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# BIODIVERS©

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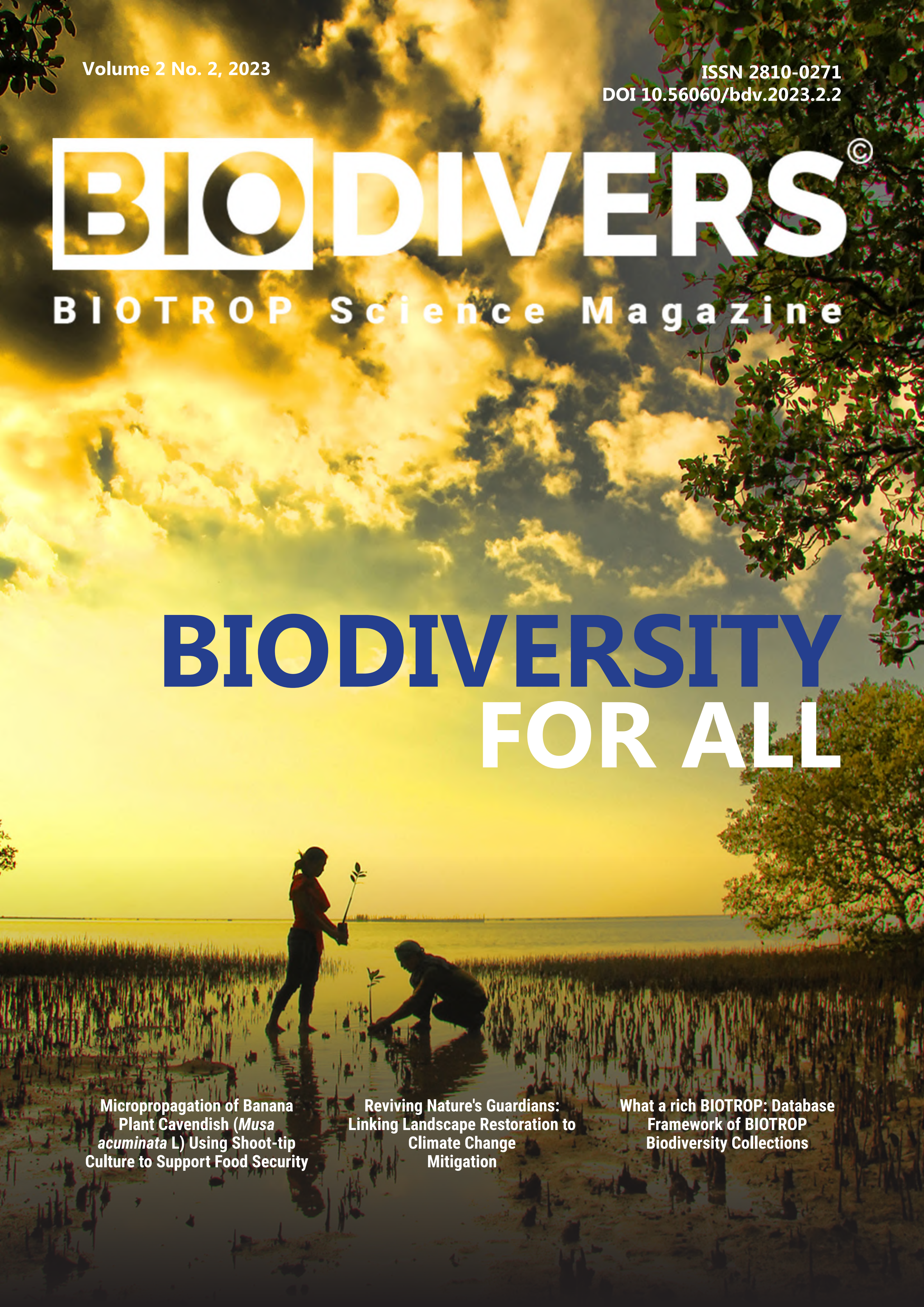

## BIODIVERSITY FOR ALL

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Reviving Nature's Guardians: Linking Landscape Restoration to Climate Change Mitigation

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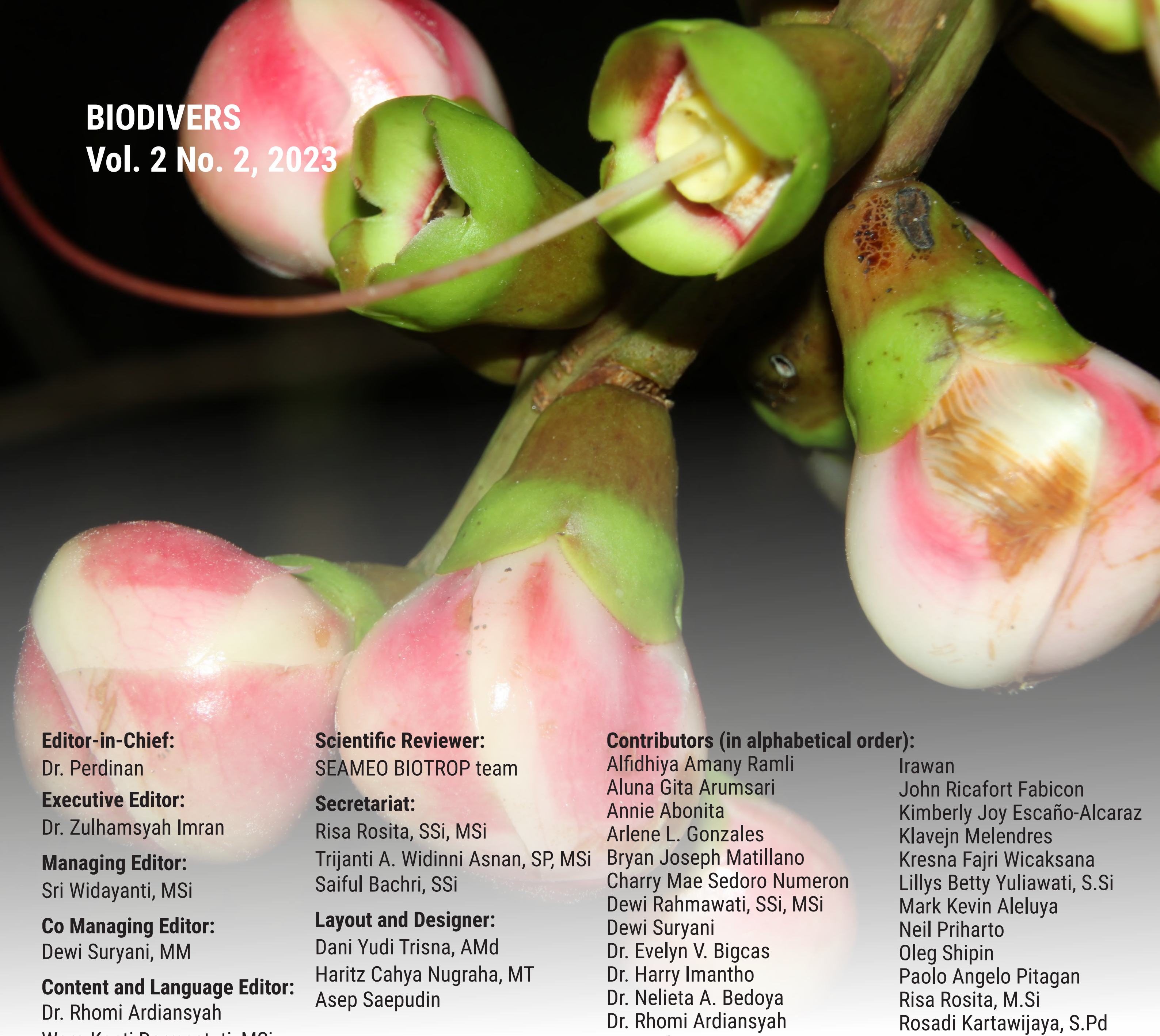

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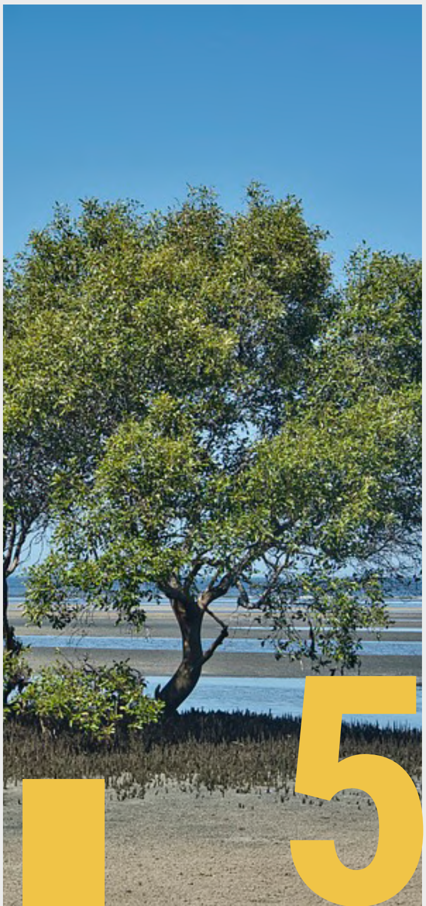





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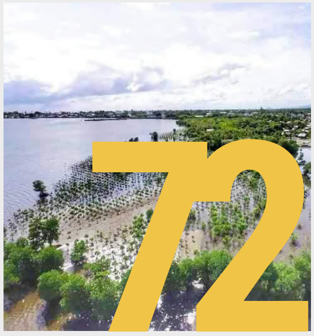
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# OVERVIEW OF BIODIVERS

BIODIVERS is a bio-science general audience magazine launched in December 2021 by the Southeast Asian Ministers of Education Organization Regional Centre for Tropical Biology (SEAMEO BIOTROP). It is designed as a scientific publication to increase awareness on issues related to Tropical Biodiversity from the Mountain to the Ocean (MOTO) and increase biodiversity literacy.

BIODIVERS is a bi-annual publication that focuses on the Restoration and Conservation of Unique and Degraded Ecosystems, Sustainable of Management and Proper Utilization of Biodiversity, Bioenergy, Biotechnology to Support Food Security and on Strengthening Ecosystem Resilience in Facing Global Climate Change. This magazine also envisions becoming a popular-scientific magazine for promoting and publishing research findings of scientists from SEAMEO BIOTROP and overseas. The articles will come from writers worldwide.

# SEAMEO BIOTROP

Southeast Asian Regional Centre for Tropical Biology


## Vision

To become a reputable center for sustainable biodiversity management in Southeast Asia

## Mission:

To deliver innovative products and technologies in science-education for saving biodiversity in transforming best practices for the betterment of Southeast Asia societies.

To promote applied science education on sustainable use of biodiversity for the well-being of society in Southeast Asia.

To build highly competent human resources for managing sustainable biodiversity in Southeast Asia.

# SEAMEO BIOTROP Program Thrust

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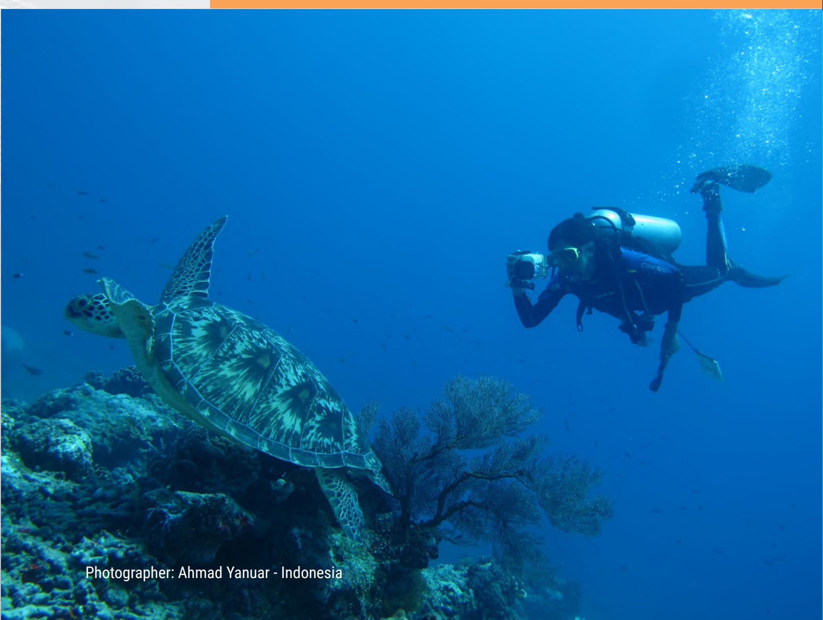
Restoration and conservation of unique and degraded ecosystems

Sustainable Management and Wise Utilization of Biodiversity, Bioenergy, Biotechnology, and Food Security

Photographer: Arnold Conlu Jumpay - Philippines



Photographer: Ahmad Yanuar - Indonesia

Strengthening ecosystem resilience to global climate change

**Dr. Zulhamsyah Imran**
Director of SEAMEO BIOTROP

# BIODIVERSITY FOR ALL

**Dear Valued Reader,**
**Greetings from SEAMEO BIOTROP, Indonesia!**

In recent years, most people talk about biodiversity, especially the importance of saving biodiversity concerning climate change. What exactly is biodiversity? Biodiversity or biological diversity is the variety of life on Earth, in all its forms, from genes and bacteria to entire ecosystems such as forests or coral reefs (https://www.un.org/en/climatechange/science/climate-issues/biodiversity; accessed on 12 August 2023). Furthermore, the United Nations explains that the biodiversity we see today results from 4.5 billion years of evolution, increasingly influenced by humans. Thus, biodiversity refers to the diversity of life forms, ecosystems, and genetic diversity on our planet. Therefore, biodiversity varies, including species diversity, ecosystems, genetic variation within species, and interactions between species.

In recent years, the Earth has been experiencing the loss of biodiversity. Now, let us dwell on what causes biodiversity loss. The first direct cause of biodiversity loss is land use change for food production, which drives an estimated 30% of biodiversity decline globally.

Overexploitation ranks second in the cause of biodiversity loss for food, medicines, and timber, which drives around 20% of biodiversity loss. Climate change is the third cause of biodiversity loss, which, together with pollution, accounts for 14% of biodiversity loss (https://royalsociety.org/topics-policy/projects/biodiversity/human-impact-on-biodiversity/; accessed on 12 August 2023). All of these drivers are stemmed from human activities, which are growing due to the increasing human population. Most natural resources are used to fulfill human necessities, such as agriculture, mining, industrial infrastructure, and urban areas.

Within several decades, climate change has become increasingly significant as a driver of biodiversity loss. Climate change was the natural long-term shifts in temperatures and weather patterns. However, due to increasing human activities concerning the use of fossil fuels, climate change has become increasingly significant in causing biodiversity loss. The leading greenhouse gases causing climate change are carbon dioxide and methane.

The severe impacts of climate change on biodiversity loss need to be tackled to secure the Earth. Concerning tackling climate change impacts, there has been a developing concept of Biodiversity for All.

Biodiversity for all encapsulates the idea of promoting and ensuring the availability and existence of biodiversity for the benefit of all living organisms, including humans, on Earth. The concept of Biodiversity for All emphasizes the importance of maintaining and conserving a wide range of species and ecosystems.

In achieving the success of the "Biodiversity for All" concept, it is pertinent to implement sustainable practices and policies that conserve and protect species and ecosystems by establishing protected areas, promoting sustainable resource management, supporting conservation efforts, raising awareness about the value of biodiversity and integrating biodiversity considerations into various sectors, such as agriculture, urban planning, and development.

These efforts should be done locally and globally, collaborating with governments, organizations, and individuals who commit to saving the Earth for the present and future generations.

The concept of "Biodiversity for All" puts forwards the interconnectedness of all life forms and the need for collective efforts to ensure the well-being of nature and humanity.


**Prof. Dr. Ir. M. Faiz Syuaib, M.Agr**
Director of Research, Technology, and Community Services
Directorate General of Higher Education, Research, and Technology
Ministry of Education, Culture, Research, and Technology

## Message from the Director of Research, Technology, and Public Services, Directorate General Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, Republic of Indonesia for BIODIVERS Vol. 2 No. 2

Scientific publication is a receptacle to inform and disseminate result of research conducted by academicians and researchers to the public. The scientific publications give benefits to transfer knowledge, to develop science, innovation and further technology, as well as to enrich learning purposes in educational side. Scientific publication is one of important criteria of quality and reputation for mostly higher education institutions. Publication that resulted from such evidence-based analysis may also beneficial for national strategic developments plan, policy making and community services as well as to develop the nation become a science-based community. Considering the importance of dissemination of research results, increasing scientific publications is necessary, in term of quantity and quality as well. The important target of beneficiaries of research dissemination is to the public, actually. So, it is necessary to shift the way of explaining the result, not just as scientific-based publication but also needed as popular scientific-based publication to make it broader and easier to understand for more people or public with broader background. SEAMEO BIOTROP, as a Regional Centre for Tropical Biology, has a mandate to conduct research, training, and dissemination of information, including increasing regional collaboration, and has contributed to enhancing the quality of education at National and Regional. Besides BIOTROPIA, as one of reputable scientific publications indexed by Scopus, SEAMEO BIOTROP has to keep their commitment to disseminate the research results to the public. Since 2022, SEAMEO BIOTROP has been committing to publish another edition of publication in the form of a magazine with several popular scientific articles named BIODIVERS. This publication is expected to deliver information or the result of research from scientists and academicians to the public in more 'popular' way and terms to make it easier to understand and more applicable for the needs of communities. more than that, it is hoped that this edition will also make it easier and more general to convey information related to the development and progress of science and research results, so that it can also contribute to the world of education.The publication of BIODIVERS is one of the commitments of SEAMEO BIOTROP to promote the Science-Education in Biodiversity Conservation Education. In this edition, BIODIVERS brings the theme "Biodiversity for all". It is expected to deliver information to the public about the importance and the real value of biodiversity. Besides the public, BIODIVERS could be the media for any scientist, student, and lecturer to publish their research results in a popular science magazine.Finally, I hope that SEAMEO BIOTROP will continue to consistently publish quality research results to be disseminated to the public by publishing BIODIVERS. In this edition, BIODIVERS can provide benefits to society through the latest advances in science and education related to Biodiversity Conservation. As a representative of the Ministry of Education, Culture, Research and Technology, I congratulate SEAMEO BIOTROP for successfully publishing high quality research results on the BIODIVER issue and appreciate SEAMEO BIOTROP's consistencyin always focusing on supporting biodiversity education, especially Indonesia, and, in general, Southeast Asia.

# EDITORIAL REMARKS


**Dr. Perdinan**
Deputy Director for Administration
of SEAMEO BIOTROP, Indonesia
Department of Geophysics and Meteorology,
IPB University

## "Biodiversity for All, Embracing Our Role in Advancing Scientific Discovery and Conservation"

**Dear Esteemed Readers and Contributors,**

Welcome to the latest edition of our scientific magazine BIODIVERS. As we delve into the depths of research and exploration, we are reminded of the profound impact that the pursuit of knowledge can have on our understanding of the natural world and our responsibility to preserve it.

Biodiversity is all living things on earth, including humans and microorganisms. Each works together in an ecosystem, like a complex web, to maintain balance and support life. The most aspects of our lives depend on biodiversity. Biodiversity has important value for human life, because it provides basic human needs to survive in nature, such as food, fuel, and medicine. Many crucial ecosystems services are provided such as pollination, seed dispersal, control of agricultural pests, nutrient cycling, climate regulation, and clean water.

Biodiversity has cultural value to humans as well. The value of biodiversity can also be understood through the lens of the relationships we form and strive for with each other and the rest of nature. We may value biodiversity because of how it shapes who we are, our relationships to each other, and social norms. These relational values are part of peoples' individual or collective sense of wellbeing, responsibility for, and connection with the environment. The different values placed on biodiversity are important because they can influence the conservation decisions people make every day.
**(https://www.amnh.org/research/center-for-biodiversity-conservation/what-is-biodiversity)**

In this issue, we turn our focus to a critical global challenge: the preservation of biodiversity. The intricate web of life that surrounds us is under threat from various human-induced factors, and it is our duty as scientists, researchers, and conservationists to shed light on these issues and propose innovative solutions.

In this special occasion, we highlighted some topics of the article such as the significance of bananas as a vital global fruit crop and emphasizes their nutritional potential for diversifying staple foods in Indonesia. It acknowledges the growing need for food due to population growth and underscores biotechnology as a solution to address world food challenges, particularly in Indonesia

Another theme about climate change mitigation and adaptation are still hot issues to discuss, addressing challenges at various levels and proposing creative remedies. The ecotechnology turns the problem of wastewater flowing into lake from adjacent restaurants into solution for many urban challenges including use of nutrient rich water as a fertilizer for mangrove trees in a Novel Urban Ecosystem.

Phytoremediation also become an interesting topic, the use of plants to remove contaminants, holds promise for addressing soil and water pollution. There some information focuses on testing the efficacy of succulents, specifically *Opuntia* spp., in phytoremediating heavy metals from soil collected near a metallurgical factory in Carmona Cavite's industrial zone.

The intersection of biodiversity science and internet-based data availability has created a reinforcing relationship. This is due to the growing global awareness of how biodiversity loss impacts human life sustainability. SEAMEO BIOTROP recognizes this and seeks to enhance the visibility of its managed biodiversity

collections. The biodiversity collections includes valuable specimens of plants, fungi, insects, and pests for research. To preserve and improve accessibility, these collections are being digitized. A study was conducted to develop an integrated framework for the BIOTROP biodiversity database that caters to national, regional, and global requirements. The study successfully created a database framework that consolidates digital data from the biodiversity collection into a management system. This initiative enhances the availability of vital biodiversity information to botanists and the public worldwide.

Talking about precious product, Sandalwood (*Santalum album*) and Teak (*Tectona grandis*) are crucial wood resources for furniture production. The implementation of sustainable micropropagation techniques holds promise for augmenting wood supply.

*Trichoderma harzianum* still become our problem in agroecosystem, we provide the information about the impact of temperature on the growth of *Trichoderma harzianum*. This fungus serves various functions, including disease prevention in plants, promotion of plant growth, and organic matter decomposition.

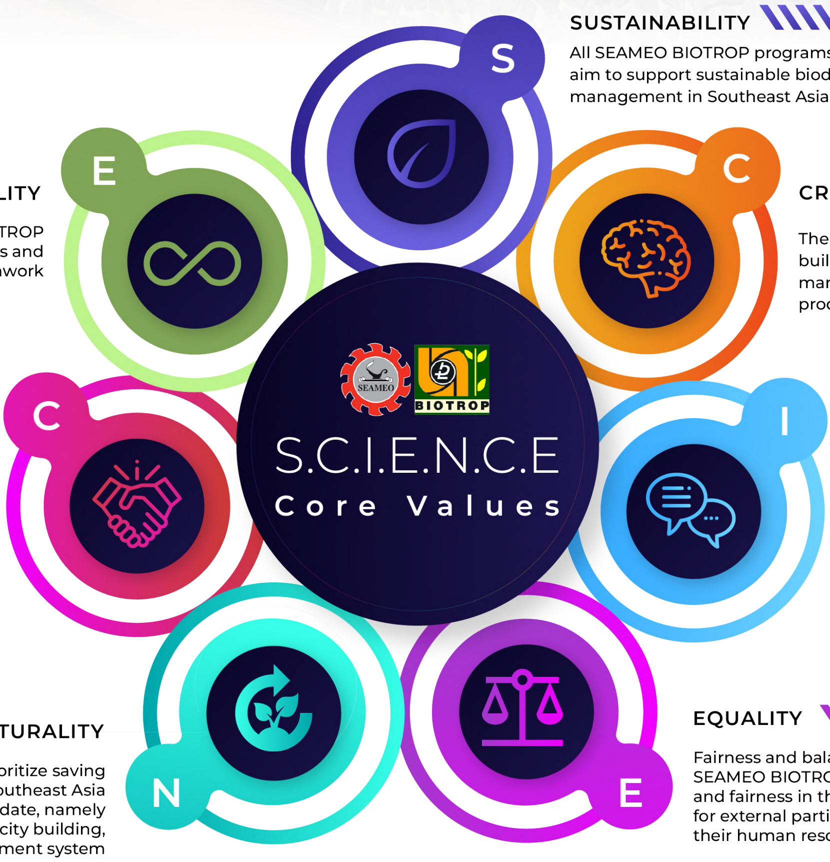
From the overseas contributor, Philippine Cinnamomum trees are known for their fragrant leaves and bark, serving various economic and medicinal purposes. Despite this, limited information exists about their protein profiles. Cinnamomum possesses substantial protein content, holding potential culinary, medicinal, and pharmaceutical applications.

