeri* (Forel, 1905)	1	1
18		Formicoxenini	*Cardiocondyla*	*Cardiocondyla* sp. 2	11	1
19		Pheidolegetonini	*Lophomyrmex*	*Lophomyrmex bedoti* (Emery, 1853)	2	2
20			*Pheidolegeton*	*Pheidolegeton* cf. *af f ini*(Jerdon, 1851)	56	3
21		Pheidoliini	*Pheidole*	*Pheidole* sp. 2	1	1
22				*Pheidole* sp. 3	119	5
23				*Pheidole* sp. 12	112	8
24				*Pheidole* sp. 13	501	8
25		Selenopsidini	*Monomorium*	*Monomorium f loricola*(Jerdon, 1851)	1263	18
26				*Monomorium* sp.	27	2
27			*Solenopsis*	*Solenopsis geminata* (Fabricius, 1804)	1549	23
28		Tetramoriini	*Tetramorium*	*Tetramorium kheperra* (Bolton, 1976)	45	6
29				*Tetramorium* cf. *bicarinatum* (Nylander, 1846)	44	5
30				*Tetramorium* sp. 1	180	4
31				*Tetramorium* sp. 2	7	1
32	Ponerinae	Ponerini	*Odontoponera*	*Odontoponera denticulata* (Smith, 1858)	28	15
33			*Hypoponera*	Hypoponera sp. 5	3	1
34			*Pachycondyla*	*Pachycondyla (Brachyponera) pilidorsalis*Yamane 2007	3	1
		Diversity index		1,78		

Myrmicinae was the highest in the number of species (15 species) followed by Formicinae (11 species), Dolichoderinae and Ponerinae (four species each). Aenictinae was the lowest with only one species collected (Table 1). Myrmicinae and Formicinae were also found as a dominant subfamilies from previous ants studies in agricultural areas [8-5-7-6]. *Pheidole* and *Tetramorium* were the genera with the highest in number of species (four species). *Pheidole* is the second largest genus of ant in the world [14] that also found as predator of banana weevel in Uganda [1]. *Tetramorium* also dominan in the number of species on banana with BBTV symptom [6].

Total number of ant subfamili, tribe, genera and spesies were varied among dragon fruit plantations (Figure 2). Elevation and variation of vegetation around edge of plantation seems affected ant diversity in dragon fruit plantation. All of plantations (except Aripan in Solok Regency that located about 400 m asl) were located in coastal areas with 2-10 m asl. Dragon fruit plantation at higher place had various vegetation compare to plantations near to the see that usualy more sandy with limitid plants that able to grow. However, the diversity index of ants in this study was

1,78. The diversity index quite low because of some species were significantly higher in number of individual such as *T. melanocephalum, S. geminata* and *M. Floricola* (Table 1).

.

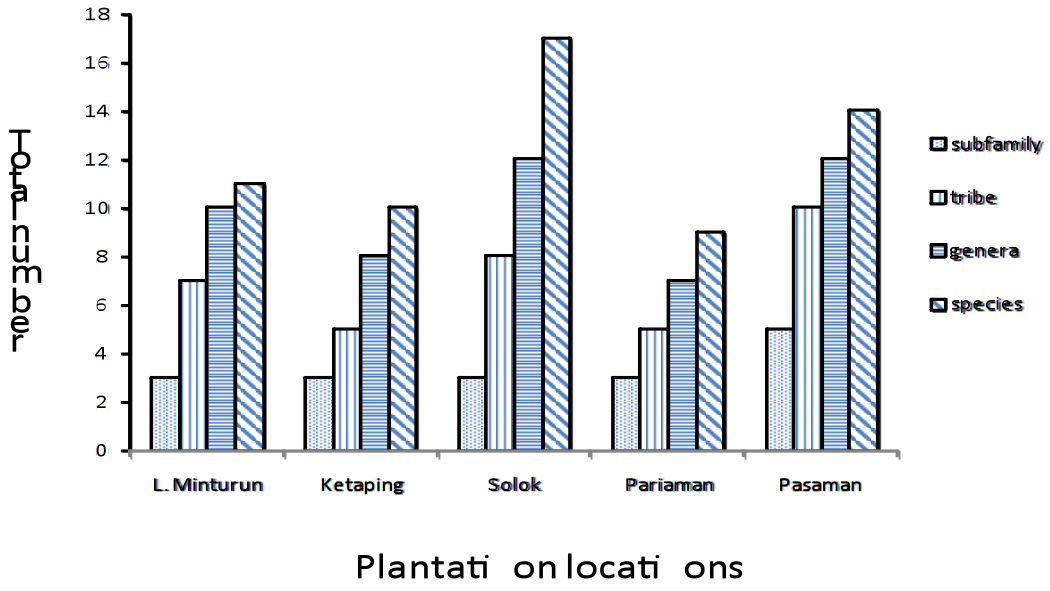


Figure 2: Distribution of number of subfamily, tribe, genera and species of ants collected from fruit plantation location in West Sumatra.

T. melanochepalum (29) was recorded more frequently followed by *S. geminata* (23) and *M. floricola (18). T. melanochepalum* (Figure 3. B) was recognize as house hold ants and tramps species [11]. During our observation this species was found more frequently on young stems and flowers. Species of *Monomorium* are very diverse in size and habits, ranging from very small generalist scevengers to large and also seed harvester [14].

Farmers were disturbed with ant visitation to dragon fruit plant, especially, red fire ant *S. geminata* that attack people during plant maintenance and harvesting. In Gasang plantation at Pariaman Regency, we found that *S. geminata* feed on dragon fruit young stems and fruits. Ants feed on mature fruit by digging fruit flesh and sometimes they nested in fruit. Ant activities on fruit caused black spots and reduced food price in market (Figure 3. C, C'). *S. geminata* also prefered young stems to feed and it caused the blackish dried abnormal stems (Figure 3. D, D'). This conditions indicated that *Solenopsis* was potencial as pest of dragon fruit [2]. Hole and damages left by insects was fungi and bacteria entrance site that can eventually kill plant [13].

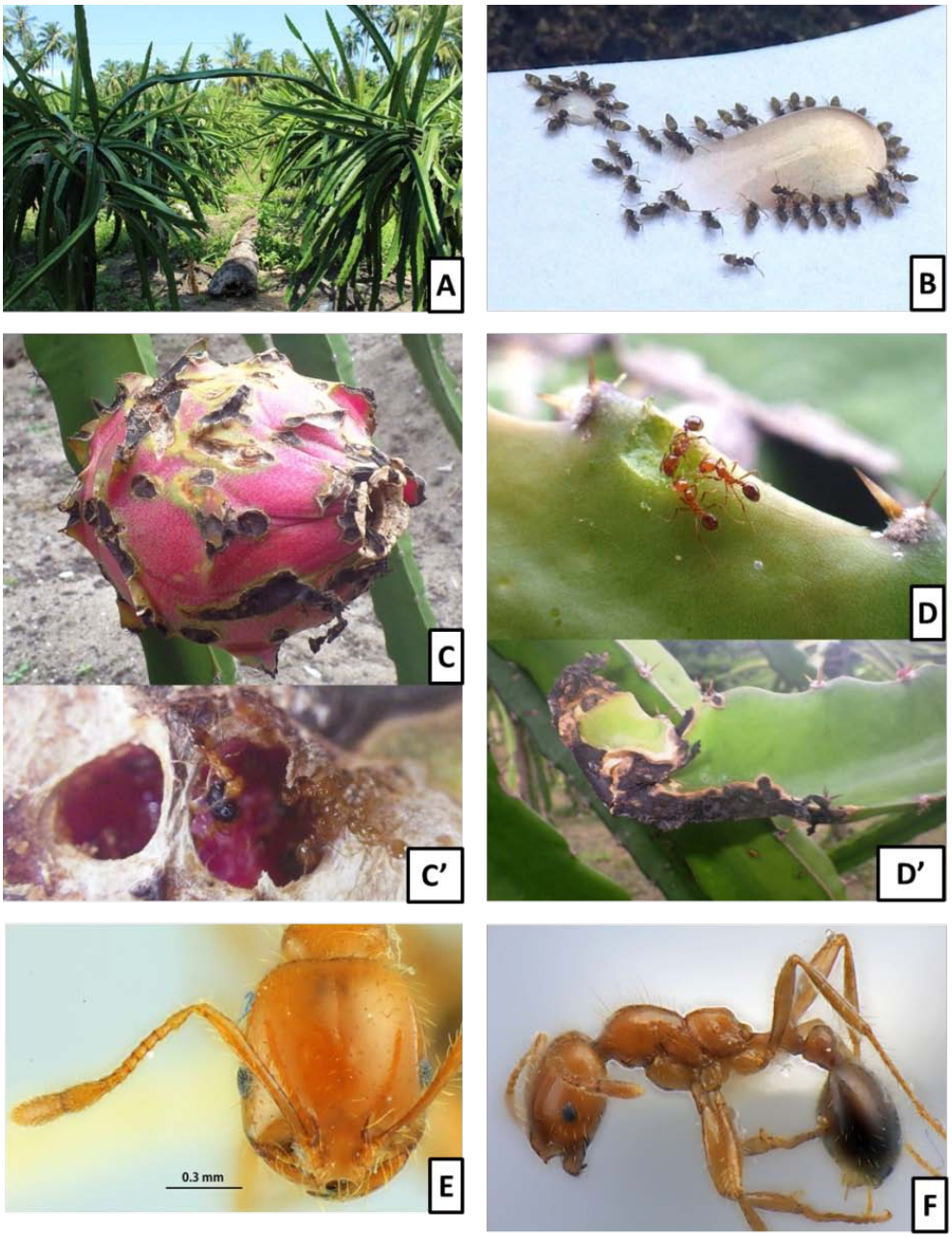


Figure 3: Dragon Fruit plantation and two dominant species of ants collected during sampling of ants in West Sumatra. A : Dragon fruit plantation, B: *T. melanochepalum* workers came to honey baited trap, C: Dragon fruit attacked by *S. geminata*, C': Entrance of the nest of *S. geminata* in dragon fruit, D: *S. geminata* is eating dragon fruit stem, D': Morfology of stem after ants attack, E: Head in full-face view minor worker of *S. geminata*, F: Habitus in profile of minor worker of *S. geminata*.

S. geminata was reported as the most significant invasive ant in term of distribution, density and damage to invaded environment [15]. In this study, especially in Gasang Plantation at Pariaman Regency, *S. geminata* was collected at all sections by all collection method except hand collection in section three. Jumjunidang (personal communication) also found that new cultivated dragon fruist that attacked by *S. geminata* were fail to grow in Solok Regencies. Future study is needed to control dragon fruit plantation from such pest.

4 CONCLUSIONS

A total of 34 species of ants that belonging to five subfamilies, 13 tribes, 22 genera and 8524 individual was collected from five dragon fruit plantations in West Sumatra. From three dominant ant species collected, *Selenopsis geminata* caused serious damage to dragon fruit because this species feed on young stem and fruit, even nested in fruit. Ants appearences also have a possibility to spread of plant deseases in dragon fruit plantation.

5 ACKNOWLEDGEMENT

Our sincerest thanks to the Directorate General of Higher Education of the Republic of Indonesia through the International Collaboration and Publication Project 2013 funding (Team Leader: Henny Herwina).

REFERENCES

[1] Abera, A. M. K, C. S. Gold, R. G. Van Driesche and P. E. Ragama. 2007. Composition, distribution and relative abundance of ants in banana farming system in Uganda. *Biological Control* 40: 168-178.

[2] Bellec, F. L., F. Vaillant and E. Imbert. 2006. *Pitahaya (Hylocereus spp.) A New Fruit Crop: a Market with a Future.*http://www.researchgate.net/publication/41713104_Pitahaya_%28Hylocereus_spp.%29_a_new_fruit_crop_a_market_with_a_future. Downloaded on 15 August 2014.

[3] Department of Agriculture of United States. 2011. Pest list for the importation of Dragon Fruit (multiple genera and species) into the Continental United Stated from Thailand. http://www.acfs.go.th/FileSPS/TextSPS%20USA%202239.pdf. Downloaded on 15 August 2014.

[4] Hashimoto, Y., S. Yamane and M. Maryati. 2001. How to design an inventory method for ground-level ants in tropical forests. Nature and Human Activities 6: 25–30.

[5] Herwina H and K. Nakamura. 2007. Ant species diversity studied using pitfall traps in a small yard in Bogor Botanical Garden, West Java, Indonesia. *Treubia* 35: 99-116.

[6] Herwina, H., N. Nasril., Jumjunidang and Yaherwandi. 2013. The Composition of Ant Species on Banana Plants with Bananaa Bunchy-top Virus (BBTV) Symptoms in West Sumatra. Indonesia. *Asian Myrmecology* 5: 149-159.

