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CONTENT
KANDUNGAN

Page
Muka
Surat

ORIGINAL ARTICLES

BORNEO SCIENCE

A JOURNAL OF SCIENCE AND TECHNOLOGY

REVIEW ARTICLE

BORNEO bsj SCIENCE
THE JOURNAL OF SCIENCE & TECHNOLOGY

Journal homepage: https://jurcon.ums.edu.my/ojums/index.php/borneo-science

A SYSTEMATIC REVIEW OF COERCIVITY MEASUREMENTS IN THE LIFT OF EFFECT FOR NON-DESTRUCTIVE TESTING METHOD

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Magnetic coercivity measurement; Lift-off effect; Magnetic materials; Non-destructive testing; Systematic literature review

ABSTRACT. *Coercivity refers to the intensity of the reverse magnetic field required to demagnetize a material after it has been magnetically saturated. It serves as a crucial indicator of the hardness and stability of magnetic materials. However, the accuracy of coercivity measurements can be compromised by the "lift-off effect," an error caused by air gaps between the sensor and the material surface. This paper proposes a novel method to address this issue by incorporating additional inductance measurements and developing a robust calibration technique. The rationale behind this approach lies in the observation that variations in air gaps influence both coercivity and inductance values. A systematic literature review was conducted following the PRISMA framework to better understand the current state of research and the implementation of such techniques. Searches across ScienceDirect, Scopus, and ProQuest databases yielded 136 documents published between 2014 and 2024 under the keywords: "lift-off effect," "coercivity measurement," "magnetic materials," and "non-destructive testing." Of these, only 17 were original research articles. The review highlights the United Kingdom as the leading contributor to research on reducing the lift-off effect in magnetic plate testing. Collaborations between the United States, Korea, and Poland follow this. The focus of recent studies includes the use of auxiliary inductance data, multi-frequency induction methods, metallic spherical geometry testing, sensor modeling through equivalent parameters, and fibre-optic eddy current sensors for defect detection in magnetic materials. Despite emerging approaches and technological advancements, current research remains fragmented and primarily concentrated on specific sensor designs and material types. There is a notable lack of comprehensive frameworks integrating auxiliary inductance measurements with systematic calibration methods to mitigate the lift-off effect across diverse testing environments. This highlights a critical gap in bridging theoretical insights with practical, scalable applications—an area where further research is urgently needed.*

INTRODUCTION

Coercivity measurements are widely used in industrial applications because they reflect the properties and structural integrity of materials. Coercivity is sensitive to changes in the microstructures of materials and may detect degradation or creep within them (Kikuchi *et al*., 2009; Liu *et al*., 2016; Rumiche *et al*., 2008). Coercivity in stainless steel trends towards Vickers hardness and indicates changes in mechanical properties during the quenching process (Kikuchi *et al*., 2020). The magnetic characteristics of high-carbon steel, such as coercivity and remanence, are connected in three distinct creep stages (Mitra *et al*., 2007), allowing for the non-destructive assessment of interior damage. Coercivity can reflect damage caused by high temperatures and estimate the remaining life of the alloy (Lyu *et al*., 2023).

However, the presence of air gaps between the probe and the test materials negatively impacts the precision and accuracy of coercivity measurement results (lift-off problem) (Stupakov, 2013). This phenomenon poses a serious threat to the coercivity measuring method. Insulation shields, non-standard activities, or abrasive contact surfaces can all contribute to the contact issue. Variations in the gap significantly impact the processes of demagnetization and magnetization (Thomas *et al*., 2012). To accurately estimate the zero-magnetization state, it is also necessary to consider electromagnetic noise effects (Matyuk *et al*., 2003). Many studies aim to minimize or even completely eliminate the lift-off effect. These studies can be divided into two groups: the first aims to reduce the sensitivity of the measuring probe to air gaps (Mazaheri-Tehrani & Faiz, 2022), while the second addresses the lift-off effect on measurement results (Bida, 2010).

This work aims to reduce the lift-off impact on measurement findings. When processing data, it is important to consider factors like magnetic induction and magnetic reluctance, which are very sensitive to fluctuations in the air gap (Balakrishnan *et al*., 1997; Stupakov *et al*., 2010). Conventional coercivity measurements require proximity between the sensor and the sample. However, the sample is always shielded during industrial measurements, and it is expensive to remove and replace the insulating shells. Therefore, using a specially made probe to measure the inductances caused by the air gap would be considerably easier.

The actual coercivity measurement of the sample can be deduced from the measurement result with the air gap by focusing on the relationship between observed coercivity and mutual inductance in the complete magnetic loop when lift-off is present (open-loop measurement). The connection between mutual inductances and coercivity is revealed by measuring the variation tendencies of coercivity and inductances with increasing air gaps for specified samples. The curve can be used to determine a sample's base coercivity from a single coercivity measurement at a random air gap (0 - 15 mm) by extrapolating this connection to subsequent samples. This paper critically evaluates the existing literature and identifies areas for further exploration, clarifying the current research landscape and highlighting promising avenues for future investigation.

LITERATURE REVIEW

Coercivity Measurements Based on Pulse Excitation

Principles and components

Coercivity refers to the strength of the reverse magnetic field required to demagnetize the sample to zero after it has been magnetized to saturation. The coercivity meter created by the University of Manchester's EM sensing group served as the basis for this article. As shown in Figure 1, the coercivity meter consists of a primary device and a measuring probe.

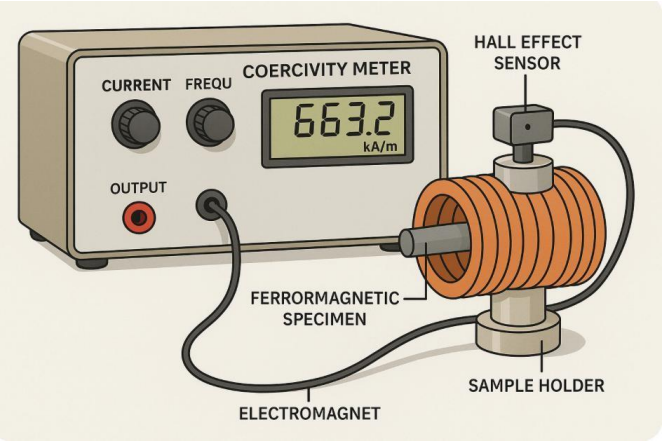

Figure 1. Coercivity meter.

Figure 2 shows the internal structure of the sensor probe, composed of a U-shaped iron core, excitation windings, and Hall-effect sensors. The U-shaped iron core's 25 mm thickness and 64 mm limb allow the magnetic flux to flow through it, greatly minimizing flux leakage. To magnetize the sample being tested to the point of saturation, excitation windings are designed to produce a magnetic field. Numerous magnetic sensors are available to quantify the target sample's magnetic characteristics (Lenz & Edelstein, 2006; Lenz, 1990). Hall-effect sensors are ratiometric devices that can record variations in the strength and direction of magnetic fields. They support wide working bands (10 - 1000 Gauss) (Lenz & Edelstein, 2006; Lenz, 1990; Ramsden, 2011). The iron yoke and the surface of the substance being tested are near the Hall-effect sensors, which are affixed at the tips of each limb. The strength of the magnetic field exerted on the sample is related to the magnitude of the current passing through the sensor.

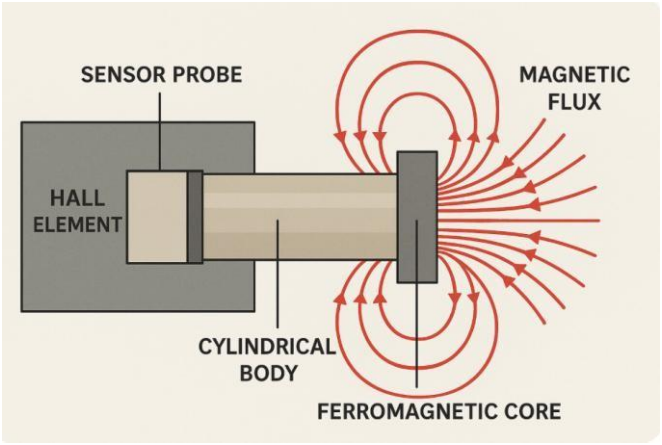

Figure 2. Internal structure of the sensor probe and magnetic flux distribution.

System description

Figure 3 depicts the magnetizing process and the position of the magnetizing stage during the measurement process. The entire measurement process can be divided into the magnetizing process and the coercivity measuring process. During the magnetizing process, the tested samples are subjected to the hysteresis loop. The measurement process starts by triggering the high-voltage excitation module and applying a high-level voltage of up to 350 V to the excitation winding, causing a pulse excitation that magnetizes the sample to reach saturation point 2. The demagnetizing module is turned on after the excitation module is switched off when the voltage output of the Hall sensors reaches its maximum value. The sample is subjected to an opposite-polarity magnetic field by the demagnetizing current,

which demagnetizes it to saturation point 3. The entire procedure, applicable to all materials, takes 3.5 seconds. The examined sample returns to retentivity point 4 when the pulse excitation current is turned off, which also causes the externally applied magnetic field to revert to zero. The coercivity measurement procedure starts when the magnetizing process is complete.

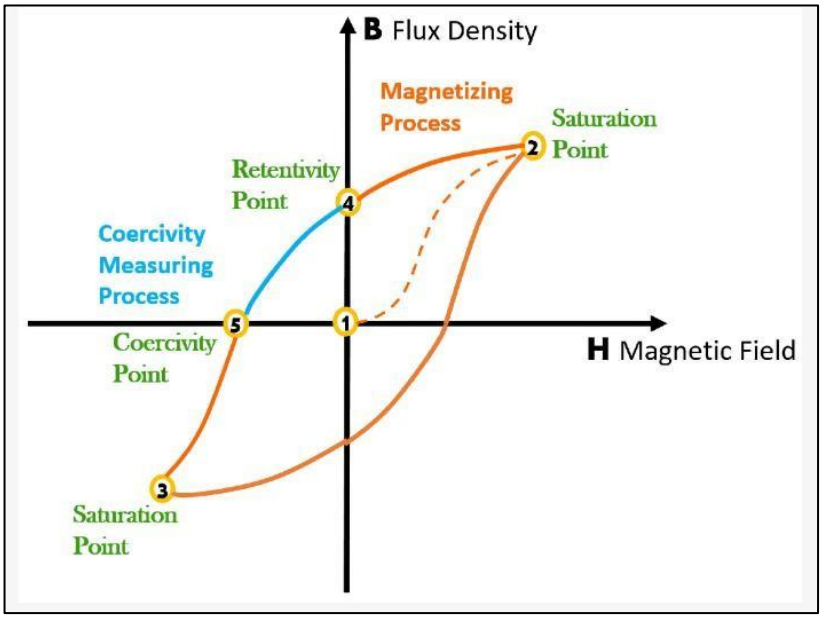

Figure 3. Coercivity meter's magnetizing process.

To measure coercivity, samples are first magnetized to a coercivity point. The coercivity is then calculated by measuring the current flowing through the winding. The material fluctuates near the coercivity point during the measurement procedure, alternating between magnetizing and demagnetizing modes and effectively tracing a very small minor loop. This is accomplished by using Hall sensor feedback to deliver a DC-biased small AC current to the excitation winding. When the sample reaches the coercivity point, the sensor output does not drop to zero. As shown in the internal structure in Figure 4, the non-zero output will be sent to the Hall sensor monitor and used to select the module connected to the windings.

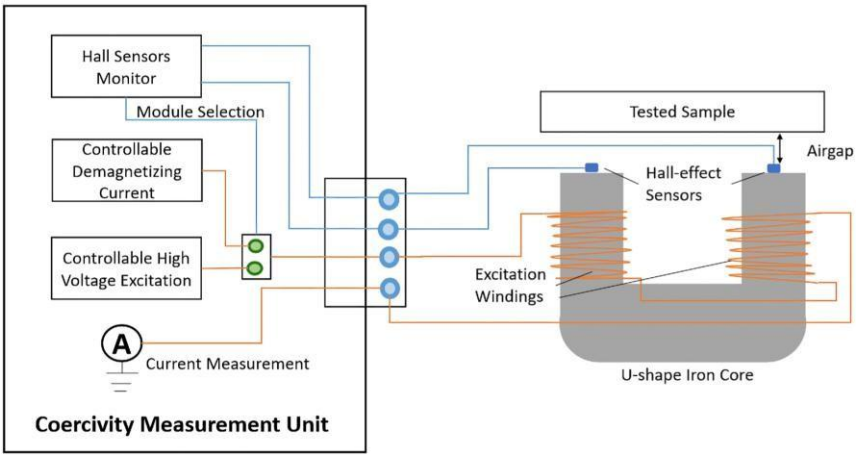

Figure 4. Internal modules of the coercivity meter (Source: Lyu *et al.,* 2023).

Coercivity- Mathematical Approach

The coercivity in magnetic materials is generally defined as the magnetic field intensity (H_C) required to reduce the magnetization (M) of a material to zero after it has been saturated. However, when considering lift-off (i.e., an air gap between the sensor and the material), this introduces a geometric error in measurement, particularly in Eddy current and inductive methods. Therefore, important equations relevant to coercivity and lift-off are given as in equation 1.

$$H_C = H_{M=0} \tag{1}$$

where H_C= coercivity, M = Magnetisation and H= Magnetic field strength.

As for the effect of Lift-Off in Eddy-Current Testing (EC), Lift-Off affects the impedance Z of the sensor due to an increasing air gap (d). The general impedance in Eddy current testing is given as equation 2.

$$Z(d) = R\,(d) + j\omega L(d) \tag{2}$$

where R(d) is the resistance dependent on lift-off distance d; L(d) is the inductance dependent on lift-off, and ω is the angular frequency of the excitation field. As d increases, L(d) decreases, which leads to an incorrect estimation of coercivity if not compensated.

Hence, calibration correction using inductance variation is needed to correct for lift-off in coercivity measurement, which is given as equation 3.

$$H_c^{corrected} = H_c^{measured} + k \cdot \Delta L(d) \tag{3}$$

where k = calibration constant, $\cdot\ \Delta L(d)$ = change in inductance due to lift-off; $H^{measured}$= uncorrected coercivity.

While the sensor signal attenuation due to lift-off is given as equation 4.

$$S(d) = S_0 + e^{-\alpha d} \tag{4}$$

where $S(d)$ = sensor signal at lift-off distance, d; $S_0 =$ signal at zero lift-off; α = decay constant.

Figure 5 shows the sensor signal attenuation due to lift-off. The key takeaway is that lift-off introduces systematic errors that attenuate the magnetic signal and shift the measured coercivity. Compensation can be achieved by analyzing how inductance or signal strength decays with the air gap and using this relationship for calibration.

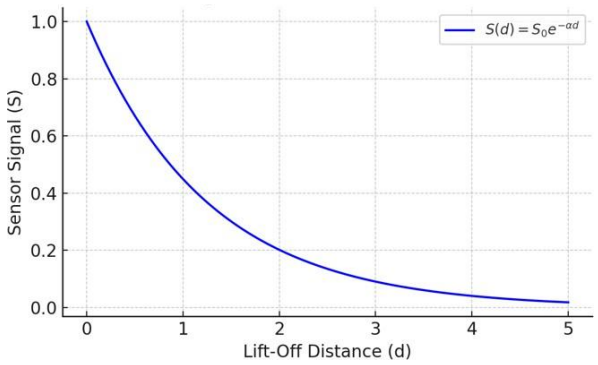

Figure 5. The sensor signal attenuation due to lift-off.

MATERIALS AND METHODS

Article Strategy

The article search for the systematic literature review (SLR) is based on four types of academic databases: Science Direct, Scopus, and ProQuest. These databases were used because they include all journal articles from various sources and are credible. Gusenbauer and Haddaway (2020) stated that the selection of the four base resources includes "search engines," which assist in obtaining appropriate and high-quality studies for systematic research. The search was also implemented with the keyword settings "Lift-off Effect," "Coercivity Measurement," "Magnetic Materials," "Non-Destructive Testing," and "Magnetic Coercivity Measurement" in all three databases. Table 1 shows the keywords used in the article selection process.

Table 1. Article search for SLR database.

Database	Keyword
	"Lift-off Effect"
Science Direct	"Coercivity Measurement"
Scopus	"Magnetic Materials"
ProQuest	"Non-Destructive Testing"
	"Magnetic Coercivity Measurement"
	TITLE-ABS-KEY /Article Title – Abstract - Keywords

Article Selection Criteria

A methodical procedure has been used to filter the search results to obtain accurate and pertinent articles for the research inquiry, as shown in Figure 6. The initial screening stage involves applying acceptance and rejection criteria. The criteria have been meticulously outlined in five steps, encompassing factors such as publication date, reference material, language, methodology, and the field of study of the journal article. This approach ensures that the selected journal article pertains to neuroscience education in the context of online learning, specifically for the SLR objective.

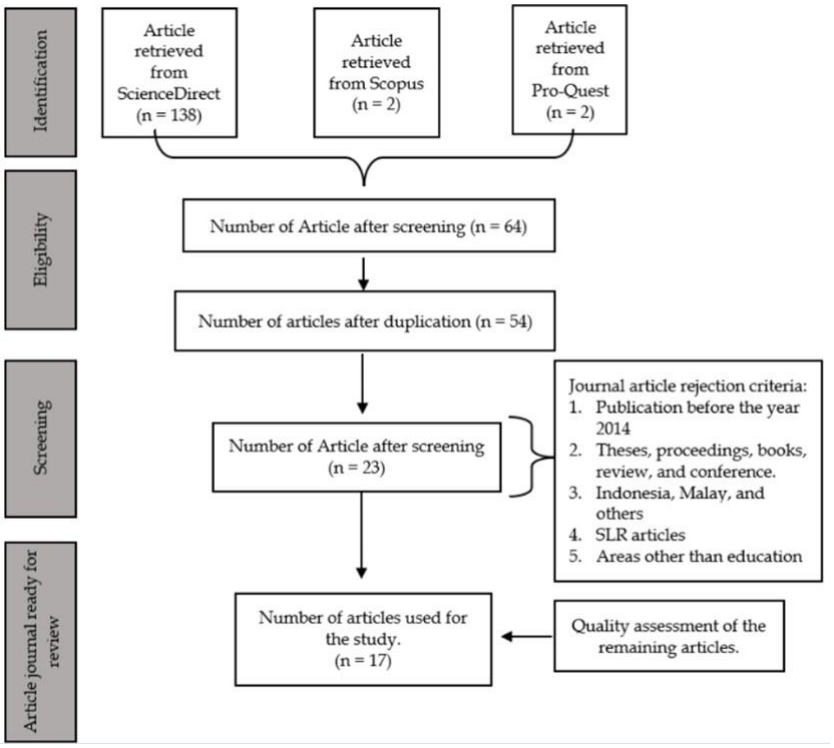

Figure 6. Article Selection Process Flow

The study follows a five-step process to select relevant articles for analysis. The first step involves choosing articles published between 2014 and 2024 to find new information about coercivity measurement and lift-off. The ten-year range is selected to limit the search. In the second step, articles with the same findings or those published repeatedly in different databases are excluded. Only journal articles are selected, while SLR articles, books, proceedings, theses, reviews, and conference papers are excluded. In the third step, only items published in English are selected, and the chosen articles are carefully read for analysis in the fourth step. Any journal article in Indonesian, Malay, or any other language other than English is excluded. The research methods used in each journal article include quantitative, qualitative, and mixed methods. In the fifth step, the study focuses on the areas of "lift-off effect," "coercivity measurement," "magnetic materials," "non-destructive testing," and "magnetic coercivity measurement." Table 2 outlines the acceptance and rejection criteria for journal articles.

Table 2. Article acceptance and rejection criteria.

Database	Acceptance	Rejection
Year of publication	Publication from 2014 - 2024	Publications before 2014
Type of reference material	Journal articles	Theses, proceedings, books, reviews, and conferences
Language	English	Malay, Indonesian, Thailand, and others
Methodology	Quantitative, Qualitative, Mixed method	Systematic Literature Review
Field of study	Non-Destructive Testing	Others
Level	Higher learning Institution Professional Development	

Article Selection Progress

Science Direct, Scopus, and ProQuest are three reputable databases utilized in the study to locate pertinent publications addressing the research topics. Each article's title, abstract, and content were carefully examined to ensure they met the research criteria before selection. The chosen articles were processed and analyzed for data. The PRISMA flow diagram (Moher *et al.,* 2009) is depicted in Figure 6 and describes the steps involved in finding and evaluating publications. In total, 17 papers from various databases were located and used.

RESULTS AND DISCUSSIONS

Out of the 23 publications initially reviewed, 17 met the inclusion criteria following a rigorous screening and quality assessment process. The excluded articles were removed due to insufficient relevance, methodological weaknesses, or duplication. The 17 selected studies, summarized in Table 3, include key details such as publication year, research focus, authorship, and country of origin. These works provide valuable insights into developing non-destructive testing (NDT) methods, particularly in reducing the lift-off effect in coercivity and magnetic property measurements.

The United Kingdom emerged as the leading contributor in this research area, producing six of the 17 selected publications (Figure 7). These studies predominantly focus on advancing sensor technologies, optimizing testing models, and mitigating the lift-off effect through novel techniques. A notable example is the study by Hu (2023), which utilized eddy current techniques for the electromagnetic NDT of metallic spherical geometries. Earlier, Lu *et al*. (2018) explored the reduction of lift-off effects on permeability measurement using multifrequency induction data, setting a precedent for technical innovation in this field. Among the most relevant to this paper is the work by Lyu *et al*. (2023), who proposed a novel approach integrating auxiliary inductance data and a calibration method to reduce lift-off errors in coercivity measurement. Their methodology

aligns closely with the objectives of the present study, particularly in addressing how air gap variations impact both inductance and coercivity readings.