Now we are going to the sea level, In the aftermath of the most powerful typhoon on record, Typhoon Haiyan, significant damage was inflicted on coastal greenbelts. Mangrove trees were uprooted, their trunks broken, resulting in complete defoliation and mangrove death. We emphasize the importance of allowing mangroves time to regenerate naturally and establish colonization. Human-assisted rehabilitation efforts should consider the patterns of natural succession when planning post-disturbance rehabilitation measures.

Through peer-reviewed contributions, we gain insights that guide policy decisions, inspire public awareness, and drive the transformation towards sustainable practices. As editors, we take pride in curating a collection of articles that reflect the diversity of research disciplines united in the common goal of preserving our planet's rich tapestry of life.

We extend our heartfelt gratitude to the authors, reviewers, and readers whose unwavering commitment to scientific excellence propels our journal forward. It is your dedication that fuels the engine of discovery and enables us to bridge the gap between knowledge and action. May our collective efforts lead to a future where biodiversity thrives, ecosystems flourish, and generations to come inherit a planet that has been nurtured and protected.

Thank you for being an integral part of our scientific community, and for joining us on this journey to make a lasting impact on the world we share.


**SUSTAINABILITY**

All SEAMEO BIOTROP programs and activities aim to support sustainable biodiversity management in Southeast Asia

**EGALITY**

All human elements in SEAMEO BIOTROP must have egalitarian attitudes and inclusive and solid teamwork

**CREATIVITY**

The innovation and creative capacity buildings, including creativity in manufacturing research and training products

**CONNECTIVITY**

All science education activities must have connectivity that supports and complements each other in a science-based learning system Applied Digital Science Technology

**INTEGRITY**

Integrity is meant consistency in the actions, values, methods, measures, principles, expectations and various outcomes for the achievement of the SEAMEO BIOTROP organization

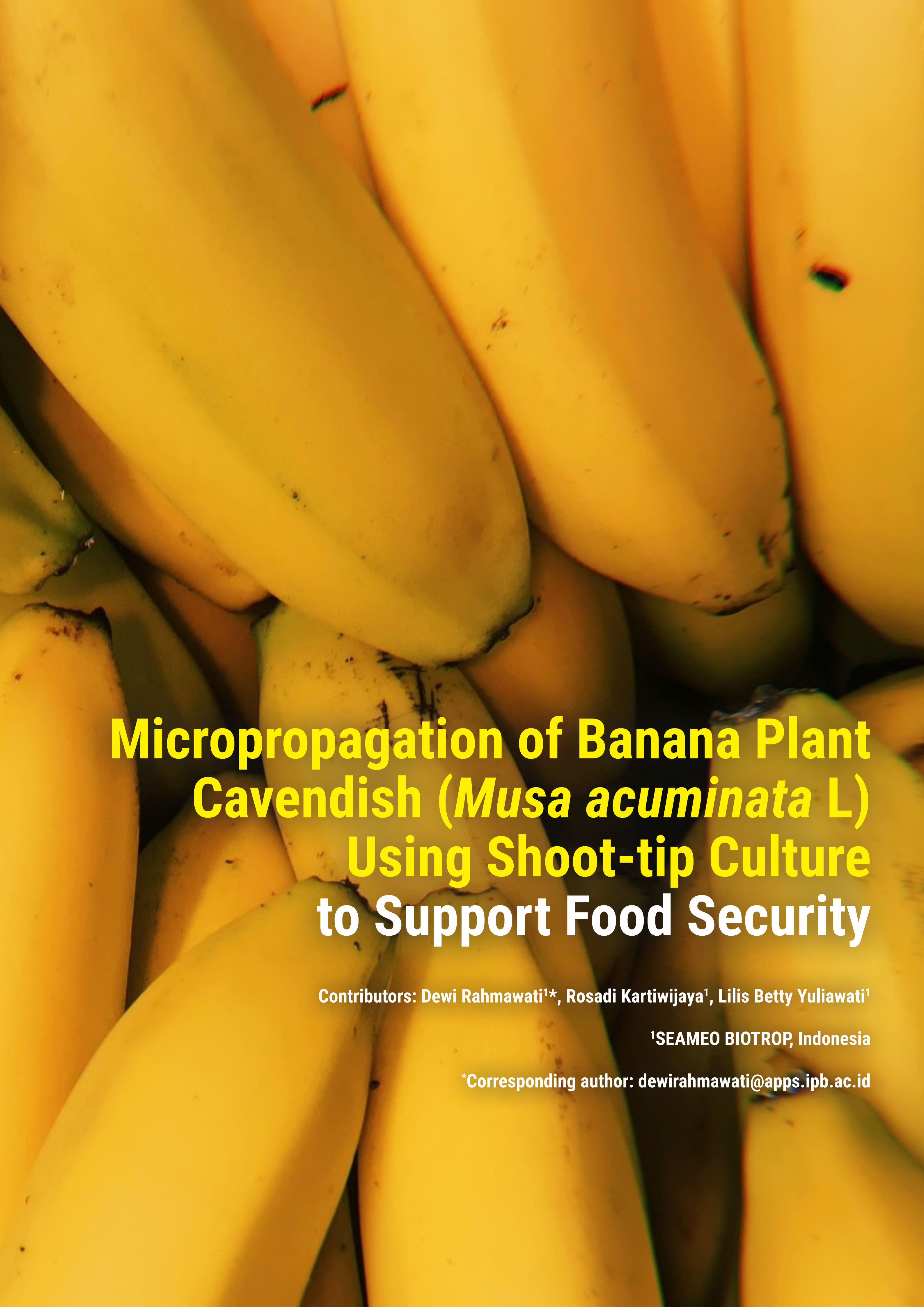
**NATURALITY**

SEAMEO BIOTROP will prioritize saving natural biodiversity in Southeast Asia through its organizational mandate, namely science education, capacity building, and a knowledge management system

**EQUALITY**

Fairness and balance in the development of SEAMEO BIOTROP's internal human resources and fairness in the opportunities for external parties to develop their human resources at SEAMEO BIOTROP

https://www.kew.org/plants/cavendish-banana



# Micropropagation of Banana Plant Cavendish (*Musa acuminata* L) Using Shoot-tip Culture to Support Food Security

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## Abstract

Banana is one of the world's most important fruit crop. From a nutritional perspective, bananas are an excellent opportunity to diversify staple foods in Indonesia. An increase in the human population will increase the need for food. The application of biotechnology is a solution to facing the challenges and threats of the world food crisis, including Indonesia. Therefore, using a propagation method to produce seedlings quickly and on a large scale and to make disease-free seeds through tissue culture techniques is necessary.


Keywords: banana, biotechnology, food, micropropagation, tissue culture.

## Introduction

An increase in the human population will increase food needs. One way to grow food productivity to achieve domestic food sovereignty is by applying biotechnology. Biotechnology solves the challenges and threats of the world food crisis, including Indonesia. Food crops with biotechnology must be carried out and developed to anticipate the danger of a world food crisis, which is predicted to peak starting in 2050. Biotechnology can also answer global climate change, the water crisis, and reducing pesticides and world carbon emissions. FAO predicts that food demand will increase by as much as 60% so that the world's population does not sink into poverty and hunger. Banana plants are the fourth most important crop in developing countries after rice, wheat, and corn. From a nutritional perspective, bananas are an excellent opportunity to diversify staple foods in Indonesia. According to the Central Statistics Agency (BPS) records, Banana production in Indonesia reached 9.60 million tons in 2022. It showed a figure 9.79% higher than the previous year, which was 8.74 million tons.

The main problem of bananas in Indonesia's decline in banana production is the high disease incidence. The expected technology includes all production systems to produce quality fruit that is safe for consumers and protects the environment. Therefore, obtaining good-quality parental donors, resistance to pests and diseases, and resistance to environmental changes is essential. In Indonesia, the development and cultivation of cavendish bananas still have many obstacles, including the need for quality seeds, *Fusarium* wilt rot, and uniform quality (Ministry of Agriculture, 2014). The limited availability of quality banana seeds in Indonesia is caused by the low quantity and quality of banana seeds produced using conventional methods. Conventional propagation of banana seeds by taking saplings from the stem of the mother plant cannot meet the demand for quality banana seeds on a large scale. In addition, the time needed to get seeds is quite long. Therefore, using a propagation method to produce seedlings quickly and on a large scale and to make disease-free seeds through tissue culture techniques is necessary.

## Methods

Figure 1 shows the in vitro propagation of bananas in SEAMEO BIOTROP. The tissue culture process included initiating cultures from sterilized shoot tips obtained from the banana plant (Fig. 1a), shoot initiation (Fig. 1b), shoot multiplication, elongation and rooting (Fig. 1c), acclimatization (Fig. 1d), and banana nursery in the field (Fig. 1e).


Figure 1. Micropropagation of banana in SEAMEO BIOTROP: a. explant sterilization, b. shoot initiation, c. shoot multiplication, elongation and rooting, d. acclimatization, e. banana nursery in the field

## Results and Discussion

One of the goals of plant tissue culture is micropropagation. Micropropagation is plant propagation in vitro by carrying out explant multiplication activities. The initial stage in explant selection is the selection of the mother plant or the so-called explant source. The choice of explant sources is essential for the success of micropropagation selection of explant sources and continuous improvement of parent plant quality. Starting banana tissue culture involves looking for and determining banana trees that will be used as quality mother plants. The mother plant factor is essential to maintain and improve its quality. Increasing the quantity and quality of bananas need quality banana seeds. If the mother of the banana plant is a superior seed, the resulting embryo will also have excellent characteristics. Mother plants are to be used as a source of explants from species or varieties with healthy vigor and are free from symptoms of pests and diseases.

Sterilization of banana explants by using 70% alcohol solution for 10 minutes, then immersing the explants in 50% NaOCl solution (Sodium hypochlorite) for 30 minutes. Multiplication of banana shoots is done by stimulating an increase in axillary shoot proliferation from the shoot hump. Shoot multiplication can be induced by adding growth regulators to the media. Media is essential in inducing shoot formation and proliferation, influenced by the concentration and type of hormone used. The multiplication stage of banana plants uses cytokinins combined with auxins. The often-used high concentration of cytokinin is BAP (6-Benzyl Amino Purine), 2-5 mg/L.

In contrast, the often used auxin is NAA (1-Naphthaleneacetic acid) with a low concentration, between 0.1-0.5 mg/L. The most suitable plant growth regulators for in vitro plant propagation are often species-specific or even specific to certain cultivars within a species (Gahan & George, 2008). Therefore, the optimal combination of growth regulator concentrations for each type of banana is often different. Optimal concentration combination obtained through empirical research. Several studies have been conducted on banana explants, including micropropagation of abaca banana (*Musa textillis* Nee) using BAP 5 mg/L gave the best results with an average of 8.6 micro shoots per explant (Avivi & Ikrarwati, 2004), on *Musa paradisiaca* L using MS media with the addition of 0.2 mg/L IAA combined with 5 mg/L BAP gave the highest shoot multiplication by forming 6-17 shoots (Fitramala et al., 2016), in *Musa paradisiaca* cv. Raja Bulu, the best concentration to increase the number of shoots is BAP 5.8 mg/L (Yosafat, 2020), banana (*Musa paradisiaca* L.) Kusto cultivar using 6 mg/L BAP treatment (Apriani et al., 2016), banana (*Musa* spp .) cv. Giant Cavendish 5.0 mg/L BAP + 0.5 mg/L NAA (Gebeyehu, 2015), multiple *Musa sapientum* shoots were induced in vitro from shoot meristems with Murashige and Skoog's medium supplemented with BAP 3.0 mg/L and NAA 0.2 mg/L (Kalimuthu et al., 2007). The best BAP concentration to induce propagules plant bananas Cavendish is 3.0 mg/L (Maulida et al., 2018), whereas using a low BAP combination of 0.2 mg/L + IAA, 0.1 mg/L in *Musa paradisiaca* L also resulted in a low number of shoots


(Dhanalakshmi & Stephan, 2016) and the same thing for Kepok Amorang Banana shoot multiplication using ¼ MS + 1 ppm BAP, the rate of shoot multiplication obtained in this study was low, which ranged from 1-3 (Supriati, 2010). BAP primarily influences the development of explants, namely in the formation of shoots, shoot multiplication, and spurring cell division in plant metabolism to form the necessary organs (Faridah et al., 2017). BAP is a type of cytokinin that is more commonly used in in vitro culture because it is more effective and stable than other cytokinin hormones. Shoot propagation of various bananas in vitro using BAP shows that the higher the concentration of BAP can produce more banana shoots at a specific optimum concentration. However, cytokinins that are too high can inhibit and become mutagens for banana plants. Auxin in the right concentration plays an active role in cell differentiation, but it can be toxic at levels that exceed the optimum concentration. In vitro rooting induction for Cavendish banana plants was MS media without ZPT with macro content half of the normal concentration, plus 1 g/L of activated charcoal.

Tissue culture is a technique used to grow plant cells, tissues, or organs under sterile conditions on a known composition nutrient culture medium. The basis of tissue culture techniques is that plant cells have totipotency, namely the ability of cells to grow and develop to form complete plants. The success of propagating banana seedlings through tissue culture is influenced by several things, including the media used, explant sterilization methods, plant varieties, sub-cultures, and acclimatization. The advantage of banana seeds from tissue culture is their uniform plant growth. Therefore, when it is time to bear fruit, banana plants originating from tissue culture will bear fruit simultaneously, making it easier to manage banana gardens. In addition, tissue culture technology does not depend on the season and other environmental factors, helps in efforts to eliminate pathogens, only a tiny part of the original plant is used as inoculum, can produce large quantities of plants in a short time, and has superior characteristics (Rahmawati & Sandra, 2021). Tissue culture technology to propagate banana seedlings promises bright prospects to support the procurement of bananas as one of the export commodities. Banana is also crucial in fulfilling food security and food sovereignty and elevating farmers' income. Indonesia has many superior varieties that can be cultivated from seedlings produced using tissue culture technology.

## Conclusions

This article describes shoot propagation and plant regeneration of the banana cultivar cavendish. Micropropagation has played a crucial role in banana improvement programs worldwide. The culture method can be developed as a micropropagation protocol. Micropropagation is one of plant biotechnology's most commercially efficient and practically oriented applications, producing fast and pathogen-free plant propagation. Banana plantlets generated through tissue culture have a higher survival rate, reduce the cost of disease and pest control, show vigorous growth, and have a shorter harvesting period. This information can support food diversification, cultivation, and sustainable banana productivity to support food security.

## Acknowledgments

Thank you to SEAMEO BIOTOP which has invited the authors as contributors to the BIODIVERS Scientific Popular Magazine. Thank you to Dr. Erina Sulistiani who has initiated banana tissue culture at SEAMEO BIOTROP, which has become learning material for in vitro plant propagation subject.

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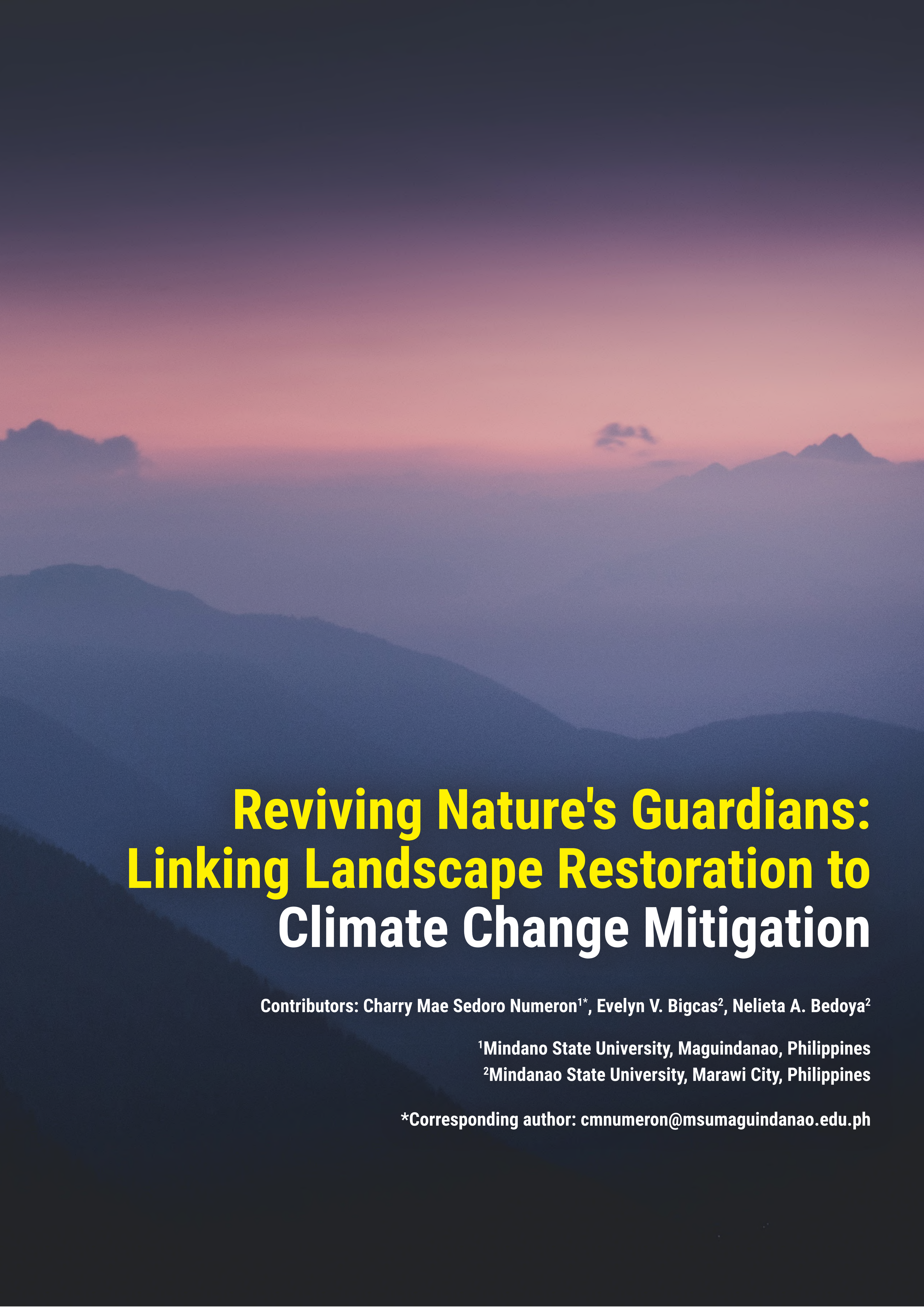

# Reviving Nature's Guardians: Linking Landscape Restoration to Climate Change Mitigation

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## Abstract

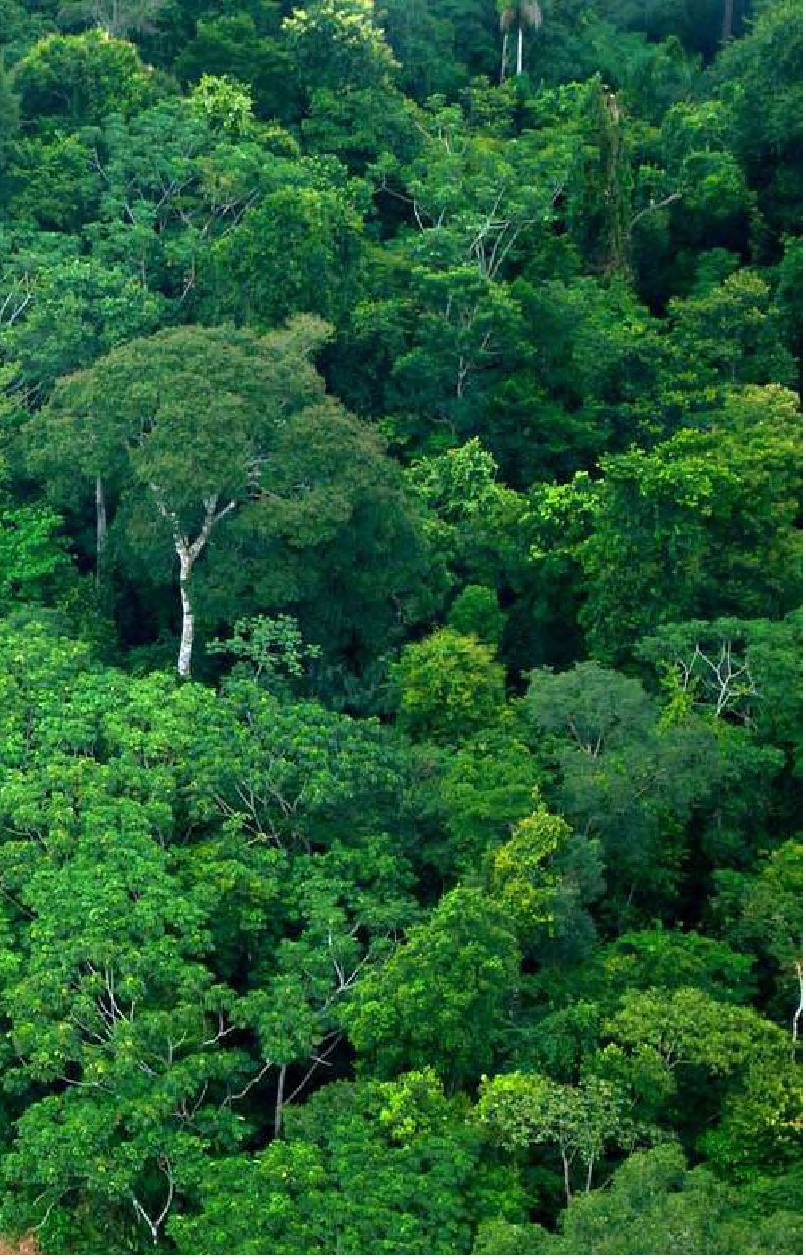
This comprehensive paper navigates the complexities of climate change mitigation and adaptation, unraveling challenges across scales and suggesting innovative solutions. It delves into indicators, tools, and methods that empower policymakers and practitioners, offering a roadmap for effective strategies. Through synthesizing literature and real-world cases, it sheds light on climate change intricacies, urging collaborative efforts and innovative approaches. The findings empower readers with actionable insights to drive impactful climate change management.