[7] Hosoishi, S., A. L. Ngoc, S. Yamane and K. Ogata. 2013. Ant diversity rubber plantations (*Hevea brasiliensis*) of Cambodia. *Asian Myrmecology* 5: 69-77.

[8] Ito F., S. Yamane, K. Eguchi, W.A. Noerdjito, S. Kahono, K. Tsuji, K. Ohkawara , K. Yamauchi, T. Nishida and K. Nakamura. 2001. Ant species diversity in Bogor Botanic Garden, West Java, Indonesia, with descriptions of two new species of the genus *Leptanilla* (Hymenoptera, Formicidae). *Tropics*10: 379-404.

[9] Jaya, I. K. D. 2010. Morphology and physiology of pitaya and it future prospects in Indonesia. *Crop Agro* 3: 44-50.

[10] Jumjunidang, Riska, D. Emilda, Sudjijo, I. Muas and Subhana. 2013. Distribusi, Karakterisasi dan Identifikasi Hama Dan Penyakit Utama Tanaman Buah Naga di Beberapa Sentra Pengembangan di Indonesia. Indonesian Tropical Fruit Research Institute Report, West Sumatra.

[11] Lee, Y. C. 2002. Tropical Household Ants: Pest Status, Species Diversity, Foraging Behavior and Baiting Studies. In: Sc.Jones J. Zhai & W. H. Robinson (eds). Proceeding of the 4th International Conference On Urban Pests, 3-18

[12] Merten, S. 2003. A Review of *Hylocereus* production in the United States. *J. PACD*: 99-105.

[13] Ortiz-Hernandez, Y. D and J. A. Carrillo-Salazar. 2012. Pitahaya (*Hylocereus* spp.): a short review. *Comunicata Scientiae* 3(4): 220-237.

[14] Shattuck S. O. 1999. *Australian Ants: Their Biology and Identification*. CSIRO Publishing. Australia.

[15] Suarez, A.V., T. P. McGlynn and N. D. Tsutsui. 2010. Biogeographic and Taxonomic Patterns of Introduced Ants. In: *Ant Ecology* (Lach L, Parr CL and Abbott K, eds). Oxford University Press, New York, 98-114.

Human Behavior Related with Cut Locus in Daily Life

Nathaphon Boonnam
Department of Mathematics
School of Science and Technology, Tokai University, Japan

Pakkinee Chitsakul
Department or Mathematics
Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Thailand

.....

ABSTRACT

The cut locus is defined by the set of all cut point along minimal geodesic segments emanating from the common point in a complete connected Riemannian manifold. This sentence is the abstract knowledge of mathematical proof that can be applied for short cut to go somewhere in our daily life. We gave the possibly case of human behaviour, collected data from the actual location, analysed with statistical tool. The result and proof are naturally similar. Furthermore, the case can be affirmatively approved by questionnaire. It is guaranteed that the cut locus may the optimal chance better than the actual path.

Keywords: Cut point, Cut locus, Geodesic

1 INTRODUCTION

The originally way of finding the shortest path between two points is routes of one airline from the country to another such as from Suvarnabhumi airport, Thailand to Hiroshima airport, Japan. This is well-known that the most obvious about the application of the shortest path problem is called a *geodesic*; a generalization of the notion of a straight line to curved spaces. This means that a curve locally minimizes the distance between two points on any mathematically defined space. For example, if we consider only two points and do not interest any point at all in the top of two mountains, we get the geodesic between two points in these mountains but it is impossible since nobody can walk in the sky. Therefore, we try finding the optimal path from the source to the destination. However, there are many geodesics indefinitely. We should be able to find uniquely one geodesic that is a *minimal* geodesic. This is one of the reasons why we need to study the geodesic in the local differential geometry.

The geodesic on a planar surface is a straight line. In the case of the surface is curved, such as a sphere, what the geodesic is. We have known the sphere having the earth-shaped geometry similarly. When we talk about the geodesic between two points on the earth's surface and consider only in confined spaces. In the sense of our mind that the geodesic mentioned above is a straight line. However, when considered on the whole earth. This is curved, not flat. Therefore, we get an interesting open problem as: what is the graph of the geodesic on the sphere look like? This is another one reasons for considering about the geodesic deeply.

In Riemannian geometry, geodesics are not the same as shortest paths between two points. The difference is that geodesics are only locally the shortest distance between points, and are parameterized with constant velocity. From now on, we consider the constant velocity that equals one called a *unit speed* as we will show in the next section.

2 MATHEMATICAL PROOF OF CUT POINT

As we explained briefly in the introduction, from now on, let M be a complete connected Riemannian manifold. A curve $\gamma:[0,\infty)\to M$ is called a geodesic if and only if γ is locally minimizing. There are many methods to find a minimal geodesic, e.g., we use isometric maps to surfaces with known geodesics, the methods for finding analytical solution by using the Euler equation [3], some properties of geodesic on some open surfaces using geodesic equation in Clairaut's relation [4], etc. The geodesics of any plane are straight lines. A straight line passing through two points has always a minimum length. Thus, we can find the existence of a unique geodesic between two points in a plane.

On the other hand, the geodesics on the sphere are the intersection of plane and sphere; it is well-known that is great circles as shown in the left of Figure 1. Each great circle is divided by a pair of antipodal points p and q into two minimal geodesics, i.e., a semi–great circle as shown in the middle of Figure 1. The geodesic extension along a semi-great circle passing through q is not minimal. Now, we get a major arc (a longer arc) and a minor arc (a shorter arc) as shown in the right of Figure 1.

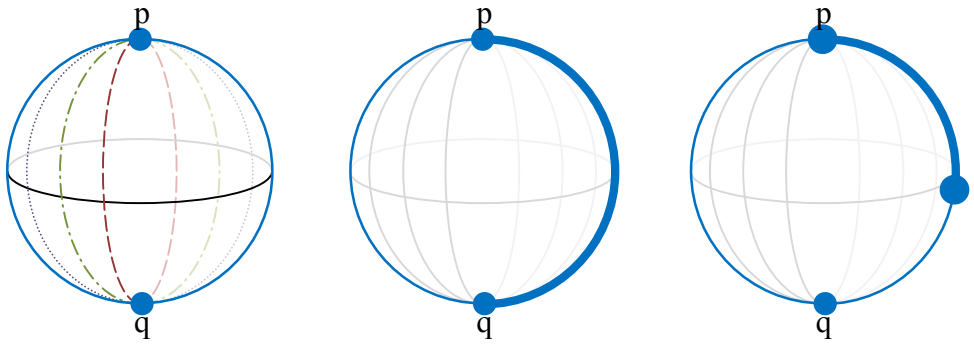


Figure 1: A Geodesic on a Sphere

In the case of cylinder, the geodesics are meridians, parallels and circular helices. It is trivial that the meridian passing through p and q is a minimal geodesic as shown in Figure 2.

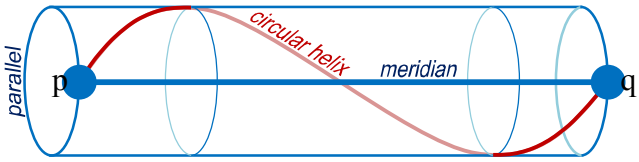


Figure 2: A Geodesic on a Cylinder

It is clearly from the above examples that all geodesics are not always minimal arcs. This is brought us to some interesting notion which are cut point and cut locus.

Let $\gamma:[0,a]\to M$ be a unit speed minimal geodesic segment joining $p:=\gamma(0)$ to $\gamma(a)$. An endpoint of $\gamma(a)$ along γ is called a *cut point* of p along γ if any extended geodesic joining p to $\gamma(a+\varepsilon)$ along γ is not minimizing arc anymore for any positive number ε.

For each point p on M, the cut locus C_p is defined by the set of all cut points along the minimal geodesic segments emanating from p. It is known (for example, see [1]) that the cut locus has a local tree structure for 2-dimensional Riemannian manifolds.

In this paper, we begin mentioning the theorem as follows:

Theorem. If the points p and q are joined by two distinct unit speed minimal geodesics α and β, then q is a cut point along α and also along β.

Proof. Assume that α and β are two distinct unit speed minimal geodesics emanating from p=α(0)= β(0) to q=α(a)=β(a). This implies that L(α)=L(β) where L (·) denotes length of a geodesic. We will get a contradiction by supposing that the geodesic extension of α is a minimal geodesic. For any positive sufficiently small ε, there exist geodesic extensions α(a+ε) and β(a–ε) containing in an ε–neighbourhood at q as shown in Figure 3.

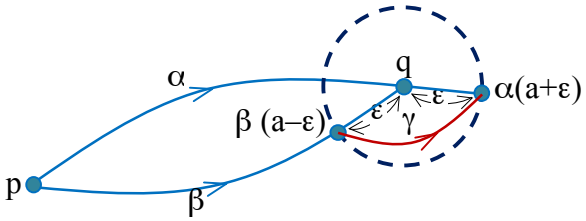


Figure 3: ε–neighbourhood at q

Here, we may suppose a unique minimal geodesic γ joining β(a–ε) to α(a+ε) such that 2ε > L(γ). Thus, L(α) + 2ε > L(β)+L(γ) or L(α)+ε > L(β)–ε+L(γ). This is a contradiction since the geodesic extension of α is not minimizing arc anymore. Therefore, q is a cut point of p along α. Similarly to q is a cut point of p along β. This completes the proof of theorem.

We refer to [2] for basic tools in Differential Geometry, and [1] for some properties of geodesics on a surface of revolution.

3 HUMAN BEHAVIOR OF CUT POINT

One of the proof's method of theorem in section 2 is easy to see everywhere having the broken geodesic β, we can immediately find another geodesic γ that is shorter than β naturally. This means that the cut locus has some relation to the shortcut that we always use in our daily life. For example as shown in Figure 4, we have the most common sense in small area about walking in the direction that no construction paths. Because of the self-awareness of our mind is not the shortest path, so many people chose to walk off the path to get the shortest distance possibly.

Figure 4: Walked by the Cut Point

Besides the small area, it can always be seen generally to walk off the path. Now, in our mathematical experiment, we have expanded the area widely around Building No. 17 to a cafe in Tokai University, Shonan Campus, Japan which were divided into 2 groups as follows:

1. The samples passed through the actual place, and
2. The samples collected from the work-sheet to trace the line.

After that, we will bring these two groups to compare the results with the corresponding theorem that we are interested by using simply statistical tools.

4 MATHEMATICAL EXPERIMENTS

4.1 Sample Group in the Actual Place

We collected data from 1,000 persons totally divided by 200 persons per day at the time when there were not a lot of people, i.e., 9:30 – 10:30 and 16:30 – 17:30, in order to mimic the behavior of the human walking. From the data in both directions, which the results are shown in Table 1, we evaluated by a percentage value. It appears that 78.3% of choosing shorter paths and 21.7% of choosing the construction path.

Table 1 The Number of People in Our Experiment

Number of People	1st day		2nd day		3rd day		4th day		5th day		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
9.30 – 10.30	81	19	83	17	81	19	85	15	80	20	410	90
16.30 – 17.30	71	29	68	32	75	25	80	20	79	21	373	127

4.2 Sample Group in the Work-sheet

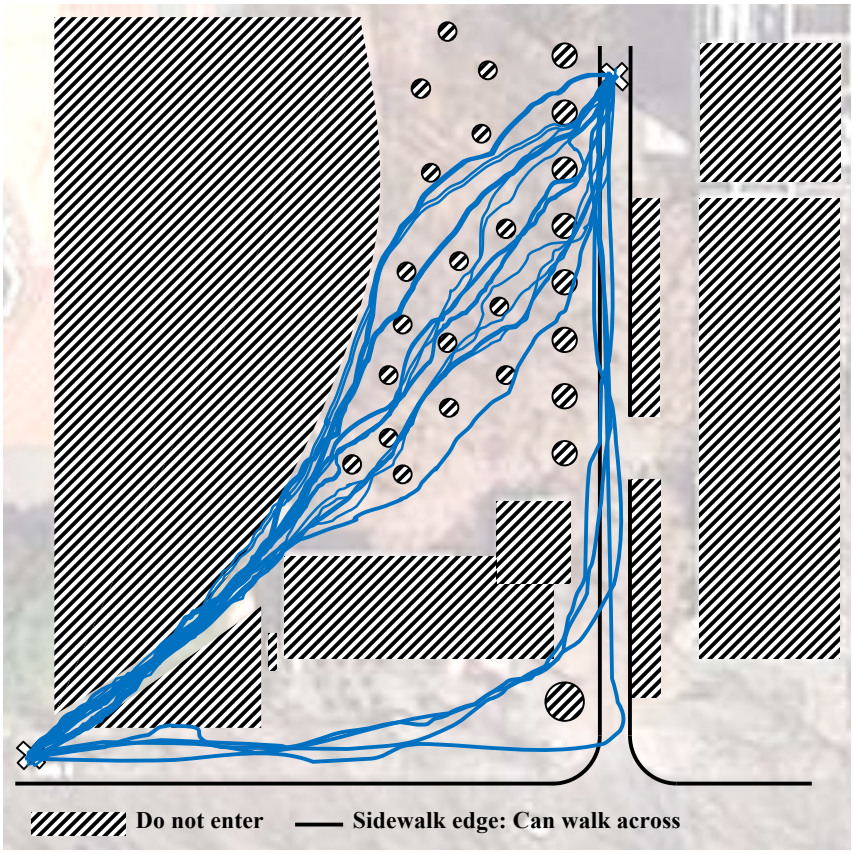


Figure 5: The Overlay of Images of the Whole Experiments.