Table 3. List of articles analysed in a systematic literature review.

No	Year	Title	Author	Research on	Country
1	2018	Reducing the Lift-Off Effect on Permeability Measurement for Magnetic Plates from Multifrequency Induction Data	Lu *et al.* (2018)	Lift-off variation causes errors in eddy current measurement of nonmagnetic plates as well as magnetic plates. For nonmagnetic plates, previous work has been carried out to address the issue	The United Kingdom
2	2019	Non-destructive testing on creep-degraded 12% Cr-Mo-W-V ferritic test samples using Barkhausen noise	Gupta *et al.* (2019)	The MBNenergy method is employed for evaluating the microstructural changes induced by creep/ageing of high chromium steel subjected to different creep test conditions as stress and temperature.	France & Japan
3	2019	A fast and non-destructive method to evaluate yield strength of cold-rolled steel via incremental permeability	Li *et al.* (2019)	This paper describes the relationship among IP, eddy-current (EC) impedance and microstructure, and discovered a new IP feature that indicates materials' average grain size and lattice friction.	China
4	2022	Magnetic indicators for evaluating plastic strains in electrical steel: Toward non-destructive assessment of the magnetic losses	Zhang *et al.* (2022)	In this study, a setup was designed to stimulate the magnetization mechanisms separately while maintaining the same experimental conditions. The magnetization processes related to the domain wall kinetics were revealed to be more correlated to plastic strain.	France & Japan
5	2022	An evaluation of non-destructive methods for detection of thermally-induced metallurgical machining defects.	Brown *et al.* (2022)	Introduces a recently developed x-ray diffraction method is shown to be capable of detecting thermally induced white layers formed during hard turning, as well the identification of grinding-induced rehardening and tempering.	The United Kingdom
6	2022	Research progress on magnetic memory nondestructive testing.	Xu *et al.* (2022)	This paper systematically reviews the progress of magnetic memory research in the past 20 years in terms of theoretical studies on the coupling of force-magnetic effects, factors influencing weak magnetic signals, defect identification, and quantification studies.	China
7	2023	Non-destructive surface and subsurface characterization of the machined parts by using a fiber optic Eddy current sensor	Kim *et al.* (2023)	Introduces a fiber-optic eddy current sensor (FECS) to enable non-destructive surface and subsurface characterization of the subtractive or additive manufactured metal parts practical way of using the method of evaluating the metrological properties of eddy current sensors.	The United States & Korea

Continue **Table 3.** List of articles analysed in a systematic literature review.

No	Year	Title	Author	Research on	Country
8	2023	Evaluation of the Properties of Eddy Current Sensors Based on Their Equivalent Parameters	Dziczkowski & Tytko (2023)	A practical way of using the method of evaluating the metrological properties of eddy current sensors. The idea of the proposed approach consists of employing a mathematical model of an ideal filamentary coil to determine equivalent parameters of the sensor and sensitivity coefficients of tested physical quantities.	Poland
9 *	2023	A Novel Method for Reducing the Lift-Off Effect in Coercivity Measurement through Auxiliary	Lyu *et al.* (2023)	This paper proposes a new method to address this issue by incorporating additional inductance measurements and formulating a calibration method. The calibration principle is based on the fact that both the coercivity and the inductance measurements change with the variation of air gaps.	The United Kingdom
10	2023	Application of Eddy Current Techniques in Electromagnetic Non-Destructive Testing of Metallic Spherical Geometry	Hu (2023)	Introduces two strategies to counteract the lift-off effect. Firstly, a method leveraging a linear eddy-current characteristic identifies the ball's diameter without contact. A key insight is the peak frequency feature relating to the lift-off spacing between the coil's center and the ball.	The United Kingdom
11	2023	Non-destructive evaluation of magnetic anisotropy associated with crystallographic texture of interstitial free steels	Jolfaei *et al.* (2023)	The findings indicate that the non-destructive technique deployed in this study - a U-shaped electromagnetic (EM) sensor that can be placed onto a sheet specimen - is promising for a rapid assessment of the magnetic anisotropy in IF steels.	The United Kingdom & Netherland
12	2023	Coercivity modulation of FeCoCrMoTi films by artificial magnetic phase defects engineering based on multilayer structure	Liu *et al.* (2023)	Demonstrated an in-plane anisotropic [Ta(20nm)/FeCoCrMoTi(100 nm)]2/Ta(10 nm) multilayer film (M20 sample) with a high coercivity of 945 Oe and a sufficient remanent magnetization of 2890 Oe, which is 92.1% and 63.1% higher than FeCoCrMoTi(200 nm)/Ta(10 nm) film (SL sample), respectively.	China
13	2024	Non-destructive testing of ferromagnetic steel components based on their magnetic response	Ducharne (2024)	Introduces the non-destructive testing (NDT) methods based on the magnetization mechanisms. Then, the targeted properties, that is, the specific information required by the industrials or NDT end-users, are described (internal stress, microstructural properties, to name a few).	France

Continue **Table 3.** List of articles analysed in a systematic literature review.

No	Year	Title	Author	Research on	Country
14	2024	Design of a multi-modal sensor for the in-process measurement of material properties based on inductive spectroscopy	Wendler *et al.* (2024)	This paper proposes a robust contactless multi-modal sensor for the in-process measurement of material magnetic properties composed of a central excitation coil surrounded by eight receiving coils.	Germany
15	2024	Characterization on multiphase microstructures of carbon steels using multi-frequency electromagnetic measurements	Shen *et al.* (2024)	This paper studied responses of electromagnetic signals on carbon steels with different phase compositions using a U-shaped and a cylindrical electromagnetic sensor.	The United Kingdom & China
16	2024	Experimental and theoretical study of the harmonic distortion in a ferromagnetic plate excited by a pair of air-cored coils.	Skarlatos & Poulakis, (2024)	This paper studies the harmonic distortion in a ferromagnetic plate. The electromagnetic field inside the specimen is excited by a couple of air-cored coils, and the field is measured at a specific location using a set of Hall sensors.	Greece & France
17	2024	Parametric design methodology for yoke-magnetization in magnetic flux leakage detection systems	Lam *et al.* (2024)	This paper proposes a systematic design procedure for the yoke-magnetization component in MFL systems utilizing permanent magnets.	Vietnam

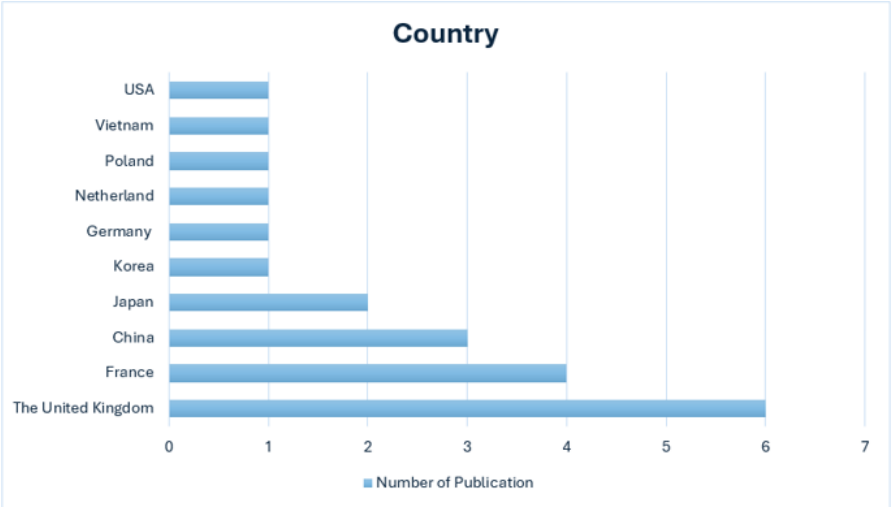

Figure 7. Tendencies of countries in article publications.

Research from France and Japan reflects a different emphasis, often involving experimental designs that isolate specific magnetization mechanisms under controlled conditions. Meanwhile, studies from China tend to centre on the correlation between eddy current impedance, incremental permeability, and material microstructure, highlighting the influence of electromagnetic properties on material evaluation. Poland's contribution introduces a mathematical modelling approach using an ideal filamentary coil to derive equivalent sensor parameters and sensitivity coefficients, indicating a strong theoretical orientation. Conversely, the United States and Korea have collaborated on fibre-optic eddy current sensors for surface and subsurface characterization, showcasing applied sensor innovation in advanced manufacturing contexts. The cumulative literature reflects a global interest in refining NDT techniques; yet gaps remain, particularly in

applying these methods to a broader range of magnetic materials. While current studies largely focus on specific alloys or configurations, there is limited empirical work on coercivity measurements across various materials such as cobalt, nickel, iron, and steel. By incorporating these materials, the present study extends the applicability of lift-off mitigation strategies, contributing novel insights to the field.

CONCLUSION

From 2014 to 2024, there were relatively few published studies on the lift-off effect in coercivity measurements of magnetic materials. The United Kingdom dominated in publishing related to coercivity measurement and the lift-off effect compared to other countries. In conclusion, the systematic review of literature on the lift-off effect in coercivity measurement of magnetic materials highlights the significant influence of non-ideal conditions during testing. The lift-off effect, which arises from the separation between the measurement sensor and the material surface, can lead to inaccuracies in coercivity readings, particularly in soft magnetic materials where precise measurements are crucial. Addressing the lift-off effect is vital for improving the accuracy and reliability of coercivity measurements. Future research should focus on enhancing sensor technology, developing standardized protocols to minimize lift-off variation, and creating robust mathematical models to correct for lift-off-induced errors. Additionally, different materials can also be suggested as samples. By doing so, the precision of magnetic material testing can be significantly improved, leading to better material performance in various industrial applications.

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RESEARCH ARTICLE

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ASSESSING CATALYTIC CONVERTER DEGRADATION IN EMISSION REDUCTION: COMPARATIVE STUDY OF CO, THC, AND NOx ACROSS MILEAGE, ENGINE CAPACITY, AND TRANSMISSION TYPE

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ABSTRACT. *Environmental concerns have led to stricter global emission standards for combustion engine vehicles. This study evaluates the degradation patterns of catalytic converters in reducing CO, THC, and NOx emissions across 20 passenger vehicles with varying mileage (up to 100,000 km), engine capacities (1.2 - 1.5L), and transmission types (manual/automatic). Four catalytic converter designs (Types A-D) were assessed using the New European Driving Cycle (NEDC) methodology for compliance with Indonesian and EURO-4 standards. Results revealed that Type D converters maintained the best performance for CO (0.2 - 0.4 g/km) and THC emissions (0.02 - 0.04 g/km) with stable degradation across their lifespan. Conversely, Type B exhibited significant degradation (degradation factor 9.0) and higher emission levels overall. Statistical analysis showed significant differences in THC emissions between converter types ($p < 0.05$), while differences in CO and NOx were not statistically significant ($p > 0.05$). NOx emissions showed the highest degradation factor (5.78) across all converter types. This research demonstrates the critical relationship between catalytic converter design, vehicle operational parameters, and emission reduction effectiveness over time, providing insights for future catalyst material development and maintenance strategies to maintain long-term environmental compliance.*

INTRODUCTION

Environmental pollution has become a significant global challenge alongside rapid industrialization and technological advancement. Urban populations increasingly fail to meet air quality standards set by the World Health Organization (WHO) (WHO, 2016a), and air pollution continues to rise globally (European Environment Agency, 2016), making it a recognized public health emergency (United States Environmental Protection Agency, 2024). The automotive transportation sector plays a major role in this issue by emitting harmful gases such as carbon monoxide (CO), total hydrocarbons (THC), and nitrogen oxides (NOx) from vehicle exhaust (Walsh, 2011; IEA, 2017; UNECE, 2023).

Governments worldwide have introduced regulations aimed at reducing automotive emissions and promoting environmentally friendly vehicles (Kholod & Evans, 2016; European Commission, 2019). These standards differ across countries based on policy readiness and infrastructure support. The

European Union has set CO_2 emission targets for new cars (Gaikindo, 2020), while Russia emphasizes black carbon reduction due to diesel transport (Kholod & Evans, 2016). Indonesia implemented Euro 4 emission standards in 2018, with permitted levels for CO max 1 g/km, THC max 0.1 g/km, and NOx 0.08 g/km (Republic of Indonesia, 2017), supported by regulations for compliance (UNCAS, 2019; Tempo, 2024) and a roadmap targeting Euro 5 adoption by 2027 (OECD, 2019). However, the success of such standards depends heavily on fuel availability and supporting distribution infrastructure (AEA Technology, 2021).

Emission standards such as Euro 4, 5, and 6 define permissible levels of CO, THC, NOx, and particulate matter (PM) (European Commission, 2007; ACEA, 2014). Euro 5 and Euro 6 introduce stricter limits, necessitating advancements in fuel quality and emission control technologies (ICCT, 2016; van Wee, 2019). Some countries also require an Air Pollution-Health Risk Assessment (AP-HRA) for policies affecting air quality (WHO, 2016b). To meet these regulations, manufacturers deploy catalytic converters that use oxidation and reduction catalysts to convert toxic gases into less harmful substances (Twigg, 2007; Heck *et al*., 2012; Ashok, 2021). These devices are designed to maintain emissions below regulated limits throughout a vehicle's lifespan (Johnson, 2015; Milku *et al*., 2024), but their performance can degrade due to mileage, wear, and varying operating conditions (Li *et al*., 2001; Abdolmaleki *et al*., 2020).

Although catalytic converters are widely used, long-term evaluations of their real-world performance remain limited (Giuliano *et al*., 2020; Barbier *et al.,* 2023). Efficiency loss over time can lead to increased emissions, reducing environmental compliance (Zotin *et al*., 2005). Research has shown that engine output, operating conditions, and fuel quality all influence emission levels. Kim *et al.* (2020) and Kim *et al*. (2021) observed increased PM and NOx with higher engine loads and high CO levels during start-up and acceleration. Other studies highlight the roles of fuel detergency and additives in reducing CO_2, CO, and HC emissions (Kean *et al*., 2003; Zhu *et al*., 2016; Zheng *et al*., 2017; Shabanov *et al*., 2020; Daud *et al*., 2022). Driving behaviour, such as acceleration and deceleration, and road gradient also significantly affect emission levels (André & Rapone, 2009; Pathak *et al*., 2016; Prakash & Bodisco, 2019; Jang *et al*., 2023). Emissions are particularly high during positive acceleration, contributing to increased particle counts (Pelkmans & Debal, 2006; Weiss *et al.,* 2013). Differences between Real Driving Emissions (RDE) and New European Driving Cycle (NEDC) testing further underscore the importance of accurate emission assessments (Fontaras *et al.,* 2014).

Life cycle studies show that emissions and fuel consumption from internal combustion engines significantly affect environmental load (Hawkins *et al*., 2013; Messagie *et al*., 2014; Wu *et al*., 2017; Bieker, 2021), with mileage and maintenance practices being key influencing factors (Sakno *et al.,* 2021). Previous research has established methodological approaches for studying catalytic converter degradation with varying sample sizes. Van der Schoot *et al*. (2001) conducted an experimental study of 24 catalysts (9 with lower mileage under 40,000 km and 15 with high mileage up to 80,000 km), concluding that degradation was primarily caused by thermal deterioration. de Almeida *et al.* (2014) analyzed a single vehicle's catalytic degradation over 30,000 km at 10,000 km intervals, finding degradation factors of 1.40 for CO and NOx emissions and 1.34 for MNHC emissions. Corvalán and Vargas (2003) utilized a database of 160 light-duty vehicles across 9 driving cycle categories, determining that deterioration depends on average speed and accumulated distance.

This study analyzes the degradation of catalytic converter performance in 20 Jakarta-based vehicles with varying engine capacities and transmission types. Using NEDC-based tests under Euro 4 conditions, emissions of CO, THC, and NOx were measured across a mileage range of almost 100,000 km. All vehicles received standard maintenance (European Commission, 2007; Toyota Motor Corporation Australia Limited, 2025), and the findings aim to inform catalytic design improvements and long-term emission reduction strategies (van der Schoot *et al.,* 2001). By examining the comparative performance of different catalytic converter types across multiple parameters, this research addresses a critical gap in understanding how these emission control technologies perform throughout their operational lifetime.

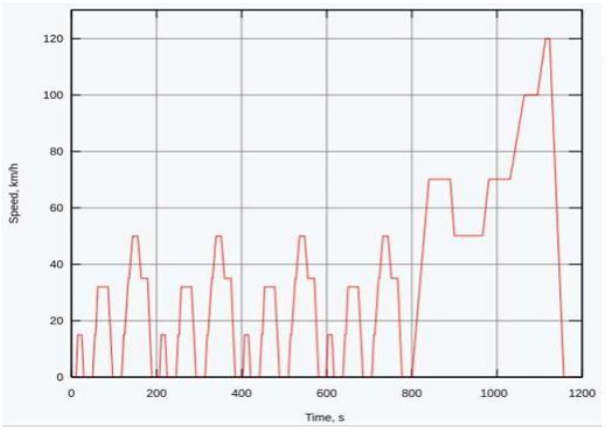
METHODOLOGY

Emission Data

Emission measurement data were collected from the laboratory certified by Vincotte Belgium, which conducted the Euro 4 emission tests. The total number of vehicles was 20 units from various models: AMPV, BMPV, SUV, and Van, consisting of different engine capacities and transmission types. Before conducting the test, it was important to ensure that each vehicle received maintenance treatment. The treatments included standard services such as changing the engine oil, fuel filter, and air filter, as well as draining the remaining fuel from the gasoline tank and replacing it with Euro 4 gasoline.

Experimental Test

The New European Driving Cycle (NEDC) test mode was a driving pattern implemented to assess vehicle emissions and fuel economy, excluding light trucks and commercial vehicles. It is also referred to as the MVEG cycle (Motor Vehicle Emissions Group). It consists of four times ECE-15 urban driving cycles (UDC) and one time Extra-Urban driving cycle (EUDC). The NEDC, intended to represent typical car usage in Europe, has faced repeated criticism for often delivering unachievable economy figures. Some preparations were needed and conducted before the test. Figure 1 shows the test cycle process.



NEDC Test

No	Activity
1	Setting vehicle to Chassis Dyno
2	Fit Exh tail to pipe CVS
3	Setting Cooling fan
3	Setting Operational vehicle data to VET System
4	Setting Road Load data at Chassis Dyno
5	Setting Test Mode Euro4 NEDC
6	Klik Start at PC control Chassis Dyno for Analysis
7	Run NEDC test for 20 Menit then idle rpm 5 menit
8	Evaluation Coasdown for 1 time run

Figure 1. NEDC Test Mode, the ECE-15, Urban Driving Cycle (UDC), and Extra Urban Driving Cycle (EUDC) (Source: van der Schoot *et al.,* 2001).

Data Collection and Analysis

Data collection in this study employed quantitative methods to analyse the performance of various vehicles with various catalytic converter types based on their service life. The research steps are as follows:

Data Collection

Emission measurement data is collected in a laboratory that adheres to the EURO 4 emission test measurement standard. Before conducting the test, the vehicle being evaluated has undergone general maintenance procedures, including the replacement of engine oil, oil filters, air filters, fuel filters, and refuelling with Euro 4 Gasoline. Emission content data collected during testing includes CO (Carbon monoxide), THC (Total hydrocarbons), and NOx (Nitrogen oxides). Testing was conducted on 25 vehicles. 5 vehicles for each catalyst type A, B, C, and D, as well as across a range of some period, driven on each catalyst type. Catalyst A, used for the 1.2-liter engine, the vehicle sample consists of 3

units with automatic transmission and 2 units were manual transmission. Catalyst B, used for 1.3 and 1.5-liter engines with all manual transmission types. Catalyst C is used for the 1.5-liter engine and consists of 4 units of automatic transmission and 1 unit was manual transmission. The last catalytic is D type, consists of 1,3 and 1,5 litres engines, and all of the vehicle samples were manual transmission.

Analysis Procedure

The output of the emission test data was the emission content of CO, THC, and NOx. Software MATLAB version R2024 was utilized to process the data to create the analysis of variance, correlation analysis, and regression. These statistical tests identified any significant differences in emissions among catalysts for CO, THC, and NOx levels. MATLAB was also used to assess and visualize the distribution and variability of emissions for each type of catalyst. The first analysis was an analysis of variance to visualize a graph showing each catalyst's effect on emission gases. The next was a correlation analysis of emission levels toward mileage and engine capacity, as well as a comparison between transmission types through a t-test. The analysis continued with the variance of transmission types and presented a graph to show the tendencies. Next, linear regression was used to analyze the data for each catalyst, and finally, analyses were conducted to examine the degradation of the catalyst through a first-order kinetic reaction equation and the second Arrhenius equation.

Analysis Model

First-order reaction kinetics models and second-Arrhenius-type models were employed to analyze the experimental data on CO, THC, and NOx emissions. By adopting these theoretical frameworks, the objective was to evaluate the degradation behaviour of each catalyst over time and the corresponding emission levels. For CO and THC emissions, a first-order reaction kinetics model was utilized (Ma *et al.*, 2009). This model is typically used to describe processes where the rate of change is proportional to the current value, such as a decline in catalyst efficiency resulting in increased emissions. We can assess how effectively each catalyst maintains low emission levels over the distance traveled by fitting the CO and THC emission data to an exponential model. The fitted models facilitate quantifying the rate of degradation in catalyst performance.