## Introduction

The pressing global issue of climate change has garnered significant attention and efforts in mitigating its impacts (Intergovernmental Panel on Climate Change [IPCC], 2014). Approaches towards climate change mitigation and adaptation entail various challenges, both globally and locally. One crucial aspect in addressing climate change is the restoration and rehabilitation of forests and landscapes (Chazdon et al., 2016). Forest and landscape rehabilitation play a vital role in mitigating climate change by sequestering carbon, conserving biodiversity, enhancing ecosystem resilience, and providing numerous benefits to communities (Hansen et al., 2001, Chazdon et al., 2016).

Addressing the implementing challenges of climate change mitigation and adaptation requires a comprehensive understanding of the interconnecting issues. First major hurdle is the need for strong political will and effective international cooperation (IPCC, 2014). Reaching consensus among nations on crucial matters such as emission reduction targets and financial support can be difficult due to differing priorities and interests. However, without collective action and collaboration, the implementation of effective strategies at a global scale becomes even more challenging, if not impossible.

Second is the technological and financial constraints associated with the transition to low-carbon technologies and infrastructure (International Energy Agency [IEA], 2020). The substantial financial investments required for clean energy and sustainable infrastructure are a real struggle, particularly for developing countries. Therefore, addressing these constraints necessitates innovative financing mechanisms, technology transfer, and capacity-building support to ensure equitable access to sustainable solutions.



world examples and insights into the challenges and successes of climate change mitigation and adaptation efforts. These case studies spanned various geographical regions, sectors, and scales, allowing for a diverse range of experiences and approaches to be considered.

The findings from the literature review and case study analysis were then synthesized to identify common themes, challenges, and best practices in addressing climate change. This synthesis aimed to provide a comprehensive overview of the key issues and problem-solving approaches in climate change mitigation and adaptation. Furthermore, an evaluation was conducted on the indicators and tools used in climate change mitigation and adaptation planning and implementation. This assessment involved examining the effectiveness, applicability, and limitations of these indicators and tools in measuring progress, assessing vulnerabilities, and supporting decision-making processes. Based on the research findings and analysis, practical recommendations were developed for policymakers and practitioners. These recommendations aimed to guide effective climate change management, including policy formulation, capacity-building initiatives, collaboration strategies, and innovative financing mechanisms.

Furthermore, the importance of widespread behavioral and cultural changes in effectively reducing climate change (Abbass et al., 2022). It can be difficult to encourage individuals and society to adopt sustainable practices and reduce greenhouse gas emissions owing to resistance, a lack of information, or deeply ingrained habits. As a result, they emphasized the need of government, organization, and community collaboration in providing the required infrastructure, knowledge, and support systems that enable sustainable choices and foster behavior change. By incorporating such behavioral changes into climate change mitigation methods, it is conceivable to have a major and long-term impact on global climate change initiatives.

## Methods

This research paper employed several methods to comprehensively review key issues and problem-solving approaches in climate change mitigation and adaptation. Firstly, a literature review was conducted, encompassing academic journals, research articles, reports, and policy documents, to gather relevant information and insights on the topic. The review provided a broad understanding of climate change mitigation and adaptation strategies. Additionally, case studies were analyzed to offer real-

## Result and Discussion

### Implementation Challenges of Climate Change Mitigation and Adaptation Strategies

Since the 1970s, escalating human impact on Earth's ecosystems, driven by a growing population and increased wealth, has led to greater resource extraction, but concurrently caused unprecedented global declines in ecosystem extent, community distinctiveness, wild species abundance, and local varieties. These changes jeopardize essential benefits derived from nature, disproportionately affecting different segments of society. The intricate link between nature and its contributions, vital for humanity, is rapidly deteriorating, posing a significant threat to future generations' well-being (Diaz et. al, 2019). Addressing the implementing challenges of climate change mitigation and adaptation requires a comprehensive understanding of the interconnecting issues. One major hurdle is the need for strong political will and effective international cooperation (United Nations Framework Convention on Climate Change [UNFCCC], 2021). Reaching consensus among nations on crucial matters such as emission reduction targets and financial support can be difficult due to differing priorities and interests. Without collective action and collaboration, the implementation of effective strategies at a global scale becomes even more challenging, if not impossible.

Technological and financial constraints (International Energy Agency [IEA], 2020) pose another significant challenge in the transition to low-carbon technologies and infrastructure. The substantial financial investments required for clean energy and sustainable infrastructure are particularly challenging for developing countries. Limited access to funds and technology hinders their ability to implement effective mitigation and adaptation measures, leaving them more vulnerable to the impacts of climate change. To address these constraints, innovative financing mechanisms, technology transfer, and capacity-building support are necessary to ensure equitable access to sustainable solutions.

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Cultural and behavioral systems science unites a diverse group of behavior scientists, incorporating various backgrounds, specialties, and areas of emphasis both within and beyond the realm of behavioral science (Cihon et al., 2019). Encouraging individuals, businesses, and societies to adopt sustainable practices and reduce greenhouse gas emissions can be challenging due to resistance, lack of awareness, or deeply ingrained habits. Initiating individual and community behavioral changes requires comprehensive education, awareness campaigns, and incentives to foster a culture of sustainability. Governments, organizations, and communities must collaborate to provide the necessary infrastructure, information, and support systems that enable sustainable choices and facilitate behavior change.

### Effect of Landscape or Forest Rehabilitation and Restoration on Climate Change

The rehabilitation and restoration of landscapes and forests play a crucial role in mitigating climate change and enhancing ecosystem resilience. One significant impact is the carbon sequestration potential of forests and healthy ecosystems. The widespread increase in vegetation greening since 1981, made possible by satellite technology. This greening trend, occurring alongside other indicators of climate change, is considered a strong piece of evidence for human-induced global shifts (Piao et. al., 2020). Landscape and forest rehabilitation/restoration efforts can enhance carbon storage capacity by implementing reforestation programs, revegetating degraded forests, and protecting existing forests. The removal of carbon from the atmosphere by trees helps reduce greenhouse gas concentrations and mitigate climate change.

Restoration activities also contribute to biodiversity conservation (Chazdon et al., 2016). Biodiversity plays a crucial role in supporting human well-being through various ecosystem services (Millennium Ecosystem Assessment, 2005). Biodiversity-rich landscapes support ecosystem resilience and sustain the adaptation of species to changing climatic conditions. By restoring and conserving forests and other ecosystems, habitats for diverse plant and animal species are created, ensuring their survival and promoting ecological balance. Preserving biodiversity is crucial for maintaining ecosystem services that are essential for human well-being and sustainable development.

Moreover, restored landscapes and forests contribute to water cycle regulation. They enhance water retention, reduce soil erosion, and promote natural hydrological processes such as groundwater recharge and streamflow regulation (Foley et al., 2005). These restoration efforts mitigate the impacts of floods, droughts, and water scarcity by improving water availability and regulating water flow. Healthy ecosystems act as natural sponges, absorbing and slowly releasing water, thereby reducing the risk of water-related disasters and supporting climate change adaptation efforts.

### Reciprocal Impact of Climate Change on Landscape/ Forest Rehabilitation/Restoration

Climate change has reciprocal impacts on landscape and forest rehabilitation/restoration efforts. Altered climatic conditions can affect the success and efficacy of these restoration activities. Changes in temperature, precipitation patterns, and extreme weather events can influence the growth and survival of newly planted trees and restoration projects (Jump & Penuelas, 2005). Thus, in selecting tree species for reforestation or native species for restoration, the changing climatic condition must play an essential role.

Shifting species ranges due to climate change can also impact the composition and functioning of restored ecosystems. As plant and animal species redistribute in response to changing climatic conditions, restoration projects need to consider the adaptability of species to future environmental conditions (Parmesan et al., 2013). Together with climate change projections, the selection of species that can thrive under changing circumstances will enhance the long-term success and resilience of restoration efforts.

Climate change also increases the risks and uncertainties associated with landscape/forest rehabilitation and restoration. Factors such as increased frequency of wildfires, pest outbreaks, and invasive species are some obstacles to the success and long-term viability of restoration projects (Laurance et al., 2011). Changing climatic conditions can alter the ecosystem dynamics, making it challenging to predict and manage these risks. Therefore, incorporating adaptive management approaches and considering climate change impacts in restoration planning are crucial in addressing these uncertainties.

## Conclusion

The implementation of effective mitigation and adaptation strategies to climate change is faced with a gamut of challenges, starting from political will, technological and financial constraints, behavioral and cultural change, and uncertainties in predicting long-term impacts. However, landscape and forest rehabilitation/restoration play a significant role in addressing climate change. By enhancing carbon sequestration, conserving biodiversity, regulating the water cycle, and promoting sustainable land management, these restoration efforts thus, contribute to climate change mitigation and adaptation. It is essential to recognize the reciprocal impacts of climate change on these restoration efforts, including altered climate conditions, shifting species ranges, and increased risks and uncertainties. Taking these factors into account and implementing adaptive management approaches are keys to ensuring the long-term success and resilience of landscape/ forest rehabilitation and restoration efforts in the midst of this global climate change.

The implementation of effective climate change mitigation and adaptation measures is closely aligned with the Sustainable Development Goals (SDGs) outlined by the United Nations. The SDGs provide a comprehensive framework for addressing global challenges, including climate change, poverty eradication, and sustainable development. Addressing climate change aligns with SDG 13: Climate Action, which aims to take urgent action to combat climate change and its impacts. Implementing mitigation strategies, such as transitioning to renewable energy sources and reducing greenhouse gas emissions, contributes to SDG 7: Affordable and Clean Energy, and SDG 12: Responsible Consumption and Production. These actions support the goal of ensuring sustainable energy access and promoting sustainable consumption and production patterns.

Adaptation measures to climate change also align with several SDGs. Enhancing ecosystem resilience through landscape and forest rehabilitation contributes to SDG 15: Life on Land, which focuses on protecting, restoring, and sustainably managing terrestrial ecosystems. Restoring landscapes can also have positive impacts on SDG 2: Zero Hunger, by enhancing food security through sustainable land management practices.

Furthermore, the implementation of climate change mitigation and adaptation measures has cross-cutting effects on other SDGs. For example, addressing climate change can have positive impacts on SDG 1: No Poverty, by promoting sustainable economic growth and creating employment opportunities in renewable energy sectors. It also supports SDG 6: Clean Water and Sanitation, by preserving water resources through landscape restoration and reducing water pollution. By integrating climate change actions into the SDG framework, countries can align their efforts towards achieving sustainable development while simultaneously addressing the urgent challenge of climate change. This holistic approach fosters synergies and maximizes the impact of climate change mitigation and adaptation measures, leading to a more sustainable and resilient future for all.

## Recommendation

A comprehensive and integrated approach is necessary to maximize the impact of landscape and forest rehabilitation/restoration and overcome implementation challenges in mitigation and adaptation strategies. Strong political commitment and international cooperation are vital to establish global frameworks for emission reduction targets and financial support. Facilitating technology transfer and providing financial assistance to developing countries can help overcome technological and financial constraints. Education, awareness campaigns, and incentivization should drive behavioral and cultural change towards sustainability. Prioritizing robust scientific research, monitoring systems, and scenario planning can reduce uncertainties and enhance adaptation strategies.

Integrating climate change scenarios into restoration planning is crucial, accounting for changing climatic conditions and species adaptability. Adopting adaptive management approaches, such as regular monitoring and feedback, scenario planning, flexible management strategies, and stakeholder engagement, will enhance the effectiveness of restoration efforts. By implementing these recommendations, we can achieve successful landscape and forest rehabilitation/restoration outcomes with significant contributions to climate change mitigation and adaptation.


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# Freshwater Mangrove: A Novel Urban Ecosystem for an Enhanced Ecosystem Services in Cities

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## Abstract

This R and D on ecological engineering using freshwater mangrove addresses a problem of urban wastewater and, at the same time, water pollution and its health concerns, climate change mitigation, as well as typically low aesthetic appearance of cities. The ecotechnology turns the problem of wastewater flowing into lake from adjacent restaurants into solution for many urban challenges including use of nutrient rich water as a fertilizer for mangrove trees in a Novel Urban Ecosystem (NUE).


## Introduction

Environmental problems in urban landscapes have become increasingly challenging, applying pressure on urban natural resources to meet the rising demand for various forms of ecosystem services (ES). Solutions to urban problems would call for nature's help, i.e., employing biodiversity to address rising urban challenges (Ahern, 2016). Integration of novel ecosystems (NE) has been proposed as an alternative approach to ecological restoration that can obtain a more realistic outcome in addressing the dilemma of ecosystem restoration in this new Anthropocene epoch (Sack, 2013).

Urban wetlands are well accepted as cost-effective for flood mitigation and treatment of stormwater and wastewater. However, emergent and free-floating macrophytes need frequent harvesting to maximize nutrient removal and avoid

nuisance due to massive growth. When left to proliferate on site, the decomposition of this vegetation may return nutrients rapidly into the water. Therefore, there may be an advantage in substituting soft-tissue plants with woody vegetation. Mangrove trees, having unique features that are superior to macrophytes for wastewater treatment, have been studied and applied in urban wetlands even in the absence of tidal flushing and salinity. This emerging technology of using freshwater mangroves for water quality improvement has recently been integrated into urban landscaping, particularly in urban wetlands. This new approach of utilizing mangroves presents promising benefits, however, there is no well-documented study that focuses on the efficiency of freshwater mangroves in delivering different ecosystem services.

Thus, this study is to investigate the potential of an eco-technology based on freshwater mangroves to deliver projected ecosystem services by following the principles of ecological engineering and NUE based on the impact path of a pilot-scaled NUE established in the campus of the Asian Institute of Technology, Thailand.

## Methodology

The success rate of planting mangroves in freshwater and their growth rate were measured. A total of 353 mangrove seedlings and saplings at ages from 8 months to 1.5 years were planted at West Lake, of which 10% (35 trees) were sampled at the age of 1.5 years old for monitoring and evaluation of the growth rate. Their height increase was monitored using a steel tape while the girth development was measured using a caliper.

The ecosystem services were assessed based from the gathered data on water quality monitoring, carbon sequestration potential through biomass estimation.

Water quality monitoring used the parameters NH3-N, TP, TKN, and TOC to determine the reduction of concentration in the water over time. The result was compared to the initial monitoring conducted when no freshwater mangrove is present at West Lake. A long-term monitoring of water quality was conducted considering that a continuous inflow of wastewater was present up to present.

The carbon sequestration was calculated using the total biomass per area multiplied by the conversion factor in obtaining carbon equivalents.

$$CS = \text{W x carbon coefficient}$$

where CS is carbon sequestration in g $m^{-2}y^{-1}$ W is dry weight estimate of biomass in g and carbon coefficients are roughly averaged at 0.50 (Kauffman and Donato, 2012). Carbon accumulation and its projection were determined by multiplying the dry weigh biomass by carbon coefficients which are 0.45, 0.48, 0.50, or roughly averaged at 0.50 (Kauffman and Donato, 2012). Since the carbon concentration rate in woody biomass is usually slightly less than 50%, it is a common practice to convert biomass to carbon by multiplying 0.46-0.50 when the local or species-specific value is unavailable.

## Results and Discussion

The ability to thrive in a new environment is the first indicator of the effectiveness of establishing an NUE. The survival and growth rates of freshwater mangroves established in a shallow lake (West Lake) on the campus of the Asian Institute of Technology (Thailand) challenge the conventional knowledge that the absence of salinity is fatal to mangroves.

Table 1 shows the results of the three-year survival of mangrove trees introduced at West Lake. Freshwater mangroves' survival and mortality rates were 87.2% and 12.8%, respectively. The survival rate indicates that the mangrove species *Rhizophora* spp. does not necessarily require saline environments for their survival and growth. The different batches of trees introduced at West Lake varied in age to determine whether age is a limitation in planting mangroves in freshwater conditions. The result indicates that the survival rate is booming regardless of age, ranging from 77.8% to 96%.

## Freshwater Mangrove

Table 1. Three-year survival and mortality rate (%) of *Rhizophora* spp. introduced at West Lake, AIT

| Batch of Trees | Total num. of trees | Survival | Mortality | % Survival | % Mortality |
|---|---|---|---|---|---|
| 1 | 27 | 21 | 6 | 77.8 | 22.2 |
| 2 | 30 | 26 | 4 | 86.7 | 13.3 |
| 3 | 30 | 46 | 4 | 92.0 | 8.0 |
| 4* | 100 | 92 | 8 | 92.0 | 8.0 |
| 5* | 120 | 98 | 22 | 81.7 | 18.3 |
| 6 | 25 | 24 | 1 | 96.0 | 4.0 |
| Total | 352 | 307 | 45 | 87.2 | 12.8 |

* Saplings less than one year old

In addition, the annual average increase in height and girth of the sample mangroves in freshwater were 16.6 ± 0.95 and 1.6 ± 0.10 cm, respectively.

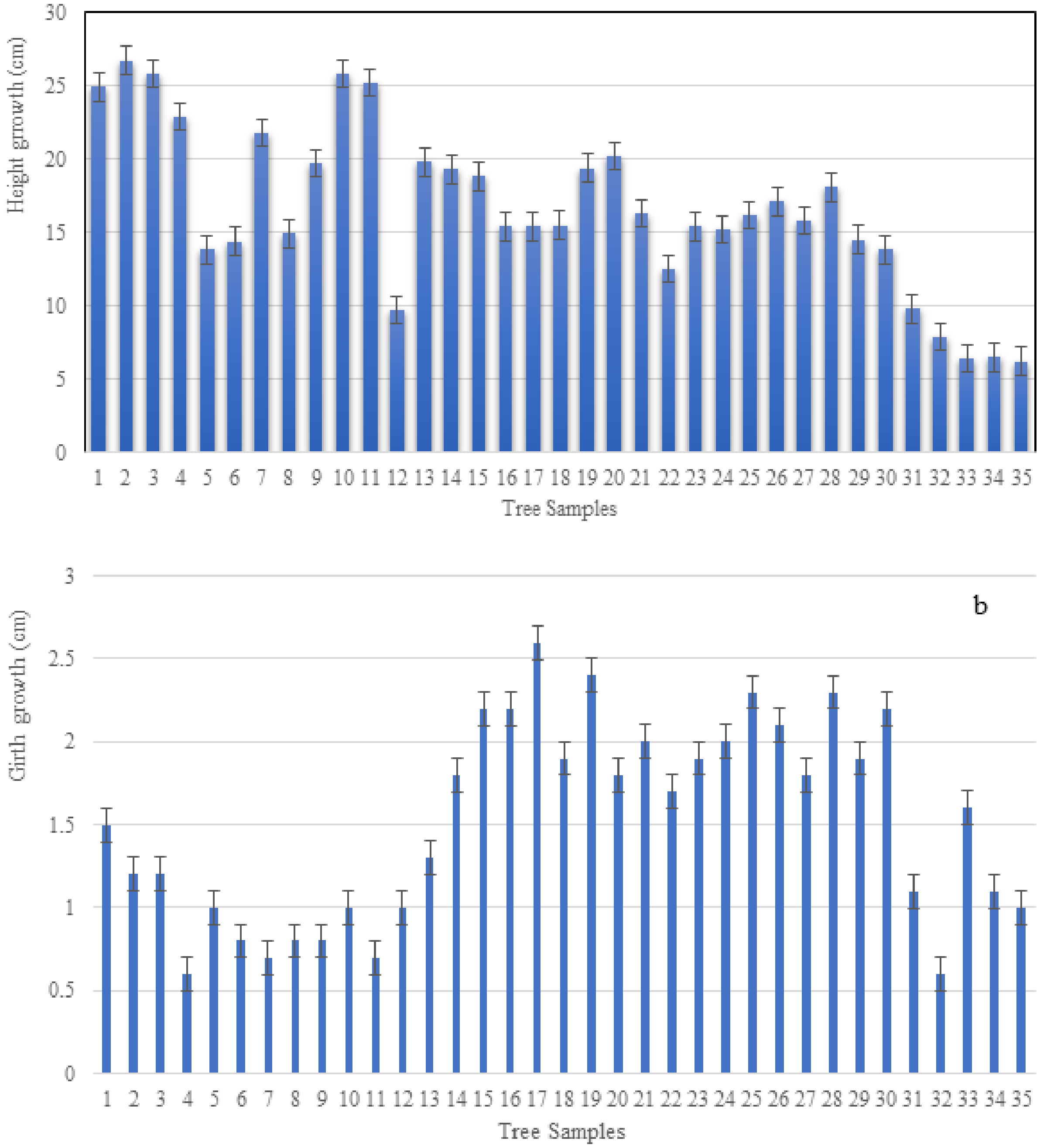


Figure 1. Annual growth in (a) height and (b) girth of *R. apiculata* planted in freshwater at West Lake.


## Potential Ecosystem Services of Freshwater Mangroves as NUE in Cities

### Water Quality Improvement in Wetlands

Initially, the pollutant ion concentrations at West Lake were 1.47 (NH3-N), 2.45 (TKN), 0.08 (TP), and 3.13 mg/L (TOC). A 44.6% reduction in NH3-N concentrations was recorded at the end of the monitoring period. The final percentage reduction in TKN, TP, and TOC concentrations was 61.6%, 41.2%, and 63.7%, respectively. The mangrove ecosystem introduced into West Lake, coupled with water circulation and aeration, was likely successful in improving the eutrophic conditions of the lake water.

Monitoring chlorophyll levels is a direct way of tracking algal growth. Surface waters with high chlorophyll conditions are typically high in nutrients, generally phosphorus and nitrogen. Initial assessment of the chlorophyll-a at the Library Lagoon had six species of microphyte at a concentration of 334 μg/L and is fluctuating according to season, such as monsoon season that, brings dilution. The long-term impact of the mangrove is the diversification of the species of microphytes while reducing their concentration in the water. For almost ten years, a change in the diversity was observed while simultaneously decreasing the concentration to 112 μg/L. A notable development in the pilot area of freshwater mangrove on-campus was the complete disappearance of *Spirulina*, significantly when mangrove trees were increased. The same observation was noted at West Lake, where Spirulina was present during the water quality baseline assessment and disappeared when the eco-engineering components were introduced, such as the mangrove introduction, soil mounds creation, and the enhancement of water circulation and aeration through a water pump. In addition, the relationship between the abundance of macrophytes, DO concentration to the population of rotifers, and the Potential Nitrification Rate (PNR). As the mangrove root's volume and spread increased over time, it helped enhance DO in the water. DO measurements indicate that water adjacent to the mangrove root systems had the DO concentration of 0.12 to 0.52 mg/L, slightly higher than in the sites distant from the mangrove trees. When oxygen is available in an almost anoxic bottom substrate, it augments the microbial activity for the bioconversion process.

### Carbon Sequestration Potential

A comparison of carbon sequestration estimates between our study and the literature shows that saline water mangrove accumulated higher biomass at early ages than freshwater mangroves. However, the difference may vary depending on the species. Among the mangrove species planted in freshwater, *Sonneratia* spp. accumulated the largest biomass during the first three years of plantation, while *Lumnitzera racemosa* revealed the least biomass gain. As a result, the carbon sequestration capacity is proportional to the biomass gain. The first three years after the mangrove plantation was projected to sequester above and below ground. *Sonneratia* spp. is expected to capture 287 ± 136 g C m-2 y-1, followed by *Rhizophora* spp. and *Lumnitzera* spp. 345 ± 66 and 30 ± 17 g C m-2 y-1, respectively. Belowground sequestration is twice lower in comparison to the aboveground sequestration capacity of mangroves.