For data in this subsection, we collected data only 200 persons in the sample who are students in King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The results were

shown in Figure 5 and appeared that 81.5% of choosing shorter paths and 18.5% of choosing the construction path.

When we compared the two samples, we can therefore conclude the most of people choose to the paths that are shorter than the construction path. Moreover, in the case of mathematical experiments as above, it can be applied to the cut locus due to some persons drawn line through the construction path on the different walkway.

5 CONCLUSIONS

The mathematical proofs and experiments are fare possibly in the same result. This concludes the humans use a logical mathematical sense and choose the shortest path. In addition to the one-sided matches, it also gives us the interesting information that is cut locus, reinforcing behavior that uses advanced mathematical tools in our life as well.

6 FUTURE WORKS

The further process is to construct the algorithm for programming and simulation in many areas such as missile controls, clinical planning, computer graphics, pattern recognition, etc. These are the cases for further study as follows:

A double gimbal is a fundamental component of pointing and tracking applications for example missile guidance system, ground–based telescope, antenna assemblies, weapon system. In case that we want the missile to intercept the target, we necessary to control the angle of the missile with respect to the axis and the plane. Thus, for each pair of gimbals configurations, there is at least one curve segment called a minimal geodesic for the optimal energy function. Suppose that there exists a cut point, we have at least two minimal geodesics, this may be caused the missile making a mistake direction to the right target.

The clinical technique of effective operation is small surgical scar for rapidly recovering. If we consider at arm-shape similarly to cylinder, we can find the minimal geodesic to plan the operation. Since there exists a cut locus, this is a chance to consider which the optimal surgery path is.

In computer graphics, the medial axis of a closed surface is the set of centres of empty balls which is touched the surface at more than one points. In mathematics, the closure of the medial axis is known as the cut locus. If the medial axis and the radii of balls at each point is given, one can approximate the surface, we may use medial axis as a skeleton to identify as a human signature.

7 ACKNOWLEDGEMENT

We wish to thank King Mongkut's Institute of Technology Ladkrabang for financial supports.

REFERENCES

[1] K. Shiohama, T. Shioya, and M. Tanaka, (2003), The Geometry of Total Curvature on Complete Open Surfaces, Cambridge tracts in mathematics 159, Cambridge University Press, Cambridge, New York.

[2] M. P. Do Carmo, (1976), Differential Geometry of Curves and Surfaces, Prentice-Hall, Englewood Cliffs, New Jursey.

[3] N. Boonnam and P. Chitsakul, (2010), "The shortest path between two points on some surfaces by using the application of Euler equation", Proceeding of the 6th Conference on mathematics, Statistics and Its Application (ICMS2010) University Tunku Abdul Rahman, Kuala Lumpur Malaysia, pp.704–711.

[4] W. Khuangsatung and P. Chitsakul, (2012), "Some Geodesics in Open Surfaces Classified by Clairaut's Relation", World Academy of Science, Engineering and Technology, volume 69, pp.1153–1157.

Emotion Estimation from Facial Expressions Using Kinect

Chaiyapruk Thongkam
Course of Information Science and Engineering
Graduate School of Engineering, Tokai University, Japan

Osamu Uchida
Department of Human and Information Science
School of Information Science and Technology, Tokai University, Japan

Nathaphon Boonnam
Department of Mathematics
School of Science and Technology, Tokai University, Japan

ABSTRACT

In recent years, many researches on emotion recognition using facial expression that are intended to improve qualitative communication between computer and human have been conducted. In human communications, not only the meanings of words but also many kinds of nonverbal information such as tones of voice and facial expressions are used. Then, for example, in order to perform smooth communication between robots and human, it is quite important to estimate the emotion of the conversation partner. Therefore, in this study, we propose two methods to recognize nine facial expressions (happiness, sadness, surprise, anger, fear, disgust, contempt, kissing, neutral) by using a Kinect in real time. In the proposed methods, several feature points on the face of the target person are used for estimating emotion. The experimental results indicate that the methods can recognize human facial expression with practical precision.

Keywords: Facial expression recognition, Kinect, Depth sensor, User interface

1 INTRODUCTION

Currently, the field of robotics that communicate with a human is attracting high attention from many areas. For example, news-reading humanoid robots, called Kodomoroid and Otonaroid [1] were invented recently. Moreover, recently there has been a movement that humanoid robots are utilized as therapists who help stroke patients recover. In order to have robots carry out human-like communication, it is necessary to realize the capability to estimate human emotion.

There have many studies on facial expression recognition [2-8]. For example, Littlewort et al. [2] developed the Computer Expression Recognition Toolbox (CERT), which is a software tool for fully automatic real-time facial expression recognition. The toolbox can code the intensity of 19 different facial actions from the Facial Action Coding System (FACS) [3] and 6 different prototypical facial expressions. Littlewort et al. insisted that CERT provides sufficiently accurate estimates of facial expression to enable real-world applications. However, to recognize each emotion expression, it is necessary to spend time. Moreover, for the system using only RGB camera, there is a problem that the system is weak for a variation of lighting conditions.

In this paper, we propose two methods to recognize nine facial emotions (happiness, sadness, surprise, anger, fear, disgust, contempt, kissing, and neutral) using a Kinect in real-time. The Kinect was released by Microsoft in 2010 as a device for the Xbox 360 having a voice and gesture recognition function. The most distinctive feature of Kinect have a depth sensor. Using the sensor, Kinect can detect and track human movements in a high accuracy. In the proposed method, the feature points of a face are detected by a depth information obtained by using a Kinect sensor and the emotion is estimated by applying the perspective of the FACS. Moreover, we implement a system to display estimated emotion based on two types of the proposed methods with gauges which show likelihood values of each emotion. The results of the verification experiments show that the proposed methods are useful sufficiently. So, if we can use the depth information from Kinect to recognize emotions through facial expressions, it is beneficial to a lot of areas certainly.

2 PRELIMINARIES

2.1 Facial Action Coding System (FACS)

Duchenne was an anatomist who first studied Human facial muscles. He had experimented with electricity through the electrodes to stimulate the muscles' working. However, the problem is some human's muscles overlap when an electrical stimulus to the muscles. It may be leaded to a bundle of muscles moving along later. Ekman and Friesen developed a system called "Facial Action Coding System (FACS)" [3]. This system records facial motion observed by eye and called the motion "Action Unit" as shown in Table 1. Each Action Unit can work together more than two muscles such as frown caused by the interaction of muscle 3 parts, Depressor Glabellae (Frown), Depressor Supercilii (Wrapped in strips), and Corrugator Supercilii (Caused wrinkles on the forehead).

2.2 Mathematical notions applied with Kinect

We use Kinect to detect feature points on a human face by using an RGB camera and depth information. The extracted about 120 feature points are plotted on the human face as shown in Figure 1.

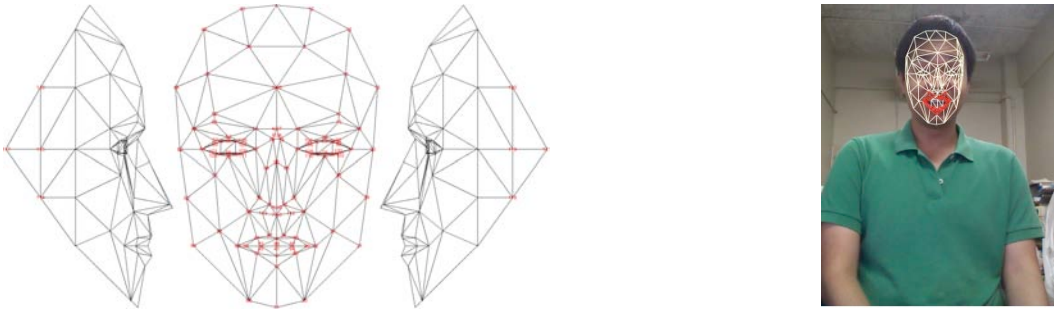
On the facial expression, we consider in the 2-dimensional plane measuring a feature area. We calculate a distance between two given points or an area between three given points on coordinate systems. A measuring on the angle defined by the lateral length and the longitudinal length of the mouth is very useful for analysing mouth-shape geometry. The angle θ is defined by

$$\theta = \tan^{-1}\left(\frac{Y}{X}\right)\times\left(\frac{180}{\pi}\right), \tag{1}$$

where X and Y denote the lateral length and half longitudinal length of the mouth, respectively. The angle given by (1) is large if $X < Y$ and small if $X > Y$.

Table 1 Action Units

AU No.	AU Content	AU No.	AU Content
1	Inner Brow Raiser	20	Lip Stretcher
2	Outer Brow Raiser	22	Lip Funneler
4	Brow Lowerer	23	Lip Tightner
5	Upper Lid Raiser	24	Lip Pressor
6	Cheek Raiser	25	Lips Part
7	Lid Tightener	26	Jaw Drop
8	Lips Toward Each Other	27	Mouth Stretch
9	Nose Wrinkler	28	Lip Suck
10	Upper Lip Raiser	38	Nostril Dilator
11	Nasolabial Furrow Deepener	39	Nostril Compressor
12	Lip Corner Puller	41	Lid Droop
13	Cheek Puffer	42	Slit
14	Dimpler	43	Eyes Closed
15	Lip Corner Depressor	44	Squint
16	Lower Lip Depressor	45	Blink
17	Chin Raiser	46	Wink
18	Lip Pucker		



(a) Feature Points Model (b) Extracted Feature Points

Figure 1: Feature Points Structure

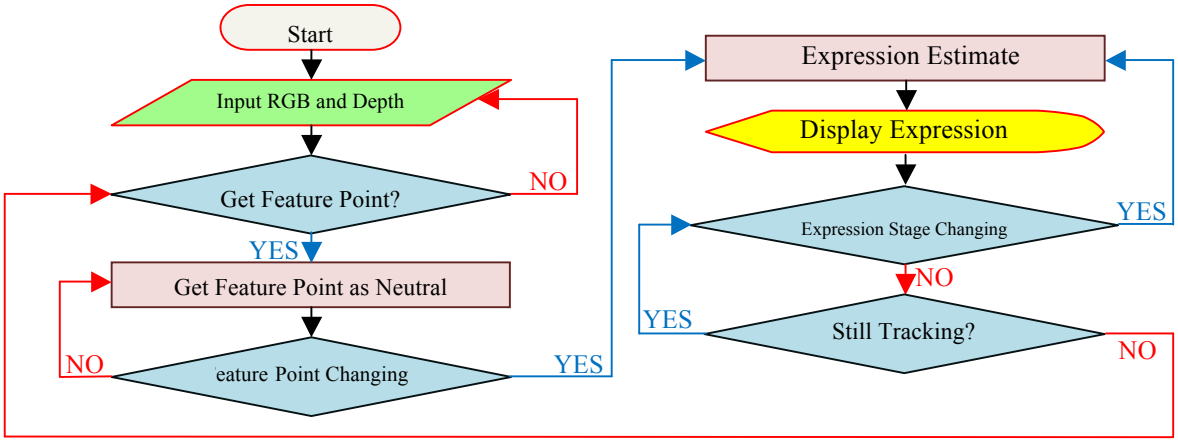


Figure 2: Flow of the Proposed Method

3 PROPOSED METHOD

We use a Kinect Face Tracking SDK [10] acquainting a feature point from the RGB and depth information. In this section, we explain two algorithms of facial expression recognition methods which are integrated the feature points on a face with mathematical notions applied into facial expression recognition application. The both algorithms apply in the same flowchart as shown in Figure 2.

Method I calculates the likelihood scores for each emotion by using a feature point as coordinates from RGB information. On the other hand, Method II utilizes the movement of facial action based on Action Units with separated 8 directions of the moving vectors of the feature points. The prediction of expression uses EMFACS (Emotional Facial Action Coding System) [9] coding that compares a relevant action unit with human expression coding.