In the case of NOx emissions, an Arrhenius-type model was employed. The Arrhenius equation is commonly used to describe the relationship between chemical reaction rates and temperature; however, in this context, it has been adapted to model the variation in NOx emissions relative to the distance travelled. This approach offers valuable insights into the degradation behaviour of the catalyst, particularly in reducing NOx emissions over extended use. The fitted Arrhenius model enables us to comprehend each catalyst's efficiency decline, which subsequently affects NOx emissions (Wang *et al.*, 2016; Huang *et al.*, 2017).

The resulting graphs for each catalyst type display experimental data and the fitted model. First-order and Arrhenius fits are plotted to compare predicted emission trends over the distance travelled with the observed values. This analysis is conducted to assess the effectiveness of each catalyst type at each specified period. To obtain good analysis results, we use the regression equation in each plot, which helps us read the data on the relationship between distance travelled and emissions for each catalyst type. The graphs generated for each catalyst type include experimental data along with the fitted model. The first-order and Arrhenius fits are plotted to compare the predicted emission trends against mileage with the actual observed values. Regression equations are also presented in each plot, which helps interpret the model parameters and understand the relationship between travel distance and emissions for each catalyst type. This analysis aims to determine the effectiveness of each type of catalyst in minimizing emissions over time, providing valuable information to improve catalytic converter technology.

RESULTS AND DISCUSSIONS

Samples of vehicle catalytic converters from around Jakarta city were obtained from one of the emission testing laboratories certified by the international body. The sample included several indicators: catalytic types, engine capacity, transmission, mileage, and emissions. The emission data presented in Table 1 comprehensively compare CO, THC, and NOx emissions across four different types of catalytic converters (types A, B, C, and D). Meanwhile, the degradation factor of the catalyst was defined by comparing emission concentration data after transitioning from used to new vehicle conditions. Results from previous and current studies are shown in Table 2.

Table 1. Catalytic converter performance test data.

No.	Catalytic Type	Vehicle Data			Emissions (g/km)		
		Engine Capacity (cc)	T/M Speck	Mileage (km)	CO	THC	NOx
1	A	1200	AT	19.792	0,235	0,059	0,015
2	A	1200	MT	23.796	0,386	0,055	0,018
3	A	1200	MT	36.106	0,408	0,047	0,012
4	A	1200	MT	51.617	0,493	0,061	0,018
5	A	1200	AT	72.319	0,502	0,066	0,021
6	B	1300	MT	24.749	0,332	0,048	0,014
7	B	1300	MT	75.068	0,411	0,062	0,027
8	B	1300	MT	91.751	0,697	0,066	0,052
9	B	1500	MT	34.700	0,513	0,048	0,022
10	B	1500	MT	91.702	0,437	0,047	0,024
11	C	1500	MT	918	0,371	0,037	0,007
12	C	1500	AT	8.853	0,489	0,050	0,015
13	C	1500	AT	37.095	0,277	0,062	0,023
14	C	1500	AT	50.317	0,316	0,059	0,028
15	C	1500	AT	60.152	0,259	0,057	0,027
16	D	1500	MT	19.491	0,354	0,028	0,023
17	D	1500	MT	20.215	0,283	0,025	0,034
18	D	1500	MT	62.795	0,303	0,030	0,027
19	D	1300	MT	21.156	0,497	0,041	0,015
20	D	1300	MT	40.164	0,384	0,035	0,015

Table 2. Comparison studies: Degradation Factor**.**

Approach	Deterioration		
	THC	CO	NOx
AP-42	1.17	1.40	1.10
COPERT	1.46	1.44	1.51
Roberto M.	3.23	1.72	3.04
Rodriguez P.	1.34*)	1.40	1.40
Present Study	1.36	1.88	5.78

Note: *) MNHC

Catalyst Performance

Figure 2A illustrates the distribution of carbon monoxide (CO) emissions. The Type B converter catalyst shows less than optimal results in reducing CO emissions, indicated by results between 0.4 to 0.6 g/km. This result is inversely proportional to the Type D catalyst, which shows the lowest CO emission level at 0.2 to 0.4 g/km. It also indicates that this converter is the most effective in minimizing CO compared to other types. Type A has moderate CO emissions, while Type C demonstrates a relatively narrow distribution with values lower than Type B but higher than Type D. These data take one test per vehicle after conducting maintenance treatments to refresh engine combustion performance as a standard, such as replacing engine oil, changing filters, and cleaning the combustion chamber.

Figure 2B focuses on total hydrocarbon (THC) emissions. Type D performs the best again, with emissions tightly clustered between 0.02 and 0.04 g/km and a significantly lower median value compared to other types. In contrast, Type A shows a moderately wide distribution, with THC emissions ranging from 0.04 to 0.06 g/km. Type B has a similar but slightly wider range of THC emissions, indicating inconsistent performance. Type C shows a moderate improvement over Types A and B, with emissions ranging from 0.03 to 0.05 g/km. Figure 2C presents the NOx emissions for each catalytic converter type. Type A converter catalysts show quite optimal results in reducing NOx emissions, indicated by a value between 0.01 to 0.03 g/km; the same occurs for Type D converter catalysts. However, unlike Type A and D catalysts, Type B catalysts show less favorable results, with the efficiency level of this catalyst on NOx emissions ranging from 0.02 to 0.04 g/km. Type C has a distribution similar to Type B, with a slightly higher median value, reflecting moderate but not exceptional performance.

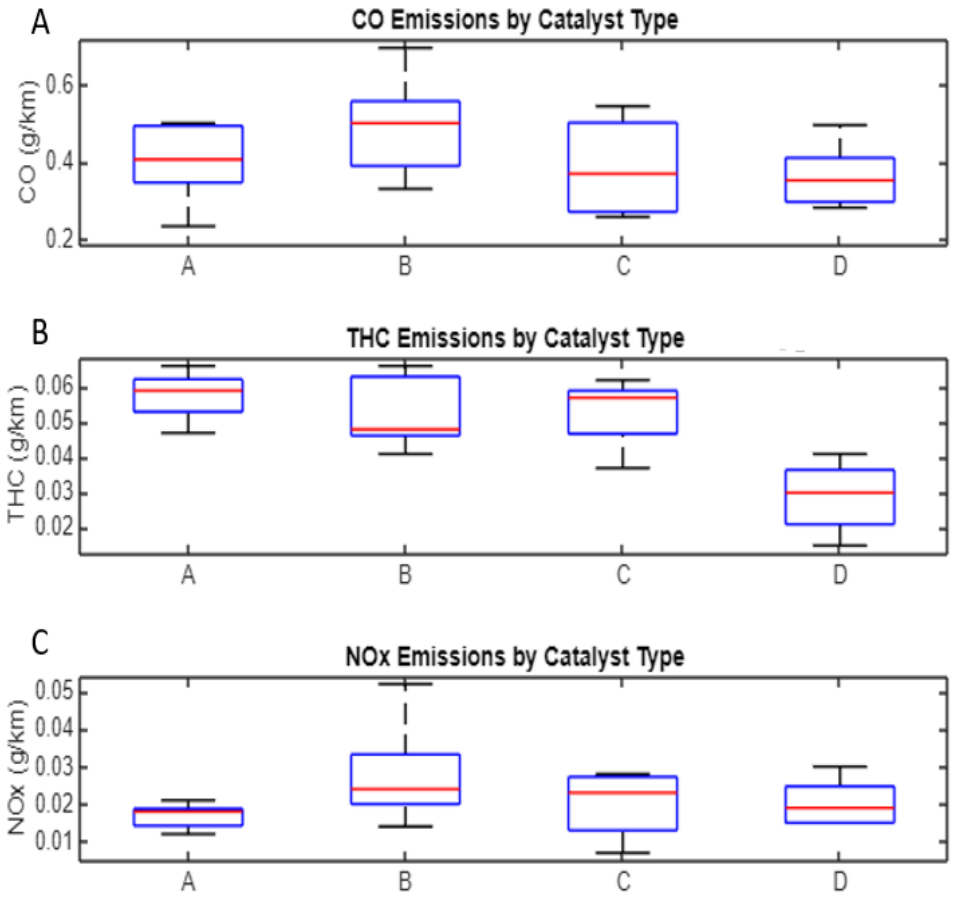


Figure 2. Performance of each type of catalyst: (A) against CO emissions; (B) against THC emissions; and (C) against NOx emissions.

Emissions Trend as a Function of Vehicle Mileage

Figures 3A, 3B, and 3C illustrate the trends in CO, THC, and NOx emissions as a function of vehicle mileage. In Figure 3A, there is a positive but not statistically significant correlation between mileage and CO emissions, with a correlation coefficient (r) of 0.361 and a p-value of 0.118. This suggests a mild upward trend in CO emissions as mileage increases, but the relationship is not strong. Figure 3B shows different results, where a significant positive correlation between distance traveled and THC emissions, with r = 0.452 and p = 0.045, indicates that THC emissions tend to increase with longer distances. Figure 3C shows a strong and significant relationship between distance traveled and NOx emissions, with r = 0.632 and p = 0.003, indicating that NOx emissions increase significantly with greater distance.

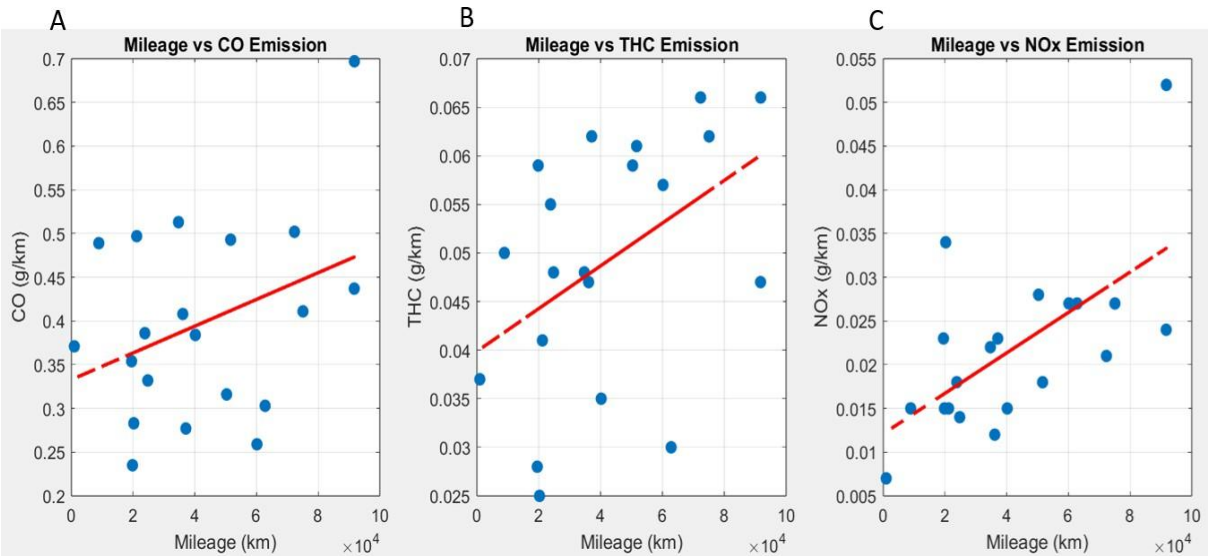


Figure 3. Illustration of emissions trend as a function of vehicle mileage: (A) against CO emissions; (B) against THC emissions; and (C) against NOx emissions.

As shown in Table 3, the correlation analysis between engine capacity and emissions reveals varying relationships. CO emissions have a negative correlation with engine capacity (r = -0.275) but are not statistically significant (p = 0.241). THC emissions also show a negative correlation with engine capacity (r = -0.427), with a p-value of 0.060, indicating a trend where larger engines might produce lower THC emissions, although this is only marginally significant. NOx emissions, however, have a weak positive correlation with engine capacity (r = 0.197) and are not statistically significant (p = 0.405). These results imply that engine capacity may not be a strong predictor of CO and NOx emissions, but could have a modest influence on THC emissions.

Table 3. Correlation of emissions against mileage and engine capacity.

Relation	CO		THC		NOx	
	r-value (correlation coefficient)	p-value	r-value (correlation coefficient)	p-value	r-value (correlation coefficient)	p-value
Mileage	0.361	0.118	0.452	0.045	0.632	0.003
Engine Capacity	-0.275	0.241	-0.427	0.060	0.197	0.405

Emissions Trend as a Function of Engine Capacity

Figure 4A shows the relationship between engine capacity (measured in cubic centimeters, cc) and CO emissions. The graph line shows a negative correlation, meaning CO emissions tend to decrease with increasing engine capacity. Although the graph shows a relatively large spread of variability, this aligns with the correlation analysis, where a weak negative correlation was identified between engine capacity and CO emissions ($r = -0.275$, $p = 0.241$) (see Table 3). However, the downward slope of the graph line implies that vehicles with large engine capacities can emit lower levels of CO. In Figure 4B, the scatter plot shows the correlation between engine capacity and THC emissions. The trend line shows a negative correlation: THC emissions tend to decrease with increasing engine capacity. The data points show less variability than CO emissions, and the downward trend is more pronounced. This aligns with the correlation coefficient of -0.427 ($p = 0.060$) (see Table 3), which indicates a stronger negative relationship, although slightly significant, between engine capacity and THC emissions. These results confirm that vehicles with larger engines are more efficient in reducing THC emissions. Figure 4C illustrates the relationship between engine capacity and NOx emissions. The trend line in this scatter plot indicates a weak positive correlation, with NOx emissions slightly increasing as engine capacity grows. However, the distribution of data points is highly scattered, and the correlation is not statistically significant, as indicated by a correlation coefficient of 0.197 ($p = 0.405$) (see Table 3). This weak relationship suggests that engine capacity does not substantially impact NOx emissions.

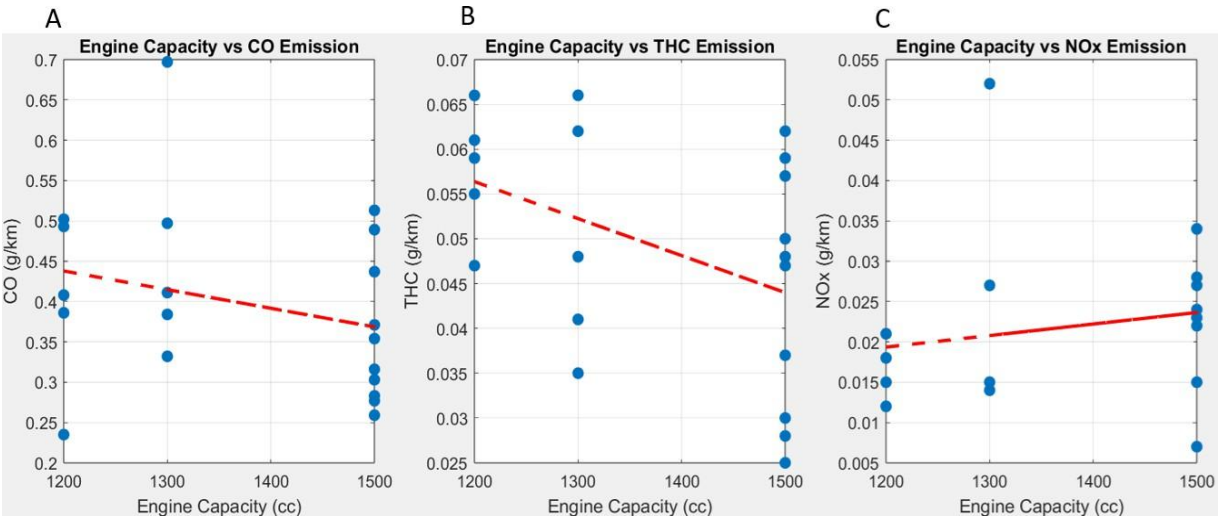


Figure 4. Illustration of emissions trend as a function of engine capacity: (A) against CO emissions; (B) against THC emissions; and (C) against NOx emissions.

Emissions Trend as a Function of Transmission Type

The analysis of the impact of transmission type (AT vs. MT) on emissions shows notable differences. As shown in Table 4, the t-test results indicate that CO emissions are higher in manual transmission (MT) vehicles (mean: 0.419 g/km) compared to automatic transmission (AT) vehicles (mean: 0.346 g/km), though the difference is not statistically significant ($t = -1.361$, $p = 0.190$). For THC emissions, the t-test reveals a significant difference ($t = 2.482$, $p = 0.023$), with AT vehicles producing higher THC emissions (mean: 0.059 g/km) compared to MT vehicles (mean: 0.045 g/km). NOx emissions, however, show no significant difference between AT and MT transmissions, with identical means of 0.022 g/km and a t-value of -0.103 ($p = 0.919$).

Figure 5A shows the distribution of CO emissions for vehicles with AT and MT. The box plot reveals that cars with manual transmissions (MT) generally have higher CO emissions than those with automatic transmissions (AT). The median CO emission for MT vehicles is greater than that of AT vehicles, with a wider interquartile range, indicating more variability in CO emissions among MT

vehicles. This observation supports the statistical analysis, which suggests a difference in emission performance based on transmission type, although it is not statistically significant for CO emissions (t = -1.361, p = 0.190). Figure 5B explains the THC emission picture for AT and MT transmission vehicles. The box plot highlights a significant difference: AT transmission vehicles show higher median THC emissions compared to MT transmission vehicles. The interquartile range for AT is narrower, indicating a less frequent emission distribution, while MT vehicles show a more spread-out THC emission distribution. The t-test confirms this difference as statistically significant (t = 2.482, p = 0.023), suggesting that AT vehicles are less effective in controlling THC emissions.

Figure 5C presents the NOx emission levels for AT and MT vehicles. Both transmission types display similar NOx emission values, with overlapping medians and comparable interquartile ranges. The distribution indicates minimal variability, and the t-test results confirm that there is no statistically significant difference between the two transmission types for NOx emissions (t = -0.103, p = 0.919).

Table 4. Relation transmission type against emission.

Transmission	Mean Value		
	CO	THC	NOx
AT	0.346	0.059	0.022
MT	0.419	0.045	0.022
Comparison AT vs MT [t-test result]	t = -1.361 p = 0.190	t = 2.482 p = 0.023	t = -0.103 p = 0.919

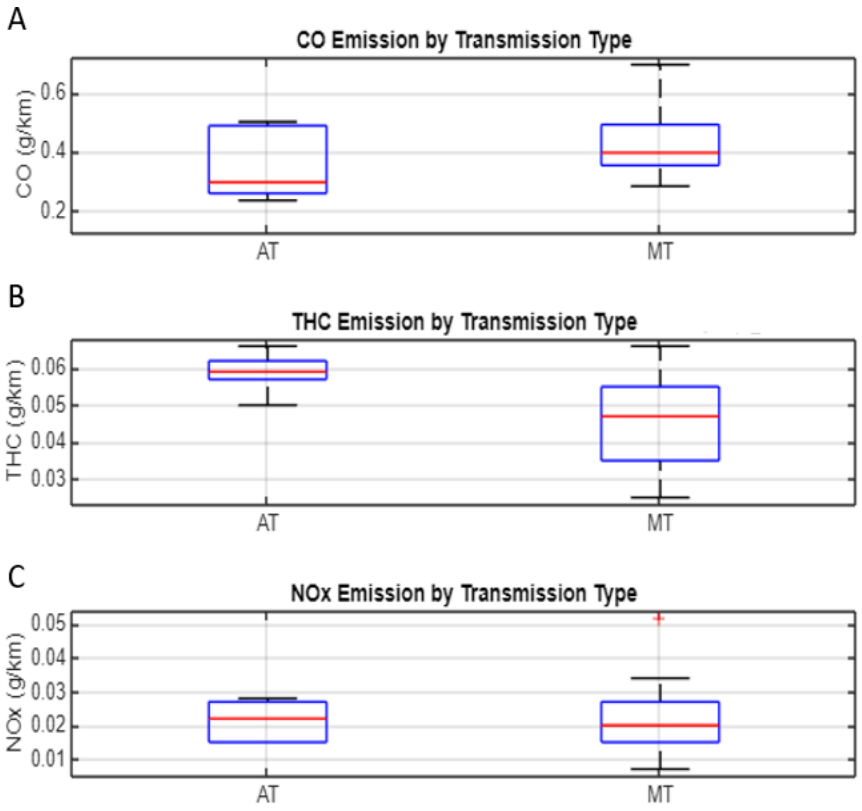


Figure 5. Distribution of emissions of each type of transmission: (A) CO emissions; (B) THC emissions; and (C) NOx emissions.

CO Emissions as a Function of Catalytic Converter Type, Engine Capacity, and Vehicle Mileage

Figures 6A, 6B, and 6C illustrate the analysis of carbon monoxide (CO) emissions concerning catalytic converter type, engine capacity, and vehicle mileage. Figure 6A examines the relationship between CO emissions and the type of catalytic converter. The trend line indicates a slight negative correlation, represented by the equation $y = -0.0257x + 0.022$, with an R^2 value of 0.0692. This low R^2 value suggests that the type of catalytic converter has a minimal impact on CO emissions, and changes in catalytic type do not powerfully explain the variation in CO emission levels. The negative slope indicates a trend where CO emissions decrease as the catalytic converter type improves, although the effect is relatively weak. Figure 6B shows the relationship between CO emissions and engine capacity. The linear regression line, represented by the equation $y = -0.0002x + 0.7152$, also suggests a weak negative correlation with an R^2 value of 0.0754. This indicates that as engine capacity increases, CO emissions slightly decrease. However, the low R^2 value indicates that engine capacity is not a significant predictor of CO emission levels. The scatter of data points suggests that other factors may influence CO emissions.