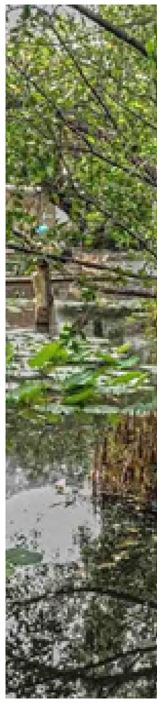

Table 2. Estimated carbon sequestration rates of freshwater mangroves in comparison with sea water mangroves.

| Species | Tree age, years | Above [Below] ground [root] sequestration rate* g C m$^{-2}$ y$^{-1}$, calculated [1] |
|---|---|---|
| *Rhizophora mucronata* and *R. apiculata* | Saline water (Xiong et al., 2019) | |
| | 1 | 18 ± 5 [4 ± 2] |
| | 3 | 345 ± 66 [89 ± 50] |
| | 5 | 357 ± 95 [164 ± 49] |
| | 10 | 498 ± 160 [234 ± 106] |
| | 25 | 527 ± 183 [248 ± 101] |
| | 40 | 696 ± 179 [327 ± 156] |
| | 85 | 637 ± 241 [300 ± 138] |
| | This study (freshwater) | |
| | 1 | 14 ± 3 [6 ± 2] |
| | 3 | 245 ± 109 [113 ± 49] |
| | 5 | 409 ± 142 [95 ± 34] |
| *Sonneratia spp.* | Saline water (Xiong et al., 2019) | |
| | 1 | 25 ± 10 [8 ± 4] |
| | 3 | 287 ± 136 [132 ± 53] |
| | 5 | 631 ± 315 [291 ± 141] |
| | 10 | 554 ± 166 [255 ± 145] |
| | 20 | 246 ± 78 [116 ± 61] |
| *S.caseolaris* | | This study (freshwater) |
| | 3 | 281 ± 138 [78 ± 29] |
| | 12 | 691 ± 244 [393 ± 156] |
| *S.ovata* | 6 | 235 ± 64 [95 ± 35] |
| *Lumnitzera racemosa* | Literature data (Saline water) (Xiong et al., 2019) | |
| | 3 | 22 ± 7 [10 ± 5] |
| | 10 | 135 ± 43 [62 ± 29] |
| | This study (freshwater) | |
| | 2 | 30 ± 17 [10 ± 6] |
| | 8 | 598 ± 270 [439 ± 199] |

Mangrove is among the trees with the highest primary productivity, accumulating and storing significant amounts of carbon in their biomass. The high primary production and slow decomposition processes in mangrove soil supposedly cause unusual carbon dynamics (Komiyama et al., 2008). The soil of mangroves can store three times as much carbon as its biomass (Kauffman & Donato, 2012), surpassing terrestrial trees' carbon storage capacity. Moreover, the literature suggests that the carbon sequestration potential of mangroves is inversely correlated with water salinity (Rahman et al., 2015; Perera et al., 2013) as higher salinity induces mangroves to spend more energy maintaining water balance and ion concentrations than primary production and growth (Perera et al., 2013).

Our results indicate that the capacity of mangroves to sequester carbon is similar between saline and freshwater environments. This implies the suitability of mangroves under urban environments to contribute to climate change mitigation while simultaneously functioning for water pollution mitigation. Different mangrove species may possess different carbon sequestration capacities. This study focused on *Rhizophora* and *Sonneratia* because *Sonneratia* is a fast-growing species (Chen et al., 2012), and *Rhizophora* has a large wood mass per unit area and relatively high bulk density (Wiarta et al., 2019), translating into higher potential for carbon absorptions than other species.

## Conclusion

It was demonstrated that novel "Freshwater mangrove ecosystem" introduced at the West Lake is one of the very few urban novel ecosystems described scientific literature. This novel ecosystem of considerable diversity was shown to develop naturally and successfully over the period of 3 years since a start-up from baseline state in March 2019. This novel ecosystem has proven to provide several ecological benefits such as: (1) nutrient absorption leading to an efficient growth of mangrove


A well-established freshwater mangrove in a wetland of AIT campus

trees and other macrophytes at the treatment area; (2) carbon sequestration in relation to the enhanced biomass accumulation at the treatment site; (3) Water quality improvement gradual decreaset of NH3-N, TKN, TP, Chlorophyll a concentration at west lake from the baseline situation to current condition. Thus, the novel urban ecosystem of freshwater mangrove is a sustainable and cost-effective approach to solving ecological crises in the urban environment.

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Source: https://www.onmanorama.com/food/features/2020/07/17/why-to-avoid-moringa-drumstick-leaves.html

# Phytoremediation of Lead, Arsenic and Chromium Polluted Soil Using *Opuntia* spp. (Dilang-Baka)

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## Abstract

Phytoremediation capabilities of *Opuntia* spp. were tested on heavy metal polluted soil collected in a metallurgical factory in Carmona Cavite. Initial soil testing shows traces of Arsenic: 8.48 mg/Kg, Chromium: 116 mg/Kg, and Lead: 79 mg/Kg. pH and moisture are neutral and dry. *Opuntia* was compared to *A. vera*, within two months. Final results show both can decrease concentrations of Arsenic and Lead but Chromium from *Opuntia* have increased concentration might be due to the reduction of Cr(VI) to Cr(III).


**Keywords:** Phytoremediation, *Opuntia* spp., *A. vera*, Arsenic, Chromium, Lead

## Introduction

Phytoremediation is a technology that uses living plants to clean up soil, air, and water contaminated with hazardous contaminants such as heavy metals (Bruni & Mcleskey, 2013). Since phytoremediation utilizes plants, it is a cheap way of removing contaminants in a certain environment. The plant roots stabilize the soil and prevents movement of pollutants. It is also done on the spot which means less transportation and off-site costs for industries that wishes to remove soil or water contaminants in their area. (Bruni & Mcleskey, 2013). Phytoremediation is an affordable technique that combines several methods to remediate soil contaminated with heavy metals, and other toxic pollutants (Belliturk et al., 2015). Despite their cost-effectiveness and environment friendliness, field applications of these technologies have only been reported in developed countries. In most developing countries, these are yet to become commercially available technologies possibly due to the inadequate awareness of their inherent advantages and principles of operation. With greater awareness by the governments and the public of the implications of contaminated soils on human and animal health, there has been increasing interest amongst the scientific community in the development of technologies to remediate contaminated sites (Wuana & Okieimen, 2011).

The researchers focused on the following heavy metals; arsenic, chromium, and lead, mainly because these are one of the heavy metals that can be seen in contaminated soil (Magahud et al., 2015).


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## Methodology

The study was conducted from April to May 2021. Proper protocols have been followed to conduct accurate results for the propagation of plants. The soil was collected from metallurgy factories around the industrial zone of Bancal Carmona, Cavite. The soil samples in different sites near the factories were collected in a zig-zag pattern to ensure homogeneity. The Plants used for this study were harvested from General Trias Cavite in a residential area. The plants were initially planted directly in the soil and transferred to separate plots two months before the experiment to ensure that care and maintenance for the plants were uniform. Museum of Natural History then authenticated the plants in the University of the Philippines Los Baños Laguna.

Watering the plants was done deeply but infrequently, and the surrounding soil was allowed to dry before watering again to avoid root rotting (Farmer's Almanac, 2020). Distilled water was also used to avoid contaminants added to the soil, and the soil was cultivated once a week. The plants were kept roofed to avoid exposure to harsh environmental factors such as extreme sunlight and heavy rains or wind.

For the pH and moisture, the researchers used the 3-way meter. The 3-way meter is stuck to the ground about 5 cm in depth, and this is done three times in each pot to get the average pH and moisture.

The collected soil was subjected to initial soil analysis before the propagation of plants to establish a baseline for this study's variables. Soil analysis in this study included basic pH and moisture measurement and specific heavy metal testing. Mach Union Laboratories did the testing.

## Results

### 1. Other Factors Affecting Phytoremediation capabilities of *Opuntia* spp.

Table 1 Initial and Final pH and Moisture test results of plots for *Opuntia* spp.

| Plant Code | Initial | | Final | |
|---|---|---|---|---|
| | pH | Moisture | pH | Moisture |
| Plant A (E1) | 7.2 | 5.5 | 7.2 | 7.0 |
| Plant B (E1) | 7.9 | 3.0 | 7.9 | 4.5 |
| Plant C (E1) | 8.0 | 2.5 | 8.0 | 3.5 |
| Plant D (E2) | 7.5 | 4.0 | 7.5 | 6.0 |
| Plant E (E2) | 7.9 | 3.0 | 7.5 | 4.0 |
| Plant F (E2) | 7.6 | 3.0 | 7.9 | 3.5 |
| μ | 7.6 | 3.5 | 7.6 | 4.75 |

*Legend:For moisture levels; 1-3 Dry; 4-7 Moist; 8-10 Wet*

Table 1 shows the initial pH and moisture test results of *Opuntia* spp. Plots. Each plot's initial and final pH remained the same; Plant A: 7.2; Plant B:7.9; Plant C:8.0; Plant D:7.5; Plant E:7.9: and Plant F:7.6. This gave a mean of 7.6. In terms of moisture, there were significant increases for all plant pots; Plant A: 5.5 to 7.0; Plant B:3.0 to 4.5; Plant C:2.5 to 3.5; Plant D:4.0 to 6.0; Plant E:3.0 to 4.0; and Plant F:3.0 to 3.5; with a mean of 3.5 for the initial testing and 4.75 for the final moisture test.

### 2. Final Heavy Metal Concentration from the Composite soil sample of Positive Control and Experimental Set-up

Table 2 Initial and final heavy metal test results

| Heavy metal | Normal Range | Initial Results | Final Results | |
|---|---|---|---|---|
| | | | *Aloe vera* | *Opuntia* spp. |
| Arsenic | μ 5 mg/Kg | 8.48 mg/Kg | <0.09 mg/Kg | <0.09 mg/Kg |
| Chromium | 14-70 mg/Kg | 116mg/Kg | 58mg/Kg | 137 mg/Kg |
| Lead | 10 – 50 mg/Kg | 79 mg/Kg | 64.5 mg/Kg | 64.6 mg/Kg |

Table 2 shows the Initial Heavy metal concentration of the soil sample. Arsenic with 8.48 m/Kg; Chromium with 116 mg/Kg; and Lead with 79 mg/Kg. All of the target heavy metals are beyond the normal levels as seen in the normal values indicated in the table. This table also presents the final heavy metal concentrations obtained from the soil samples of both the positive and experimental set up. Both *A. vera* and *Opuntia* spp. had a significant decrease into <0.09 mg/Kg; Chromium decreased for *A. vera* at 58mg/Kg but increased for the *Opuntia* spp plots with 137 mg/Kg; Lastly lead had a significant decrease for both plants; *A. vera* with 64.5 mg/Kg and *Opuntia* spp. with 64.6 mg/Kg.

### 3. Percent Difference Between Positive and Negative Control

Table 3 Percent difference between the positive control *A. vera* (Sabila) and *Opuntia* spp. (Dilang-baka) treated soil in terms of final heavy metal concentration

| Heavy Metal | Concentration With Positive Control *Aloe Vera* | Concentration With Experimental *Opuntia spp.* | Difference | Average | Percent Difference |
|---|---|---|---|---|---|
| Arsenic | <0.09 | <0.09 | 0 | 0.09 | 0.00 |
| Chromium | 58 | 137 | 79 | 108 | 73.15 |
| Lead | 64.5 | 64.6 | 0.10 | 64.55 | 0.155 |

Table 3 presents the percent difference between the positive control *A. vera* and the experimental *Opuntia* spp. The first column shows the heavy metals in the soil sample Arsenic, Chromium, and Lead. The second column shows the final concentration of heavy metals in *A. vera*: As <0.09; Cr 58; Pb 64.5; The third column shows the final concentration of heavy metals in *Opuntia* spp.: As <0.09; Cr 137; Pb 64.6; the fourth column indicates the difference between the heavy metal concentration of the positive control *A. vera* and the experimental *Opuntia* spp.

The results are As 0; Cr 79; Pb 0.10; the following findings can be computed by subtracting the heavy metal concentration value of experimental *Opuntia* spp. to the value of the positive control *A. vera*. The fifth column shows the heavy metal average concentration value of the *Opuntia* spp. with the *A. vera*, As 0.09; Cr 108; Pb 64.55; it can be done by adding the heavy concentration value of experimental *Opuntia* spp. to the value of the positive control *A. vera*, and dividing its sum by 2. The last column shows the percent difference between *A. vera* and *Opuntia* spp.


## Discussion

The soil sample has a mean pH of 7.6, neutral for the initial and final testing. In the study of Lenart and Wolny-Koladka (2012), the soil pH can be alkaline to neutral but still have high concentrations of heavy metals, particularly zinc, cadmium, chromium, lead, nickel, iron, and manganese. The solubility of heavy metals is inversely proportional to pH of the soil; the higher the solubility, the lower the pH, thus resulting in a much higher metal absorption of the plants (Fassler & Robinson, et al., 2010). Moisture levels, on the other hand, varied in the initial and final testing; 3.5 or dry during the initial measurement and 4.75 or moist in the final testing. This change in moisture can be attributed to the plant maintenance technique of the researchers, and it also helped the plant uptake heavy metal contaminants that are present. According to the study by Angle et al. (2013), the higher the moisture content is, the easier it is for plants to do phytoextraction of soil contaminants.

According to ATSDR, 2007, the natural concentration of Arsenic in the soil is usually around 1 to 40 mg/kg with a mean of 5 mg/kg; the results from the collected soil were elevated, meaning there is an increased amount of Arsenic in the soil compared to the normal mean value. This is also high for the soil to be considered healthy and suitable for plant growth, as the ideal level of arsenic for plant flourishing should only be within 0.61 mg/kg to 0.7 mg/kg (Wade, 2019). The initial result for chromium is relatively high above the normal value as per the WHO, 2000, chromium concentrations should range from 14 to about 70 mg/kg, and the results from the collected soil came back at 116 mg/kg. The initial result of lead shows elevated amounts from the normal values at 79 mg/kg. Lead naturally should be 10 – 50 mg/Kg (University of Massachusetts Amherst, 2020).

On the other hand, in the final testing results, *A. vera*, the positive control of the study, decreased all heavy metal concentrations significantly. In a 2018 study conducted by Elhag et al., they evaluated the effectivity of *A. vera* on heavy metal-contaminated soil. It was shown that succulent species of plants, such as the Aloe, have an ability called phytovolatilization, where the plant takes up water-containing contaminants through their roots, converts them to gaseous form, and then releases them into the atmosphere. Lead might not be within the normal range, but it significantly decreased from the original amount of 79 mg/Kg. In the same study by Elhag et al. (2018), the duration of their experiment was one year, and they proved the efficiency of Aloe as a long-term phytoremediator of Lead.

*Opuntia* spp., on the other hand, significantly decreased Arsenic levels of the soil at <0.09 mg/Kg and Lead at 64.6 mg/Kg, the same as the positive control. Another genus of cacti, such as the *Nopalea*, have proven to be effective phytoremediators of chemicals used in textile manufacturing. In the study of Adki et al. (2012), they used cell cultures of *Nopalea cohenillifera* Salm. Dyck to phytoremediation textile dye. These proponents have successfully used cell cultures of *Nopalea* to transform various toxic textile dyes into less phytotoxic metabolites. Chromium, on the other hand, increased to 137 mg/Kg from the initial 116 mg/Kg. In congruence to another study by Adki et al. (2013), they used *Nopalea cochenillifera* as a hyperaccumulator of Chromium (VI) in in-vitro cultures. It was shown in the study that *Nopalea* can assimilate enormous amounts of Cr(VI) without significant changes in root growth; in the study it has accumulated 25, 263.369 mg/kg of Cr(VI) without any negative effects to the plant. In another study conducted by Zayed and Terry in 2015, they concluded that some plants accumulate Cr(VI) but release Cr(III) in the soil as an output. Cr(VI), the hexavalent form, is dangerous and carcinogenic, while Cr(III) is tetravalent, considered stable and safe for living organisms. In the heavy metal testing conducted in this study, the chromium that was obtained is not specified; it is the Cr(total) or Total Chromium content of the soil. This might be the possible reason why there is an increased amount of chromium in the soil sample of *Opuntia* spp.

In Arsenic, there is no difference between *A. vera* and *Opuntia* spp. with 0.00 %. This can prove that the two plants used for the study have phytoextracting abilities for phytoremediation soil. As stated in the study of Elhag et al. (2018), succulents have a high capacity to absorb heavy metals.

There is a negligible difference between the *A. vera* and *Opuntia* spp for Lead. computed at 0.15%. As explained by Elhag et al. (2018), the longer the duration of planting, the higher the chance of the plant removing contaminants in the soil, Lead for instance can be absorbed at higher rates once the roots are a stable rate of growth.

Lastly, chromium differs by 73.15% between *A. vera* and *Opuntia* spp. The computation was done by subtracting the absolute value of the heavy metal concentration of experimental *Opuntia* spp. to the value of the positive control *A. vera* and then dividing it by the quotient of the heavy metal concentration value of experimental *Opuntia* spp. plus the value of the positive control *A. vera*. The answer is then multiplied by 100. The high percent difference of 73.15% of Chromium between *A. vera* and *Opuntia* spp. might be due to the reduction of Cr (VI) to Cr (III), the reduction is possible even at slightly alkalic soil, and it is most common in aerobic soil as stated in the study of Zayed &Terry in 2015. Upon the absorption of Cr(VI), the plant, instead of doing phytovolatilization and releasing the product in the atmosphere, does a different pathway and rereleases the byproduct in the soil as a safe form.

## Conclusion

Based on the findings of this research, *Opuntia* spp., the experimental plant, can be used for phytoremediation because it can reduce the amount of certain heavy metals in the soil, making the soil suitable for planting. However, specific recommendations should be made to improve this technique, such as longer duration and addition of trials for the experiment; trying onsite phytoremediation or collecting more soil samples from multiple locations; using extract or cell culture to phytoremediation; conducting specific chromium testing to support the literature cited; and consider the effect of other factors to the phytoremediation capabilities of the *Opuntia* spp., such as the microorganisms present in the soil and the presence and effect of fertilizers or other chemicals in the sample.

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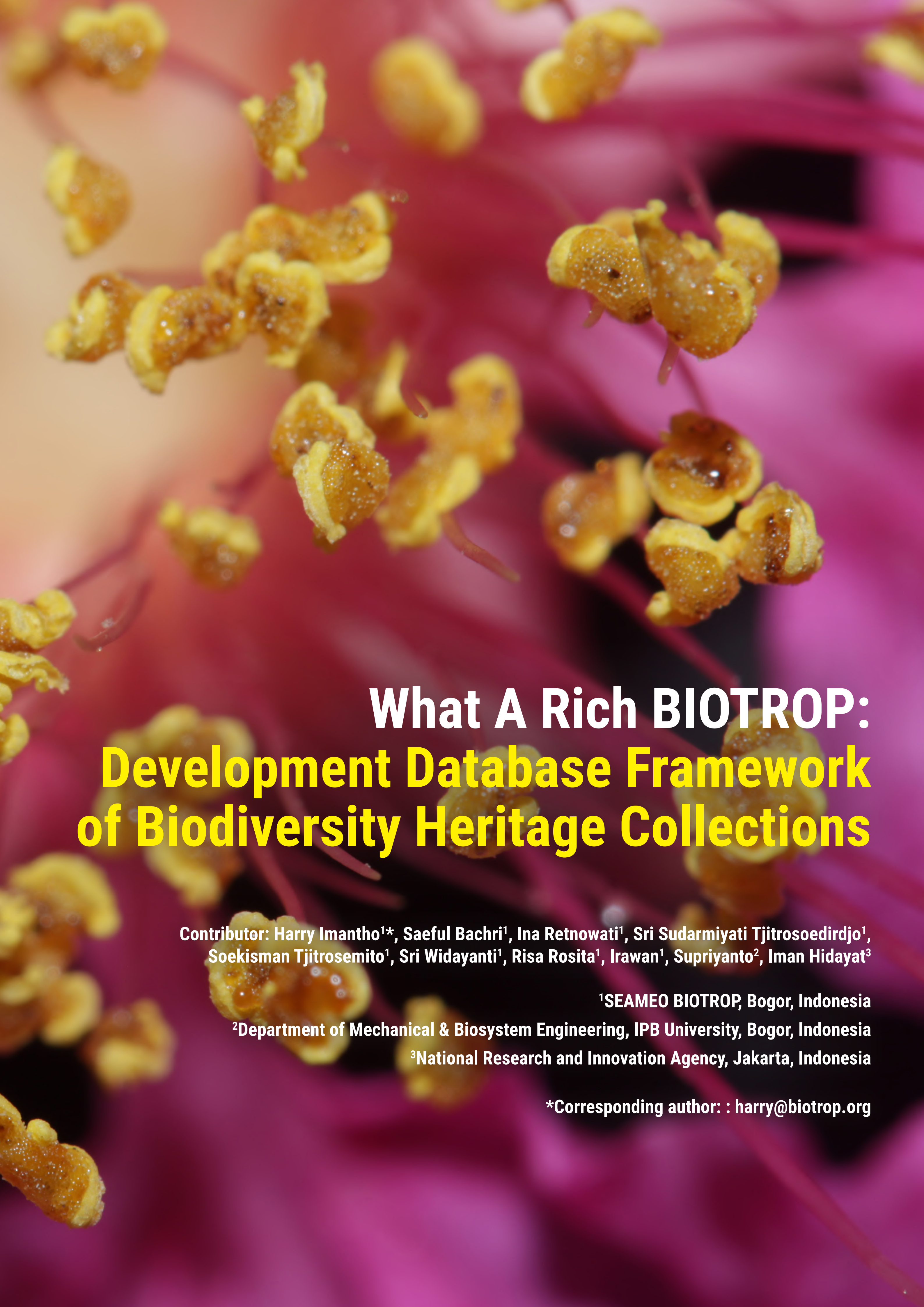

# What A Rich BIOTROP: Development Database Framework of Biodiversity Heritage Collections

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## Abstract

The need for biodiversity science with the availability and interoperability of data on the internet reinforces each other. This is driven by the increasing literacy of the world community regarding the impact of loss of biodiversity on the sustainability of human life. High-quality biodiversity data is needed to demonstrate that spatio-temporal loss of biodiversity has led to a reduction in quality of life. This facts have underpinned SEAMEO BIOTROP to increase the visibility of managed biodiversity collection data. SEAMEO BIOTROP maintains thousands of valuable herbarium collections of weeds, trees and invasive plants; fungi, insects and pests for research and testing purposes. These historic and valuable collections of biodiversity need to be preserved and better managed in digital format so that the information is available to botanists and the general public around the world. A study regarding the development of an integrated biodiversity collection database framework has been carried out by SEAMEO BIOTROP. This study aims to formulate and develop an initial framework for the BIOTROP biodiversity database that is relevant to national, regional and global needs. This study succeeded in developing an integrated database framework that brings together all digital data from the SEAMEO BIOTROP biodiversity collection into a database management system. This database management system also adopts Darwin Core metadata to ensure easy exchange and sharing of data with existing biodiversity data management systems in the world.