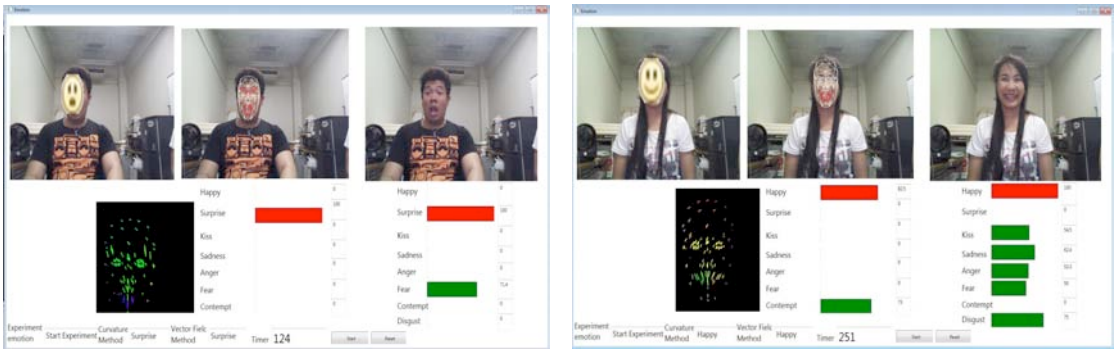
In Method I, we use a mathematical combination with coordinates on the facial surface. We calculate distances, spaces, and angles by using facial feature points. Table 2 shows feature areas and mathematical calculations for each emotion. For example, in the case of the estimation of the emotion of happiness, we use the mouth and cheek coordinates, because EMFACS of the happiness emotion considers two actions; cheek riser and mouth corner puller. Practically, we use the distance between the lip corner and the cheek coordination for estimating the emotion of happiness.

Table 2 Feature Area for Taking Advantage of Estimation for Each Emotion (Method I)

Emotion	Feature Area	Feature Quantity
Happiness	Mouth and Cheek	Distance
Surprise	Mouth and Eye brow	Distance and Angle
Kiss	Mouth	Angle
Sadness	Mouth	Angle
Anger	Mouth and Inner Eye Brow	Distance and Angle
Fear	Mouth, Eyebrow, and Chin	Angle
Contempt	Mouth and Cheek	Space and Angle

(a) Happiness Emotion (b) Sad Emotion

Figure 3: Feature Point Movement Vector



(a) Surprise Emotion (b) Happiness Emotion

Figure 4: Output of the Proposed Emotion Recognition System

In Method II, we use a movement of vector based on a facial feature point on a face. The vector will be changed by time and angle from a starting point and separating each direction with color depending on the angle of direction as shown in Figure 3. We estimate each emotion using a different facial feature region. Table 3 shows a number of feature points in the mouth, brow chin, and cheek region with a different amount points. The directions of vectors are used for analysis a human emotional based on EMFACS coding action. The moving vectors of feature points are displayed with color depending on the direction of them. The system estimate the emotion using scores for each emotion. The scores for each emotion are displayed by bar graphs as shown in Figure 4. The emotion which has the maximum score is regarded as the estimation result and displayed the related emoticon. The bar graph which has the maximum score is turned to be red. For example, in the case of the estimation of the happiness emotion with Method II, 26 feature points are used. In this case, if more than or equal to 13 moving vectors of the feature points are judged as having directions written in Table 3, the score exceeds 50%, and the color of the score bar of the happiness emotion turns into red.

Table 3 Feature Area for Taking Advantage of Estimation for Each Emotion (Method II)

Emotion	**Left eye brow** (2 points)	**Right eye brow** (2 points)	**Centre of mouth** (4 points)	**Left of mouth** (7 points)	**Right of mouth** (7 points)	**Chin** (4 points)	**Left cheek** (2 points)	**Right cheek** (2 points)	**Nose bridge** (3 points)	**Nose** (3 points)	**Inner eye brow** (2 points)
Happiness (26 points)	-	-	Up	Upper left or Left	Upper right or Right	Up	Upper left	Upper right	-	-	-
Surprise (30 points)	Up	Up	Up or Down	Up or Down	Up or Down	Down	Up	Up	-	-	-
Kiss (22 points)	-	-	Up	Upper right	Upper left	Up	-	-	-	-	-
Sadness (28 points)	Down	Down	Up	Upper right or Up	Upper right or Up	Up	-	-	-	-	Upper left or Upper right
Anger (26 points)	Lower right	Lower left	Up or Down	Upper right or Up	Upper right or Up	Up	-	-	-	-	-
Fear (26 points)	Up	Up	Up or Down	Upper left or Lower left	Upper right or Lower right	Down	-	-	-	-	-
Contempt (26 points)	-	-	Upper right or Upper left or Left or Right	Upper right or Upper left or Left or Right	Upper right or Upper left	Upper right or Upper left	Upper right or Upper left	Upper left or Upper right	-	-	-
Disgust (8 points)	-	-	-	-	-	-	-	-	Up	Up	Lower left or Lower right

4 EXPERIMENTS AND RESULTS

In our approach, the testing is on real–time video stream with 640x480 RGB resolution with 30 fps frame rate and 320x240 Depth Resolution. A Kinect was placed far from the subject about 1m with fixed face orientation. The subjects are 9 people; 6 men and 3 women, we test for changing state from neutral to each emotion with 15 times per emotion. In the case of Method I, the average precisions of the emotions of happiness, surprise, and fear are more than 90%. However, the precisions of the emotions of sadness and contempt are less than 80%. Whereas, in the case of Method II, the average precisions of the emotions of happiness, surprise, disgust, anger, contempt, and neutral are more than 90%. But, sadness, fear, and kiss are less than 90%. Overall, Method II can estimate emotions about 2% better than Method I. As shown in Table 4, it can be said that Method I is better for a lot of expressions because the calculated value can be used to distinguish each emotion easily. In contrast, Method II is useful in the case for estimating the emotion having a little movement of the face such as sadness.

Table 4 Experimental Results

Emotion	**Method I**	**Method II**	**Difference**
Happiness	97.0%	95.6%	+1.4%
Surprise	94.8%	94.1%	+0.7%
Kiss	86.7%	85.9%	+0.8%
Sadness	72.6%	80.7%	–8.1%
Anger	83.0%	94.1%	–11.1%
Fear	93.3%	85.2%	+8.1%
Disgust	–	91.9%	–
Contempt	78.5%	91.1%	–12.6%
Neural	100%	94.8%	+5.2%
Average	*88.2%*	*90.3%*	

5 CONCLUSIONS

In this paper, we proposed a method to recognize facial expression using a depth sensor, specifically Microsoft Kinect by two methods to estimate the facial expression based on facial feature point in real-time. Experimental results show that the proposed methods can estimate human facial expressions with practical precision. In the future, we will plan to estimate an emotion under various conditions (far distance from camera, different user's head orientation) or multiple user's at the same time.

REFERENCES

[1] National Museum of Emerging Science and Innovation, Meet the world's Most Advanced Android Robot, Kodomoroid and Otonaroid, http://www.miraikan.jst.go.jp/en/press/docs/20140609_E.pdf

[2] G. Littlewort, J. Whitehill, T. Wu, I. Fasel, M. Frank, J. Movellan, and M. Bartlett, "The Computer Expression Recognition Toolbox (CERT)", Proc. 9th IEEE Conf. on Automatic Face and Gesture Recognition, pp.298-305, 2011.

[3] P. Ekman, W. Friesen, The Facial Action Coding System: A Technique for the Measurement of Facial Movement, Consulting Psychologists Press, Inc., San Francisco, 1978.

[4] T. Chaiyapruk, O. Uchida "Facial Expression Recognition System Using Kinect", IEICE General Conference 2014, 2014.

[5] H. V. Kuilenburg, M. Wiering, M. den Uyl, "A Model Based Method for Automatic Facial Expression recognition", Machine Learning: ECML 2005, pp. 194-205, 2005.

[6] H. Drira, B. B. Amor, M. Daoudi, A. Srivastava, S. Berretti, "3D Dynamic Expression Recognition based on a Novel Deformation Vector Field and Random Forest", Proc. 21st International Conference on Pattern Recognition, pp. 1104-1107, 2012.

[7] R. C. Luo, P. H. Lin, Y. C. Wu, C. Y. Huang "Dynamic Face Recognition System in Recognizing Facial Expressions for Service Robotics", 2012 IEEE/ASME International Conference on Advanced Intelligent Mechatronics, pp. 879-884, 2012.

[8] A. Ryan, J. F. Cohn, S. Lucey, J. Saragih, P. Lucey, F. D. la Torre, R. Adam, "Automated Facial Expression Recognition System", 43rd Annual 2009 International Carnahan Conference on Security Technology, pp.172-177, 2009.

[9] EMFACS -- Scoring For Emotion With FACS, http://face-and-emotion.com/dataface/facs/emfacs.jsp

[10] Face tracking, http://msdn.microsoft.com/en-us/library/jj130970.aspx

Transmission Mechanism of *Ralstonia solanacearum* Phylotype IV by Insects on Banana Plant in West Sumatra

Mairawita Marlis, Department of Biology, Andalas University, Indonesia
Habazar, Nasir, Suswati, Hasyim

Abstract

Trigona minangkabau Sakagami et Inoue flower visiting insect and *Cosmopolites sordidus* Germar and *Oidoporus longicollis* Olivier insect or the banana stem weevil identified as insect vectors of *Ralstonia solanacearum* Phylotype IV in highland banana plantation of West Sumatra, Indonesia. However, information of *R. solanacearum* Phylotype IV transmission mechanism by insect presented in limited records. This study intended to identify the transmission mechanism of *R. solanacearum* Phylotype IV by *C. sordidus* and *O.longicollis* in generative and *T.minangkabau* in vegetative phase. Factorial complete randomized applied with two factors expanded into twelve treatments and three replications. First factor encompassed population density of *C. sordidus* and *O. longicollis* with five levels: 0, 2, 4, 6, 8 and 10 insects/seedling. Second factor divided into *R. solanacearum* Phylotype IV injections with two levels: control and *R. solanacearum* Phylotype IV suspension injection (*106* cfu/ml); *T.minangkabau* population density extended into 0, 10, 20, 30, and 40 insect/ banana flower combined with bacterial suspension sprayer to the female flower. Observations parameters included to incubation period, diseases incidence, attack and population density of *R. solanacearum* Phylotype IV on stem tissue and fruits. The result indicated *C. sordidus*, *O. longicollis* and *T. Minangkabau* effectively improved the infection of *R. solanacearum* Phylotype IV for banana. Bacterial transmission mechanism encountered through insects body surface contamination and visceral part especially in digestion tracts. More, the treatment combination of insect density and bacterial injection increased the damage on plant by *R.solanacearum* Phylotype IV. The density of both *C. sordidus* and *C. longicollis* in seedling and *T. Minangkabau* in generative phase effectively improved blood disease attack. Strain of *R. solanacearum* Phylotype IV transmitted by three vectors insects virulent caused wilt symptom and plant death in three and 30 days after infection.

Keywords *C. sordidus* Germar, *O. longicollis* Olivier, *T. minangkabau* Sakagami et Inoue, transmission mechanism, *R. solanacearum* Phylotype IV. blood disease bacterium

Introduction

Blood disease bacterium caused by *Ralstonia solanacearum* Phylotype IV contributed to decreased banana population in West Sumatera. This bacteria lethal caused and systematically infected the body tissue of banana (Eden-Green, 1992) that firstly reported on 1907 on Kepulauan Selayar (Wardlaw, 1972). In 1999, the blood disease bacteria dissemination discovered in West Sumatera (Hermanto *et al*., 1998; Setyobudi dan Hermanto, 1999). Six years at next revealed Nagari Tabek Panjang, Bungo Koto Tuo, Simarasok, Padang Tarok and Koto Tinggi, Baso subdistrict as the central of banana planting on Agam district of West Sumatera heavily attacked by *R. solanacearum* Phylotype IV.

· Corresponding author
E-mail address: mairawitamarlisrahman@gmail.com

The spreading and developing of blood disease bacterium discovered very fast disseminated. Its geographical distribution in Indonesia occurred about 100 km/year (Eden-Green,1994) and particularly in Sumatera about 189-203 km/year (Setyobudi dan Hermanto, 2000). Nowadays, entire of banana planting in West Sumatera infected by *R. solanacearum* Phylotype IV.