Figure 6C analyzes the relationship between CO emissions and vehicle mileage. The regression line $y = 0.0000x + 0.3330$ shows a positive correlation, with an R^2 value of 0.1300. While the correlation is slightly stronger compared to the other two analyses, the R^2 value remains low, indicating that mileage explains only a small portion of the variability in CO emissions. The positive slope implies that CO emissions increase as vehicle mileage rises, reflecting potential degradation in the vehicle's emission control systems over time.

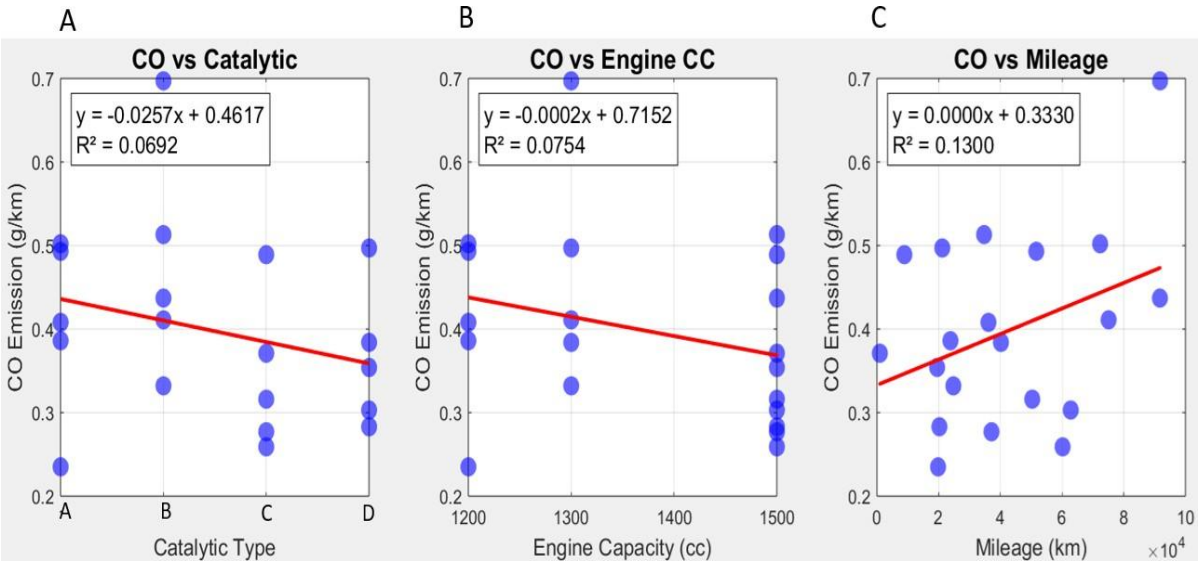


Figure 6. Illustration of CO emissions: (A) on catalytic type; (B) on engine capacity; and (C) on vehicle mileage.

NO_x Emissions as a Function of Catalytic Converter Type, Engine Capacity, and Vehicle Mileage

Figures 7A, 7B, and 7C analyze nitrogen oxide (NOx) emissions concerning catalytic converter type, engine capacity, and vehicle mileage. Figure 7A illustrates the relationship between NOx emissions and the type of catalytic converter. The regression line equation $y = 0.0010x + 0.0193$ shows a slight positive correlation with an R^2 value of 0.0146. This very low R^2 value indicates that the catalytic converter type has an almost negligible impact on NOx emissions, and the variability in NOx emissions needs better explanation by changes in catalytic type. The slight upward trend suggests a minimal increase in NOx emissions as the catalytic type number increases, but the effect is statistically insignificant. Figure 7B examines the correlation between NOx emissions and engine capacity. The regression line $y = 0.0000x + 0.0222$ indicates a weak positive relationship, with an R^2 value of 0.0388. This suggests that NOx

emissions increase slightly with larger engine capacities, but the relationship is not strong. The scatter of data points highlights the minimal impact of engine capacity on NOx emission levels, indicating the need for additional factors to explain the variability in NOx emissions.

Figure 7C presents the relationship between NOx emissions and vehicle mileage. The regression equation y = 0.0000x + 0.0121 reveals a more pronounced positive correlation, with an R^2 value of 0.3996. This indicates a moderate relationship, suggesting that NOx emissions increase as vehicle mileage accumulates. The R^2 value is considerably higher than in the previous analyses, indicating that mileage is a more significant factor influencing NOx emissions. The positive slope reflects the effect of wear and aging on the vehicle's emission control systems, contributing to higher NOx emissions over time.

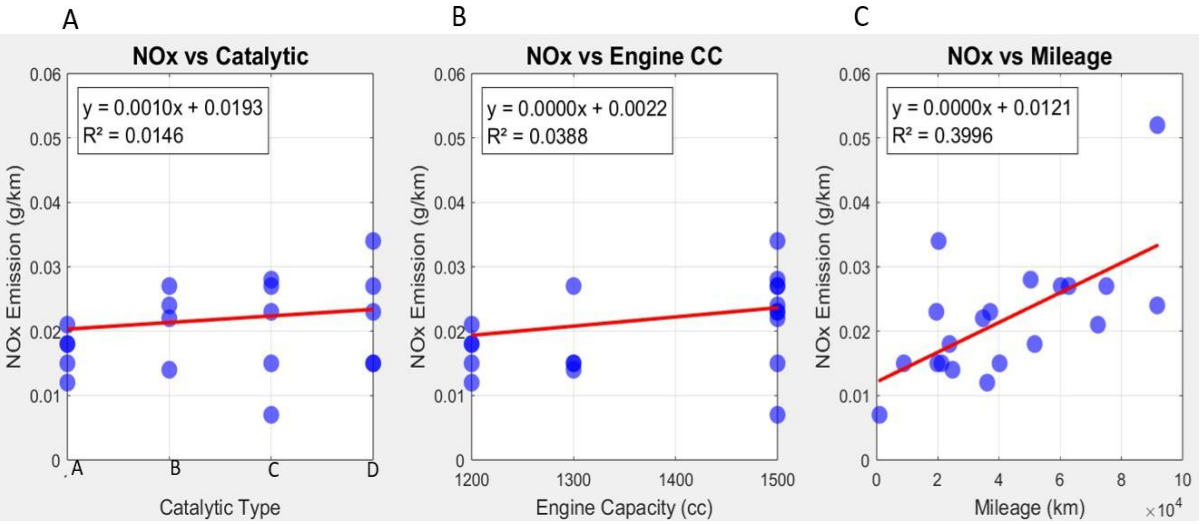


Figure 7. Illustration of NOx emissions: (A) on catalytic type; (B) on engine capacity; and (C) vehicle mileage.

THC Emissions as a Function of Catalytic Converter Type, Engine Capacity, and Vehicle Mileage

Figures 8A, 8B, and 8C analyze total hydrocarbon (THC) emissions related to catalytic converter type, engine capacity, and vehicle mileage. Figure 8A depicts the relationship between THC emissions and the type of catalytic converter. The regression line y = −0.0079x + 0.0688 indicates a strong negative correlation, with an R^2 value of 0.4899. This relatively high R^2 value indicates that the type of catalytic converter significantly influences total hydrocarbon (THC) emissions, with emissions decreasing as the catalytic converter type number increases. This trend suggests that more advanced catalytic converter types effectively reduce THC emissions, underscoring the importance of utilizing high-performance converters to meet emission standards.

Figure 8B shows the relationship between THC emissions and engine capacity. The equation y = −0.0000x + 0.1059 reflects a weak negative correlation with an R^2 value of 0.1822. Although there is a slight trend of decreasing THC emissions with larger engine capacities, the relationship is not particularly strong. The low R^2 value indicates that engine capacity is not a major determinant of THC emission levels, and other factors may play a more significant role in influencing THC emissions. Figure 8C examines the correlation between THC emissions and vehicle mileage. The regression line y = 0.0000x + 0.0399 shows a moderate positive correlation, with an R^2 value of 0.2043. This suggests that THC emissions tend to increase with higher vehicle mileage, although the correlation is not as strong as that observed in Figure 8A. The increase in THC emissions with mileage may be due to the gradual degradation of the vehicle's emission control system, resulting in reduced catalytic efficiency over time.

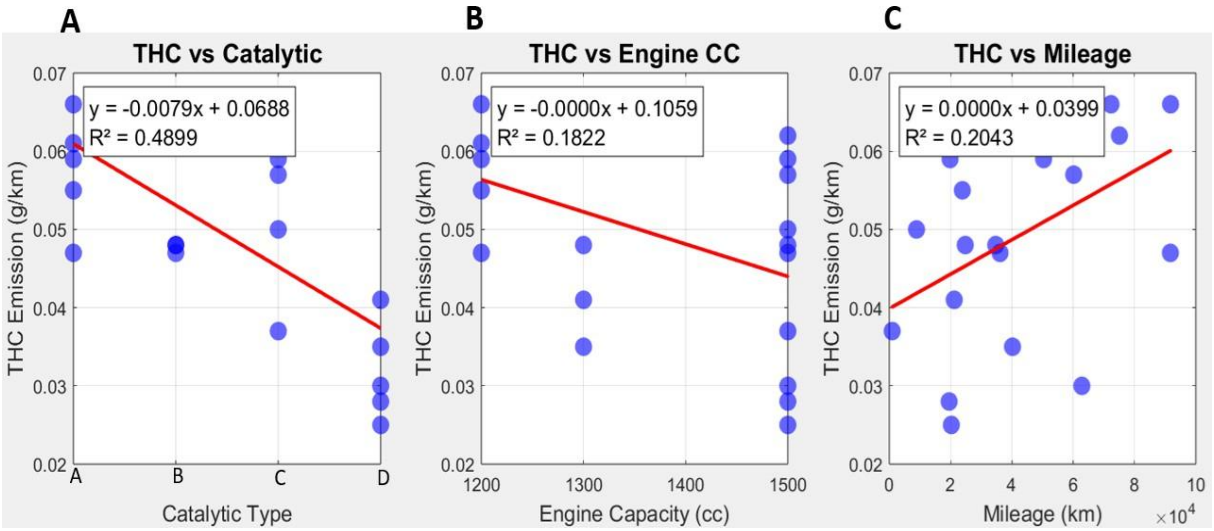


Figure 8. Illustration of THC emissions: (A) on catalytic type; (B) on engine capacity; and (C) vehicle mileage.

Catalytic Performance Degradation on CO, THC, and NO_x Emissions

This analysis aims to understand how different types of catalysts (A, B, C, and D) affect emissions of CO, THC, and NOx as the mileage of a vehicle increases. For CO and THC emissions, a first-order reaction kinetics model is applied. This model is commonly used to describe processes where the rate of change is proportional to the current value, such as the degradation of catalyst efficiency leading to increased emissions. By fitting the CO and THC emission data to an exponential model, we can assess how effectively each catalyst maintains low emission levels as mileage increases. The fitted model helps quantify the rate at which catalyst performance decays. The First-Order Reaction Model equation 1 is written as:

$$y(t) = y0\, e^{-kt} \tag{1}$$

where *y(t)*: the emission level at time *t* (e.g., mileage), y_0: The initial emission, *k*: The rate constant, which dictates how quickly the emission level decays, and *t* the time of mileage in this context. This equation describes an exponential decay. In the case of catalytic degradation, y(t) could represent the emission of a pollutant, and the constant k represents how fast the catalytic converter loses its efficiency in reducing emissions.

Figures 9A, 9B, 9C, and 9D illustrate the relationship between mileage and CO emissions for catalytic converters A, B, C, and D, respectively. Each plot shows experimental data points and a first-order exponential fit to highlight trends in CO emissions as vehicle mileage increases. In Figure 9A, the relationship between mileage and CO emissions for Catalyst A is depicted. The exponential fit equation $y = 0.2714e^{0.0000x}$ indicates a slight positive trend, suggesting that CO emissions increase marginally as mileage accumulates. However, the increase is relatively small, indicating that Catalyst A maintains reasonable performance over time, but there is a gradual degradation in emission control efficiency as mileage increases. Figure 9B presents the data for Catalyst B, with the equation $y = 0.3495e^{0.0000x}$. The trend line indicates a more pronounced increase in CO emissions with mileage than Catalyst A. This suggests that Catalyst B experiences a more significant decline in performance as the vehicle's mileage increases, resulting in higher CO emissions over time. In Figure 9C, the trend for Catalyst C is shown with an equation $y = 0.4334e^{-0.0000x}$, indicating a negative relationship. This suggests that CO emissions decrease as mileage increases, which is counterintuitive and may point to inconsistencies in the data or the possibility of other external factors influencing emission levels. The trend implies that Catalyst C may have a unique characteristic or effect that leads to reduced emissions over time, though further

investigation is needed to validate this behavior. Figure 9D shows the data for Catalyst D, with the equation $y = 0.4139e^{-0.0000x}$. Like Catalyst C, the trend line indicates a decrease in CO emissions with increased mileage. This negative relationship suggests that Catalyst D maintains or even improves its emission control effectiveness over time, although this trend may also be due to data variability or external factors affecting the results.

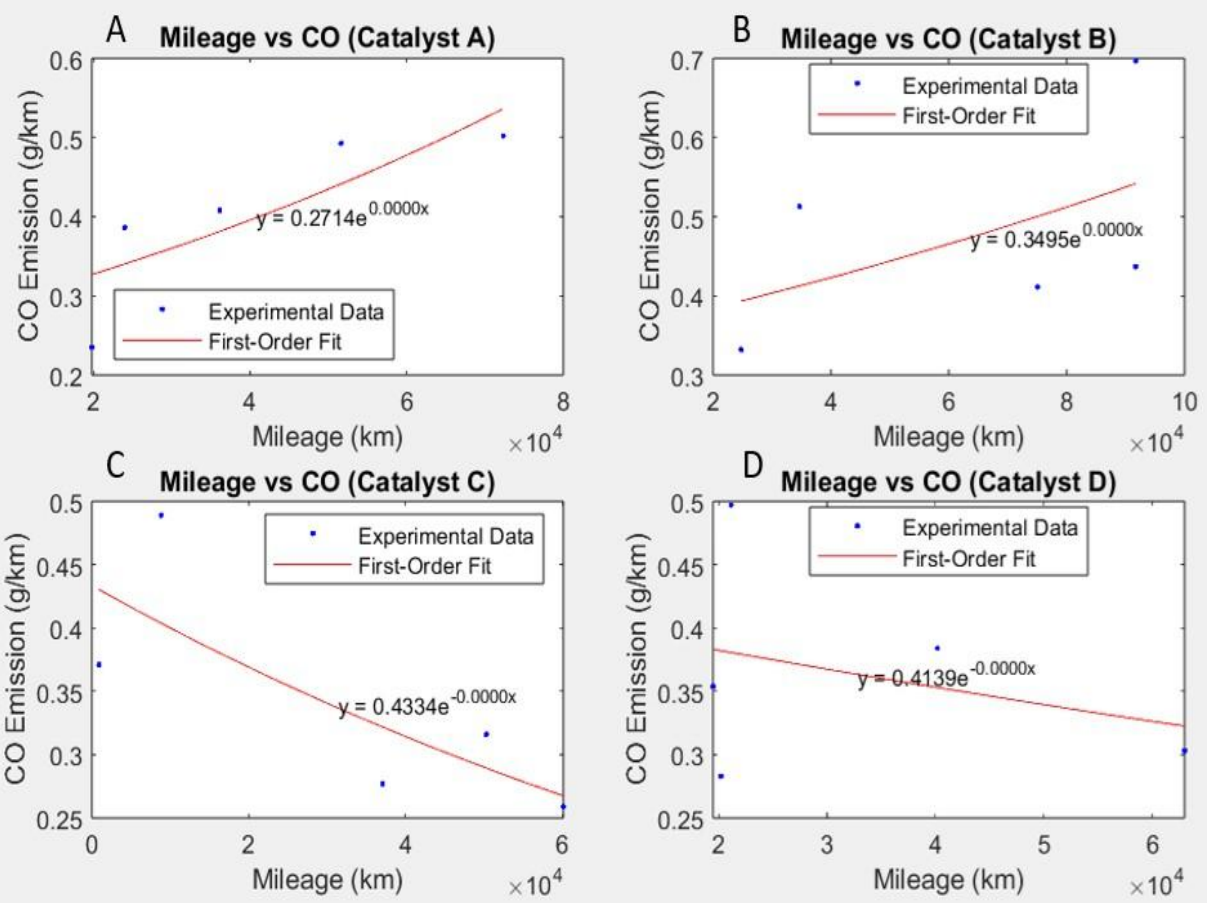


Figure 9. Illustration of catalytic performance degradation on CO emission: (A) Catalyst type A; (B) Catalyst type B; (C) Catalyst type C; and (D) Catalyst type D.

Figures 10A, 10B, 10C, and 10D illustrate the relationship between mileage and THC emissions for different types of catalytic converters: A, B, C, and D, respectively. Each plot shows experimental data points and a first-order exponential fit to highlight trends in THC emissions as vehicle mileage increases. Exclude Catalyst C. Overall, the image of THC emission compared to CO was nearly the same, excluding Catalyst type C, which, as shown in Figure 10C, increased. The differences in THC compared to CO emissions are the Constanta, where in Figure 10A, the relationship between mileage and THC emissions for Catalyst A, the exponential fit equation $y = 0.0498e^{0.0000x}$ = indicates a slight positive trend. Figure 10B presents the data for Catalyst B, with the equation $y = 0.04495e^{0.0000x}$. The trend line indicates almost the same in THC emissions with mileage compared to Catalyst A. In Figure 10C, the trend for Catalyst C is shown with an equation $y = 0.0439e^{0.0000x}$, indicating a positive relationship. This suggests that THC emissions increase as mileage increases; the trend implies that Catalyst C may have a unique characteristic or effect that leads to reduced CO emissions over time, but not for THC.

Further investigation was needed to validate this behavior. Figure 10D shows the data for Catalyst D, with the equation $y = 0.0426e^{-0.0000x}$. Similar to Catalyst B, but slightly decreased, the trend

line indicates a decrease in THC emissions with increased mileage. This negative relationship suggests that Catalyst D maintains or even improves its emission control effectiveness over time, although this trend may also be due to data variability or external factors affecting the results.

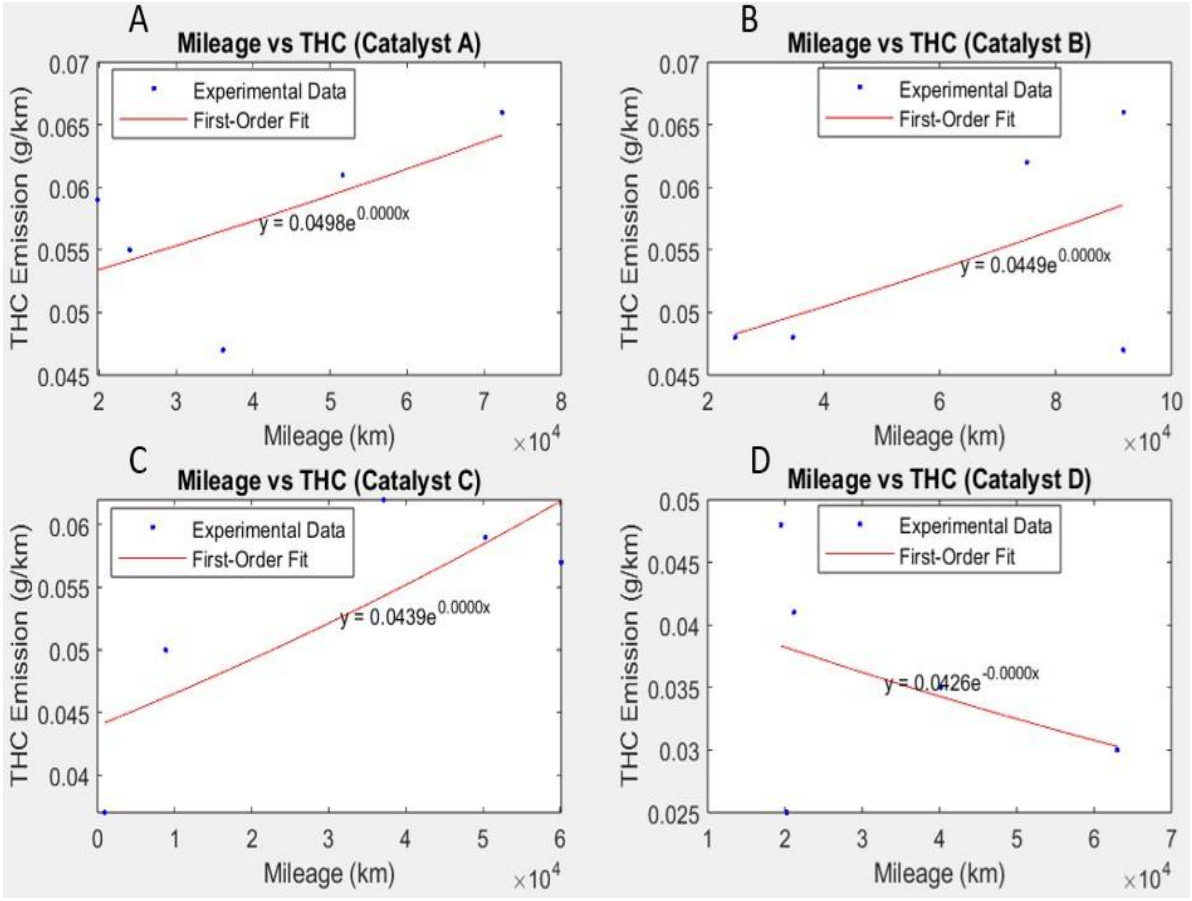


Figure 10. Illustration of catalytic performance degradation on THC emission: (A) Catalyst type A; (B) Catalyst type B; (C) Catalyst type C; and (D) Catalyst type D.