**Keyword:** biodiversity, database framework, herbarium specimen, insect collection, fungi collection

## Introduction

Currently, the resonance between the needs of biodiversity science and the availability and interoperability of data on the internet is getting stronger. This situation is driven by the awareness that biodiversity is essential for the sustainable development of human society (Cardinale et al., 2019). Increasing public literacy and knowledge is important in saving, preserving, and managing biodiversity wisely and sustainably.

Biodiversity refers to the various life forms that exist on earth, including animals, plants, microorganisms, and the entire ecosystem they live in. The different components of biodiversity in an environment work together to maintain the balance of the ecosystem. These interactions create several functioning systems called ecosystem services, consisting of provisioning, regulating, cultural, and supporting services (Hooper et al., 2005). However, biodiversity's sustainability, quality, and quantity continue to decline due to various pressures such as over-exploitation and utilization, waste pollution, air pollution, microplastic pollution, habitat destruction, alien species invasion, climate change, and forest fires (Reid et al., 2019; Dudgeon et al., 2005; Hooper et al., 2012).

High-quality biodiversity data is needed to show that the spatio-temporal biodiversity loss has led to a decline in ecosystem services (Sutherland et al., 2013). Furthermore, high-quality biodiversity data are also indispensable for research and management in biodiversity conservation (Jin & Yang, 2020). The growth of data digitization in the last centuries has made billions of biodiversity records from various sources (e.g., museums, herbaria, and field surveys) available to the public (Guralnick et al., 2016; Nelson & Ellis, 2019). Organizations and individuals have used this development to build databases containing massive species occurrence records. These databases have contributed to a wide range of applications, such as predicting the distribution of focal species (Lin et al. 2017), estimating the extinction risk of plants (Darrah et al., 2017), controlling plant invasions (Banerjee et al., 2021), evaluating conservation and protected areas (Pelletier, 2018), and analyzing the impacts of climate change on biodiversity (Cabrelli et al., 2014).

Over five decades, SEAMEO BIOTROP has made significant contributions to biodiversity and ecosystem conservation, such as improving plant productivity, nutrient cycling efficiency, and reforestation programs in the region. The Centre also provided knowledge and understanding of the ecology and management of tropical terrestrial and aquatic weeds, vertebrate pests, insects, and fungi that may affect stored products. One evidence of SEAMEO BIOTROP's significant contribution to biodiversity and

ecosystem conservation is its thousands of valuable herbarium collections of weeds, trees, and invasive plants. The center also maintains a high-value collection of fungi, insects, and storage pests for research and testing purposes. This valuable collection of historic biodiversity must be preserved and better managed digitally to make information available to botanists and the general public worldwide. This study aims to develop an initial framework for the SEAMEO BIOTROP biodiversity database relevant to national, regional, and global needs.

## Methods

### Time and location

The study was carried out from July until November 2022. Historic biodiversity data was collected from SEAMEO BIOTROP Herbarium, Entomology Laboratory, and Phytopathology Laboratory. The database framework formulation, analysis, development, and finalizing document of SEAMEO BIOTROP heritage collections were done in the Remote Sensing and Ecology Laboratory, SEAMEO BIOTROP, Jalan Raya Tajur Km. 6 Bogor.

### Tools and material

Hardware and software were used to develop the database framework of SEAMEO BIOTROP heritage collections, as presented in Table 1.

Tabel 1. Softwares used in the study

| No | Name | Function |
|---|---|---|
| 1 | Scanner | Digitalizing of specimen and picture of collection |
| 2 | Microsoft Office | Digitalizing and develop guide book |
| 3 | Figma Interface Design Tool | Online and collaborative web interface design |
| 4 | PostgreSQL | Object Relational Database Management System |

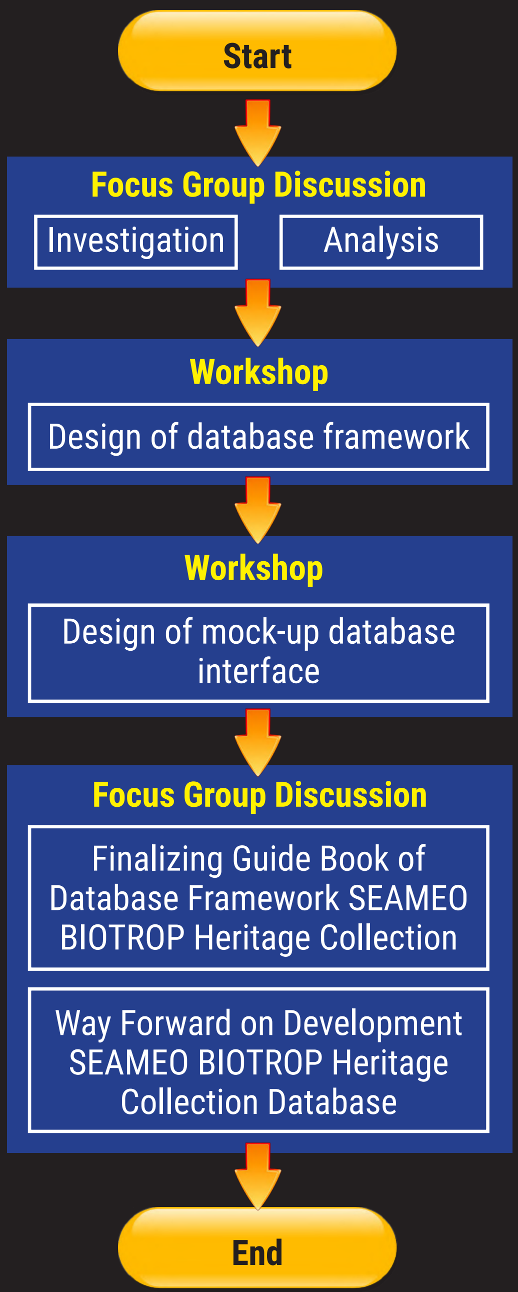



Figure 1. Stages of activity in the design of a biodiversity heritage collections' database framework.

## Method

The modified waterfall method is used as a guide in the process of designing a biodiversity database framework. The waterfall method is a systematic and sequential information system development model. Each stage of the method is carried out collaboratively by involving data owners, taxonomists and biologists, information systems experts, and database managers through FGDs and workshops, as shown in Figure 1.

### Investigation

Investigation is a process of exploring constraints and challenges in managing data and information on biodiversity. Consultation activities with stakeholders are carried out through focus group discussions (FGD). This activity also focuses on gathering information from experts regarding national, regional, and global needs for biodiversity data and information that can be handled by SEAMEO BIOTROP expertise. The results of this stage serve as a reference for the need to develop biodiversity data and information management systems, objectives, and system specifications required by stakeholders.

### Analysis

Needs analysis is a detailed process of the results of the investigation. The analysis is carried out on the system currently running/in use, standard operating procedures used, and manual, semi-manual, or automated processes using existing application systems. The analysis phase produces detailed requirements recommendations for developing existing application systems or even developing new application systems. The analysis phase also examines the resource requirements in developing an application system that fits the purpose.

### Design of database framework

The database framework design phase focuses on developing a conceptual data model from a collection of specimens of weeds and invasive plants, insects, and fungi concerning taxonomic structure. The conceptual data model is then translated into tables (entities) to build a relational database model.

### Design of mock-up database

This stage focuses on making prototypes of the database management system interface. The interface design provides features according to the needs of stakeholders, both internal users (SEAMEO BIOTROP) and users at the national, regional, and international levels.

Development of guide book and identifying way forward on development of biodiversity heritage database management system

This stage is intended to produce a very important manual for data managers, database management system developers, and general users. The guidebook is a standard reference for taking samples of collections or specimens in the field, a reference for recording collection or specimen data, and a reference for the development of a sustainable database management system.

# Result and Discussion

## Baseline Data

The International Association of Plant Taxonomy has internationally recognized the SEAMEO BIOTROP Herbarium and has been listed in the Herbarium Index with the abbreviation BIOT since 1990. The SEAMEO BIOTROP Herbaria maintains collections of specific weeds and invasive plant species specimens for research and reference. Data for 2022 show that the SEAMEO BIOTROP Herbarium (BIOT) collection consisted of 6,121 weeds and invasive alien plants, 7,720 specimens of forest herbarium, and 2,970 specimens of moss and lichen. These collections are stored as specimens, scanned images, and tabular data records. From the total collection of weeds and invasive alien plants, it is known that 1,936 species from 187 families were plant species introduced to Indonesia (Invasive Alien Species, IAS). About 17.5% of the introduced plant species were classified as weeds. Most of the SEAMEO BIOTROP herbarium (BIOT) digital collection data has been stored in a separate database.

Furthermore, SEAMEO BIOTROP Laboratory phytopathology maintains a collection of fungi (fungi and mold) consisting of 390 cultured fungi, including preharvest and postharvest species of fungi and 5 uncultured mycorrhizal species. This valuable collection data has not been fully managed using a database approach, so the availability and accessibility are still limited for microbial experts (mycologists) and the public. Currently, limited use of the fungi collections is for research and testing. SEAMEO BIOTROP also manages and maintains a collection of warehouse insects and pests in the form of collections of live insects, dry-preserved insects, and wet-preserved insects, which are stored in the Entomology Laboratory. Insect collecting activities started from 1979 to 1984, resulting from the research project TDRI (TPI) - BIOTROP. The recording of insect data refers to the book "Identification Records of Insects and Arachnids from Stored Products in Indonesia" compiled by C.P. Haines. Some of the digital insect collection data has been stored in a separate database but is still limited to internal use and has not been developed into institutional explicit knowledge.

Although most of the valuable collection data at SEAMEO BIOTROP has been stored in digital form, it is managed using a separate database approach and only oriented towards internal data management needs. This valuable collection of historic biodiversity must be preserved and better managed in an integrated database format to make the information available to botanists and the general public worldwide. This effort also supports the Center's program initiative to strengthen its visibility, contribution, and role in saving biodiversity by researching and developing tropical biology learning models in Southeast Asia An integrated biodiversity database system. It can be used as a reference in assessing the condition of ecosystems prior to land conversion and over-exploitation in several regions in Indonesia. An initial framework for the SEAMEO BIOTROP biodiversity database has been proposed and initiated for development, responding to national, regional, and global needs, as presented in Figure 2.

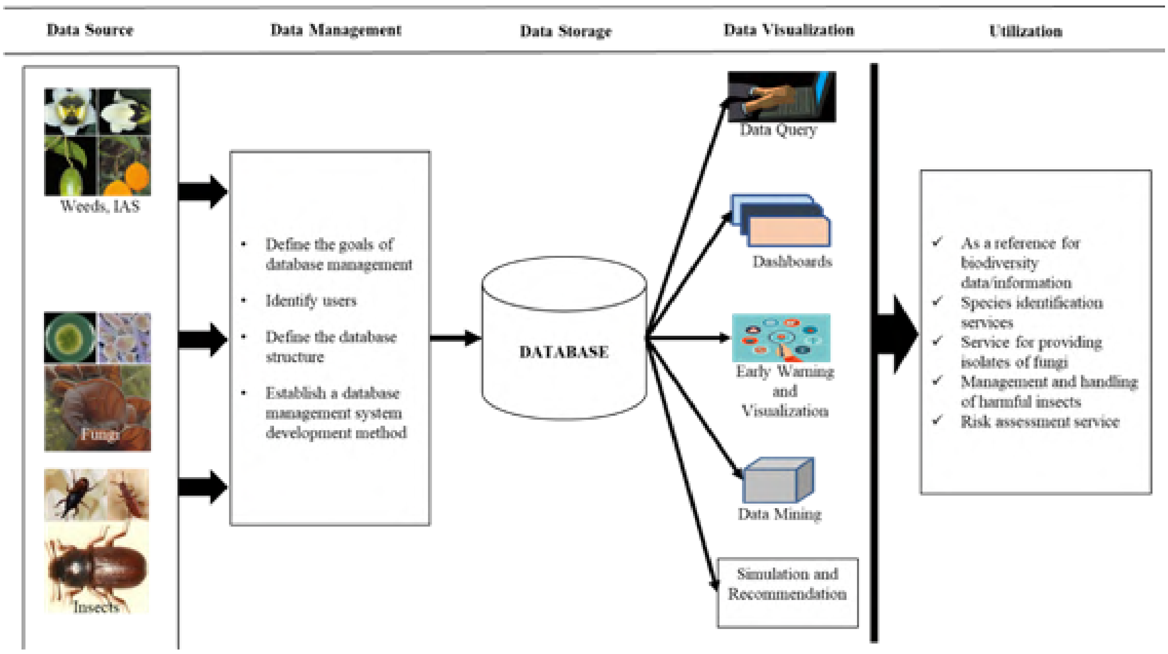



Figure 2. Biodiversity heritage collections' database framework.

## System Development

The development of the database framework has been carried out by identifying specific and general data and information in detail from each type of collection that has been recorded and is available in each laboratory, as presented in Tabel 2 - 4. Information of the same nature, contained in the three taxonomy types of data collection, has become a reference for building an integrated conceptual database. Specific information is retained as information tied to each type of collection. Some information and metadata are added with reference to Darwin Core guidelines to ensure ease of data sharing and potential for developing integration with existing database management systems in the world, such as the Global Biodiversity Information Facility (GBIF).

Table 2. Conceptual database for weed and IAS specimens data management.

| No | Table name (entity) | Information |
|---|---|---|
| 1 | Kingdom | Contain Kingdom Plantae code |
| 2 | Family | Contain family and family name code |
| 3 | Genus | Contain genus and genus name code |
| 4 | Species | Contain species code and species name, description and references |
| 5 | Origin | Plant origin and its ecosystem |
| 6 | Collector | Contain collector code, collector name and initials Contain specimen code, collection number |
| 7 | Location | Contain the location code where the specimen was collected, the coordinate position |
| 8 | Specimen | Contain duplicate specimen information in other herbariums, drawings, references |
| 9 | Specimen photo | Contain herbarium specimen photo codes or collection photos from the field |
| 10 | Determined by | Contains the code name that performs the identification and identification date |
| 11 | Notes | Benefits, control and other important information |

Table 3. Conceptual database for fungi collection data management.

| No | Table name (entity) | Information |
|---|---|---|
| 1 | Kingdom | Contain kingdom code and kingdom name |
| 2 | Phylum | Contain phylum code and phylum name |
| 3 | Class | Contain class code and class name |
| 4 | Ordo | Contain order code and order name |
| 5 | Family | Contain family code and family name |
| 6 | Genus | Contain genus code and genus name |
| 7 | Species | Contain species code and species name, description and references |
| 8 | Collector | Contain collector code, collector name and initials |
| 9 | Isolate | Contain specimen code, collection number, duplicate number, origin, date received, date of isolation, specimen photo, micrograph photo, Sequencing information, description, symptoms, references |
| 10 | Host Plant | Contain host code plants, origin isolates (host and substrate) e.g. corn, peanuts, etc |
| 11 | Growth Condition | Contain kode dan growth condition (Medium, Cultivation Temp, Rehydration Fluid, Relative humidity, pH, Water activity, Aeration ($O_2$ and $CO_2$), light) |

Table 4. Conceptual database for insect collection data management.

| No | Table name (entity) | Information |
|---|---|---|
| 1 | Kingdom | Contain kingdom code and kingdom name |
| 2 | Filum | Contain phylum code and phylum name |
| 3 | Class | Contain class code and class name |
| 4 | Ordo | Contain order code and order name |
| 5 | Family | Contain family code and family name |
| 6 | Genus | Contain genus code and genus name |
| 7 | Species | Contain species code and species name, description and references |
| 8 | Collector | Contain collector code, collector name and initials |
| 9 | Status specimen | Status specimen: W, wet collection in fluid preservative; C, culture of live insects; P, pinned dry collection |
| 10 | Specimen | Contain specimen code, collection number, image, location, coordinate position, description, collection date |

The resulting conceptual database is translated into a more technical format as a logical framework database. An object-relational database approach is used to create relational diagrams of database management systems for weed, IAS, insect, and mushroom collections, as shown in Figure 3.

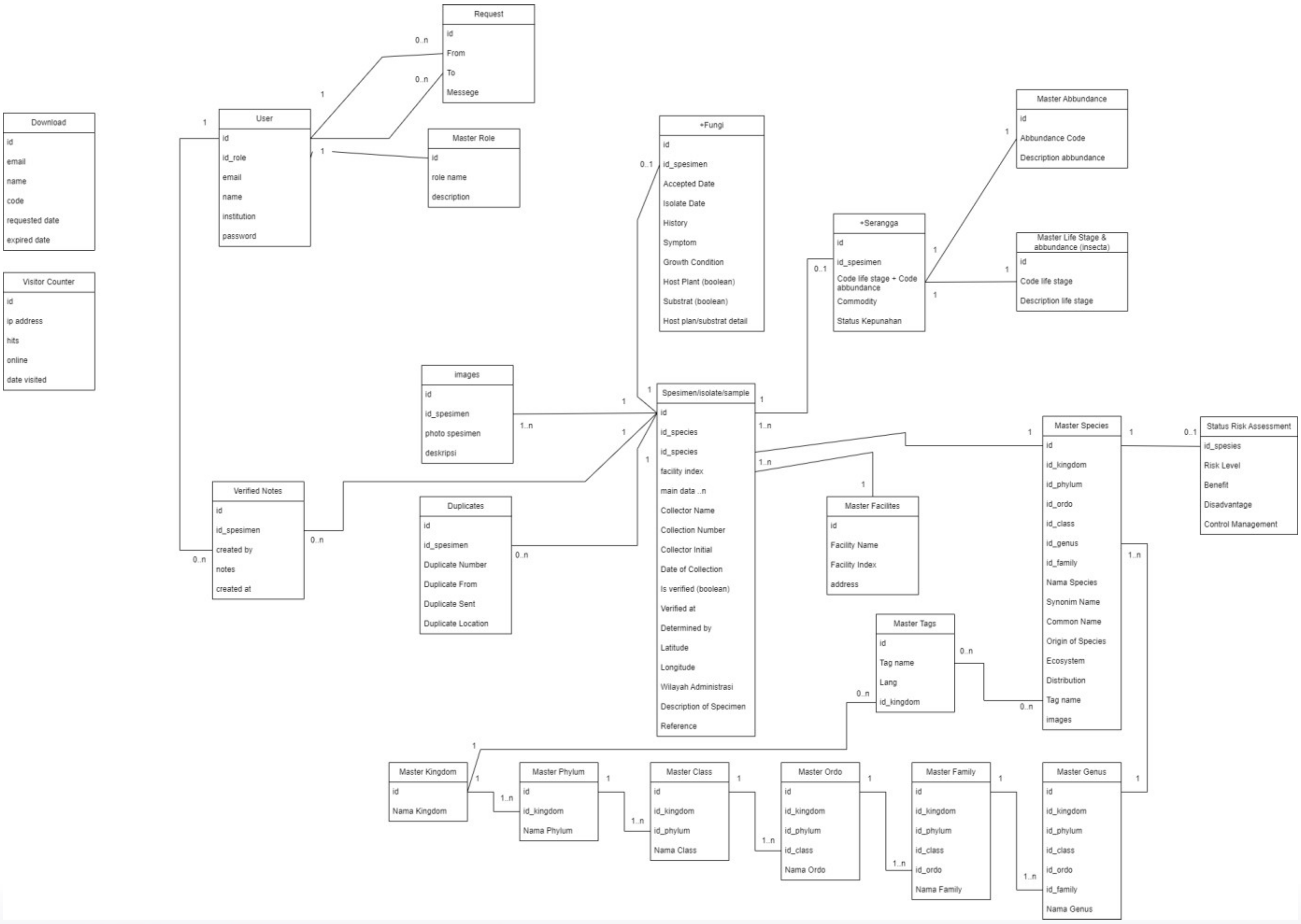



Figure 3. ER Diagram of SEAMEO BIOTROP integrated biodiversity heritage collection database.

The intensity and quality of user interaction with the SEAMEO BIOTROP collection database is the key to the success of the developed database management system. An interactive prototype of the interface has been designed, considering the identified stakeholders' needs. An attractive, interactive, responsive, easy-to-read interface and easy-to-find information that users are looking for will have a positive impact on the level of database utilization by users widely. Input from experts has been collected through FGDs, analyzed, and internalized into the features and menus available on the database management system interface prototype. Figure 4 presents an interface prototype that has been generated based on the input and opinions of experts.