Vector insect inferred to contribute the massive distribution. Some experts assumed strongly indication its big roles in disease promulgation (Wiyono *et al.,* 1993; Maryam *et al.,* 1994, Soguilon *et al* ., 1995; Setyobudi dan Hermanto, 1999). Observation result on heavily attack plants by *R. solanacearum* Phylotype IV (Tabek Panjang, Subdistrict Baso, Agam District, North Sumatera Province) inspected some insects included to *Trigona minangkabau*, *Drosophila* spp, hump weevil (*Cosmopolites sordidus Germar*) and stem weevil (*Odoiporus longicollis* Oliver) associated with the sick plant (Mairawita *et al*, 2009) and identified as vectors for the bacteria (Mairawita et al.,2011). Propagules of *R.solanacearum* Phylotype IV highly dense observed on the caput, elytra and abdomen of C.*sordidus* and *O.longicollis.* Isolated bacteria virulence of insect body very highly affected disease symptom to Kepok banana seeding after 3-4 days inoculation. However, information about contributions of *T. minangkabau* and *C. sordidus O. longicollis* and *R. solanacearum* Phylotype IV transmission mechanism to banana planting less literally described. This research intended to identify propagule transmission mechanism of *R. solanacearum* Phylotype IV by *C. Sordidus* and *O. longicollis* in vegetative as well as *T. minangkabau* in generative phase of banana

Methods

This research extended into two phases;

A. Transmission mechanism determination of *R. solanacearum* Phylotype IV on banana seeding by *C. sordidus* dan *O. longicollis*
B. Transmission mechanism determination of *R. solanacearum* Phylotype IV through banana flower by *T. minangkabau*

A. Transmission mechanism determination of *R. solanacearum* Phylotype *IV* on banana seeding by *C. sordidus* dan *O. longicollis*

Research Setting

This research conducted on trial garden Gadut and bacteriology laboratory of Agricultural faculty of Andalas University, Padang.

Research Design

Factorial completed randomized design employed in two factor with twelve treatments and three replications. First factor included to population density of *C. sordidus* contaminated by *R. solanacearum* Phylotype IV. (C) five levels: C0 (without *C. sordidus)*; C1 (2 insects); C2 (4 insects); C3 (6 insects); C4 (8 insects); C5 (10 insects). Second factor consisted of *R. solanacearum* Phylotype IV suspension. (B) two levels: B0 (without bacteria suspension injection*)* and B1 (with bacteria suspension injection (10^6 upk ml^{-1})). The similar treatment also applied for *O. longicollis*.

Multiplication of hump and stem weevil insect on banana planting

The multiplication of *C. sordidus* and *O. longicollis* integrated with modification method of Mitchel *et al*., (1978). Imago, instar larva 2 and 3 collected from weevil insect infected hump and stem on open field. Hump and stem with larva laid on holed plastic

containers. Fifty imago pairs put into those containers that full of pseudostem logs of healthy banana. Those covered by screen and tied on the top. Watered trays settled beneath the containers bases to maintain the moisture and well covered by banana leaves before entirely secured by 70% paranet. Addition or replacement of new pseudostem applied every two days.

***R. solanacearum* Phylotype IV inoculation of banana planting through insects**

Insects fasted for 48 hours and contaminated by propagule *R. solanacearum* Phylotype IV with dipping method into bacteria suspension (10^6 upk/ml). Contaminated bacteria insects pairs penetrated into banana seed after wounded on hump by sterilized needle. Banana seed covered by screen until 75% part of polybag. It applied for both *C. sordidus* and *O. longicollis*

B. Transmission mechanism determination of *R. solanacearum* Phylotype IV through banana flower by *T. minangkabau*

Research Setting

Treatment conducted in blood disease epidemic area on lowland Pasar Usang (21 m osl), subdistrict of Batang Anai, Padang Pariaman district, trial garden Gadut and bacteriology lab of Agricultural Faculty of Andalas University, Padang.

Research Design

Factorial completed randomized design also employed and expanded into two factor with ten treatments and three replications. First factor included to population density of *T.minangkabau* contaminated by *R. solanacearum* Phylotype IV. (T) five levels: T0 (without *C. sordidus)*; T1 (10 insects); T2 (20 insects); T3 (30 insects); T4 (40 insects); Second factor consisted of *R. solanacearum* Phylotype IV suspension. (B) two levels: B0 (without injection of *R. solanacearum* Phylotype IV*)* and B1 (injection of bacteria suspension (10^6 upk ml^{-1})). Every treatments applied in three replications

Multiplication of *T. minangkabau*

Multiplication of *T. minangkabau* treated by removing the colony from native house wall lobric in Lubuk Minturun into wooden box with size 15 x 20 x 30 cm. Glass slide set on the top to control insect colony development. Wooden cover put on it before wires and placed on stem. The cultivation conducted on 2-3 months for regarding the quantity of needed insects.

***R. solanacearum* Phylotype IV inoculation of banana flowers**

T. minangkabau feed with *R. solanacearum* Phylotype IV before inoculated to banana flowers. Fifteen ml bacteria suspension (population 10^6 upk/ml) mixed with 2 ml honey and steered up before poured into sterilized petri dishes and customized into box 60 x 70 cm. The colony of *T. minangkabau* positioned in the opposite side and screen applied in the outer of the box before plastic slide covered on top to prevent sun burn and rain. It hang to a tree for three days before brought to the field with cotton obstructed insect entrance hole. Some *T.minangkabau* injected into 1-2 weeks banana flower that obviously secured by transparent shield. Tied rope of shield tip strongly pull to prevent the escaped insect and finished by labelling. Treatment of bacteria suspension sprayer conducted to female flowers in the early blossom. Noting need for bunches with treatment. *R. solanacearum* Phylotype IV population calculation recorded on 1, 3, 6 and 9 days after inoculation to the fruit which arose from the bunches flower

Data Analysis

Entire of parameter observation: incubation period, disease incidence, attack intensity and population density of *R. solanacearum* Phylotype IV analysed by software SPSS ver. 20 presented on tables and graphics.

Result

A. Transmission mechanism determination of *R. solanacearum* Phylotype *IV* on banana seeding by *C. sordidus* dan *O. longicollis*

Density of *R.solanacearum* Phylotipe IV in Plant

Highly dense Bacteria population notified for all treatment of 1, 3, 6 and 9 days after inoculation. Analysis data revealed strengthening mutual interaction occurred on suspension *R.solanacearum* Phylotype IV with heavy population of *C. sordidus* or *O. longicollis* that significantly different on 1, 3, 6 days after inoculation (dai) (P. 0.05) (Table 1) in spite of insignificantly for nine days.

Table 1. The density of *R. solanacearum* Phylotype IV (log upk/ml) in 1, 3, 6 and 9 days after inoculation by *C. sordidus* and *O. longicollis*

Treatment	The density of *R. solanacearum* Phylotype IV (log upk/ml)									
	O. longicollis					*C. sordidus*				
	1	3	6	9	r	1	3	6	9	r
Control (without insect or bacteria injection)	0.00e	0.00e	0.00f	0.00e	0.00	0.00h	0.00d	0.00c	0.00	0.00
Bacteria injection	7.57bc	7.30b	6.42e	8.53a	0.20	6.69f	7.38c	8.52a	8.35	0.61
Two insects without bacteria injection	7.09c	6.93c	6.36e	8.47a	0.36	7.82b	6.51b	8.41a	8.19	0.30
Two insects with bacteria injection	6.98d	6.00d	7.32c	8.35b	0.54	7.72c	7.14c	8.10a	7.85	0.14
Four insects without bacteria injection	7.33c	7.35b	7.36c	7.89c	0.20	8.20a	6.84b	8.45a	8.31	0.19
Four insects with bacteria injection	7.37c	7.42b	7.44e	7.56d	0.6	7.31d	8.07a	8.54a	8.49	0.40
Six insects without bacteria injection	8.05a	7.69a	6.56e	8.32b	0.9	7.83b	7.40c	8.30a	8.30	0.20
Six insects with bacteria injection	7.95a	7.36b	7.20d	7.95c	0.14	7.86b	7.32c	7.99b	7.79	0.04
Eight insects without bacteria injection	7.76b	7.29b	7.15d	8.28b	0.14	7.25d	7.19c	8.02a	7.95	0.29
Eight insects with bacteria injection	7.06c	6.60d	7.63b	8.44a	0.14	6.47g	6.98c	8.29a	8.03	0.60
Ten insects without bacteria injection	7.14c	7.40b	7.77a	8.00b	0.51	8.28a	7.57c	8.28a	8.23	0.06
Ten insects with bacteria injection	7.67b	7.40b	7.62b	8.54a	0.29	7.00e	7.30c	8.40a	8.47	0.55

r = increasing rate of R.solanacearum Phylotype IV *density*

The cultivation of *R. solanacearum* Phylotype IV colony on TTC medium inspected in 72 hours after incubation. Colony sizes were varies between 1-10 mm, spherical shape with/without red spot on the cores and surrounded by white mucus in distinct colour intensity; pink to red. The colony size and colour related to pathogen strain virulence. In general, strain of *R.solanacearum* Phylotype IV with pink dot considered to highly virulence.

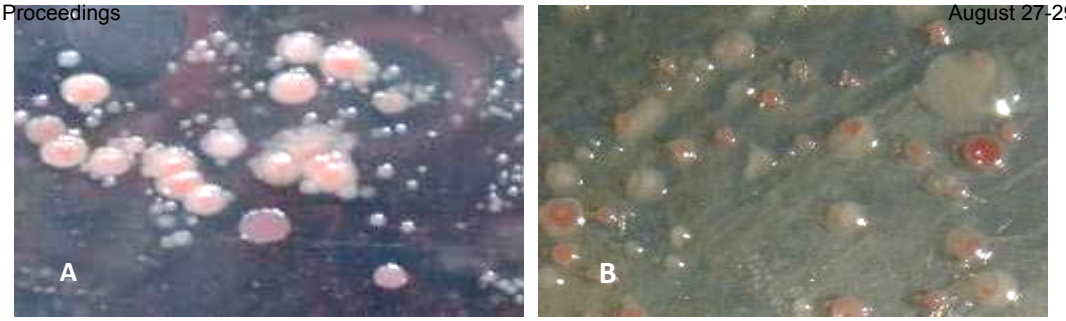


Figure 1. The diversity of shape and size of colony *R. solanacearum* Phylotype IV infected by *C. sordidus* and *O. longicollis* on banana planting. A. Pink colony, B. Colony with red mucus. A and B = virulence strain

Incubation period of *R. solanacearum* Phylotype IV infected by *C. sordidus* and *O.longicollis*

Suspension injection of *R. solanacearum* Phylotype IV and bacterial contamination insect with diverse density either combined or not combined formulation considered to disease symptom with vary of incubation period. Data analysis by software SPSS ver.20 and advanced test (Duncan) with alpha 0.05 analyzed as significant different (P.0.05) between *R. solanacearum* Phylotype IV suspension application and insect population density to bacteria incubation period on banana seedling and real interaction between both (P.0.05).

The fastest incubation period figured on treatment with 2 insects *C. sordidus* combined with bacteria suspension injection (bsi) on 3.67 days with 76.06% effectiveness while *O. longicollis* investigated on 6 insects without bsi on 3 days with 79.06% effectiveness as well, despite of insignificant different for two insects completed with bsi (Table 2). It also insignificantly different without bsi or insect, 4 insects without bsi, 8 insects with bsi and 10 insects with/without bsi in spite of six insects *O. longicollis* treatment without bsi.

Table 2. Incubation period and acceleration effectiveness incubation period of *R.solanacearum* Phylotype IV infected by *C.sordidus* on banana seedling

Treatment	Incubation period (day)		Effectiveness (%)	
	O.longicollis	*C.sordidus*	*O.longicollis*	*C.sordidus*
Control (without insect or bacteria injection)	0.00	0.00		
Bacteria injection	14.33a	8bc	0	47.81
Two insects without bacteria injection	12.33a	14.67a	13.96	4.30
Two insects with bacteria injection	4.67c	3.67c	67.41	76.06
Four insects without bacteria injection	11.33a	8bc	20.93	47.81
Four insects with bacteria injection	6.33c	13.33a	55.82	13.04
Six insects without bacteria injection	3.00c	13.67a	79.06	10.82
Six insects with bacteria injection	6.33c	15.33a	55.82	0
Eight insects without bacteria injection	11.00b	4.67c	23.23	69.53
Eight insects with bacteria injection	7.67c	9.67b	46.47	36.92
Ten insects without bacteria injection	10.00b	10.33b	30.21	32.61
Ten insects with bacteria injection	13.00a	8bc	9.28	47.81

The initial symptom of blood disease on banana seedling discovered as wilted leaves followed by yellowish colouring on young leaves. Vascular tissue showed discoloration into broken chocolate on whole part of plant and ooze produced when stem sliced (Figure 2). Foliage damage considered as another case. At the other hand, disease symptom of *O. longicollis* inspected as broken stalk because of larva development. Transparent mucus with

yellowish colour leak out from leave stalk, stem and auger hole along stem (Figure 3). The harmed banana plant tissue by both larva and imago intruded on translocation of nutrient and water that obstructed the plant development.