In the case of NOx emissions, the Arrhenius-type model is utilized. The Arrhenius equation is often used to describe the temperature dependence of reaction rates, but in this context, it is adapted to model the change in NOx emissions over mileage. This approach provides insights into the degradation behavior of catalysts, specifically their efficiency in reducing NOx emissions as they are subjected to prolonged use. The fitted Arrhenius model allows us to understand how the efficiency of each catalyst decreases, which in turn impacts NOx emissions. Arrhenius Equation 2 (Wang *et al*., 2016).

$$k = Ae^{-Ea/RT} \tag{2}$$

where k: The rate constant of the reaction, A: The pre-exponential factor (a constant), Ea: The activation energy required for the reaction, R: The universal gas constant, and T: Temperature (in Kelvin).

The Arrhenius-type model helps describe how external factors, such as increased mileage, might accelerate the production of certain emissions, especially NOx, which is often sensitive to temperature or engine load changes. The simplified exponent model used in the MATLAB script is equation 3:

$$y = ae^{bx} \tag{3}$$

where *y*: The emission level (e.g., NOx), *a:* A scaling factor, *b:* Represents the impact of mileage (proxy for time), and *x*: mileage (proxy for a stressor like temperature or aging).

Figures 11A, 11B, 11C, and 11D depict the relationship between mileage and NOx emissions for different catalytic converters: A, B, C, and D. Each graph displays experimental data points and an Arrhenius fit to analyze how NOx emissions change as vehicle mileage increases. In Figure 11A, the Arrhenius fit equation $y = 0.0130e^{0.0000x}$ indicates a clear positive trend, showing that NOx emissions increase as vehicle mileage accumulates. The data points and the fitted curve suggest that Catalyst A experiences a noticeable decline in its efficiency in controlling NOx emissions over time, pointing to potential wear and aging effects on the catalyst's performance.

Figure 11B illustrates the relationship for Catalyst B, with the Arrhenius equation $y = 0.0118e^{0.0000x}$. The trend is similar to Catalyst A, showing an increase in NOx emissions with higher mileage. The curve indicates a steady rise, suggesting that Catalyst B also undergoes a reduction in NOx emission control efficiency as the vehicle is used for longer periods. In Figure 11C, the data for Catalyst C is represented with the equation $y = 0.0119e^{0.0000x}$. The positive trend confirms that NOx emissions increase with mileage, albeit slightly less steeply compared to Catalysts A and B. This implies that Catalyst C has a moderate decline in NOx control efficiency, but the effect remains significant as the vehicle accumulates mileage. Mileage vs NOx Emission for Catalyst D was shown in Figure 11D. The performance of Catalyst D, with the Arrhenius fit $y = 0.0133e^{0.0000x}$, the graph reveals a clear positive correlation, indicating that NOx emissions rise as vehicle mileage increases. The trend for Catalyst D is comparable to that of Catalyst A, reflecting a similar decline in performance over time.

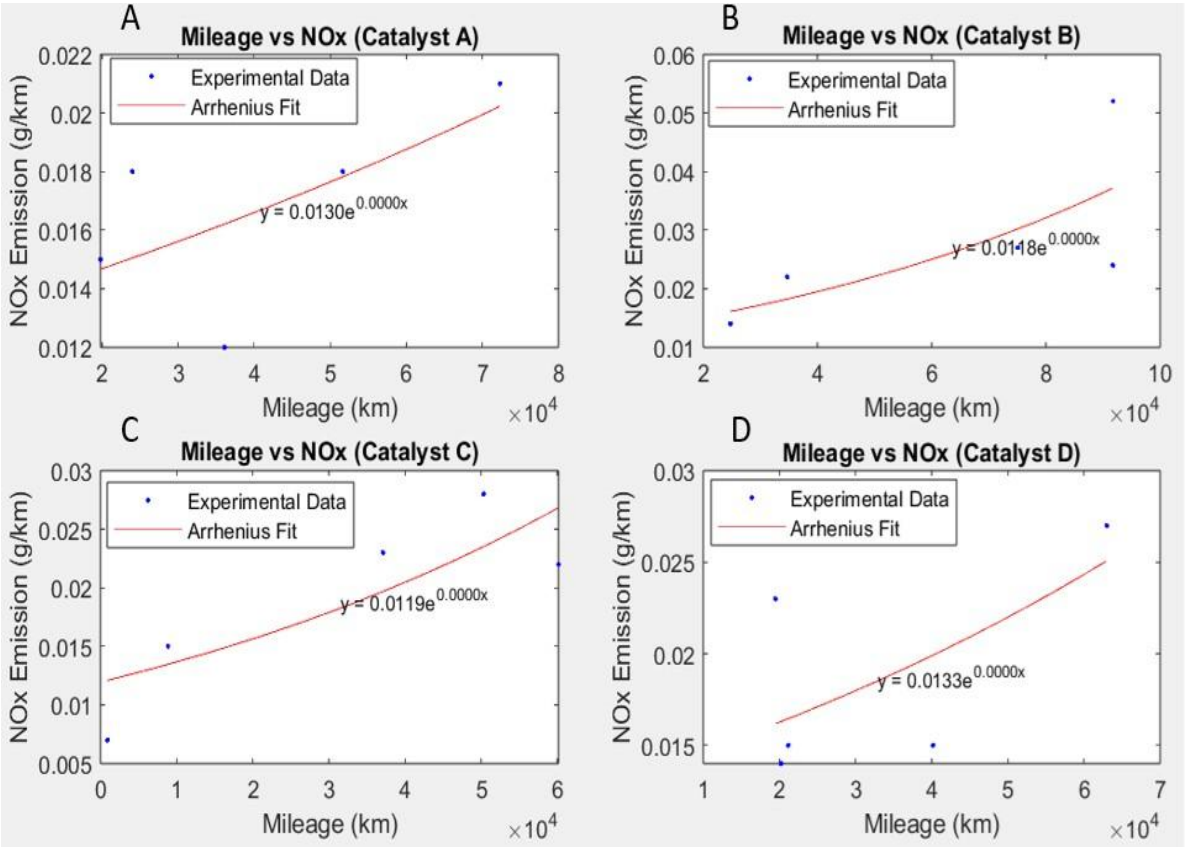


Figure 11. Illustration of catalytic performance degradation on NOx emission: (A) Catalyst type A; (B) Catalyst type B; (C) Catalyst type C; and (D) Catalyst type D.

CONCLUSION

This study shows that the performance of the catalytic converter was degraded by operational lifetime. The NOx degradation factor (DF) was the highest, at 5.78, at a mileage near 100 km. This study also showed that catalytic converter type D has the best performance for CO and THC emissions, with stable degradation across its lifespan. On the other hand, the catalytic converter type B degraded significantly, at 9.0. NOx emissions indicate that all types of catalytic converter performance were decreasing at almost the same rate of deterioration. Future research may provide a deeper analysis of the catalytic material, which impacts the reduction of CO and THC, and is more significant for NOx. Selecting vehicle samples that have received the same maintenance treatment throughout the vehicle's life may enable the collection of a homogeneous sample and reduce unnecessary factors that influence the degradation performance of catalytic converters.

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RESEARCH ARTICLE

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COMPARATIVE GROWTH PERFORMANCE OF *NEOLAMARCKIA CADAMBA* ROXB. BOSSER (WHITE LARAN) UNDER DIFFERENT SITE CONDITIONS IN FOREST PLANTATION AT JAWALA PLANTATION INDUSTRY, SAPULUT FOREST RESERVE, NABAWAN

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ABSTRACT. *This study investigated the growth of Neolarmakia cadamba Roxb Bosser (White Laran) across various site conditions characterised by differing elevations, slopes, distances from roads, and forest fringes. Plots meeting specific criteria were established in two distinct sites, one year and five years old, of planted N. cadamba trees. The findings indicate that the one-year-old trees at higher elevations on flat terrain show a greater mean diameter at breast height (DBH = 8.75 cm), mean total height (5.84 m), and volume (0.16 m³) compared to those at lower elevations on hilly land. Conversely, for the five-year-old trees, higher average DBH and total height were observed in flat areas near roads and forest edges. Analysis using the Kruskal-Wallis test demonstrated significant differences in DBH, height, and volume among the plots, with $p < 0.05$. This study suggests that variations in the growth of N. cadamba arise from different site conditions related to slope, altitude, and proximity to roads and forest edges, likely due to differences in nutrient availability and light access. These findings may prompt further research in this area, particularly regarding treatment recommendations and predictions for the early growth of the species in diverse site conditions.*

INTRODUCTION

Forest plantations are becoming increasingly important in the global forest estate. The plants chosen for the restoration area include fast-growing exotics, such as *Acacia mangium*, *Shorea leprosula*, *Neolarmakia cadamba* (*N. Cadamba*), Binuang (*Octomeles sumatrana* Miq.), and others. Despite indigenous species being a priority for restoration programs, several factors must be considered to optimise growth performance, including site conditions such as soil type (Kanowski *et al*., 2005). Different tree species exhibit varying growth rates due to their unique surroundings, which in turn affects their growth. Some factors that may influence tree growth include nutrient availability (Binkley, 2003), light intensity and absorption (Poorter *et al*., 2012; Dieler & Pretzsch, 2013), soil quality (Kozlowski, 1999), and water availability (Lebourgeois *et al*., 2013). To accurately record the growth, measurements

of the tree must be taken, such as its height and diameter at breast height (DBH). The growth is quantified by assessing the tree's diameter at breast height (DBH), approximately 1.3 meters above the ground, and its height (Walker *et al*., 2011). In a tree plantation, keeping track of its growth is crucial for carrying out pruning and care according to the prescribed schedule. The growth rate is widely used in the plantation to monitor the growth of the trees planted (Hodge *et al*., 2001). It ensures that the tree growth meets the requirements and characteristics needed for high-quality timber. Tree height is also an indicator typically used in sites to assess tree growth rate performance (Skovsgaard *et al*., 2008). The DBH and height of tree data can generally be used to demonstrate that the growth rate of the same tree species at different locations varies due to differences in nutrient intake, tree competition, soil conditions, light intensity, and water availability.

Numerous research studies and publications have focused on the growth of *N. cadamba* across various countries; however, there is still limited evidence regarding its growth performance in Sabah. Until recently, little was known about how specific site characteristics, such as soil type and quality, elevation, light conditions, and other environmental factors, affect the growth metrics (including above-ground dimensions and biomass) of *N. cadamba* in a rainforest plantation environment. The lack of silvicultural data on native species and insufficient growth and yield information hinders their use in large-scale plantations (Hashim *et al*., 2015). Understanding these factors is crucial for enhancing forest management practices and promoting the efficient growth of this valuable species under diverse conditions. Due to market demand and its unique traits, such as adaptability to intermittent waterlogged conditions (M. Khatta *et al*., 2023), *N. cadamba* has been more frequently utilized in Sabah compared to other fast-growing species (Yulianti & Sudrajat, 2016). According to Lee *et al*. (2005), the internal rate of return (IRR) for *N. cadamba* ranges from 12% to 29%, which is considered high based on financial analysis. Thus, comprehending the growth of this species in Sabah is essential.

This study examined the growth of the indigenous species *N. cadamba*, commonly known as Laran, at Jawala Plantation Industries Sdn. Bhd. in the Sapulut Forest Reserve, Nabawan, Sabah. The research aims to explore how the growth of *N. cadamba* varies under different site conditions. Specifically, it seeks to assess the effects of various surrounding factors on the growth of *N. cadamba* in forest plantations, such as elevation, slope, and soil compaction. The study has two primary objectives: 1) To compare the growth of one-year-old *N. cadamba* across different slope and elevation conditions, and 2) To evaluate the relationship between the growth of *N. cadamba* and varying site conditions, including slope, distance from the road, and proximity to forest edges. All chosen variables related to site conditions were considered to assess their influence on the growth of *N. cadamba*. This research aims to enhance the limited knowledge regarding *N. cadamba* early growth in diverse site conditions.

MATERIALS AND METHODS

This study was carried out at Jawala Plantation Industries Sdn. Bhd. situated in the Sapulut Forest Reserve (FR), Nabawan, Sabah. The company is a wholly owned subsidiary of Jawala Corporation Sdn. Bhd., and oversees roughly 11,043 hectares, which includes areas designated for production, non-production, and conservation. The total production area spans approximately 8,930 hectares, consisting of 8,442 hectares of conventional industrial tree plantation (ITP) and 488 hectares designated for reduced-impact logging (RIL) harvesting practices. The non-production area covers about 1,364 hectares, which includes 84 hectares for roads and clearings, 1,192 hectares for streams, ponds, and riparian reserves, 868 hectares of steep terrain, and 20 hectares for infrastructure. Additionally, the conservation areas encompass 749 hectares. To achieve the research objectives of exploring the relationship between the early growth of *N. cadamba* and its surrounding environment, several methods were employed.

Study the Growth Performance of One-Year-Old Planted *N. cadamba* Under Different Slopes and Elevations.

Four plots were set up in the industrial tree plantation (ITP) for data collection, each containing 40 one-year-old laran trees (*N. cadamba*). The trees were planted with a spacing of 5 × 5 meters, and the plots were aligned with the planted trees, measuring 20 meters by 35 meters each. Two plots (A and B) were positioned at a higher elevation of 475 meters, while the other two (C and D) were situated at a lower elevation of 375 meters, as shown in Figure 1.

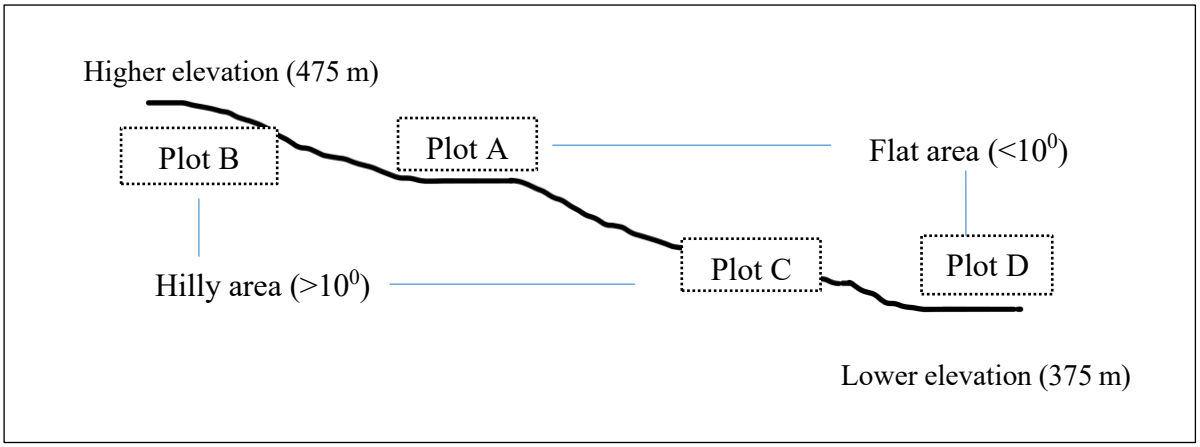

Figure 1. Illustration of the site location of study Plots (A, B, C, and D).

Each elevation features two distinct slope types: flat (plots A and D) with a slope angle of less than 10 degrees, and hilly (plots B and C) with a slope angle greater than 10 degrees. The distance between plots A and B at the higher elevation and plots C and D at the lower elevation is approximately 500 meters. Additionally, the distance between plots A and B, as well as C and D, is around 20 meters each. A total of 160 one-year-old *N. cadamba* trees were planted on-site, having previously been nurtured in a nursery before their transplant to the ITP area. Data regarding diameter at breast height (DBH), height, slope, and elevation were recorded and collected.

Study of the Growth of Five Years *N. cadamba* Planted Under Different Site Conditions.

This study was carried out by choosing study locations that fulfilled the criteria for the specific site conditions necessary for the investigation (refer to Table 1). Three plots were established, each designed with a sampling area of 100 m × 20 m. The plots differ based on slope, distance from the road, and proximity to the forest fringe, as depicted in Figure 2. Plot E is situated in a flat area (≤ 10°), close to the road (≤ 20 m), and near the forest fringe (< 20 m). In contrast, Plot F is in a hilly region (> 10°), near the road (≤ 20 m), and somewhat distant from the forest fringe (20-40 m). Finally, Plot G is also in a hilly area (> 10°) but is positioned farther from both the road (> 20 m) and the forest fringe (> 40 m). Field data collection was conducted using a diameter tape and a Trimble device; the DBH of the trees was measured with the diameter tape, while the Trimble was used to assess tree height. Soil compaction readings were obtained using a soil compaction meter.

Table 1. The different characteristics of the selected study plots.

Plot	Slope	Distance from the road (open area)	Distance from forest fringe (closed area)
Plot E	≤ 10°	≤ 20 m	≤ 20 m
Plot F	> 10°	≤ 20 m	20 – 40 m
Plot G	> 10°	> 20 m	> 40 m

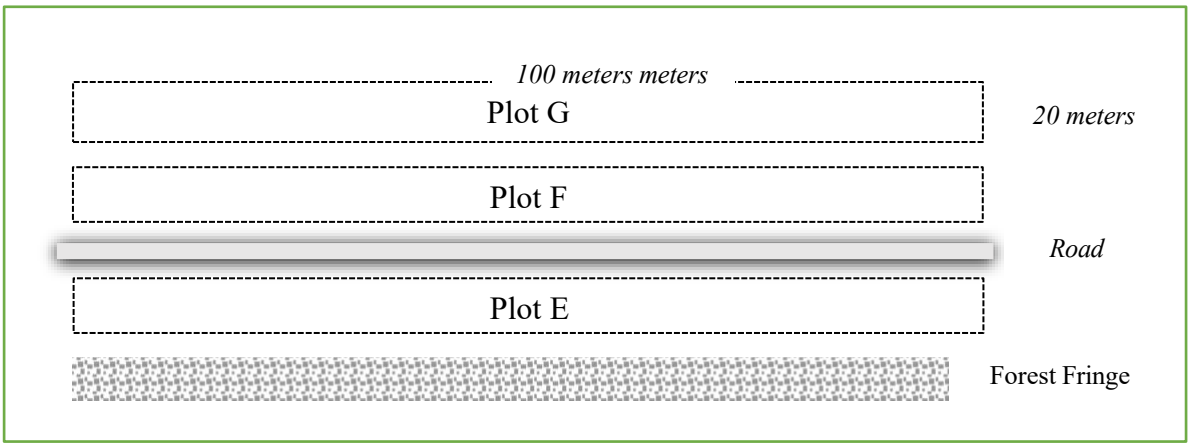

Figure 2. Illustration of different site characteristics of study Plots (E, F, and G).

RESULTS AND DISCUSSIONS

The Growth Rate of One-Year-Old *N. cadamba* at Different Elevations and Slopes

The mean diameter at breast height (DBH) of *N. cadamba* in the first year of growth is higher in the flat areas (Plots A and D) compared to the hilly areas, as indicated in Table 2. Specifically, the mean DBH for Plot A at the higher elevation is 8.746 cm, while for Plot D at the lower elevation, it is 7.635 cm. In the hilly areas (Plots B and C), where the slope exceeds 10 degrees, the mean DBH is lower, measuring 7.428 cm in Plot B and 7.005 cm in Plot C. The mean total height of *N. cadamba* is greater in Plots A and B at higher elevations than in the lower elevation plots. Plot A records the highest mean total height at 5.84 m, followed by 5.53 m in Plot B, 5.39 m in Plot D, and 4.81 m in Plot C, respectively. In terms of volume, the highest mean is observed in Plot A (0.0164 m³), followed by Plot B (0.0102 m³), Plot D (0.0094 m³), and Plot C (0.0075 m³).

Table 2. Growth of one-year-old *N cadamba* at different slopes and elevations.

	Plot (PSP)	N	Minimum	Maximum	Sum	Mean		Deviation
		Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
Plot A (flat)	DBH (cm)	40	3.80	14.00	349.82	8.7455	0.4281	2.7075
	Height (Total) (Meter)	40	2.50	9.52	233.63	5.8408	0.2604	1.6471
	Volume (m³)	40	0.0011	0.0494	0.6547	0.0164	0.0018	0.0112
	Valid N (listwise)	40						
Plot B (hilly)	DBH (cm)	40	3.00	13.00	297.10	7.4275	0.3348	2.1176
	Height (Total) (Meter)	40	2.18	10.10	221.11	5.5278	0.3157	1.9965
	Volume (m³)	40	0.0010	0.0465	0.4092	0.0102	0.0014	0.0086
	Valid N (listwise)	40						
Plot C (hilly)	DBH (cm)	40	2.40	9.70	280.20	7.0050	0.2689	1.7007
	Height (Total) (Meter)	40	2.52	7.06	192.55	4.8138	0.1754	1.1095
	Volume (m³)	40	0.0007	0.0184	0.3016	0.0075	0.0006	0.0040
	Valid N (listwise)	40						
Plot D (flat)	DBH (cm)	40	2.70	12.80	305.40	7.6350	0.3524	2.2288
	Height (Total) (Meter)	40	2.20	8.93	215.70	5.3925	0.2445	1.5461
	Volume (m³)	40	0.0004	0.0284	0.3761	0.0094	0.0010	0.0065
	Valid N (listwise)	40						

A Kolmogorov-Smirnov and Shapiro-Wilk test were conducted to evaluate normality, yielding a p-value greater than 0.05 ($p > 0.05$), which indicates that the distributions in the four plots are not normal. The Kruskal-Wallis test revealed significant differences in the diameter at breast height (DBH), height, and volume of *N. cadamba* trees among the plots. The results for DBH are as follows: $H(3) = 11.559$, $p = 0.009$, with mean ranks of 100.70 for Plot A, 74.58 for Plot B, 67.24 for Plot C, and 79.49 for Plot D. For height, the results are $H(3) = 8.696$, $p = 0.034$. Regarding volume, the statistics are $H(3) = 14.548$, $p = 0.002$, with mean ranks of 103.48 for Plot A, 77.16 for Plot B, 65.74 for Plot C, and 75.63 for Plot D. Spearman's correlation analysis revealed a weak significant correlation between tree height and soil compaction ($rs = -0.412$, $n = 40$, $p = 0.000$).