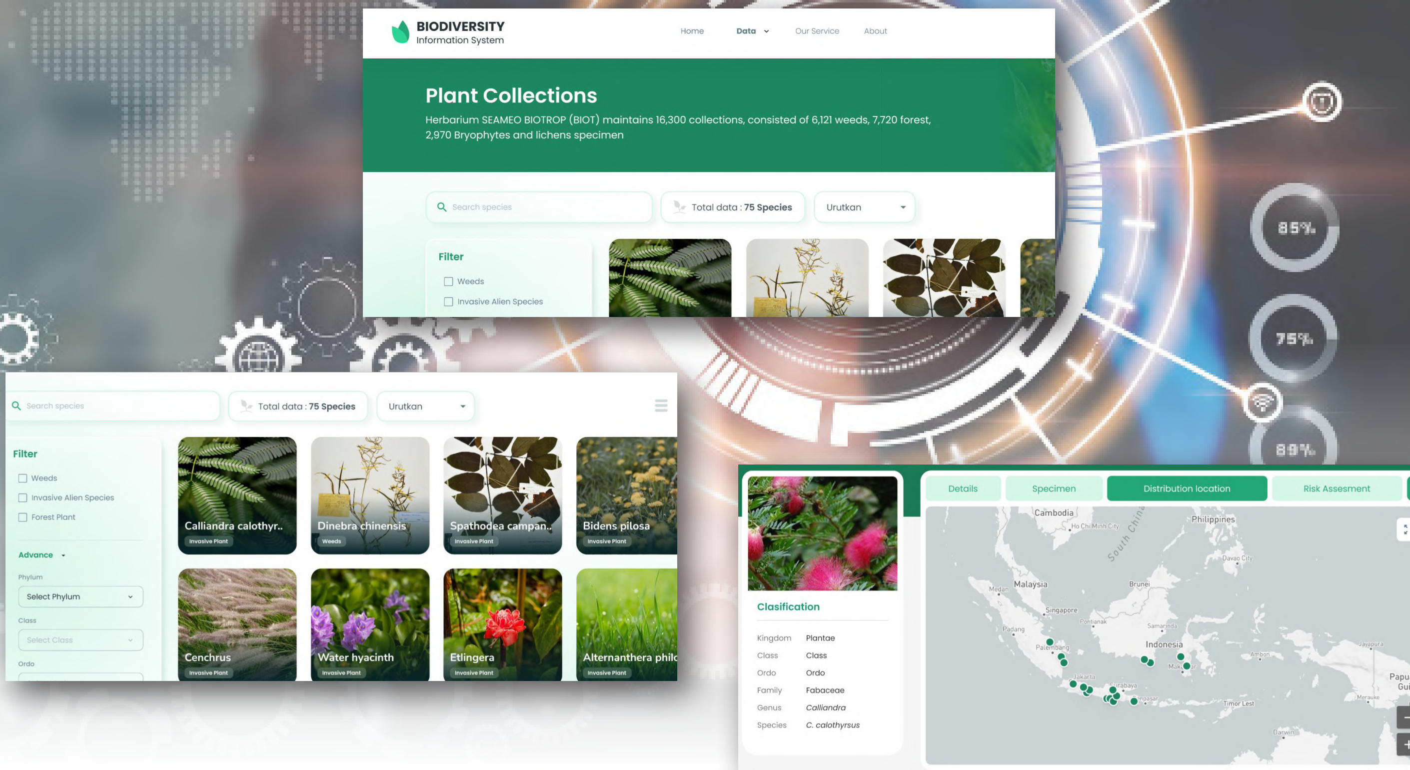
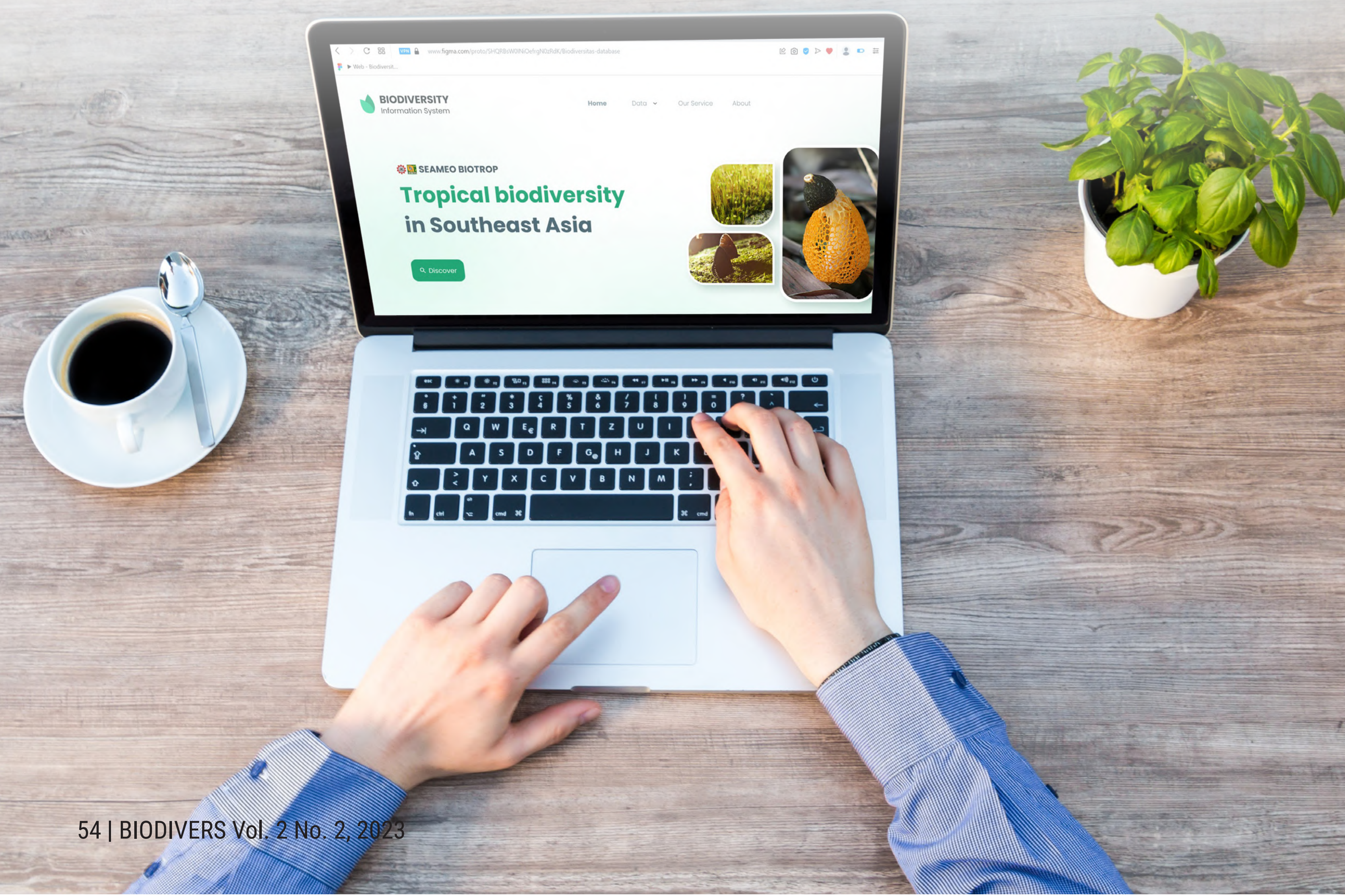



Figure 4. Prototypes interface of SEAMEO BIOTROP integrated biodiversity heritage collection database management system



## Conclusion

The study has successfully developed a framework for an integrated database of heritage biodiversity collections at SEAMEO BIOTROP. A guidebook has been written, which is expected to become a standard reference in taking samples of collections or specimens in the field, a reference in recording collection or specimen data, and a reference in developing a sustainable database management system.

## Acknowledgements

The authors thank the Ministry of Education and Culture, Research, Technology and Higher Education, the Republic of Indonesia, for providing financial support through SEAMEO BIOTROP DIPA for the fiscal year 2022. The authors also thank PiAREA and staff for providing technical assistance in the development of the database management system prototype.

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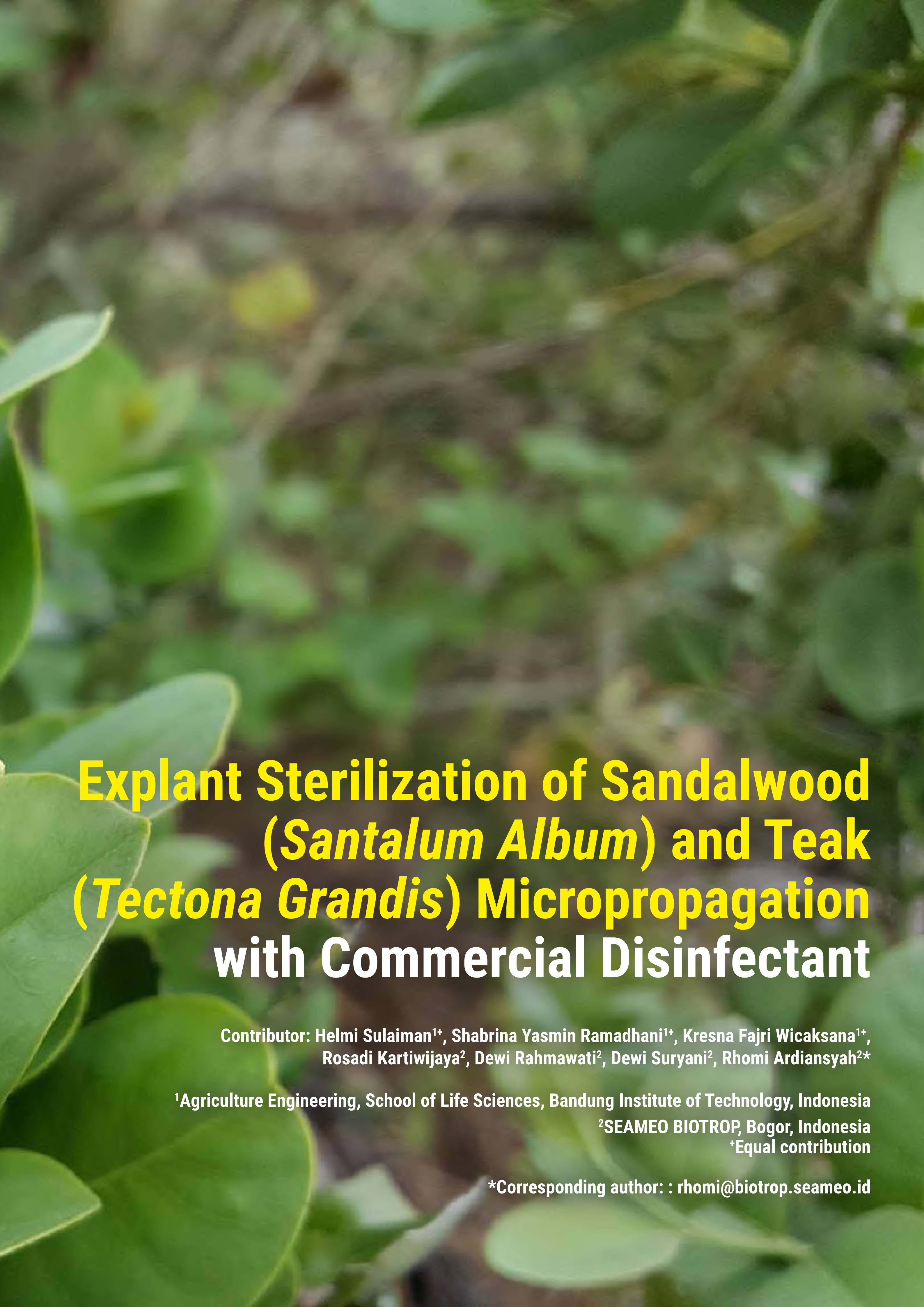

# Explant Sterilization of Sandalwood (*Santalum Album*) and Teak (*Tectona Grandis*) Micropropagation with Commercial Disinfectant

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https://open.sourcemap.com/maps/633a229f3292c3b6209d48bb/things

## Abstract

Sandalwood (*Santalum album*) and Teak (*Tectona grandis*) are essential wood sources for furniture. Sustainable micropropagation methods can benefit wood availability. This study evaluates NaOCl and Povidone-iodine effects, immersion duration, and concentrations on teak and sandalwood micropropagation. Teak is treated with 10% & 15% NaOCl and 10% & 15% Povidone-iodine. No significant difference was observed between teak treatments; however, povidone-iodine shows potential as an effective teak sterilizing agent. Sandalwood's best sterilization: NaOCl at 10% & 15%.

## Introduction

Sandalwood (*Santalum album*) is a prized tree known for its aromatic properties. It belongs to the Santalaceae family and is native to the Indian subcontinent, particularly India, Sri Lanka, and parts of Southeast Asia. These evergreen trees can reach up to 10 meters in height and have fragrant leathery leaves. The most valuable part is the heartwood, used in perfumes, incense, and traditional medicine. Sandalwood thrives in tropical and subtropical regions with well-drained soil and moderate rainfall (Kumar et al., 2012). Teak (*Tectona grandis*) is a valuable tropical timber native to Indonesia. The tree belongs to the family Verbenaceae. Teak dominates dry deciduous forest, with 5-7 months of dry season and 1000-1400 mm mean annual rainfall (Sankaran & Ratnam, 2013). The teak forest in Java also represents a lowland subhumid tropical forest ecosystem (Hartshorn, 2013). It has high quality wood and versatile uses. Evaluations on plant parts exhibit biological activities of antioxidant, antipyretic, analgesic, hypoglycemic, wound healing and cytotoxic (Asdaq et al., 2022). Conventional breeding is limited by seed availability and genetic variation, which can lead to differences in appearance. Woody tree plantation requires large amounts of uniform and high-quality planting materials obtained through vegetative propagation methods like shoot cutting or micropropagation. Micropropagation grows entire plants from small plant tissue sections in a controlled laboratory setting with a nutrient-rich medium (Pant & Husen, 2022). Sterilization is crucial to prevent contamination, which can hinder growth and cause mortality. Commercial disinfectants like bleach and Betadine® are commonly used for sterilizing plant cultures (Wamaedeesa et al., 2021; Pratiwi et al., 2020; Neliyati et al., 2019; Wiyastuti et al., 2018). This study examines the effects of different disinfectants and their concentrations on teak and sandalwood tissue culture to improve propagation efficiency.

## Methods

The experiment was conducted at the Biotechnology Laboratory, SEAMEO BIOTROP Bogor. The Murashige-Skoog (MS) (Murashige and Skoog, 1962) media was made using the following components in Table 1. Hormone 6-benzylaminopurine (BAP; Sigma®) were added into the media. BAP stock solution was made by diluting 100 mg with HCl 1 N and 100 mL $dH_2O$. The optimum pH range for MS medium was adjusted to 5.8.

The basic sterilization techniques were employed to ensure the cleanliness of the explants. They were first rinsed three times with sterile water, followed by soaking in Tween-20 solution for 30 minutes, and then rinsed again with sterile water. Subsequently, explants were soaked in Fungicide and Bactericide solution for each one hour, followed by three more rinses with sterile water. After completing these sterilization steps, the explants were ready for further treatments and experimental procedures inside the Laminar Air Flow (LAF) chamber.

Table 1. Composition of Murashige and Skoog (1962) Stock Medium

| Stock | Chemical name | Concentration (mg/L) | Stock Solution | | Volume (mL) for one litre media |
|---|---|---|---|---|---|
| | | | g/L | Concentration | |
| A | $NH_4NO_3^-$ | 1650 | 41.25 | | 20 |
| B | $KNO_3$ | 1900 | 47.5 | 50 | 20 |
| C | $KH_2PO_4$ | 170 | 17 | 100 | 10 |
| | $H_3BO_3$ | 6.2 | 0.62 | | |
| | KI | 0.83 | 0.083 | | |
| | $Na_2MoO_4.2H_2O$ | 0.25 | 0.025 | | |
| | $CoCl.6\ H_2O$ | 0.025 | 0.0025 | | |
| D | $CaCl_2.2H_2O$ | 440 | 44 | 100 | 10 |
| E | $MgSO_4.7H_2O$ | 370 | 37 | 100 | 10 |
| | $MnSO_4.4H_2O$ | 22.3 | 22.3 | | |
| | $ZnSO_4.7H_2O$ | 8.6 | 0.86 | | |
| | $CuSO_4.5H_2O$ | 0.025 | 0.0025 | | |
| F | $FeSO_4.7H_2O$ | 27.8 | 1.39 | 100 | 10 |
| | $Na_2.EDTA$ | 37.3 | 3.73 | | |
| Myoinositol | | 100 | Measured when making the media | | |
| Vitamin | Niacin | 0.5 | 0.05 | 100 | 1 |
| | Pyridoxine.HCl | 0.5 | 0.5 | | |
| | Thiamine-HCl | 0.1 | 0.01 | | |
| | Glycine | 2 | 0.2 | | |
| Sucrose/Sugar | | 30000 | Measured when making the media | | |
| Agar | | 7000 | | | |

The experiment was conducted to observe the success rate of sterilization of teak explant using several commercially available disinfectants, such as Betadine® (contains Povidone iodine 10%, Registration Number Kemenkes RI: PKD20501710078) and Bayclin® (contains 5,25% sodium hypochlorite, made in Indonesia). Fresh shoots were isolated from the axillary shoots of young Teaks in the nursery then kept in solution of three drop of tween per 100 ml sterilized water (Figure 1). First is the control group (A), following the standard procedure of double disinfection in NaOCl at 15% (v/v) and 10% (v/v) for 15 minutes and 10 minutes respectively. The second group (B) used NaOCl 15% (v/v) solution for 15 minutes, then povidone-iodine (PI) 10% (v/v) solution for 10 minutes. The last group (C) used PI solution at 15% (v/v) and 10% (v/v) for 15 minutes and 10 minutes, respectively. For each treatment with NaOCl and PI, explants were followed by three rinses in sterile water. Afterward, shoots were rinsed with alcohol 70% for one minute and then rinsed five times with sterilized water. Next, shoots were cut so each explant has one or two axillary buds.

## Explant Sterilization


Figure 1. The process of teak sterilization. (Clockwise from top left: Harvesting teak shoots from the mother plant; immersion of the shoots in a povidone-iodine solution; immersion of the shoots in a NaOCl solution; condition of the explant after establishment).

To begin with the experiment for sandalwood (Santalum album). The necessary tools and materials were prepared, including a Tween-20 solution made by mixing 3 drops of tween-20 per 100 ml of sterile water. Suitable plants meeting specific criteria, such as being healthy, having usable shoots, and appropriate size and age, were selected as the source of explants. The plant shoots were carefully taken from the lateral shoots, preferably from the first or second plant branch from the bottom for easy collection. During the explant collection process, no additional treatments like pesticides or fungicides were applied, and the apical buds were left intact. The obtained explants were then placed into the prepared Tween-20 solution and brought into the lab.

Inside LAF, advanced sterilization techniques were applied. Tools and materials needed for this stage were prepared and placed inside LAF, and the UV light was turned on for 15 minutes and then turned off. The lights and blower of the LAF were turned on before use, and the cabinet shelves were opened. For the control group, explants were soaked in 70% (v/v) alcohol for one minute and then rinsed five times with sterile water. For treatment 1, explants were soaked in 15% (v/v) NaOCl solution for 15 minutes, rinsed with sterile water, then soaked in 10% (v/v) NaOCl solution for another 10 minutes, and finally rinsed again with sterile water. They were then soaked once more in 70% (v/v) alcohol for one minute and rinsed five times with sterile water. For treatment 2, the explants followed a similar procedure with soaking in 30% (v/v) NaOCl solution for 15 minutes and then in 20% (v/v) NaOCl solution for 10 minutes, followed by rinsing and alcohol treatment.

After all the treatments were completed, plant explants were ready for planting. Inside LAF, tools and materials required for the planting stage were prepared. Explants were carefully taken with sterile tweezers and placed in a petri dish containing tissue. They were then trimmed to ensure cleanliness and ease of planting in the media. This process was repeated for all explants, and each media jar was labelled with the corresponding experimental treatment code for easy observation during the experiment. Finally, the explants were placed in culture medium containing MS culture medium supplemented with 2,2 µM BAP and 30 $g.l^{-1}$ of sucrose. Culture medium pH was adjusted to 5.8. The cultured explants are then incubated in culture room at 22 ± 2 °C for seven days under continuous fluorescent light.

## Results and Discussion

### Sterilization technique for the Micropropagation of Sandalwood

The sterilization test for sandalwood was conducted with a total of 12 samples for each treatment. There are three treatments in total for the experiment that is control (without any treatment), treatment 1, & treatment 2. Treatment 1 is a sandalwood explant sterilization treatment by immersion in a solution of Bayclin® 15% (v/v) concentration for 15 minutes and Bayclin® 10% (v/v) for 10 minutes. While treatment 2 is the treatment of sandalwood explants sterilization by immersion in a solution of Bayclin® 30% (v/v) concentration for 15 minutes and Bayclin® 20% (v/v) for 10 minutes.

Table 2. Sandalwood Sterilization Experiment Result Data

| Sample | G0 | G1 | G2 |
|---|---|---|---|
| U1S1 | X + XB | V + shoot | XJ |
| U1S2 | X + XB | X | X + XB |
| U1S3 | XB | X + XB | X + XB |
| U1S4 | XJ | X + XB | X + XB |
| U2S1 | X + XJ | X + XB | X + XJ |
| U2S2 | X | V + shoot | V |
| U2S3 | X + XJ | V + shoot | V |
| U2S4 | X + XJ | V | X + XB |
| U3S1 | V | X + XB | X |
| U3S2 | V | V | XB + XJ |
| U3S3 | X + XB | XB | X |
| U3S4 | X | X + XB | X |

Description: v = Sterile, x = browning, xb = bacterial contamination, xj = fungal contamination

Results of the experiment are shown in Table 2 and Figure 2, respectively. Each result can have multiple symptoms happening in a single sample; for example, sample U1S1 has bacterial infection while also inflicted with a case of browning. Based on the results shown it can be concluded that treatment 1 is the best treatment for the sterilization of the sandalwood because the treatment provides the best overall result in the experiment, while the control treatment and treatment 2 provide good enough results, but not the best one. The results showed through the experiment are with control treatment, the sample of sandalwood has a high percentage of being inflicted with browning cases while also resulting in some equal cases of bacterial and fungal contamination (Figure 2). In treatment 1, fungal contamination is absent with a high level of browning and bacterial contamination but still has high percentage for sterile cases too. While in treatment 2, the result shows not much difference with the control treatment. Some sample showed more than one condition, for example, a sample could be contaminated with bacteria and shown browning at the same time or contaminated by both bacteria and fungi. To comprehensively evaluate the explants, two evaluation types were employed: one based on the total number of explants under specific conditions and the other based on the final condition (Table 2; Figure 2). The purpose of this differentiation is to determine the predominant cause of contamination and facilitate further studies. Total explant with the condition counts samples with two or more condition more than one time. Meanwhile final condition counts based on these premises; (1) all fungi contaminated sample would also have bacterial contamination, therefore classified to fungi contamination; (2) browning samples with contamination will be classified as contaminated; (3) If successfully inducted samples show contamination, they will be classified as contaminated, even if they are still potentially salvageable.

## Explant Sterilization

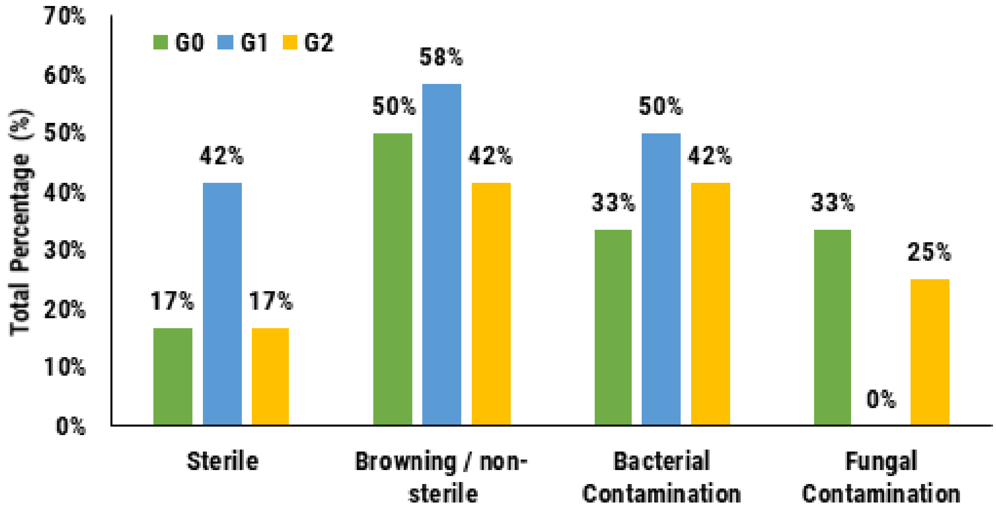


Figure 2. Bar Chart of Percentage of Sandalwood Sterilization Experiment Results

NaOCl is a strong oxidizing agent commonly found in household bleach. It is effective at killing a broad spectrum of microorganisms, including bacteria, fungi, and viruses. When used in tissue culture, NaOCl is typically diluted to a specific concentration, such as 1-10%, to ensure effective sterilization while minimizing potential damage to the explants. The treatment helps eliminate surface contaminants on plant material, reducing the risk of introducing unwanted microbes into the tissue culture environment (Teixeira et al, 2016). Some reason for its frequent usage is that NaOCl is effective against a wide range of microorganisms, including bacteria, fungi, and viruses. NaOCl is commercially available in various concentrations and forms, including household bleach, which makes it accessible in many laboratories. Dilutions of NaOCl can be easily prepared to achieve the desired concentration for effective sterilization. NaOCl is also a relatively inexpensive sterilizing agent compared to some other chemical disinfectants, making it an affordable option for tissue culture laboratories. Finally, when used at appropriate concentrations, NaOCl can effectively sterilize explants while minimizing damage to the plant tissues. It is relatively less harmful to the cells compared to certain other disinfectants, allowing for the successful initiation and growth of cultures. (Weber et al, 2015). NaOCl acts as a strong oxidizing agent and exhibits broad-spectrum antimicrobial activity. The concentration and exposure time of NaOCl is critical to achieving effective sterilization while minimizing tissue damage. Generally, a lower concentration (e.g., 1-10%) is used for tissue culture sterilization. (Teixeira et al, 2016).