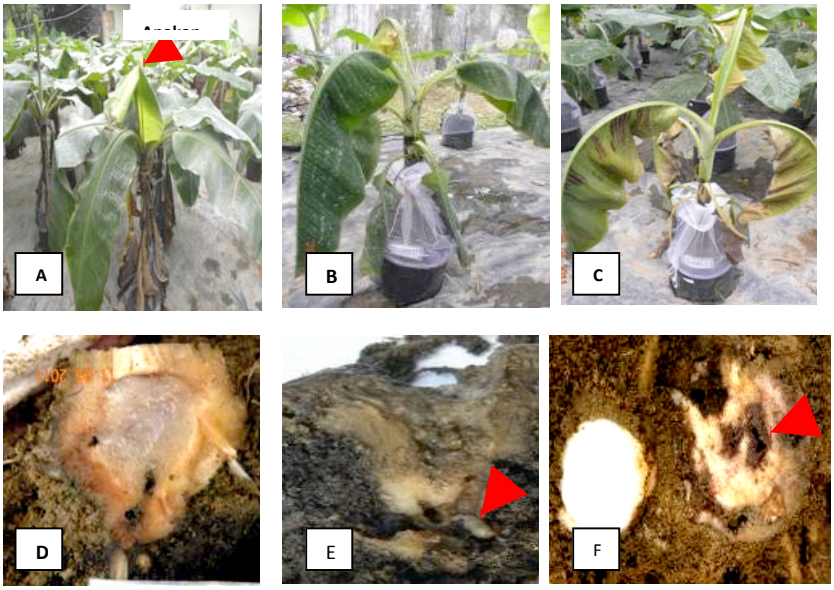


Figure 2. Blood disease attack symptom associated with *C. sordidus* to the damage of banana planting. A. Flaw foliage. B. Wilting symptom. C. Yellowish leave. D. Rotten hump E. Larva *C. sordidus* on infected banana hump F. Filial vertical slide and beetle auger hole

Figure 3. *R.solanacearum* Phylotipe IV sympthom in bananan plant transmited by *Odoiporus longicollis*. : A,B,C,D.

B. Transmission mechanism determination of *R. solanacearum* Phylotype IV through banana flower by *T. minangkabau*

Density of *R.solanacearum* Phylotype IV in plant

The improvement population density of *R. solanacearum* Phylotype IV on female flowers thoroughly noticed from first to ninth days after inoculation. Bacteria suspension sprayer directly did to bunch of banana flowers and *T. minangkabau* contamination similarly effected on bacterial infection. Bacteria population density exponentially increased after three days on 30/40 insects/flower bunch treatment with highly intensity of improvement rate; 23%-80% (Table 3).

Table 3. The density of population *R. solanacearum* Phylotype IV on (log upk/ml) on 1,3,6 and 9 days after inoculation

Treatment	Population density of *R.solanacearum* Phylotype IV (log upk/ml) on …dai				Density improvement rate (r)
	1	3	6	9	
Control (without insect, without bacteria spraying)	0i	0f	0e	0h	0.00
Bacteria spraying	6h	6e	7.45d	7.86g	0.70
Ten insects without bacteria spraying	6h	6.48d	8.02c	8.31e	0.85
Ten insects with bacteria spraying	6.48g	6.48d	8.04c	8.47c	0.76
Twenty insects without bacteria spraying	6.78f	7.30c	8.10b	8.46d	0.58
Twenty insects with bacteria spraying	6.85e	7.20c	8.13b	8.56b	0.61
Thirty insects without bacteria spraying	7.26d	7.38c	8.21b	8.48c	0.45
Thirty insects with bacteria spraying	7.64c	7.64b	8.35a	8.10f	0.36
Forty insects without bacteria spraying	7.85b	7.97a	8.30a	8.49c	0.23
Forty insects with bacteria spraying	7.99a	8.07a	8.40a	8.66a	0.23

The observation results of *R. solanacearum* Phylotype IV colony on banana planting in 1, 3, 6 and 9 days after inoculation examined as; pink coloured colony, size 0.5-4.5 mm, irregular shaped, dome shaped and non fluidal without pink formation core (Figure 4). Colony of *R. solanacearum* Phylotype IV infected by insect categorized very virulence that caused wilting symptom after three days inoculation

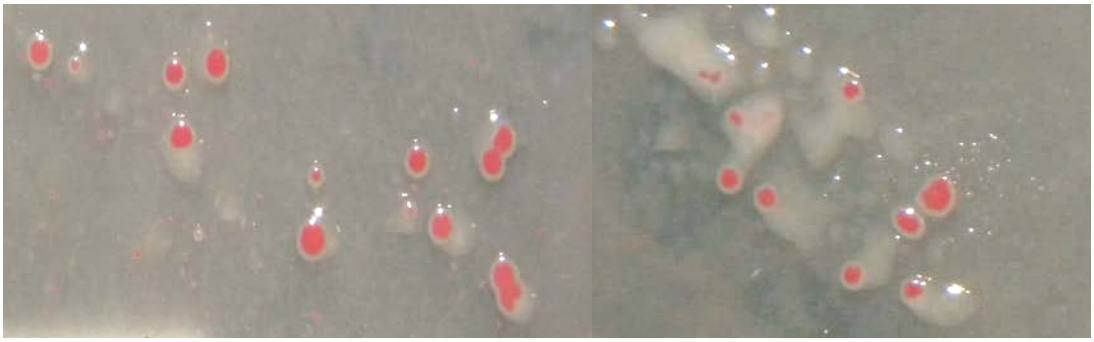


Figure 4. Colony of *R. solanacearum* Phylotype IV isolated from banana fruit contaminated by *T.minangkabau*.

Those characters accordance with *R. solanacearum* Phylotype IV colony description by Eden-Green (1994), Soguilon *et al.,* (1995) , Schaad *et al*., (2001) and Baharuddin (1994). Gram test classified bacteria colony as hydrolyzed pectin gram negative. Hypersensitive reaction test revealed positive necrotic on suspension injection area. More, pathogenic test categorized it as pathogen to banana plant with 3-4 days incubation on scale 4 (all leaves wilted) and high level virulence (scale 5). The initial symptom of blood disease bacterium found on whole wilted leaves occurred in 3-4 days after inoculation.

Incubation period

The treatment of bacteria injection or insect contamination of *R.solanacearum* Phylotype IV propagules either combined or not combined formulation contributed to blood disease symptom in generative phase of banana plant. It observed earlier than 30 *T.minangkabau* treatment with bacteria suspension injection/bsi (T3B1) included to 3 dai followed by T4B1 (40 insects with bsi) 4.5 days while other 6 days treatments laid on Table 4. Analysis statistic of software SPSS var.20 identified an insignificant different (P.0.05) between treatments on incubation period of *R.solanacearum* Phylotype IV

Table 4. The effectiveness of *R.solanacearum* Phylotype IV infection by *T.minangkabau* and bacteria suspension period on generative phase banana plant

Treatment	Code	Incubation period (day)	Effectiveness (%)
Control (without insect, without bacteria spraying)	T0B0	0	
Bacteria spraying	T0B1	6	0
Ten insects without bacteria spraying	T1B0	6	0
Ten insects with bacteria spraying	T1B1	6	0
Twenty insects without bacteria spraying	T2B0	6	0
Twenty insects with bacteria spraying	T2B1	6	0
Thirty insects without bacteria spraying	T3B0	6	0
Thirty insects with bacteria spraying	T3B1	3	50
Forty insects without bacteria spraying	T4B0	6	0
Forty insects with bacteria spraying	T4B1	3	50

Indicator of *T. minangkabau* infection of blood disease initial symptom on banana plant often figured without wilted or yellowish leaves. Almost 50% *R.solanacearum* Phylotype IV contaminated plant of *T.minangkabau* on field planting showed the absence of yellowish leave while fruits badly devastated in once time. The initial period only indicated by colour chancing/discoloration on slashing fruit tip that penetrated inside while maceration obtained on advanced damage fruit (Figure 5)

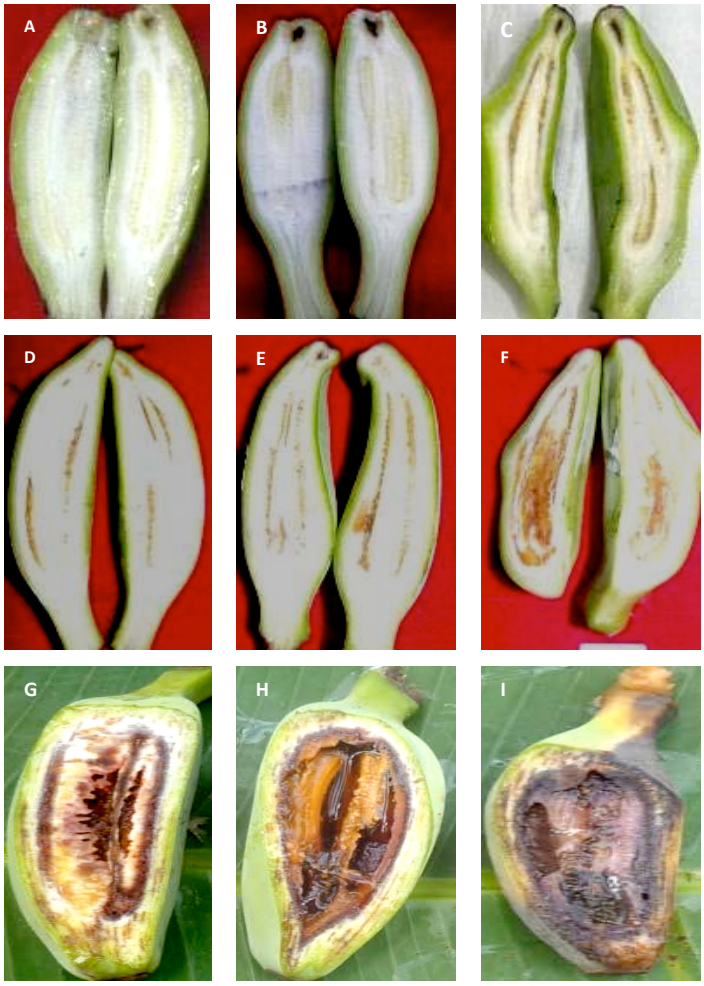
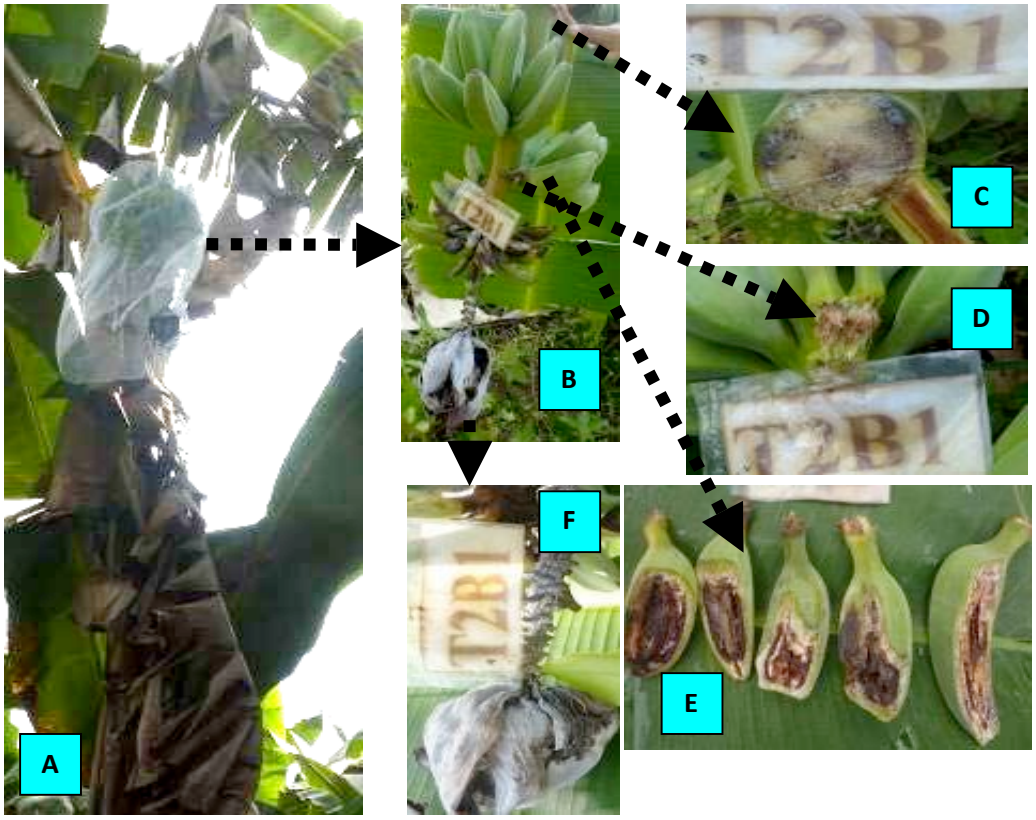