The early growth of the fast-growing species *N. cadamba* is influenced by various factors. This study highlights a significant difference in the growth of *N. cadamba* based on differing site conditions, including altitude and slope characteristics. The findings demonstrate that trees planted in flat areas show higher growth rates compared to those in hilly areas. Slope is a critical factor affecting tree growth; often, steeper slopes can hinder growth due to increased soil erosion in these areas. This erosion typically removes the nutrient-rich topsoil, especially during rainfall events. As the slope degree increases, the potential for soil erosion also rises. Previous research by Nguyen *et al*. (2012) suggested that trees on lower slopes tend to grow faster than those on steeper gradients. Additionally, a study by Hui *et al*. (2012) indicated a strong correlation between slope and DBH ($r = 0.912$). The significant negative correlation ($r = -0.634$) between slope and height observed in this study suggests an inverse relationship, indicating that a lower slope degree is associated with improved tree growth.

Study the Growth of *N. cadamba* Under Various Site Conditions.

The study investigated the impact of various environmental conditions on the growth of five-year-old *N. cadamba* trees, specifically in Plot E with 118 trees, Plot F with 100 trees, and Plot G with 84 trees, as detailed in Table 3. The mean DBH was highest in Plot E at 17.36 cm, followed by Plot F at 13.57 cm and Plot G at 9.20 cm. In terms of total height, Plot E also recorded the greatest mean at 14.53 m, while Plot F measured 10.050 m and Plot G had the lowest mean at 7.30 m. Consequently, Plot E exhibited the highest mean volume at 0.3899 m^3, followed by Plot F at 0.1826 m^3 and Plot G at 0.0729 m^3.

Table 3. Growth of 5-year *N cadamba* at five different site conditions.

Plot		N	Minimum	Maximum	Mean		Std.
		Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
E (Flat)	DBH (cm)	118	6.60	26.30	17.3568	0.4327	4.7005
	Height (Total) (m)	118	5.16	27.43	14.5341	0.3245	3.5248
	Total Volume (m^3)	118	0.0200	0.9080	0.3899	0.0208	0.2264
	Valid N (listwise)	118					
F (Hilly)	DBH (cm)	100	4.70	21.60	13.5650	0.4282	4.2824
	Height (Total) (m)	100	3.14	19.09	10.0501	0.3393	3.3931
	Total Volume (m^3)	100	0.0050	0.6870	0.1826	0.0146	0.1462
	Valid N (listwise)	100					
G (Hilly)	DBH (cm)	84	2.00	19.20	9.2024	0.4283	3.9255
	Height (Total) (m)	84	1.10	16.98	7.3048	0.3343	3.0639
	Total Volume (m^3)	84	0.0010	0.4920	0.0729	0.0093	0.0856
	Valid N (listwise)	84					

The Shapiro-Wilk test ($p > 0.05$) (Shapiro & Wilk, 1965), along with a visual inspection of the histograms and normal Q-Q plots, indicates that the diameter at breast height (DBH) and height of the trees across the three plots do not follow a normal distribution. The Kruskal-Wallis test conducted on

total height revealed a statistically significant difference among the plots ($p < 0.000$), with mean ranks of 220.53 for Plot E, 132.53 for Plot F, and 77.12 for Plot G. Additionally, there was a statistically significant difference in DBH among the plots as well ($p < 0.000$), with mean ranks of 207.14 for Plot E, 148.29 for Plot F, and 77.15 for Plot G.

These findings align closely with previous research conducted in South Kalimantan, which indicated that *N. cadamba* trees younger than five years have a diameter ranging from 6.0 cm to 16.4 cm, with an average of 25.3 cm, and heights varying from 4.1 m to 14.6 m, reaching a maximum of 17.1 m (Krisnawati *et al*., 2011). A Spearman's rho correlation test was performed to examine the relationships between DBH and slope, DBH and distance from the road, DBH and distance from the forest fringe, distance from the road and total height, distance from the forest fringe and DBH, and distance from the forest fringe and total height. All correlation coefficients were highly significant ($p < 0.001$) at the 0.01 level (2-tailed). There was a moderate negative correlation between DBH and slope ($rs = -0.511$, $n = 302$, $p = 0.000$), as well as between DBH and distance from the road ($rs = -0.529$, $n = 302$, $p = 0.000$) and distance from the forest fringe ($rs = -0.598$, $n = 302$, $p = 0.000$). The relationship between total height and slope was also significantly correlated ($rs = -0.634$, $n = 302$, $p = 0.000$), as was the relationship between total height and distance from the road ($rs = -0.530$, $n = 302$, $p = 0.000$) and distance from the forest fringe ($rs = -0.678$, $n = 302$, $p = 0.000$).

The findings reveal that trees located near the road exhibit a higher growth rate compared to those situated farther away. Fast-growing species planted close to the road demonstrate superior growth compared to their counterparts planted at a greater distance from the roadside. *N. cadamba*, being a light-demanding species, thrives and adapts well to degraded lands, particularly in logged forests, making light a crucial factor for its growth. The more sunlight these trees receive, the larger their diameter and height. In this study, the distance from the road shows a moderate negative correlation with DBH ($rs = - 0.529$). Fox (1971) noted that Laran trees prominently contribute to the initial growth in secondary forests, especially in open areas post-logging and in moist sites. This indicates that trees receiving adequate sunlight are likely to grow better than those with limited exposure. Supporting this, Kocher and Harris (2007) observed that light-demanding trees thrive in environments with ample light penetration, while they struggle when overshadowed by other trees. Additionally, a study by Naghdi *et al*. (2017) confirmed that trees near the road exhibit greater diameter increments compared to those planted farther away.

The forest fringe, or edge, serves as a transitional zone between open and closed landscapes, acting as a boundary for living spaces (Tripathi *et al*., 1993). It functions as a protective barrier against the wind. In addition to providing a habitat that supports a wide variety of fauna, the forest fringe also supplies vital nutrients to the surrounding ecosystem. A study by McDonald *et al*. (2004) found that the forest edge does not significantly influence tree growth when compared to topographical and soil factors. However, this study revealed a substantial correlation between the forest fringe and both the diameter at breast height (DBH) ($rs = - 0.598$) and height ($rs = - 0.678$) of *N. cadamba*. This can be attributed to the interactions and competition for sunlight among various tree species at the forest fringe, particularly affecting the light-demanding *N. cadamba*. Ajik (2005) also noted similar observations regarding the early growth performance of *N. cadamba* in Segaliud Lokan, Sabah.

CONCLUSION

A crucial aspect of research is to analyze the growth patterns of trees in different site settings to evaluate their potential for thriving in varying soil types, climatic conditions, and elevations. This information is instrumental in determining optimal planting sites and maximizing tree growth potential. By investigating the growth of *N. cadamba* under various conditions, one can identify the ideal requirements for soil, climate, and elevation for its optimal development. Such data can lead to improved cultivation techniques and enhanced tree productivity. *N. cadamba* is highly valued for its timber, medicinal properties, and ability to improve soil fertility while reducing erosion. Studying its growth across different environmental conditions helps pinpoint the best locations for planting the tree and maximizing

its economic benefits. Additionally, while *N. cadamba* is recognized for its invasive tendencies in some areas, analyzing its growth patterns can help identify regions where the tree is less likely to exhibit invasive behavior, thus reducing potential ecological impacts.

Understanding the growth of *N. cadamba* in various site conditions is key to optimizing its growth, enhancing its economic advantages, and minimizing ecological consequences. This study will provide insights into tropical forest dynamics and the management of specific forest species by offering empirical data on the site factors influencing the growth and health of *N. cadamba*, thereby addressing gaps in knowledge about the growth responses of this species. Such understanding is vital for identifying strategies to effectively conserve and control *N. cadamba* in timber plantations, contributing to enhanced reforestation and sustainable forest management. It involves determining site conditions that improve resilience to climate variations, leading to a better comprehension of how this species can aid in managing climate change. Moreover, it will shed light on the tree's interactions with other plant species and wildlife within and around the plantation, as well as *N. cadamba* role in influencing species richness within the plantation system. Ultimately, the data gained from this study can be utilized by policymakers and land managers in making informed decisions regarding land use and conservation strategies in forest reserves, particularly in plantation areas. Furthermore, additional studies focusing on the growth performance of fast-growing species are recommended, especially concerning any treatments applicable during the early growth phase that consider different site conditions. The research should also be expanded to encompass various age stages of the planted trees.

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ASSESSING FLUSHING DYNAMICS AND EUTROPHICATION VULNERABILITY IN SALUT-MENGKABONG LAGOON, SABAH, MALAYSIA

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ABSTRACT. *Flushing time and volume are important initial measurements for understanding the susceptibility of a lagoon to microalgal eutrophication and pollution. The goal of this study is to estimate the flushing time and vulnerability to eutrophication over spring and neap cycles. The MIKE 21 model was used, and eight monitoring points were selected in four areas within the Salut-Mengkabong Lagoon in Sabah, Malaysia - an estuary with a narrow entrance. After calibration, the advection-dispersion module was used to calculate residence times using a virtual dye tracer method. In the period of investigation spanning from October 2015 to August 2016, it was noted that residence times were notably shorter in January 2016, particularly during spring tides, lasting less than a day compared to other months. This phenomenon was attributed to the smaller volume and poor dilution within the inner Mengkabong region, increasing its vulnerability to eutrophication and pollution. The findings of the lagoon hydrodynamics modelling suggest that relocating potential sources of pollutant discharge away from high-risk areas could mitigate the impacts of coastal eutrophication. The model's results have the potential to assist local authorities in effectively managing lagoon water quality by informing development plans in the lagoon vicinity and implementing controls on pollutant discharges into the lagoon.*

INTRODUCTION

Semi-enclosed bar-built lagoons are highly productive ecosystems; however, they are also susceptible to excessive eutrophication and pollution. He and deMarchi (2010) identified that most impairments in coastal areas stem from non-point sources, including agricultural runoff, contaminated sediments, urban runoff, and atmospheric deposition. Compounding these land development pressures, lagoons frequently support extensive aquaculture operations, which can further contribute to eutrophication in surrounding waters (Sara, 2007). Consequently, quantifying the degree of nutrient enrichment and pollutant loading in these water bodies has become a critical focus for effective management. This data can be integrated with lagoonal or estuarine hydrodynamic models to predict the fate of these stressors. On a moderate analytical scale, metrics such as flushing times or residence times are employed to evaluate the dilution capacity and retention rates of nutrients or pollutants. This allows for an assessment of their concentration potential before any biological or physical processes take effect (National Research Council, 2000; Wang *et al.,* 2013; Maraqa *et al.,* 2012).

This assessment rests on the premise that isolated areas of the lagoon - characterized by smaller volumes and longer water retention times - are likely to be more susceptible to nutrient accumulation (National Research Council, 2000). Such evaluations can be examined both in relative and absolute terms. Any significant deviations in nutrient levels from expected hierarchies could justify targeted remediation efforts focused on the lagoon's aquaculture practices or shoreline developments. Should the retention of lagoon water approach or exceed the doubling capacity of microalgae, it could lead to algal blooms, necessitating appropriate management interventions (Braunschweig *et al*., 2008; Duarte & Vieira, 2009; Defne & Ganju, 2014; Wang & Yang, 2015).

Eutrophication results from two interrelated processes, making it essential to assess both water flushing times and dilution when evaluating vulnerability to eutrophication (Braunschweig *et al.,* 2008; Duarte & Vieira, 2009; Defne & Ganju, 2014; Wang & Yang, 2015). Flushing times are crucial in determining whether conditions are suitable for algal blooms to develop, while dilution influences the potential nutrient concentrations resulting from elevated nutrient loading rates, which ultimately affect the size of the blooms. This information is vital, especially considering that many algal blooms can be toxic to aquatic life and pose risks to users of the waterway, regardless of their cell density.

Traditionally, the calculation of flushing time has relied on bathymetric volume measurements and salinity distributions as a conservative tracer, assuming a near-steady state. However, this methodology necessitates a substantial investment in catchment monitoring services. In systems with significant freshwater inputs, a comprehensive database is essential to accurately describe how the salt content of water bodies reacts to freshwater river flow (Sheldon & Alber, 2006). Conversely, in bar-built lagoons lacking significant freshwater influx, rapid and extensive seasonal intertidal monitoring of resulting hyper-salinities is crucial, along with synchronized measurements of local evaporation rates at a landscape scale (Mudge *et al.,* 2008). In intermediate scenarios, the reliability of the results may diminish significantly (Sheldon & Alber, 2006).

An alternative methodology entails the deployment of robust and rigorously validated hydraulic numerical models, exemplified by the DHI MIKE 21 Hydrodynamic (HD) flexible mesh (FM) model. These models are characterized by their lower data demands, providing a versatile and precise framework for estimating flushing times. Their intuitive user interface, a hallmark of proprietary software, necessitates calibration predominantly through the acquisition of current velocity and directional measurements. Moreover, pertinent data on fluctuations in ocean boundary conditions and tidal influences can be easily sourced, enhancing the model's reliability and applicability. The primary technical requirement for implementation is a bathymetric survey, which, due to its non-urgent nature, can be conducted without extensive time constraints, thereby facilitating timely integration into hydrodynamic analysis workflows.

In less developed regions such as Sabah, Malaysia, which is situated on Northern Borneo, there is a strong incentive to maintain its relatively untouched environment to support both local and regional ecosystem services. The Salut-Mengkabong Lagoon, specifically located in the Tuaran District, has traditionally played a vital role in sustaining the livelihoods of local communities while also attracting tourism. However, recent developments within these communities have sparked concerns about their environmental impact (Mohammad Raduan *et al*., 2008). Compounding these issues, inadequate funding and inconsistent monitoring of nutrient and pollutant loadings in the lagoon's catchment area hinder the availability of crucial data necessary for addressing these environmental challenges, especially in light of ongoing changes leading to eutrophication due to excessive nutrient loading.

Nevertheless, employing a hydrodynamic modeling approach offers significant insights into the vulnerability of lagoons to eutrophication. To accurately assess the current state of this vulnerability, we systematically measured nutrient concentrations in the water column across annual and tidal cycles at multiple locations within the Salut-Mengkabong Lagoon system. This comprehensive nutrient monitoring serves as a foundation for our primary objective: estimating flushing times. By integrating hydrodynamic models with advection and dispersion models, we seek to understand the transport processes affecting nutrient distribution and retention. This integrated approach not only clarifies the

dynamics of nutrient cycling but also underscores the lagoon's resilience and capacity to mitigate eutrophic conditions. Furthermore, the findings from this modelling effort can inform effective lagoon management strategies, such as optimizing water flow, implementing nutrient reduction initiatives, and establishing monitoring programs to enhance overall ecosystem health and sustainability.

MATERIALS AND METHODS

Study Area

The Salut-Mengkabong Lagoon is primarily characterized as a bar-built, tide-influenced estuarine system (Wong, 2005; Hoque *et al.,* 2010), with a notable absence of vertical salinity stratification (Osman *et al.,* 2017). Situated in the Tuaran District of Sabah, Malaysia, just north of the state capital, Kota Kinabalu, this lagoon features a distinctive geomorphological layout comprising a single entrance that bifurcates into two principal sections: Salut and Mengkabong (Figure 1). Over the past decade, the surrounding region has experienced significant land use transformations, transitioning from pristine mangrove ecosystems and coastal forests to various developments, including aquaculture, residential neighborhoods, resorts, commercial establishments, and industrial sites (Osman *et al*., 2017). This rapid change underscores the lagoon's vulnerability to anthropogenic pressures and highlights the need for sustainable management strategies to preserve its ecological integrity.

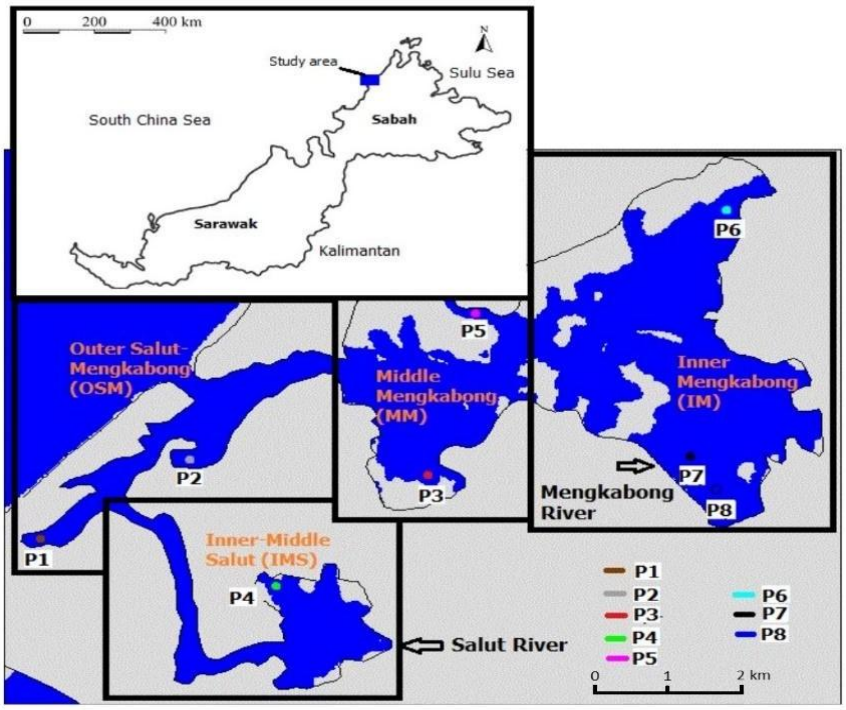

Figure 1. Map showing the study area within the Salut-Mengkabong Lagoon, divided into four main sub-lagoon catchments: Inner Mengkabong (IM), Middle Mengkabong (MM), Outer Salut Mengkabong (OSM), and Inner Middle Salut (IMS). It features eight strategically located monitoring stations (P1-P8) for data collection and analysis.

Water Flushing Measurement

The entire lagoon system is organized into four primary sub-lagoon catchments: Inner Mengkabong (IM), Middle Mengkabong (MM), Outer Salut Mengkabong (OSM), and Inner Middle Salut (IMS). Eight monitoring stations, designated as P1 to P8, were set up to assess water flushing times over a week (Figure 1). Two monitoring points are situated in the Outer Salut-Mengkabong (OSM) area, which is

closest to the open sea; one point is located in Inner-Middle Salut (IMS), characterized by a narrow channel and a wide water surface area within the inner lagoon; two points are found in Middle Mengkabong (MM), which has a large water area connected by two narrow channels to the inner and outer lagoons; and three points are in Inner Mengkabong (IM), the deepest section of the Mengkabong lagoon, featuring a wide surface area that receives freshwater from the Mengkabong River.

Hydrodynamic Module Setup

The hydrodynamic simulation of water flow within the Salut-Mengkabong Lagoon was executed using the DHI MIKE 21 FM modeling framework (DHI, 2016). This model incorporates detailed bathymetric data and encompasses an extensive marine area to mitigate boundary disturbances (Figure 2). To establish the computational domain, a Zero Mesh Generator was employed, creating a grid with triangular cells that range in size from 45 meters to 1215 meters, extending to the lagoon's water boundary. This modeling system utilizes a flexible mesh methodology, delivering a numerical solution to the two-dimensional shallow water equations, based on the depth-integrated and incompressible Reynolds-averaged Navier-Stokes equations. In this analysis, adjustments were made solely to the bed resistance parameter (Manning's number) to ensure the accuracy of the results. The effects of wind were deemed negligible, given the area's geomorphological characteristics, so wind data were excluded from the study. Therefore, the primary focus was placed on calibrating the flow dynamics.

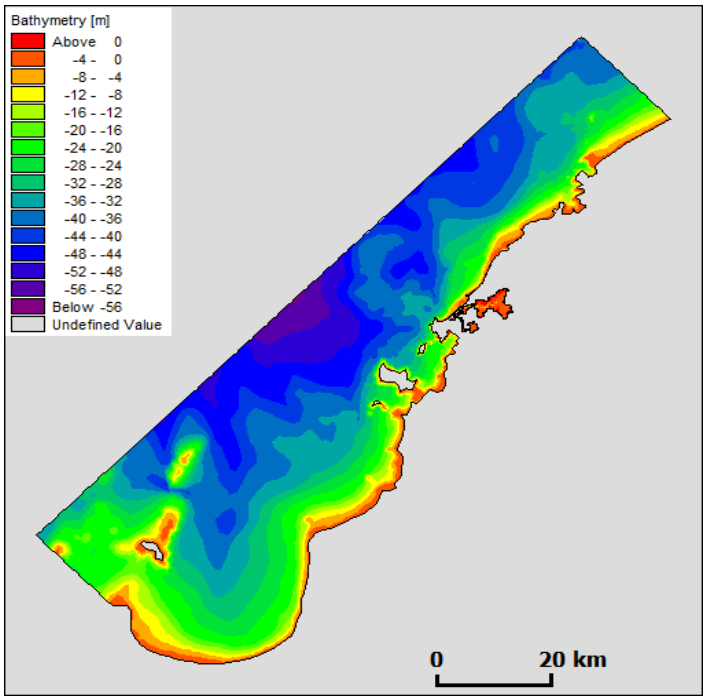

Figure 2. Bathymetry of the regional and local areas used for the MIKE modeling setup.