The usage of NaOCl for the sterilization of sandalwood is used extensively globally. Many other research and production purpose for the culture or micropropagation of sandalwood uses NaOCl as the sterilization agent in the process. General techniques of sandalwood sterilization are advised to use NaOCl for the surface sterilization of the explant (Krishnakumar et al., 2018; Warakagoda et al., 2013). For the optimum dosage used in the sterilization, the experiment that we conducted showed that the dosage is 15% (v/v) and 10% (v/v) sourced from the bleach Bayclin® (which contains NaOCl 5,25%). Previous studies showed that the optimum dosage of NaOCl used for sterilization purposes was 0.01% of pure NaOCl (Krishnakumar et al, 2018), or if sourced from a commercial bleach (such as Bayclin® or Chlorox® which generally contain 5,25% of NaOCl) the usual dosage is 15% (v/v) (Warakagoda et al., 2013).

### Sterilization technique for the Micropropagation of Teak

The result of teak sterilization showed that the treatment B, which uses both NaOCl and Povidone-iodine, showed the highest sterile explant percentage, followed by treatment A, that followed standard teak sterilization procedure (Figure 3). Treatment C that uses double sterilization with povidone-iodine showed poor sterilization by 0%. Bacterial contamination is predominantly found in treatment A, while fungi are more commonly found in treatment B. Treatment C showed a similar contamination rate for fungi and bacteria. Bacterial contaminations were found on the agar surrounding the explants, exhibiting a circular shape. Establishment phase in teak micropropagation is the most sensitive because the explants are the most vulnerable to contamination and limited in vitro response (Aguilar et al., 2019). The highest browning rate was observed in treatment C, followed by treatment A. Meanwhile, treatment B had no browning explant. The ANOVA analysis showed no significant difference between each treatment with respect to final condition at α=0.05 level of significance.

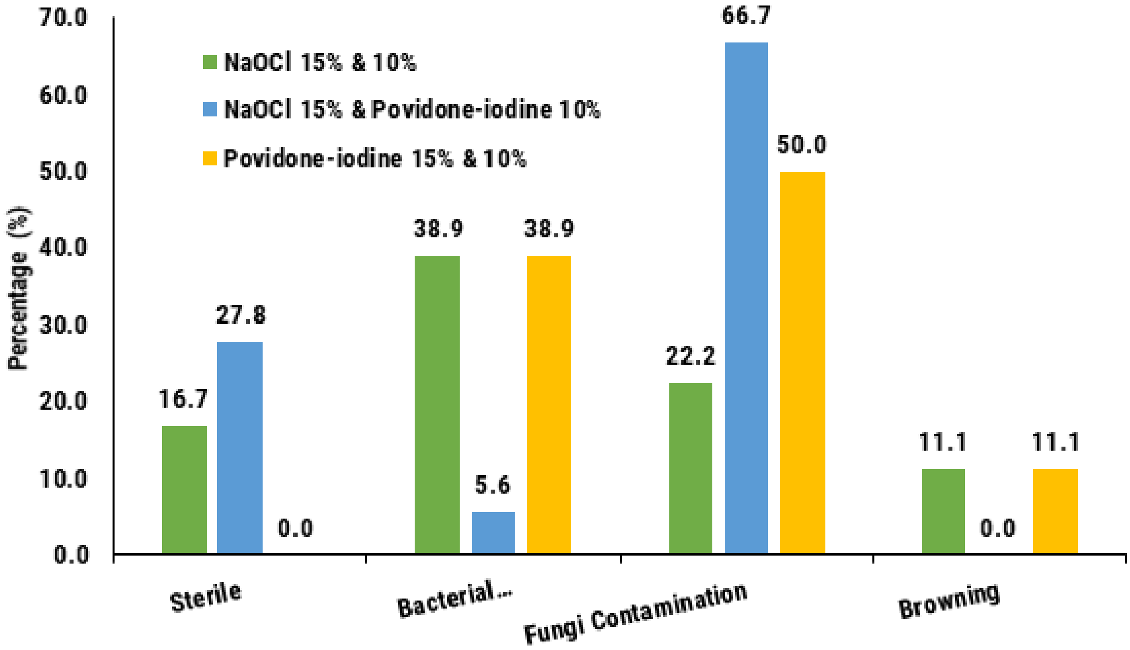


Figure 3. Bar chart of percentage of teak sterilization result based on final condition.

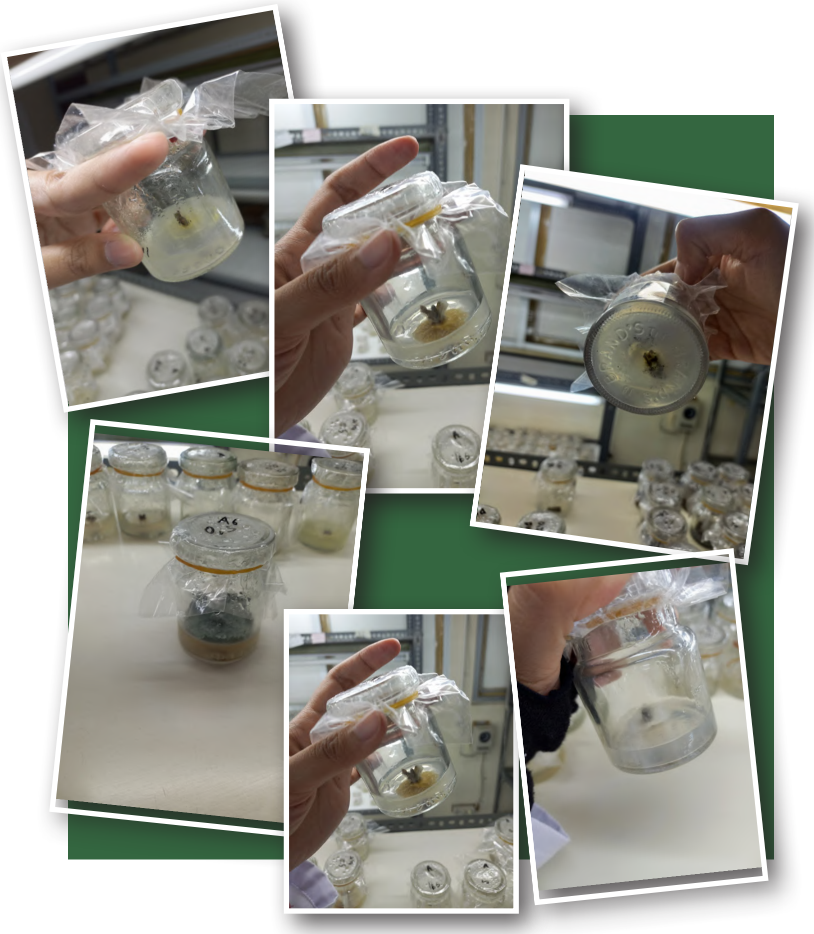

Figure 4. Examples of contaminated explant by bacteria (up) for each treatment (A, B, C); examples of contaminated explant by fungi (down) for each treatment A, B, C)

Sodium hypochlorite commonly used as broad-spectrum disinfecting agent that is effective for bacteria, viruses, fungi, and mycobacterium. It is not effective against bacterial spores and prions (Girotti, 2015). In the sterilization process, chlorine present in sodium hypochlorite operates via an oxidation mechanism. The chlorine ions stimulate inactivation of enzyme and degrade lipids and fatty acids (Pais et al., 2016). Povidone iodine is a water-soluble iodine-releasing agent, also known as iodophor. It consists of a complex between iodine and polyvinylpyrrolidone, a solubilizing carrier. Free iodine ($I_2$) act as the active bacterial agent, capable of rapidly penetrates microorganism and oxidizing vital proteins, nucleotides, and fatty acids. This process eventually results in cell death (Lepelletier et al., 2020).

Based on the result, treatment A (control) has the highest induction quality and number. Callus formations were observed at the bottom of the explant as a mass of white tissue (Figure 4). Some inducted explants grow one shoot from the axillar/lateral bud. Hormones that were added into the Murashige-Skoog media can affect explant growth. For instance, BAP is used to initiate bud formation and vegetative growth acceleration (Simanjuntak et al, 2015), while NAA (1-Naphtalenacetic acid) is used to initiate root growth (Arya & Husein, 2019). Hormones can also be added during the mixing process of stock solution. Although they may degrade during the heating process on stove and the sterilization process in the autoclave, leading to double degradation of hormones. Hormones that have fully degraded won't be effective to initiate growth on explants.

## Explant Sterilization

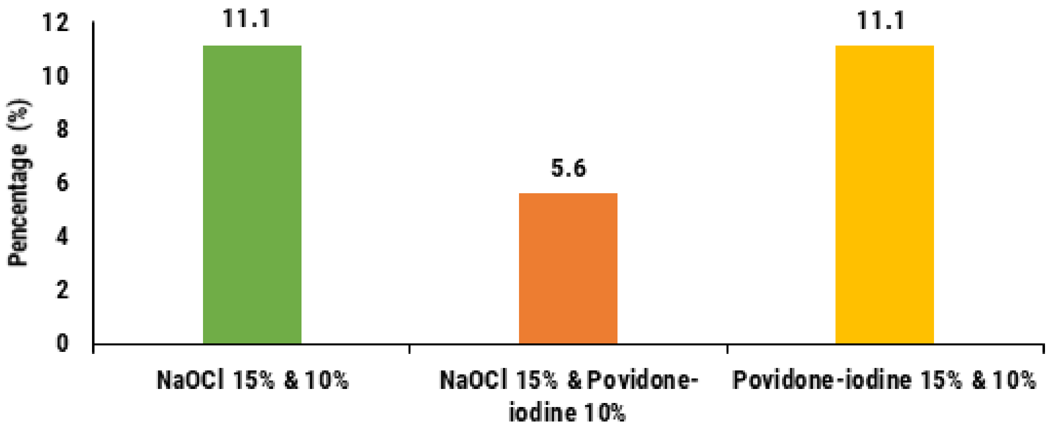


Figure 5. Bar chart of percentage of successfully inducted explant. Note the chart showing the percentage of successfully inducted explants, including the contaminated ones.

It was also observed that teak explants were still capable of growing calluses and shoots even in a contaminated state, either by bacteria or fungi (Figure 5 and Figure 6). Successful explants with bacterial contamination still had the chance to be salvaged, but the ones with fungi contamination needed to be discarded immediately. This aligns with the review by Permadi et al. (2023), which suggests that while bacterial proliferation can cause substantial damage to plant material, some explants are able to survive and thrive even when bacteria are present. Contaminants such as endophytic bacteria are difficult to eliminate because systemic steriliser could potentially harm the explants and can emerge after multiple subcultures. The explant can be salvaged after new shoots elongated and then cut into segments with one nodal. Each segment then can be used as new inoculum (Witjaksono et al., 2020). Fungi contamination has faster expansion than the explant's growth. Most species also capable of producing and releasing secondary metabolites that negatively affect the explant (Zhao et al., 2014).

The low rate of successful sterilization could be attributed to several factors. In micropropagation, NaOCl is typically used for surface disinfection, but this method has demonstrated limited effectiveness in controlling explant contamination (Widyastuti et al., 2018). Surface sterilization alone is insufficient to reach contaminants that reside dormant within the explants (Permadi et al. 2023). Another factor would be the cross contamination within the culture room. Fungi releases air borne spore, which can transfer from one explant to another. Any explant that exhibits signs of fungal contamination should be discarded immediately. During the experiment, contaminated explant wasn't immediately separated, thus increasing the risk of cross contamination. Another factor, according to Widyastuti et al., (2018), each explant may have different surface contamination levels. This is especially true since the teak mother plant was not subjected to any pretreatments, such as the application of fungicides or bactericides, and was grown in a semi-open environment.

Figure 6. Examples of initiated explants. Left to right: explant with callus formation from treatment A, explant with growing shoot from treatment A, explant with callus formation from treatment B, explant with callus formation from treatment D but contaminated.

## Conclusion

Sterilization is an essential step for a successful micropropagation. In this research, for the sterilization of sandalwood explants it was evaluated using two treatments. The study confirmed the effectiveness of Sodium hypochlorite (NaOCl) as the preferred sterilizing agent for sandalwood, with the optimal dosage being 15% (v/v). Meanwhile, sterilization tests for teak were conducted using varying concentrations of NaOCl (15% (v/v) and 10% (v/v)) and povidone-iodine (15% (v/v) and 10% (v/v)). The results revealed no significant differences between the treatments. Combination of NaOCl 15% (v/v) and Povidone-iodine 10% (v/v) showed the best sterilization outcome with 27,8% sterile rate. However, control treatment with double NaOCl immersion showed higher number of successfully inducted explant of 11,1%.

## Acknowledgement

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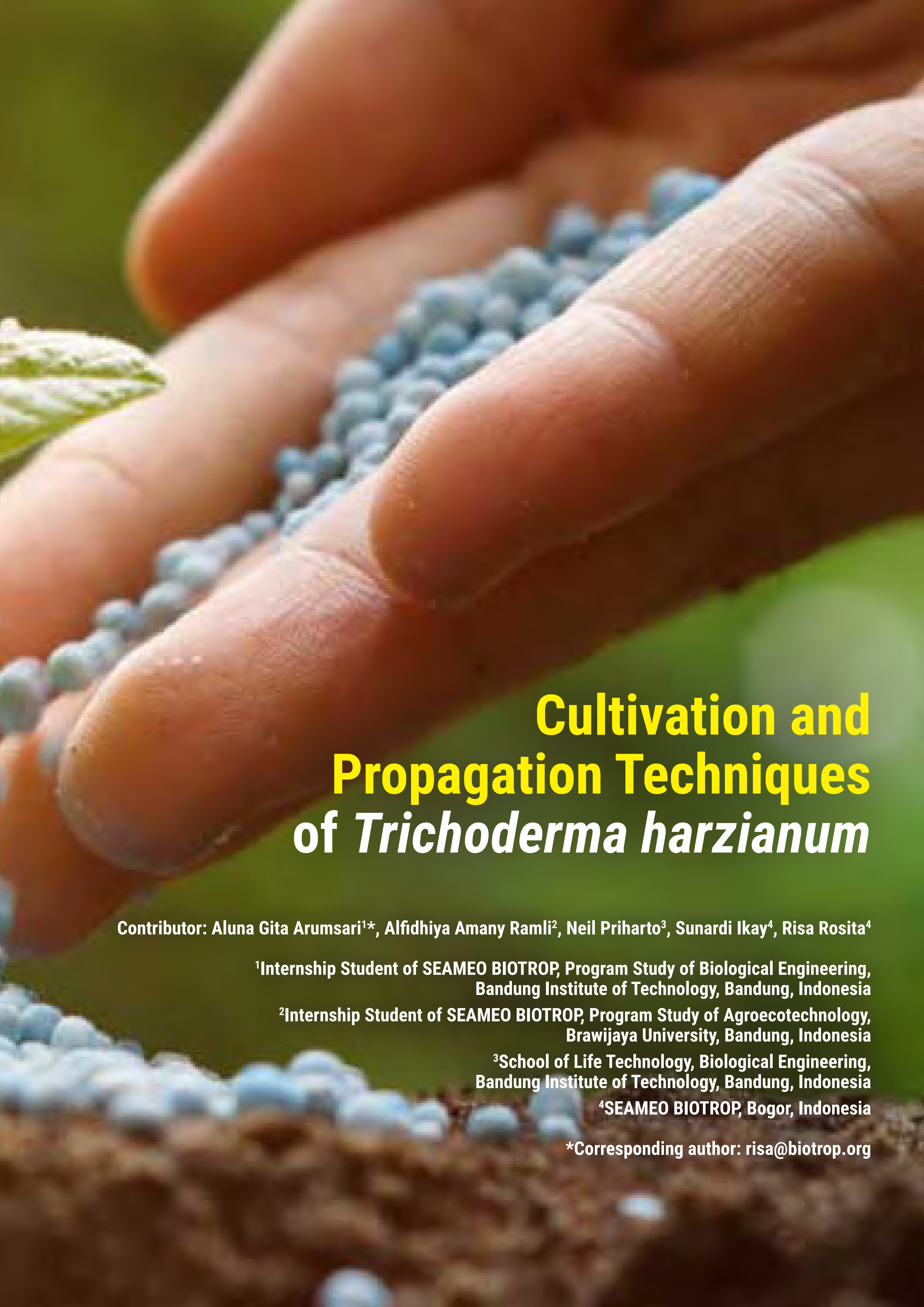

# Cultivation and Propagation Techniques of *Trichoderma harzianum*

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## Abstract

This research was conducted to determine the effect of temperature on the growth of *Trichoderma harzianum*. *Trichoderma harzianum* has functions to prevent the growth of diseases in plants, increase plant growth, and decompose organic matter. The research was conducted at the Phytopathology and Biosystem and Landscape Management Laboratory at SEAMEO BIOTROP, Bogor. The study was analyzed using a Completely Randomized Design (CRD) with different temperature treatments with five replications. The parameter observed was the diameter of the fungus growth after 6 days incubation period. Based on the research results, *T. harzianum* grew well when the fungus was incubated at 23 °C compared to 40 °C.


**Keywords:** Decomposition, fungal growth

## Introduction

*Trichoderma harzianum* is commonly known as a fungus that has various benefits, especially for agricultural activities. This fungus is able to provide benefits that can prevent plants from disease and increase plant growth. Studies conducted by Fitria et al. (2021) stated that *T. harzianum* has several benefits such as producing the IAA (Indole Acetic Acid) hormone, which helps plant growth and development. In addition, according to Wardahni et al. (2022), the fungus produces secondary metabolites in the form of gliotoxin and glioviridin, which are toxic to the *bacterium Xanthomonas campestris* pv. *vesicatoria* causes bacterial spot disease on tomato plants. These metabolites will inhibit bacteria growth, thereby preventing plants from disease attack.

Besides being beneficial to plants, *T. harzianum* has good environmental benefits as a decomposer. According to Akmal et al. (2021), *T. harzianum* can be used as a decomposer in the composting process of empty palm oil bunches. As waste from the process of utilizing oil palm, the existence of empty fruit bunches that are not processed can pollute the environment through the resulting greenhouse gas (GHG) emissions. Composting with the help of the fungus *T. harzianum* can produce compost, which is used as an organic fertilizer.

The variety of benefits that can be obtained from using the fungus *T. harzianum* makes it essential to know how to cultivate and increase its number. However, environmental problems such as climate change, which causes an increase in temperature, can be one of the obstacles to the multiplication of the fungus *T. harzianum*. For this reason, it is necessary to study the impact of temperature on the growth and propagation of the fungus *T. harzianum*.

## Method

This research was conducted from 1 to 30 June 2023 at the Phytopathology and Biosystem and Landscape Management Laboratory, SEAMEO BIOTROP, Bogor, West Java, Indonesia. Geographically, SEAMEO BIOTROP is located between 106°48' East Longitude and 6°26' South Latitude with an average annual rainfall of around 3,500-4,000 mm. The instruments used in this study consisted of analytical scales, spatulas, ruler (meter), Erlenmeyer flasks, autoclaves, test tubes, measuring cups, pipettes, ovens, Petri dishes, tweezers, cotton, paper, bunsen, loop needles, ovens, hot plates, Laminar Air Flow Cabinet (LAF), lighters and thermometers. The materials used in this study included nutrient agar media for PDA (Potato Dextrose Agar), 70% alcohol, distilled water, aluminum foil, cling wrap, spirits, sterile tissue, label paper, heat-resistant plastic, and paraffin. The research stages of this study are as follows:

### Preparation of *T. harzianum* Culture Media

The culture and propagation media used were PDA (Potatoes Dextrose Agar) as an agar tube. The media volume made was 1000 mL; 39 grams of Potato Dextrose Agar media and 1000 mL Aquades were then put into the Erlenmeyer and covered with cotton. The solution is heated using a hotplate until it boils and mixes perfectly. A total of 4 ml of PDA solution was put into a test tube, covered with aluminum foil, then sterilized using an autoclave at 1 atm 121 $^{0}$C for 15 minutes. After sterilization, the tube is placed on a slanted board until it solidifies. The oblique agar medium is ready to be used for culturing *T. harzianum*. Periodically check the condition of the fungal culture to ensure that the growth of the fungus is going well and is not contaminated by other fungi or bacteria. Making long-term cultures requires the addition of glycerol or paraffin to extend the shelf life. If the mushroom growth has reached the desired stage and is growing well, the fungus isolate can be collected and used for its purpose, in this case, for the use of fertilizers.

### Propagation of *Trichoderma harzianum*

Petri dishes that have been filled with PDA media and have been sterilized are prepared. Fungal isolates that had been previously cultured were opened carefully under sterile conditions. The loop needle is used to transfer the isolate from the tube to the prepared petri dish. Transfer the fungus isolate by sticking it on a flat surface. Make sure that the isolate is evenly distributed. The petri dish that was filled with *Trichoderma* was closed tightly and wrapped with cling wrap. Store the petri dish in an appropriate incubator at 40 °C and 23 ºC. Each treatment was repeated five times.

### Observation Growth of *Trichoderma harzianum*

The diameter of the isolate is one of the parameters in observing the growth of the fungus. Diameter can predict how much the isolate has grown since it was first isolated. The wider the diameter, the more fungal biomass can be used as a parameter for fungal growth. Observation of fungal growth was carried out 1 day after cultivation. The isolate diameter measurements were repeated every day until 7 days after planting. The diameter of *Trichoderma* was measured using a ruler by making two diagonal lines across the petri dish by crossing them. Then the two diagonals were averaged.

### Research Design

The research design used was a Completely Randomized Design (CRD) with 1 factor, namely temperature, which consisted of 2 levels, namely temperature was 23 °C to 40 °C. The experiment was repeated 5 times so that there were 10 experimental units. The independent variable in this study was room temperature, while the dependent variable influenced by the independent variable was the growth rate of the diameter of the fungus *T. harzianum*. Quantitative data from research results were analyzed using analysis of variance (ANOVA). If there is a significant difference between the treatments, a further test will be carried out using the Duncan Multiple Range Test (DMRT) at the alpha (α) level of 5%. Research data were analyzed using SAS software version 9.0.

## Results and Discussion

The results of the variance of the increase in the diameter growth of *T. harzianum* after being given a heating treatment in the oven at different temperature levels showed a very significant difference in the diameter of the growth of *Trichoderma* (P>0,05). Based on the research results, after 6 days incubation period, *T. harzianum* grew well when the fungus was incubated at 23°C compared to 40°C (Figure 1). Meanwhile, *Trichoderma* colony is shown in Figure 2.