Figure 5. The development of blood disease symptom on fruit infected by *T.minangkabau*: A. Symptom on 1 dai. B. 3 dai. C. 6 dai. D. 9 dai. E. 12 dai. F. 15 dai. G. 18 dai. H. 21 dai. I. 24 dai

In the end of investigation, 50% plant with *R.solanacearum* Phylotype IV well outsider looked with green leaves and normal fruit development. This condition enlarged disease distribution through visiting flower insects activity. Disease development integrated with the broken fruit development on three days observation (Figure 6)



6. The symptom of blood disease bacteria on banana plant infected by *T.minangkabau*. Treatment on banana flower (A) Symptom on banana fruit (a month after treatment) (B) On bunch (C) On fruit tip (D) On fruit (E) and On banana blossom (F)

Discussion

Mechanism of bacteria transmission into banana plant tissue occurred through wounded area of suspension injection or hugging of insect activity (eating and egg placing) as well as natural holes such as nectaroda on banana flowers. Also, bacteria on the surface and inside of insect body injected into by either injection or its activities. Previous research discovered propagule of *R.solanacearum* Phylotype IV in high dense associated with banana plant in West Sumatera. Bacteria on surface and inside of the insect (caput, elytra and abdomen) of banana stem weevil (*C.sordidus* and O.*longicollis*) collected as (614.8 x 10^9)±57.312 and (961.5x10^9)±41.24, while *T.mingakabau* with (200.6x 10^3)±404.85 which the highest observed inside of abdomen as (140x10^3)±17.60 upk/ml. Gold and Bandyopadhyay (2006) revealed BXM bacteria cell isolated from *P.denoiti, A.melifera,* fruit flies and grass flies collected from male flower of infected and healthy plant on Uganda planting area. Colony of *Xanthomonas campestris* pv. *Musacearum* (Xcm) isolated form *P.denoiti* body; caput (524±185), thorax (581±193) and abdomen (180±175). More, propagul of *R.solanacearum* Phylotype IV was on bees on Bluggoe banana plating of Honduras (Buddenhagen and Elsasser, 1962). Based on Huffaker and Rabb (1984), *P.savastanoi* propagule olive knot in Mediterania resided on digestion system of Olive flies (*Dacus oleae*) through contaminated secret and eggs and *Pantoea stewartii* of corns Stewart disease consisted onside gut flea bugs (*Chaetocnema sp.*).

Bacteria propagule of *C.sordidus* and *O.lingicollis* vastly developed inside of plant tissue. Bacteria reproduction rate (r) on banana plangent through infection agent *C.sordidus*, *O.longicollis* and flower visiting insect *T.minangkabau* on generative phase highly categorized 4-61% , 6-54%, and 23-85%. According to Mairawita *et al* (2012) the high population of T minangkabau visited the sick banana flower determine the rapid development and spread of blood diseases in the highlands Tabek Panjang. During one flowering period infected banana flower visited by more *T. minangkabau* imago (140.70 ± 68.75) than healthy flower (109.05 ± 33.15). Gnanamanickman et al (1999) stated bacteria succeed infected plant involved the mobility to host, pathogen and host contact, bacteria penetration and proliferation into plant tissue followed by its distribution. Nectartoda, stigma and wounds of eating on banana flowers, oviposition and development of *C.sordidus* and *O.lingicollis* became an pathogen entrance into banana plant. The bacteria increased after inserted and spread on into vascular tissue, destruction cell wall and middle lamella producing pectinesterase, sellulase and protease enzymes (Goto, 1992; Hayward, 1995) and polysaccharides (EPS) (Demery et al 1992; Hayward, 1995). Furthermore, space between cell inside of tissue full filled by bacteria caused stuck and increasing viscosity contributed to water distribution disorder of plant (Holloway et al , 1994; Habazar, 1989).

Bacteria population massive by ecological condition on Baso highland and Pasar Usang lowland supported bacteria *R.solanacearum* Phylotype IV development. Average temperature of Tabek Panjang highland revealed as 25.8-26.8 ^{0}C, RH 82-90%, precipitation 12.48-42.3 mm, rainy 15-21, light intensity 38-67% while Pasar Usang lowland 28-29.6^{0}C, 80-87%, 9.56-31mm, 12-20 and 35-63%, orderly (BMKG Sumbar, 2013). Ecological condition on Tabek Panjang highland also very supported pollinator insect activity. Nismah (1983) considered to bees activity had positive correlation to temperature and air moisture. Activities peak occurred on 17-27^{0}C and RH relative 54-90%.

CONCLUSION

Insects contributed very much on blood disease bacterium distribution on banana planting area of Sumatera Barat. Banana corm borer *C.sordidus* and *O.lingicollis* and flower visiting insect *T.minangkabau* so actively supported the promulgation.

References

Baharuddin B. Pathological, Biochemical and Serological Characterization of the Blood Disease Affecting Banana and Plantain bacterium (Musa spp.) In Indonesia. Cuvillier Verlag Gottingen. 129p. 1994.

Buddenhagen.ZW and T.A Elasser. An Insect Spread of wild Epiphytotic Bluggoe Bananas. *Nature* 194: 146-165. 1962.

Eden-Green SJ. 1992. Characteristics of *Pseudomonas solanacearum* and related bacteria from banana and plantain in South East Asia. 51-57. In: M. lemattre, S. Freigoun, K. Rudolph and J.G Swings (*Eds*): Plant Phatogenic Bacteria. IRNA.

Gold CS, Bandyopadhyay R, Tinzaara W, Ssewiko F, Eden-Green SJ. 2006. Identifying Insect Vectors and Transmission Mechanisms for Banana *Xanthomonas* Wilt.International Institute Of Tropical Agriculture Oyo Road, PMB 5320, Ibadan, Nigeria.

Hayward AC and P. C Fahy. Media and Methods for Isolation and Diagnostic Test In: PC Fahy and GJ Persley. *Bacterial Disease A Diagnostic Guide.* Academic Press. Australia. 1983.

Mairawita,Suswati, Habazar T, Hasyim A and NasirN. 2012. *Trigona Minangkabau* Potential as Bacterial Spreader Agent of *Ralstonia solanacearum* Phylotype IV Cause Blood Disease on Banana Plants. International Proceedings of Chemical, Biological & Enviromental Engineering. Biological and Life sciences. ISBN*978-981-07-2909-7.IPCBEE Vol.402012. ISSN : 2010-4618.www.ipcbee.com International Conference on Biological and Life Sciences. ACSIT Press, Singapore

Mairawita, T. Habazar, A. Hasyim and N. Nasir. 2009. Flower visiting insects and ground diversity as spreading agents of Blood Disease bacterium on banana in West Sumatra. The 2nd International seminar and workshop on advanced molecular biology. 18-20 August 2009. Padang, West Sumatra. 2009.

Maryam Abn, Tata Rasta O, W and D Handayani Sihombing. 1994. Beberapa jenis serangga pengunjung bunga pisang yang diduga sebagai penular penyakit layu bakteri (*Pseudomonas solanacearum* E.F.Smith). Presented in the *Proceedings of the Meeting of Priority Development and Design of Horticultural Research,* Solok, November, 17-19. Agricultural Research and Development Center, Center for Horticulture research and development.

Schaad NW, JB Jones and W Chun. 2001. *Laboratory Guide For Identification of Plant Pathogenic Bacteria*. APS Press.Minnesota.

Soguilon CE, Magnave LV and Natural MP. 1995. *Bugtok disease of banana. Musa fact sheet No.5 INIBAP.*

Setyobudi, L dan Hermanto, C. 1999. Rehabilitation of cooking banana farms; Base line status of *Banana Disease Bacterium* (DARAH) distribution in Sumatera, p117-120. In: A.B. Molina and V.N Roa (eds) *Advancing Banana and Plantain R&D in Asia and The Pasific.* Proc of the 9 th INIBAP-ASPNET Regional Advisory Committee Meeting, Guangchou

Wardlaw CW. 1972. Banana Disease. Including Plantains and Abaca. Logman.

Elkhana Riabova

The International University of Nature, Society and Man "Dubna"

Creating the model of the rehabilitation of Russian towns

ABSTRACT. In this article the reasons of the rehabilitation of Russian towns and mid-sized cities are considered and the main opportunities of their rehabilitation are pointed. Also in this work the approximated model of the rehabilitation is suggested, with the help of which is possible to point towns conditions at every step of the rehabilitation.

Key words: model, rehabilitation, sustainable development, urbo-ecosystem, blocks, Russian towns.

INTRODUCTION

It isn't a secret, that nowadays ecological problems move to the fore in the global problems list. Some of them are the problem of transition to the sustainable development and improving human's habitat. And bout of these problems are strongly interlinked to each other.

So, in 1987 the Brundtland Comission firstly used this term in the report "Our Common Future", giving its definition as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs." But to achieve the sustainable development in future we need to work just now, creating a safe, sustainable and comfort habitat such for living generation as for future one.

The Article 42 of the Constitution of the Russian Federation states the following, that every person has a right to a healthy environment, the true information about its condition and a compensation of damage to his health or property by the environmental offence. And in the Article 3 of the Federal Law Concerning the Protection of the Environment pointed that business or any other activity of Russian Federation central, regional and local government agencies, such as individuals and entities' activity, having influence to the environment, should be carried out, basing on the next principles:

- observance the human right to a healthy environment;
- enabling favorable conditions for human life and activities;
- scientific rationalized harmonization of ecological, economical and social interests of human, society and state to enable the sustainable development and the healthy environment.

That way endorsement of the decision to transition to the sustainable development is legislated in the Russian Federation. But make a decision and pass the law is a lot and a few at the same time. Making the real steps is needed. And one of such steps should be the rehabilitation of the human's urban habitat to the more comfortable, safer and dynamically stable urbo-ecosystem, which can develop sustainably with minimum harm to the environment and citizens.

This article is dedicated to trying to suggest such kind of model of the towns and mid-sized cities' rehabilitation. Why are towns and mid-sized cities? There are a lot of reasons to it. So, nowadays, towns and mid-sized cities compose nearly 78 per cents from all Russian urban territories, but there live only 28 per cents of all citizens in our country.

In such a way, rehabilitation of these towns, firstly, helps to deconcentrate the human population in the megalopolises and it'll decide such problems as traffic jams, queues to the hospitals and kindergartens and schools and other problems, connected with highly-concentrated population. The second reason of the towns and mid-sized cities' rehabilitation is the property of even economical and social development of all Russian territory, not only some of its parts.

Rehabilitating towns, which were industrial, agricultural and local scientific centers, creating there new, environment and human-friendly manufacturing we decide such serious problems as unemployment in many our towns and cities, economical and industrial regression inside the country, and also we'll have the opportunity to develop complex high technologies, alternative energy and etc. Also, at this moment we have methods, allowing valuing the environment and economic potential of each town, to decide what industry segment we should develop in it to change town in sustainably developing urbo-ecosystem.

The third reason isn't so clear, but also important. It'a paying an attention to low-rise building and saving the historical look of the town. From videoecologists' point of view buildings' highness shouldn't be higher than the nearest trees' highness. Consequently low-rise building, reconstruction of the ancient buildings, creating single-family detached homes with the gardens and sizable areas of the community landscapes – all of it let to create psychologically comfortable look of the town. Also, it's possible to extend the list of existing towns – museums in the open air and open new towns. One of the most famous of such towns is Suzdal' town, in the Vladimir region. This way, in consideration of the premises, it could to say that rehabilitation of Russian towns and mid-sized cities is actual and well-timed task and it can be a serious step on the way to sustainable development of the country.

To the extent that towns' problems are complex and multidimensional, their rehabilitation should be delivered not only in one specific domain, but in all directions at the same time. It needs to create such kind of a model, which could consider as much towns' problems as possible and their special aspects, and give maximum correct estimate of their conditions during all the process of rehabilitation. And rehabilitation on its own suggests planning step by step transformation of urban milieu.