The boundary conditions for the water flow model in the Salut-Mengkabong Lagoon, known as the Salut-Mengkabong Transport Model (SMTM), were defined using a technique called transfer boundary. Details of the setup for the hydrodynamic (HD) simulation of the SMTM are provided in Table 1. This method establishes the interactions occurring at the lagoon's periphery, ensuring that the model accurately represents the hydrodynamic processes involved. By implementing transfer boundary conditions, the model effectively simulates the inflow and outflow dynamics, taking into account various external factors such as tidal movements, rainfall, and freshwater contributions. A comprehensive specification of these boundary conditions is essential for generating reliable and realistic outcomes in the simulation of water movement throughout the lagoon system. In addition to the local Salut-Mengkabong Lagoon area, the study domain has been expanded to include the coastal region of western Sabah, as shown in Figure 3.

Table 1. Description of the setup for the hydrodynamic (HD) simulation of the Salut-Mengkabong Transport Model (SMTM).

Features	Description
Data simulation	Regional: 1 Oct 2015 – 1 Oct 2016 Local: 1 Oct. 2015 – 16 April 2016 (8 days)
Domain	Mesh file: 1. Nodes: 31121 nodes 2. Elements: 59456 elements 3. Time step Interval: 300 seconds Consists of 5 different grid sizes: 1. 45 m (triangular) and, 2. 135 m (quadrangular) grid cells in the lagoons. 3. 135 m, 540 m, and 1215 m (triangular) grid cells towards the water boundary of the domain. The domain extends approximately: 1. 132698.66 m along the Sabah coast, 2. 21618.89 m offshore
Bathymetric data	Regional data - derived from the MIKE C-MAP (version 2010) developed by Jeppesen Marine AS, Norway. Local data - surveyed in 2008
Boundary condition	Open boundaries: sea area Land boundary: the mainland of Sabah
River source	The river discharge based on (DHI, 2013) rates of 1.03 m^3/s and 2.27 m^3/s in Sg. Salut and Sg. Mengkabong respectively.

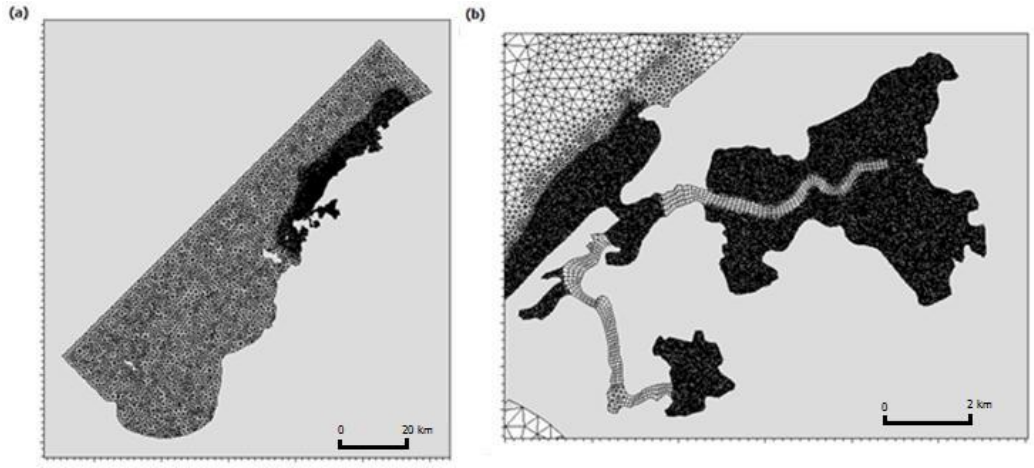

Figure 3. The model grid in MIKE 21 hydrodynamic model: (a) regional scale domain, western coast of Sabah; and (b) local scale domain of Salut-Mengkabong Lagoon.

The assessment of flushing time and vulnerability in the lagoon, particularly for tracer studies, was conducted using the HD and AD models (Li *et al*., 2015; Li & Yao, 2015; Yanagi, 2009). For flushing time calculations, we utilized simplified models based on the methodologies described by Monsen *et al*. (2002) to examine idealized scenarios. To gauge the lagoon's vulnerability, we adopted the estuarine export potential (EXP) classification system established by the National Research Council (2000). This approach facilitates a thorough understanding of the lagoon's dynamics and its susceptibility to external factors. The research framework for these analyses is depicted in Figure 4, providing a representation of both flushing times and vulnerability classifications, enhancing clarity and insight into the study's findings.

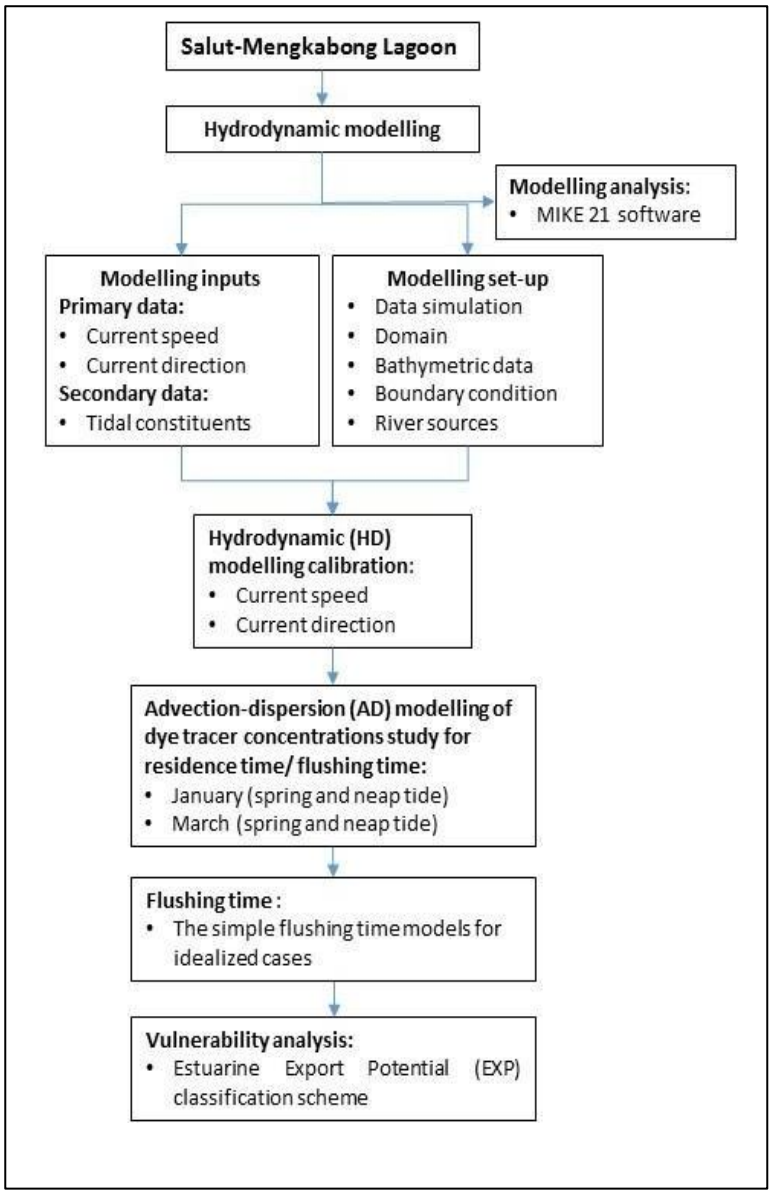

Figure 4. A framework for examining flushing time and susceptibility in the Salut-Mengkabong Lagoon.

Advection Dispersion Module Setup

The advection-dispersion model was utilized to calculate and estimate the water residence time in the Salut-Mengkabong Lagoon. In this approach, the only variable that was altered was the tidal cycle. A set of 100 virtual particles served as tracers, evenly distributed throughout the lagoon to replicate dispersion during four specific tidal periods: spring tides on January 10 and March 9, 2016, and neap tides on January 17 and March 16, 2016. Figure 5 illustrates the predicted characteristics of the highest spring tide and the lowest neap tide in the Salut-Mengkabong Lagoon.

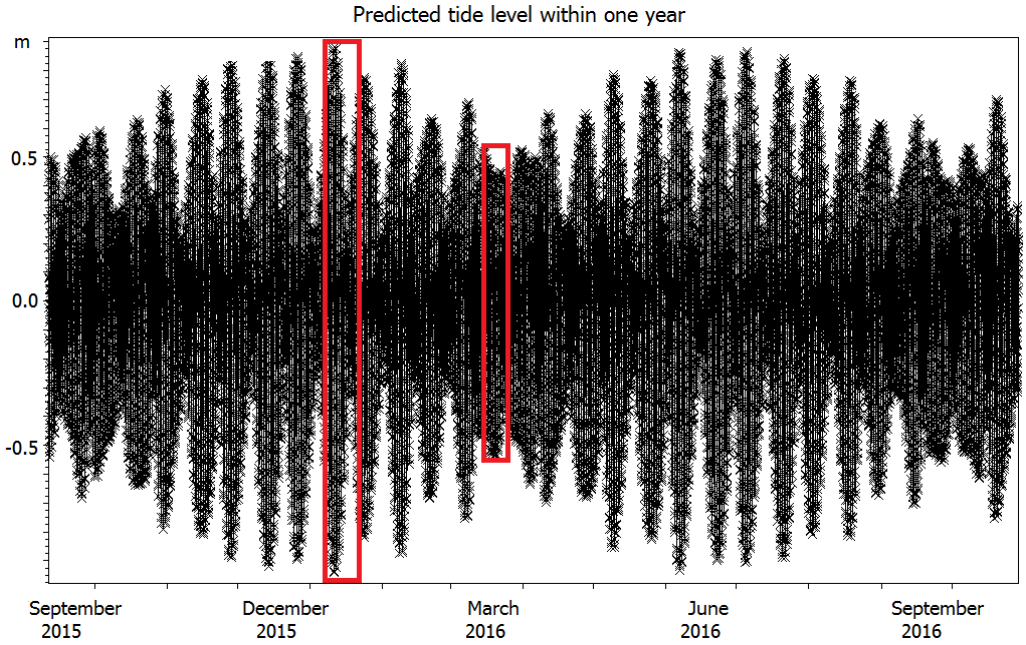

Figure 5. The highest spring and the lowest neap tides in the Salut-Mengkabong Lagoon (red box). (Source: RMN, 2015; RMN, 2016).

The simulation aimed to quantify the e-folding flushing time of the water in the lagoon, offering critical insights into its hydrodynamic behavior and water exchange mechanisms. The time required for the concentration of the tracer in the modeled area to decline served as a metric for assessing the system's flushing efficiency. To accurately determine the e-folding flushing time for each section, a thorough series of 16 simulations, each lasting seven days, was conducted. These simulations took place during the spring and near tides of January and March, building upon foundational data from previous MIKE 21 runs. This structured approach facilitated an in-depth evaluation of flushing dynamics, allowing researchers to gain a better understanding of the lagoon's ability to renew itself and regulate pollutant levels over time. By analyzing various tidal conditions, the study sought to provide a comprehensive overview of flushing efficiency across different regions within the lagoon.

In calculating the flushing time for the selected area in the Salut-Mengkabong Transport Model (SMTM), a key assumption was made: any mass introduced into the system would be uniformly and instantaneously distributed throughout the entire domain. Consequently, the concentration of a substance exiting the area would reflect the concentration throughout the SMTM. To pinpoint eight specific locations around the Salut-Mengkabong Lagoon, it was assumed that a known mass was introduced into the SMTM at time t = 0, resulting in an initial concentration represented as C0. Additionally, it was presumed that no further mass would be introduced after t = 0, and that both the flow and volume of the SMTM would remain constant over time. The concentration within the SMTM was then computed using Equation 1. According to Equation 1, at time t = T_f, the concentration diminishes to the e-folding level of approximately 37% or 1/e of the initial concentration value. The flushing time for the Continuous Stirred-Tank Reactor (CSTR) is represented by the average duration that the "fraction of tracer (water parcels) from the selected region" remains within the system (Monsen *et al*., 2002).

$$C(t) = C0 \times e\text{-}t / T_f \quad (1)$$

$$T_f = M / Mt \quad (2)$$

To determine the e-folding flushing time through the same hydrodynamic simulation, a conservative tracer was introduced into the Salut-Mengkabong Lagoon using the same data and model

configuration as the HD model. Initially, the tracer was released at a concentration of 100 kg/m³ in each of the four designated areas, while the concentration outside these areas was set to 0 kg/m³. The flushing time was calculated using the total tracer mass at the initial condition (M) and the average tracer loss rate over time (Mt) as it moved through the system, as expressed in Equation 2. This equation was primarily used to compare the differences among the lagoon areas and was subsequently applied in the "estuarine export potential" (EXP) classification by the National Research Council (2000) to evaluate the lagoon's vulnerability to eutrophication. The EXP framework includes two essential variables: flushing time and lagoon dilution, with the latter being calculated using modeling software to assess the volume of each lagoon area.

RESULTS AND DISCUSSIONS

Simulation and Model Evaluation

There was a notable agreement between the measured and simulated current speeds in the Salut-Mengkabong Lagoon after adjustments were made to the bed resistance (Manning's number) in the simulated data (Figure 6). The correlation coefficient (R-squared) for the current speeds was 0.17, accompanied by a root mean square error (RMSE) of 0.07 and a percentage RMSE of 33.50%. Additionally, the average deviation in current directions between the measured and simulated values was 33.02 degrees. Several limitations contributed to the elevated RMSE and discrepancies in the current direction, particularly the distinct geomorphology of the lagoon. This unique geomorphological feature diverges from typical open coastal waters, which restricts the flow of water from the coastal region into the lagoon.

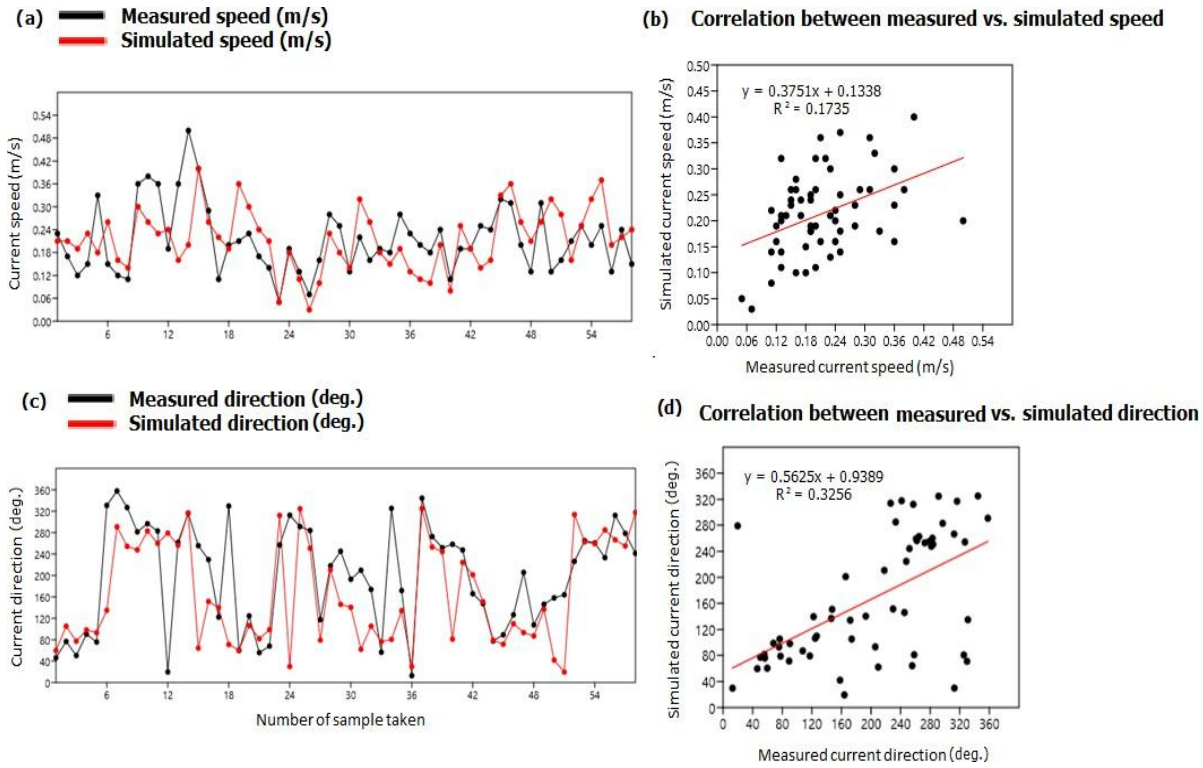

Figure 6. (a) Comparison of measured and simulated current speed; (b) Correlation between measured and simulated current speed; (c) Comparison of measured and simulated current direction; and (d) Correlation between measured and simulated current direction in Salut-Mengkabong Lagoon.

The average measured current speed in the lagoon was recorded at 0.21 m/s, while the average simulated current speed from the hydrodynamic modeling was slightly higher at 0.22 m/s. This close alignment suggests that the model's current speed predictions are valid for simulation purposes. However, a discrepancy was observed when evaluating the average current direction: the average measured current direction was 198.42 degrees, while the average simulated current direction from the hydrodynamic model was relatively lower at 165.40 degrees. This variation in current direction can be attributed to the complex hydrodynamic conditions prevalent within the lagoon, exacerbated by various obstacles at most sampling stations, which stem from the lagoon's unique geomorphological features. These physical barriers can significantly impact water movement, resulting in variations in both direction and speed across different areas of the lagoon. Consequently, while the model's current speed predictions are reliable, the intricacies of the lagoon's structure may explain the observed differences in current direction.

Flushing Analysis

In this study, flushing times were estimated at eight monitoring stations distributed across four sub-lagoon catchments: Outer Salut Mengkabong (OSM), Inner Middle Salut (IMS), Middle Mengkabong (MM), and Inner Mengkabong (IM). The data were presented as a time series for four distinct spring and neap tidal events (Figure 7). The findings indicate that the January Spring tide (Figure 7a) exhibited a higher flushing capacity than the other tidal cycles. According to Laws (2013), phytoplankton in tropical and subtropical regions typically have a doubling time of about one day. When the doubling time is extended while the flushing time remains short, phytoplankton is likely to be washed out from the isolated areas of MM (P3 and P5) and IM (P7 and P8), especially during the January Spring tide. There is also potential for washout in certain parts of MM (P5) and IM (P7) during the March Spring tide (Figure 7b).

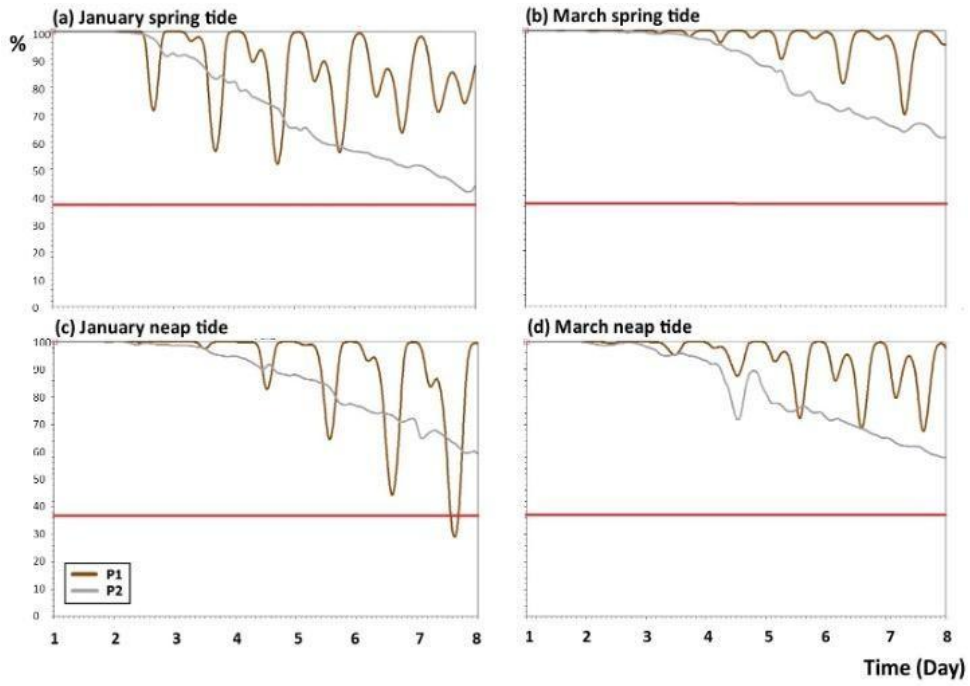

Figure 7. Time series analysis of flushing times in the Salut-Mengkabong Lagoon entrance (P1 and P2), simulated using OSM during four distinct spring and neap tide cycles.