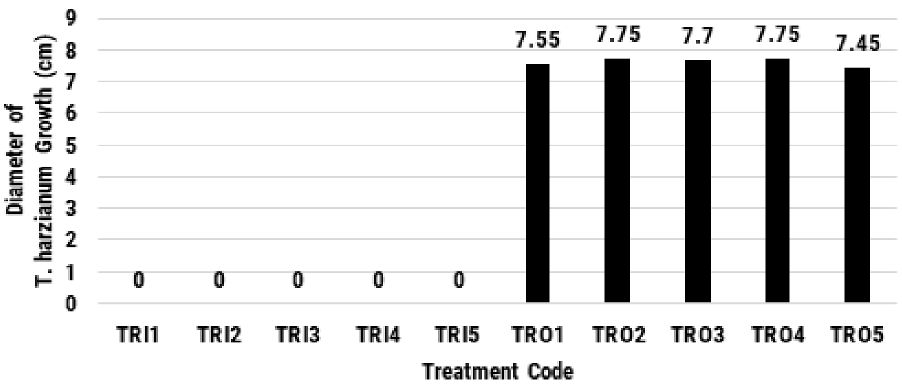



Figure 1. Diameter growth of *Trichoderma harzianum* after treatment (TR= *Trichoderma harzianum*; I= temperature 40°C; O= temperature 23°C)

Figure 1 showed a significant difference in the growth of diameter *T. harzianum* that had been treated with heating treatment at 23°C and 40°C. Based on the result, in the experiment at 40°C, no growth and diameter increased in *T. harzianum*. It happened because it had passed the optimal temperature limit for growing fungi. The results are supported by Zali & Purdiyanto (2011) that stated high temperatures could inhibit the production of carboxymethylcellulose and xylanase enzymes, whereas, in the treatment at 23°C, the diameter was increased by 7.55 cm, 7.75 cm, 7.7 cm, 7.75 cm, and 7.45 cm. In relation to Zali & Purdiyanto (2011), which stated that the growth of *Trichoderma* has a balanced quality to grow well at room temperature (28°C). According to Prabowo et al. (2006), apart from the increase in the diameter of *T. harzianum*, there is also an increase in the length of the hyphae if placed at an optimal temperature. The results of the long hyphae analysis after heating in the oven at different temperature levels showed a very significant difference in the length of the stem (hyphae) of *Trichoderma* (P>0,01). The highest growth in length of the stem (hyphae) of *Trichoderma* was achieved by treatment at room temperature (28°C). In addition to the increase in diameter and length of the stem (hyphae), several surviving spores also exist. The number of spores that survive at room temperature is better than at higher or lower temperatures. The results of the temperature treatment variance had a significant effect (P>0.05). It shows that the resistance of sports within certain temperature limits is very influential. At high temperatures, the spores could not develop properly or could not increase the number of spores present in each treatment.

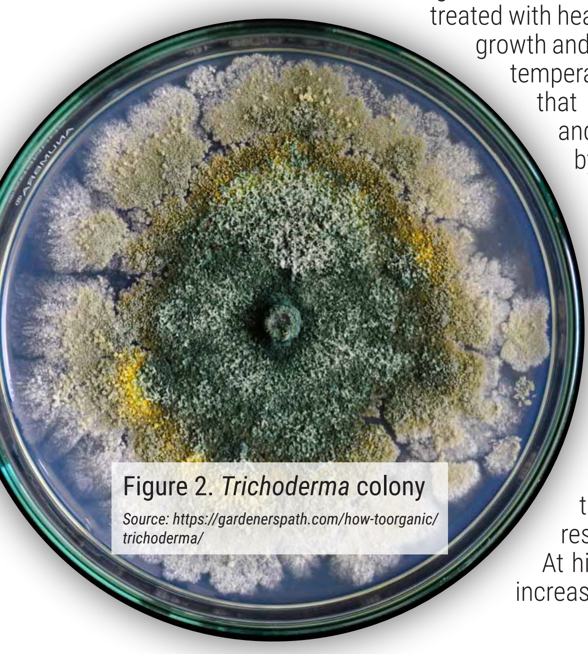
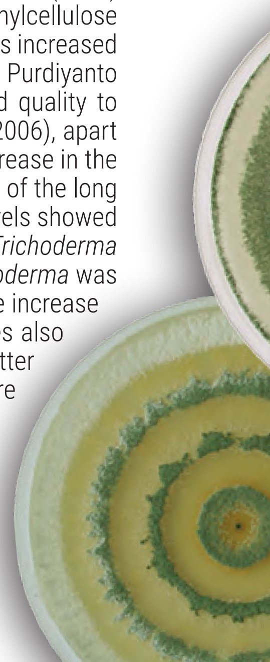


Figure 2. *Trichoderma* colony

Source: https://gardenerspath.com/how-toorganic/trichoderma/



## Conclusion

According to the study, the fungus *T. harzianum* showed better growth and increased diameter size in a petri dish at 23°C. However, at 40°C, *T. harzianum* was unable to grow properly.

## Acknowledgment

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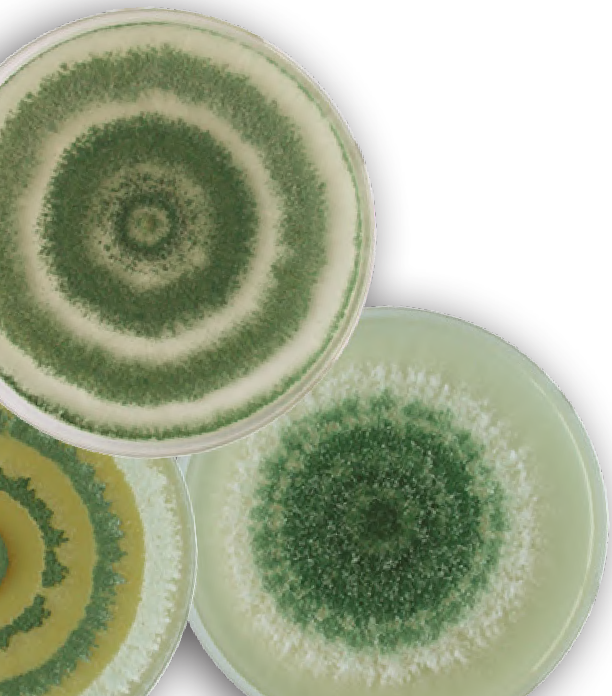


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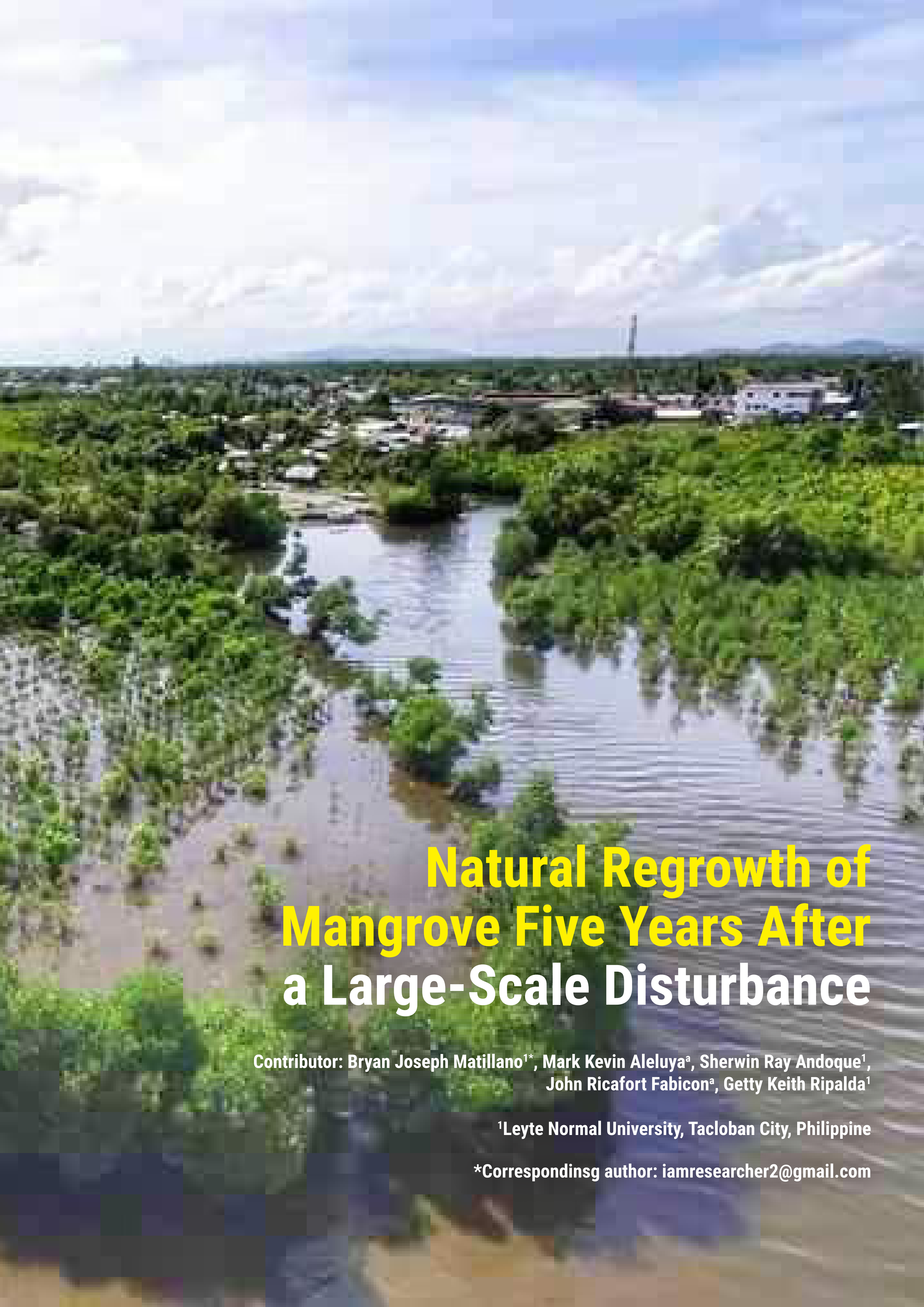

# Natural Regrowth of Mangrove Five Years After a Large-Scale Disturbance

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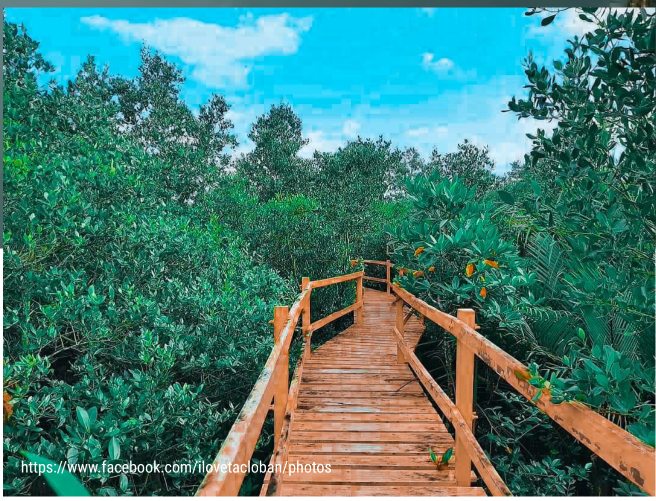

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## Abstract

Haiyan, the strongest typhoon on landfall damaged mangrove covers resulting to uprooting, cutting off trunks, total defoliation and eventually death. After five years, Cancabato Bay were monitored for mangal succession and natural regrowth. Basal area was computed using diameter by breast height to estimate recovery. New species reappeared compared to the baseline study of GIZ (2014). Mangroves manifested regrowth and should be given time for natural regeneration and colonization. Natural succession patterns should be considered in human-assisted rehabilitation after disturbance.


**Keywords:** ecological succession, succession patterns, large-scale disturbance, human-assisted rehabilitation

## Introduction

For decades, Philippine mangrove cover declined from 400,000-500,000 hectares/year and 120,000 hectares/year in 1994, attributed to unsustainable human-induced practices- aquaculture, land conversion, and settlements (Canopio et al., 2021; Primavera & Esteban, 2008; Garcia et al., 2014). As anthropogenic pressures proliferate, typhoons also contribute to mangrove decline (Hakim et al., 2017; Long et al., 2016; Primavera et al., 2016). Mangroves became an interest in climate change as a sustainable mitigating solution.

Mangroves are the coastal defense against typhoon, wave surges, and storm impacts (Primavera et al., 2016; McIvor et al., 2012). Synergism of anthropogenic and natural pressures is least understood on ecosystems and recovery, whether natural, human facilitated or combinations are dearth in literature. Nevertheless, mangrove protection by the Department of Environment and Natural Resources (DENR) recorded an increase of 247,362 hectares in 2007 (Garcia et al., 2013).

On November 8, 2013, the strongest typhoon on landfall, Haiyan generated strong winds and storm surges, causing

total devastation in Tacloban City (NDRRMC, 2013; Matillano, 2016). Haiyan damaged coastal greenbelts, uprooting large trees and breaking off main trunks, which created total defoliation and death among mangroves. The Philippine government provides PHP 167.86B Haiyan Rehabilitation Fund (Villamayor et al., 2016), including PHP 38M allocation of the DENR for mangrove planting alone (r8.denr.gov.ph).

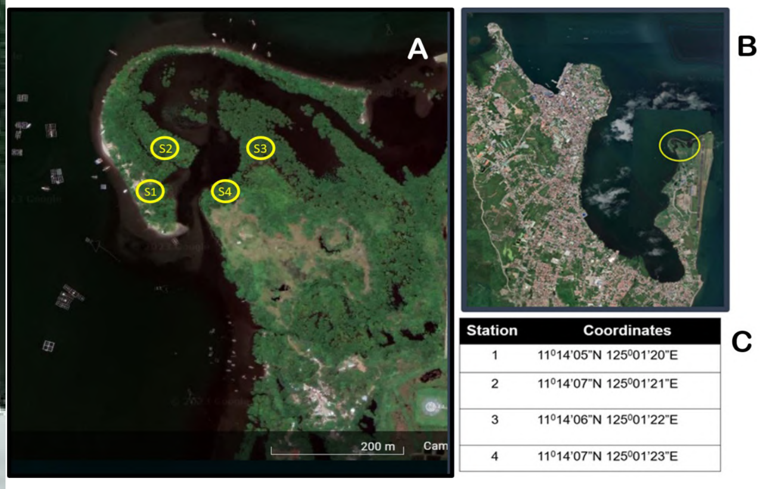
Mangrove survived along San Juanico Strait, Anibong Bay, and Cancabato Bay exhibiting regeneration (Matillano et al., 2018; Matillano et al., 2020). GIZ (2014) monitored surviving mangrove patches along Brgy. Cabalawan and Brgy. Alimasag withstanding the super typhoon and manifested regrowth even without restoration.

Monitoring recovery and regrowth, comparing from the baseline study, offers succession outlooks on ecosystem recovery. Mangroves respond to stress differently (Primavera et al., 2016), and human-assisted rehabilitation should pattern natural succession frameworks. Mangroves recover after disturbance depending upon the magnitude of damage (Primavera et al., 2016).

## Materials and Methods

### Study Site

Four permanent transect lines were laid in Brgy. Alimasag, Tacloban City, is part of the Kataisan Point, a tapering piece of land extension from mainland Leyte Island distending to Cancabato Bay and Leyte Gulf. This area is at the tip of Tacloban City DZR Airport, impending the construction of the Causeway Project embankment. During Haiyan, this area was totally obliterated, generating 7m+ surges.




| Station | Coordinates |
|---|---|
| 1 | 11⁰14'05"N 125⁰01'20"E |
| 2 | 11⁰14'07"N 125⁰01'21"E |
| 3 | 11⁰14'06"N 125⁰01'22"E |
| 4 | 11⁰14'07"N 125⁰01'23"E |

Figure1. Research Locale (A)The four permanent transect lines established in the sampling sites were labeled S1, S2, S3, and S4, respectively. (B) A map of Tacloban City, emphasizing the sampling site, encircled its exact position and proximities from water bodies. (C) A table was inserted to stress the GPS coordinates of the sampling sites.

### Methods

Four 50-meter transect lines were laid in the four identified sites (Figure 1), setting two transect lines in seaward and landward zones. A 10x10 m observational quadrat was also established every 10 m on both sides of the transect line. A total of 40 quadrats were covered for the actual observation sites. The sampling site identification was based on the extent of mangrove cover, access to the area, and land ownership delineation.

### Species Identification

The taxonomic key found in the Field Guide to Philippine Mangroves by Primavera, 2009 was the basis for the identification. Photo documentation was confirmed by the Department of Environment and Natural Resources (DENR).

### Computing the Basal Area

The diameter of surviving mangrove's trunks was measured for the computation of basal area by Diameter at Breast Height (DBH). Basal area is needed to determine forest stand density to estimate mangrove recovery. Seedlings were not included in the computation.

## Results and Discussions

As a basis for the re-emergence of mangrove species after Haiyan, GIZ (2014) data served as the baseline for surviving species list. They became a source of comparison of new species, which will be identified in this study. In 2014, 8 species of mangroves were identified compared to 22 species in this study. No other references are available after Haiyan, as most of the literature talks about the socio-political perspectives of rehabilitation (Matillano, 2016; Daga, 2019).

Table 1. Comparison of mangrove and mangrove associate species found in 2014 and 2019

| Mangrove Species | GIZ, 2014 | This Study, 2019 |
|---|---|---|
| *Acanthus ilicifolius* | | √ |
| *Acanthus volubilis* | | √ |
| *Aegiceras corniculatum* | √ | √ |
| *Aegiceras floridum* | | √ |
| *Avicennia marina* | √ | √ |
| *Avicennia rumphiana* | | √ |
| *Breyia vitis ideae* | | √ |
| *Bruigera cylindrica* | | √ |
| *Bruigera gymnorrhiza* | √ | √ |
| *Ceriops decandra* | √ | √ |
| *Ceriops tagal* | | √ |
| *Lumnitzera littorea* | | √ |
| *Lumitzera racemosa* | | √ |
| *Nypa fruticans* | | √ |
| *Pemphis acidula* | | √ |
| *Rhizophora apiculata* | √ | √ |
| *Rhizophora mucronata* | √ | √ |
| *Rhizophora stylosa* | | √ |
| *Scyphiphora hydrophyllacea* | √ | √ |
| *Sonneratia alba* | √ | √ |
| *Sonneratia caseoralis* | | √ |
| *Xylocarpus granatum* | | √ |

Table 1 shows that fourteen additional (14) mangrove species were added to the list of species present on the premise of the GIZ (2014) study. The mangrove species are *Acanthus ilicifolius, Acanthus volubilis, Aegiceras floridum, Avicennia rumphiana, Breyia vitis ideae, Bruigera cylindrical, Ceriops tagal, Lumnitzera littorea, Lumnitzera racemosa, Pemphis acidula, Rhizophora stylosa, Sonneratia alba* and *Xylocarpus granatum*. The emergence or reemergence of mangrove species after a large-scale disturbance may increase species richness as an attribute of an ecological succession of biological communities (Chang & Turner, 2019). Depending upon the severity of the disturbance, succession is site specific depending upon ecological conditions contingent on the disturbance intensity, site conditions, competition, and demographic trade-offs (Chang et al., 2019; Fischer et al., 2019). Though the data is limited to increased species richness and recovery of mangrove species after Haiyan, a manifestation of succession is already in its primary stage. It is a challenge for researchers to persistently monitor ecosystems' coping mechanisms in order to establish patterns and drivers of ecological succession.

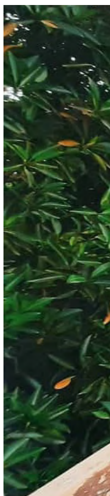
Table 2. Comparison basal area occupied mangrove and mangrove associates in GIZ, 2014 and this study 2019

| Mangrove Species | Basal Area GIZ, 2014 m²/ha | Basal Area This Study, 2019 m²/ha |
|---|---|---|
| *Acanthus ilicifolius* | | |
| *Acanthus volubilis* | | |
| *Aegiceras corniculatum* | 0.016 | 3.41 |
| *Aegiceras floridum* | | 38.27 |
| *Avicennia marina* | 0.077 | 93.71 |
| *Avicennia rumphiana* | | 6.22 |
| *Breyia vitis ideae* | | |
| *Bruigera cylindrica* | | 1.37 |
| *Bruigera gymnorrhiza* | 0.023 | 2.55 |
| *Ceriops decandra* | 0.017 | 14.46 |
| *Ceriops tagal* | | 4.5 |
| *Lumnitzera littorea* | | 2.61 |
| *Lumitzera racemosa* | | 2.56 |
| *Nypa fruticans* | | |
| *Pemphis acidula* | | 0.51 |
| *Rhizophora apiculata* | 0.029 | 11.33 |
| *Rhizophora mucronata* | 0.025 | 30.85 |
| *Rhizophora stylosa* | | 30.52 |
| *Sonneratia alba* | 0.016 | 105.7 |
| *Sonneratia caseoralis* | | 8.12 |
| *Scyphiphora hydrophyllacea* | 0.129 | 1.43 |
| *Xylocarpus granatum* | | 3.45 |
| Total | 0.332 | 361.57 |

Creepers Juvenile

The basal area occupied by surviving mangroves after super typhoon Haiyan GIZ (2014) and this study was compared to indicate recovery after five years. In Brgy. Alimasag, a total of .332 m²/ha were recorded occupied by mangroves indicating regrowth one year after the super typhoon. Resprouting from defoliation, stumps, and fallen trunks with sprouting meristems were included in the sampling. Otherwise, shoots were not included and considered dead if the trunk was cut, fallen, and uprooted but with no signs of sprouting. As there are no follow-up studies on the status of mangroves, monitoring their conditions entails ecological succession.

After five years, the basal area occupied by mangroves had reached 361.57 m²/ha, dominated by *S. alba, A. marina, A. floridum, R. mucronata, and R. stylosa*. Compared with



GIZ's (2014) study, mangrove density flourished, indicating natural regeneration without human-assisted intervention. Much is attributed to S. alba, from 0.016 $m^2$/ha in 2014 to 105.7 $m^2$/ha in 2019, indicating slow sprouting after one year, with few mangrove stands exhibiting regeneration. After five years, more mangrove trees had shown regrowth, including large trees, stumps cut, and trunks fell had already displayed recovery. Most of the mangroves did not show an indication of regrowth in 2014 but exhibited recovery after five years.

Similar to A. marina, 0.077 $m^2$/ha in 2014 to 93.71 $m^2$/ha in 2019, had shown natural recovery indicating a high increase in basal area. Though regrowth was not immediate, natural recovery patterns allow previously thriving species to regenerate and restore themselves, if not the same systems as before, may mirror natural succession mechanisms that may be recovering to their original system or, maybe, deviate into a new one.

Much is attributed to *A. floridum*, which did not manifest regrowth one year after Haiyan but flourished after five years. This implies the dawdling recovery of *A. floridum* compared to other mangrove species. If there would be stands of *A. floridum* before the disturbance, leaving defoliated trunks, cut, and fallen stumps should be given time to recover and resprout in their means prior to human-assisted rehabilitation efforts. Mangrove planting after Haiyan was so rampant as Philippine Government allocated many funds for mangrove planting in the pretense of restoration efforts. Without considering the natural succession patterns and replacing natural thriving species after disturbance, human-assisted restoration efforts may divert functional systems that had been established prior to the disturbance.

## Conclusion

After five years, mangroves reappeared: *Acanthus ilicifolius, Acanthus volubilis, Aegiceras floridum, Avicennia rumphiana, Breyia vitis ideae, Bruigera cylindrica, Ceripos tagal, Lumnitzera littorea, Lumnitze raracemosa, Pemphis acidula, Rhizophora stylosa, Sonneratia alba* and *Xylocarpus granatum* that were not identified in the study of GIZ (2014). S. alba, A. marina, and *A. floridum* have the highest basal area signifying natural regeneration among defoliated stands, cut, fallen, and uprooted trunks five years after Haiyan. Mangroves should be given time for regrowth and establish natural regeneration and colonization. Natural succession patterns should be considered in human-assisted rehabilitation after disturbance.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Natural Regrowth of Mangrove Five Years

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