The model of the Russian towns' rehabilitation could be generally shown as some interlinked and interplayed blocks, each of them has its own set of sub-blocks. They are "Geomorphic data" block, "Environmental conditions" block, "Eco-economic data" block, "Socio-economic data" block and "Social data" block, containing two sub-blocks like "Medical-demographic data" and "Socio-psychological data". The blank drawing of the model is shown below.

It should be pointed, that Geomorphic data" block is contained with data, characterizing not only town's territory itself, but also in complex with the surrounding area, while "Environmental conditions" block directly describes only ecological conditions inside the town.

Also it should be said a few words about "Environmental conditions" block, because it's only one block, which has two levels. Every sub-block of this block has its own set of sub-systems, that allowed to make full-scale range of data and receive the entire gamut of town's conditions. The figure of "Environmental conditions" block is also shown below.

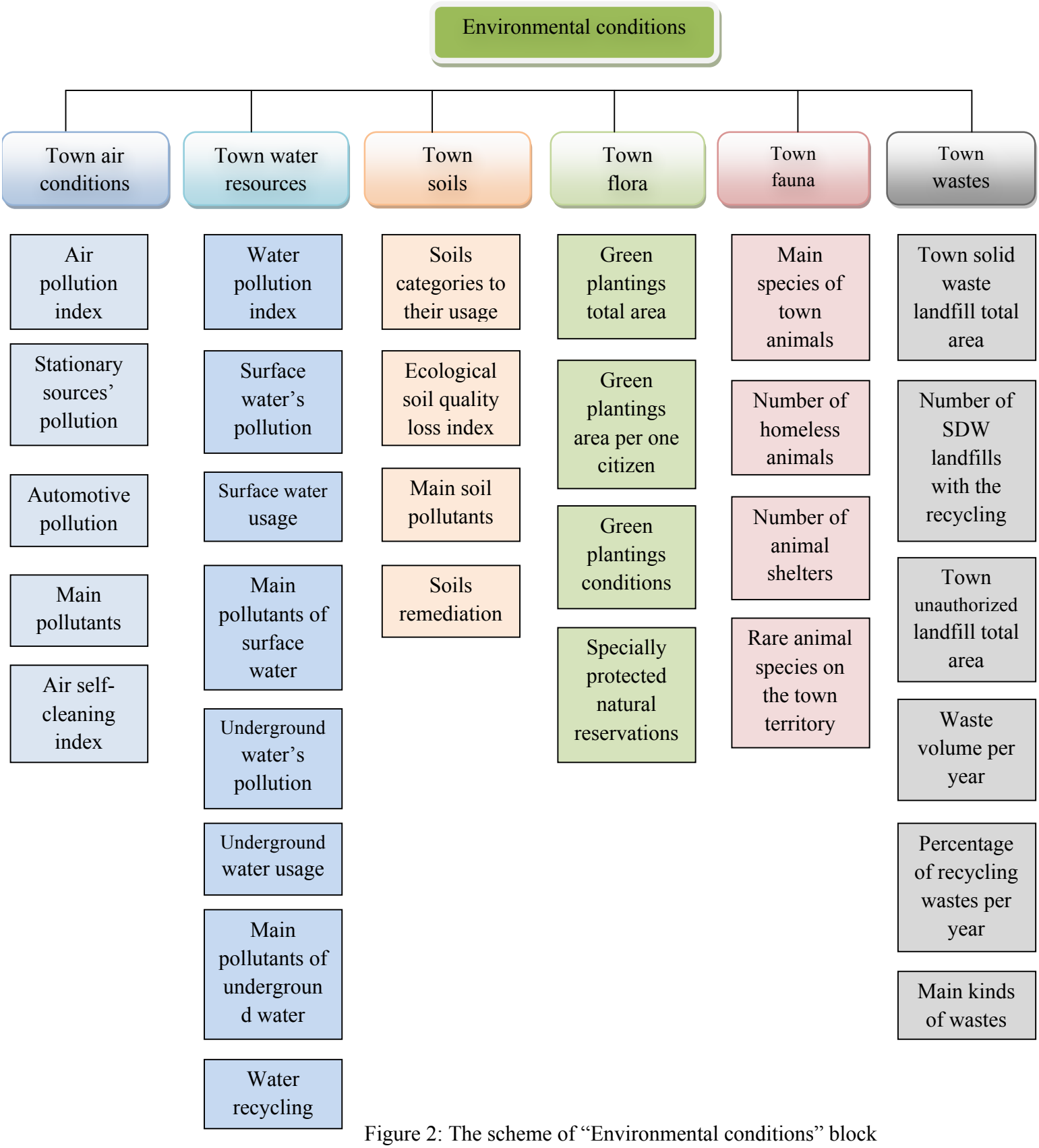


Figure 2: The scheme of "Environmental conditions" block

So, the work of this model consists from stages. On the first stage it takes place data collecting and blocks filling. Then, on the second stage takes place data processing and analyzing. Data analysis will be done with the help of correlation analysis methods, because it helps to find any interlinks between different data pairs.

Another important step is the calculation of weight coefficients for each factor to receive the most truthful vision of town's condition. At this time a calculation methodology of weight coefficients resides in generative stage, though there is a suggestion of calculating weight coefficients after correlation coefficients. The final result of the second stage will be detailed description of the town conditions, its "hot" points, and its comfort-of-life characteristics. After that the third stage comes.

The third stage suggests giving recommendations about rehabilitation of the town, taking into account its educed problems. On this stage could be such kinds of measures as:

- recultivation of lands disturbed by SDW landfills, including unauthorized landfills;
- rehabilitation of polluted water bodies and their transformation into recreation areas;
- installation of modern cleaning equipment into the contaminators, to curb the pollution;
- carrying out some environment and people-friendly architecture and planning changes into town image, such as relocation manufacturing facilities below the town; organization social infrastructure at a walking distance; changing town image with preservation of historically important objects and taking into account citizens' wishes and suggestions.

After carrying out all of prescribed recommendations, it's possible to step to the final – fourth stage – the changeover from rehabilitated town to eco-town as a dynamically sustainable and harmonically developing urbo-ecosystem. On this stage it takes place the next:

- transferring all the manufactures to the closed cycle of water usage;
- active usage of energy-saving and resource-saving technologies, alternative energy technologies development;
- transferring all the manufactures from simple purification of emissions and effluents to the closed cycle with the maximum recycling all of solid, liquid and gaseous wastes;
- opening eco-centers for citizens' ecological education, and etc.

In such a way, even nowadays we can do really steps to the sustainable development, starting with the rehabilitation our towns, mid-sized cities, and then cities and megalopolises. And this model of the rehabilitation allows not only receive the first view about town conditions, but also it allows to trace the dynamics of changes during the time, upon condition of timely data updates.

CONCLUSIONS

Following the results the next conclusions will be done:

1. Towns and mid-sized cities are a good base to creation in Russia comfortable-for-life and sustainably developing eco-towns, but firstly we should estimate their conditions and rehabilitate them;
2. It's due to the model of estimate ecological, economical and social town conditionals and its rehabilitation, taking into account maximum possible common factors for each town.

3. At present time the main problem of this model is the calculation of weight coefficients for each factor in every block.
4. The eventual result of this model work should be the full picture of every town conditions, based for which, we can give some recommendations to its rehabilitation. Besides that, existed model allows to trace the changes of town conditions during all the time of its rehabilitation.

REFERENCES

1. Brundtland Commission, 1987. Report of the World Commission on Environment and Development. United Nations.
2. Constitution of the Russian Federation (passed by referendum of 12.12.93), (edit of 30.12.2008). Article 42.
3. Federal Law Concerning the Protection of the Environment of 10.01.2002. N 7- Federal Law (passed by State Duma Federal Assembly of the Russian Federation of 20.12.2001). Article 3.
4. Gorokhov V.A., 1991. Urban green building. Line: Architecture. Educational guidance for higher education institutions. 416p. – M.: Stroyizdat, Moscow.
5. Ilin I.O., Iontsev V.A., Kashuro I.A., Kiktenko N.M., 2011. Mechanisms of life comfort improvement of citizens in big cities in the globalization (by the example of Moscow). 38p. –MSU, Moscow.
6. Mashintsov E.A., 2000. Development of the integral assessment of Tula's territory environmental conditions. Thesis for candidate's degree in engineering science. – 230p. – Tula.
7. Mashintsov E.A., 2007. Environmental assessment impacts to the morbidity of antropogenically stressed territories based on systemic approach. – 350p. – Tula.
8. Sokolova A.A., 2011. Towns in the providing of sustainable development of raw region. – Synopsis of a thesis of Cand. Sc. (Economics), – 22p. – Murmansk.
9. Solntseva A.A., 2013. Social and ecological factors of morbidity in urban towns on the south-west part of Russia. – Synopsis of a thesis of Cand. Sc. (Geography), – 25p. –Belgorod.

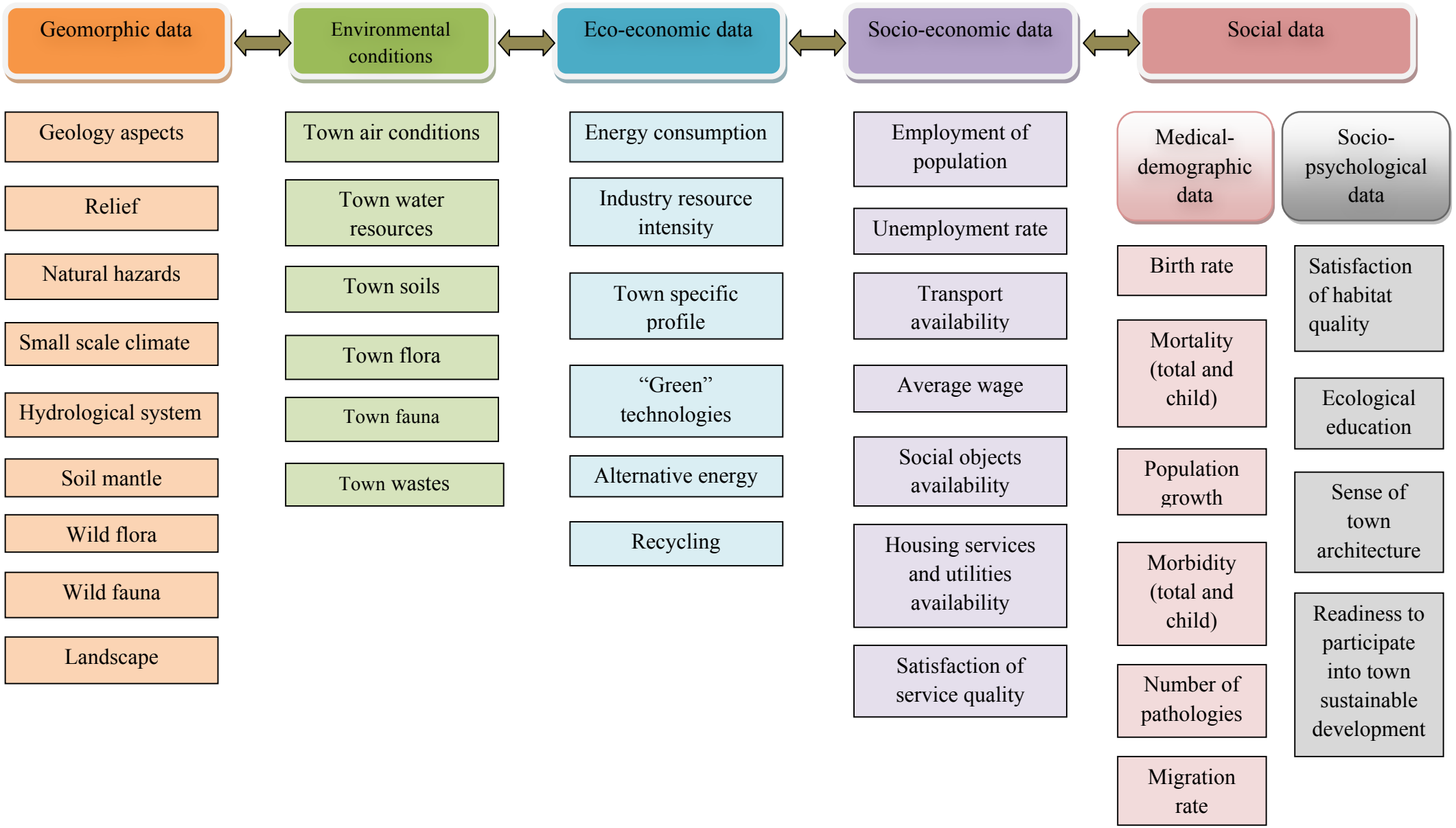


Figure 1: The scheme of rehabilitation model of Russian towns

PRESDA Foundation

Education for Sustainable Development—People, Planet, Profit