Additionally, two observations of flushing time were conducted in the OSM sub-lagoon. Monitoring station P1 is located deeper within the sub-lagoon, while P2 is situated closer to the outer edge. During the January Spring tide (Figure 7a), P1 exhibited significant fluctuations on day 2, followed by a decrease in tracer concentration from day 5 to day 7. In contrast, P2 showed a steady decline in tracer concentration. The January neap tide (Figure 7c) revealed a 37% reduction in tracer

concentration at P1 by day 7. However, during the March Spring (Figure 7b) and neap (Figure 7d) tides, both P1 and P2 exhibited minimal changes, although their fluctuation patterns differed. The point at which the tracer concentration dropped to 37% of its initial value (100%) is indicated by a thick red line in Figure 7. Regardless of the prevailing tidal conditions, it is expected that phytoplankton will proliferate in the isolated zone of OSM.

A single monitoring station, P4, was established in the inner section of the Inner Middle Salut (IMS) due to its isolation from the flow of the Salut River (see Figure 8). The simulation, which explored variations in flushing times, indicated that P4 suffered from poor circulation, leading to the accumulation of pollutants. During the January Spring tide (Figure 8a), P4 exhibited more frequent fluctuations over the seven-day observation period compared to other tidal cycles, although the decrease in tracer concentration was relatively small. In the January neap tide (Figure 8c), fluctuations began on day 5, resulting in a more pronounced reduction in tracer concentration by day 7 than noted in earlier tidal cycles. Minimal variation was observed during the March Spring (Figure 8b) and neap (Figure 8d) tides. Consequently, phytoplankton will probably thrive in the isolated area of IMS, irrespective of the existing tidal conditions.

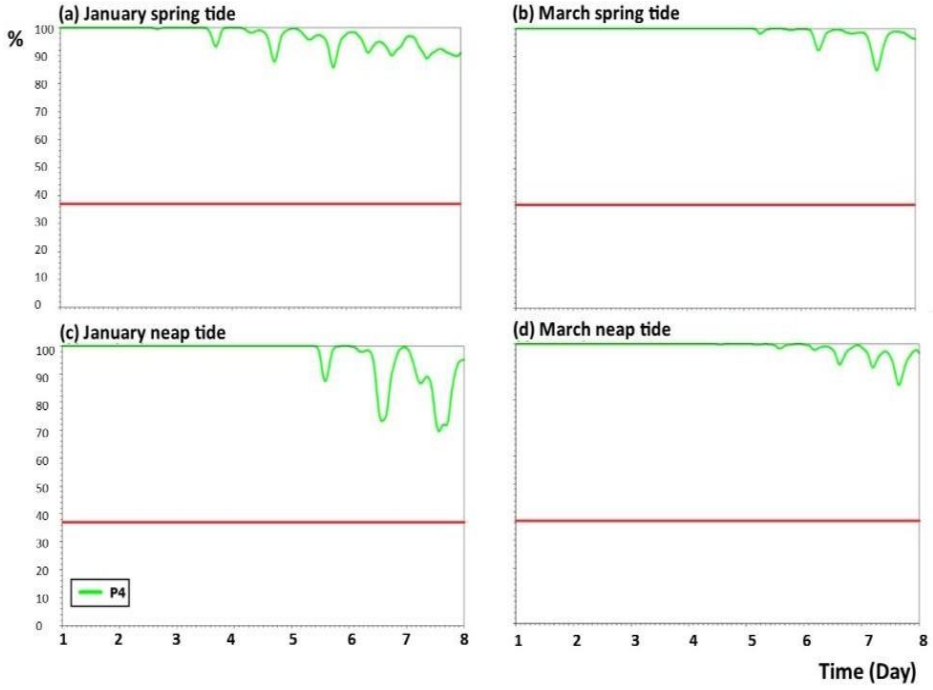

Figure 8. Time series analysis of flushing times in the IM (P4), simulated across four different spring and neap tide cycles within the Salut Lagoon.

Two monitoring stations were selected within the Middle Mengkabong (MM) sub-lagoon: P3, situated near a seagrass area, and P5, located close to the Prawn Farm Channel in Mengkabong Lagoon. During the January Spring tide, the tracer concentration at both P3 and P5 declined to 37% by day one (Figure 9). In the case of P3, during the January neap tide (Figure 9c), as well as the March Spring (Figure 9b) and March neap (Figure 9d) tides, the concentration also reached 37% by day five. For P5, the concentration dropped to 37% by day two in both the January neap (Figure 9b) and March neap (Figure 9d) tides, indicating a more rapid flushing response during the spring tide. Interestingly, despite P5 covering a smaller area, P3 exhibited a longer flushing time. This suggests that P3 may be more prone to retaining pollutants if they are discharged during neap tides. The point at which the tracer concentration fell to 37% of its initial value (100%) is marked by a thick solid black line in Figure 9. Consequently, phytoplankton are likely to be flushed out of the isolated MM areas (P3 and P5) during the January Spring tide, as well as from specific regions of MM (P5) during the March Spring tide.

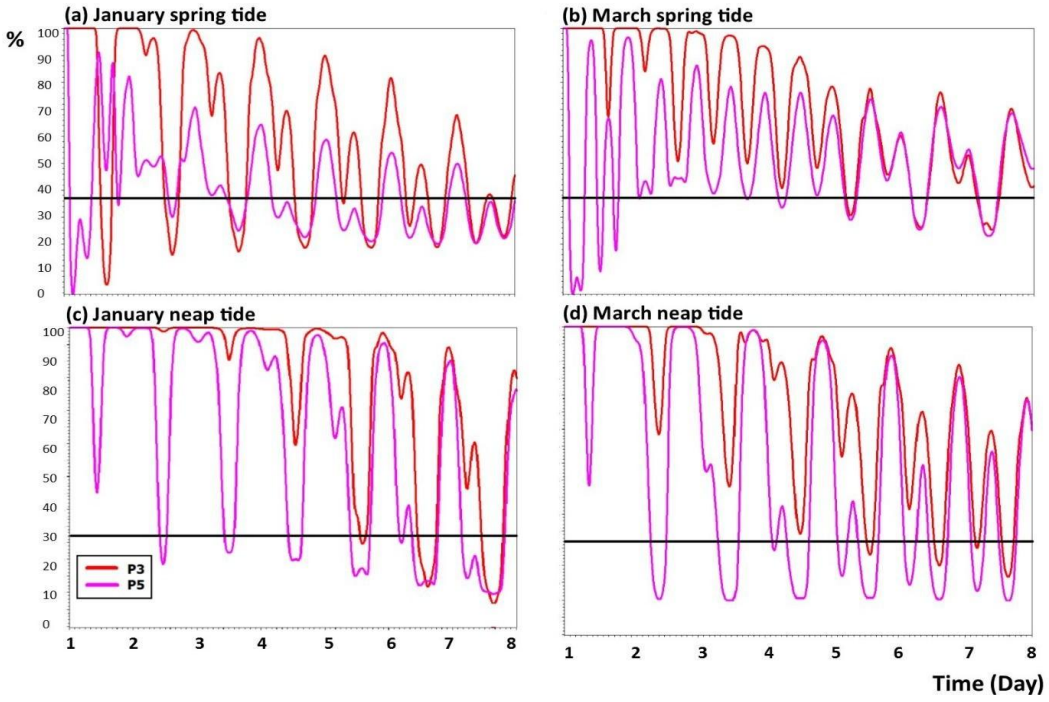

Figure 9. Time series analysis of flushing times in the MM (P3 and P5), simulated across four distinct spring and neap tide cycles within the Mengkabong Lagoon.

P6 is positioned in the northern section of the Inner Mengkabong (IM), while P7 is located in the southern part, where it benefits from robust water flow from the Mengkabong River. In contrast, P8 represents an isolated area with minimal influence from river flow. The flushing times observed during the January (Figure 10a) and March (Figure 10b) spring tides exhibited notable differences: both P7 and P8 reached a 37% concentration by day one during the January Spring tide.

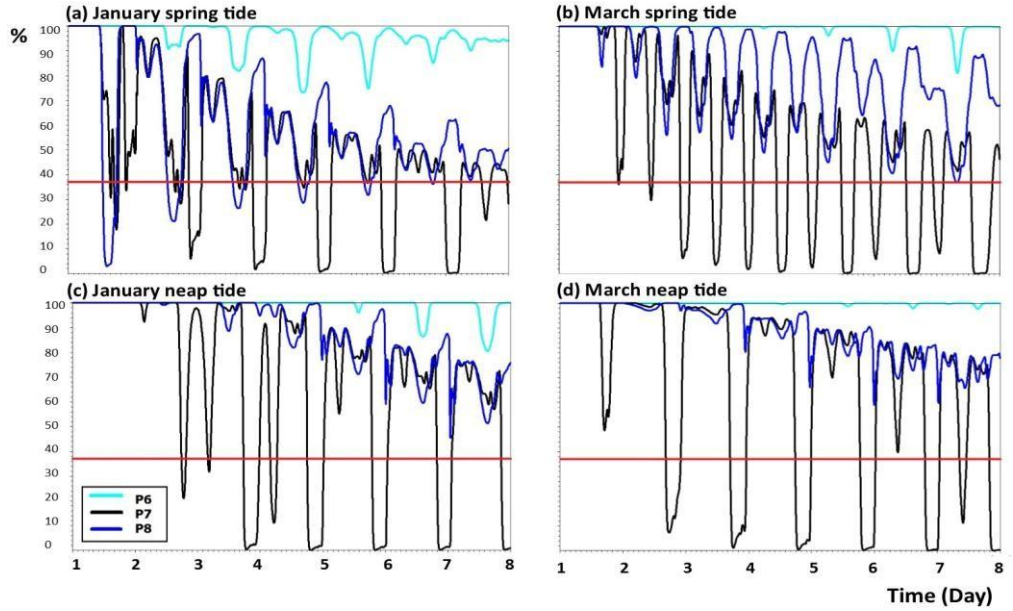

Figure 10. Time series analysis of flushing times in the IM (P6, P7, and P8), simulated across four distinct spring and neap tides within Mengkabong Lagoon.

However, during the March Spring tide, P7 achieved a 37% concentration by day two, whereas P8 took over a week to attain the same level. For both the January (Figure 10c) and March (Figure 10d)

neap tides, P7 had a flushing time of just two days, while P8 required significantly longer to be completely flushed. P6 demonstrated particularly poor flushing characteristics, especially during the March neap tide (Figure 10d), suggesting it is unsuitable for aquaculture or waste disposal. The point at which the tracer's concentration diminished to 37% of its initial value (100%) is indicated by a thick solid red line in Figure 10. Consequently, phytoplankton is likely to be flushed out from the isolated areas of IM (P7 and P8) during the January Spring tide and only minimally from specific regions of IM (P7) during the March Spring tide.

Flushing Time and Vulnerability of the Lagoons

Flushing time serves as an integrative measure within the system, and the choice of an appropriate transport scale depends on guiding questions. In this context, flushing time is used to compare the general characteristics of four different sub-lagoons within the Salut-Mengkabong Lagoon system across four distinct tidal cycle scenarios. Calculations of flushing time suggest that the lagoon is particularly vulnerable to pollutants, especially in the IM and IMS regions. These findings underscore critical insights regarding transport and flushing dynamics in both lagoons and provide support for field-based hypotheses. According to previous research (Osman *et al*., 2017), higher concentrations of PO_4 and NO_3 were found in Inner Mengkabong compared to other areas, which can be attributed to the system's capacity to flush out pollutants. This indicates that the Outer Salut-Mengkabong (OSM) experiences a rapid exchange of water with lower nutrient concentrations than other regions. Assessing a system's flushing capability is a valuable indicator of its relative potential for phytoplankton blooms in certain areas. However, determining bloom concentrations is contingent on dilution; the volume of the system influences the density and ultimately the toxicity of pollutant loads or bloom discharges.

This framework enables a comprehensive assessment of vulnerability to eutrophication and pollutant toxicity, particularly relevant in estuarine and semi-enclosed coastal areas, which are especially sensitive due to their limited water exchange with open coastal waters. By analyzing flushing times alongside system vulnerabilities, stakeholders can acquire essential insights that inform their operational strategies in fish farming and aquaculture. Understanding the flushing dynamics of a particular area allows aquaculture operators to optimize their feeding schedules, ensuring that nutrient inputs are managed effectively to mitigate the risk of triggering harmful algal blooms. Moreover, detailed information regarding flushing times can inform decisions about the optimal timing for discharging waste or conducting maintenance activities, allowing such operations to take place when the system is better equipped to assimilate or dilute potential contaminants. This approach is crucial for maintaining water quality; poorly timed discharges can result in nutrient accumulation and subsequent eutrophication, which negatively impacts aquatic life and diminishes overall ecosystem health.

For instance, Table 2 illustrates that during the March neap tide in the Inner Mengkabong (IM) sub-lagoon, the extended flushing time renders it the least favorable period for any type of discharge, emphasizing the heightened risk of pollutant accumulation within the system. With this information, operators can adopt the best management practices aligned with tidal cycles, thereby promoting the sustainability of their operations while safeguarding the ecological integrity of the lagoon system. Overall, this framework not only aids in enhancing operational efficiency but also supports the long-term health and resilience of sensitive coastal ecosystems.

To accurately assess the lagoons' responses to nutrient enrichment, it is essential to consider several key factors that influence their eutrophication. These factors include geomorphological setting, the primary production base, nutrient load, dilution, flushing time, stratification, hypsography, phytoplankton grazing, suspended material load, light extinction, denitrification, as well as the spatial and temporal distribution of nutrient inputs and allochthonous organic matter (National Research Council, 2000). However, this study found that the vulnerability analysis employed a simple classification scheme of EXP for assessing eutrophication (and other forms of pollution), serving as a reference to prevent excessive nutrient discharge into the lagoons. The susceptibility indices developed by Bricker *et al*. (1999; 2003) (Table 3) aid in evaluating the degree of nutrient over-enrichment in coastal systems, focusing primarily on dilution and flushing characteristics. According to the modified

matrix for assessing the susceptibility levels of estuaries, the Salut-Mengkabong lagoon is categorized as microtidal (< 2.5 m) and exhibits a high level of susceptibility, with an average flushing time ranging from 7 to 30 days and a smaller volume (10^{-09} to 10^{-07}).

Table 2. Flushing time of four sub-lagoon catchments in four different simulations.

	Outer Salut-Mengkabong (OSM)				Inner-Middle Salut (IMS)				Middle Mengkabong (MM)				Inner Mengkabong (IM)			
	Js	Jn	Ms	Mn	Js	Jn	Ms	Mn	Js	Jn	Ms	Mn	Js	Jn	Ms	Mn
Mean dye Tracer (%)	23.1	33.2	27.4	29.5	59.9	76.9	70.0	77.5	27.4	45.3	34.8	42.9	54.4	80.2	63.8	32.8
Est. volume (m^3)	7×10^7				4×10^7				7×10^7				1×10^8			
Flushed (days)	9.1	10.5	9.7	9.9	17.5	30.4	23.3	31.1	9.6	12.8	10.7	12.3	15.3	35.4	19.4	40.6

Note: Js=January-Spring; Jn=January-Neap; Ms=March-Spring; Mn=March-Neap.

Table 3. Matrix for assessing the susceptibility of the Salut-Mengkabong Lagoon to nutrient over-enrichment, based on flushing time and the inverse of volume.

Flushing Time (Days)	**Index**		
Slow (> 30)	Low	Low	Moderate
Moderate (7 - 30)	Low	Moderate	High
Faster (< 7)	Moderate	High	High
1/Dilution (Volume)	**Larger (10^{-13} ~ $^{-11}$)**	**Moderate (10^{-11} ~ $^{-09}$)**	**Smaller (10^{-09} ~ $^{-07}$)**

Source: Modified from Bricker *et al*. (1999); National Research Council (2000)

Utilizing the modified classification scheme for 138 estuaries outlined in the Estuarine Eutrophication Assessment (Figure 11) (National Research Council, 2000), systems located in the lower right area of the graph, characterized by smaller volumes and faster flushing rates, demonstrate a higher susceptibility to eutrophication.

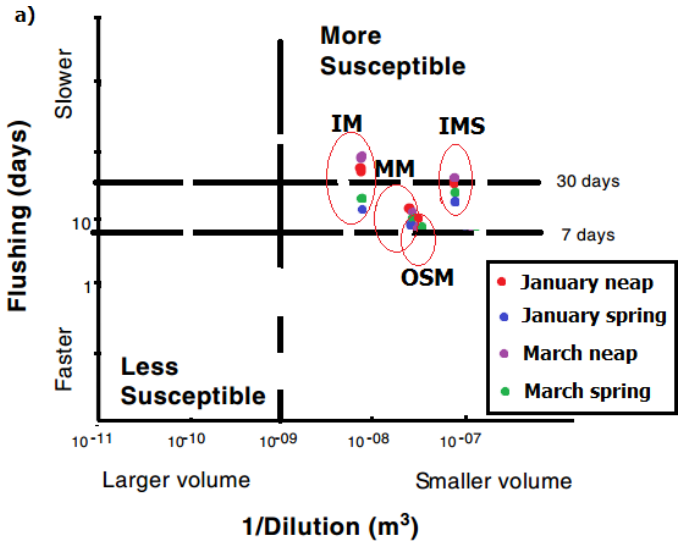

Figure 11. Relationship between expected flushing time (days) and dilution volume (m^3) for assessing system vulnerability, modified from the Estuarine Eutrophication Assessment by NOAA.

Conversely, systems exhibiting extremely large dilution volumes and shorter flushing times are deemed the least susceptible to nutrient-enhanced eutrophication. In contrast, systems anticipated to be highly susceptible to eutrophication are represented in the upper right area of the graph, where the dilution volumes are smallest and flushing rates are slowest. For the Salut-Mengkabong Lagoon classification, Inner Mengkabong (IM) is predicted to be particularly vulnerable to eutrophication, especially during the March neap tide, followed closely by the January neap tide.

CONCLUSION

The dye tracer study conducted to evaluate the e-folding flushing time revealed that the monitoring stations P4 (located in the Inner Middle Salut, IMS) and P6 (in the Inner Mengkabong, IM) demonstrated inadequate flushing characteristics. This finding underscores the critical need for regular monitoring of the Mengkabong Lagoon due to the significant risks associated with deteriorating water quality and extended residence times of pollutants. Specifically, the flushing times recorded in the inner lagoon areas (IM and IMS) - ranging from approximately 15 to 40 days - are considerably longer than those observed in the outer lagoon (approximately 9 to 10 days) and in the intermediate lagoon (approximately 9 to 13 days). These extended flushing times in the inner regions can lead to the accumulation of nutrients and pollutants, resulting in detrimental effects on water quality and aquatic life.

A deeper understanding of the hydrodynamic processes within and surrounding the watershed enhances our ability to develop more effective management strategies for land managers and regulatory authorities. Such strategies are imperative for maintaining and improving water quality throughout the lagoon system. Furthermore, the implications of prolonged flushing times in the inner sections of the Salut-Mengkabong Lagoon suggest that elevated concentrations of thermal energy, phytoplankton, and dissolved substances are likely to build up. The presence of dead-end areas combined with weak water circulation exacerbates this problem, potentially leading to eutrophication and its associated ecological repercussions. Consequently, proactive measures must be implemented to mitigate these risks and promote the overall health of the lagoon ecosystem.

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RESEARCH ARTICLE

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SUSTAINABLE PAPER FROM AGRICULTURAL WASTE: A STUDY ON PINEAPPLE LEAF FIBRE USING ORGANSOLOV PULPING

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Nonwood pulp; Organic acid pulping; Surface morphology; Tearing resistance; Tensile strength; Natural fibre

ABSTRACT. *The use of non-wood fiber sourced from agricultural waste for papermaking has attracted the interest of many researchers. In this study, paper made from pineapple leaf fiber (PALF) was developed using environmentally friendly approaches. This study aimed to prepare a PALF paper using organosolv pulping with acetic acid (AcOH) and 0.1% hydrochloric acid as a catalyst. The PALF was treated with varying AcOH concentrations (16%, 20%, 24%, 28%, and 32%) for 9 hours to study the effectiveness of delignification in producing high-quality pulp. Additionally, the morphological and mechanical properties of the PALF paper were characterized to analyze its potential as a quality paper. The study discovered that organosolv pulping could produce fibers that can be made into paper with properties comparable to those produced by other conventional methods. Based on Fourier Transform Infrared (FTIR) analysis, the emergence of cellulose-associated peaks and the reduced intensities of peaks attributed to lignin and hemicellulose suggest effective delignification. The Scanning Electron Microscope (SEM) analysis revealed that the treated PALF consists of well-separated cellulosic microfibrils. Meanwhile, mechanical analysis using the Universal Testing Machine (UTM) showed that the tensile strength of the papers (0.25, 0.23, 0.27, 0.20, and 0.19 MPa) varied, while the tearing resistance showed an increasing trend (2.07, 5.15, 6.86, 10.03, and 11.1 mN·m²/g) with increasing AcOH concentration. These findings suggest that PALF is a viable alternative for sustainable paper production.*

INTRODUCTION

It is undeniable that paper is an indispensable part of our lives. However, the increasing demand for paper has led to higher production rates, which in turn require more trees to be cut down for wood pulp. This has significant environmental consequences. Wood remains the primary raw material for producing various types and qualities of paper. However, the use of wood fibers for pulping has become increasingly problematic due to their scarcity in recent years. This has prompted interest in alternative sources, such as non-wood lignocellulosic fibers. Currently, non-wood fibers account for only about 10% of the global papermaking industry (Sibaly & Khadoo-Jeetah, 2017). In response to these challenges, researchers have explored various non-wood fibers for paper production. Among these, pineapple leaf fiber (PALF) has emerged as a promising candidate. Malaysia, with its substantial pineapple cultivation, offers an abundant supply of pineapple leaves, which are often discarded as agricultural waste. According to the Malaysian Pineapple Industry Board (MPIB), the country produced

about 416,000 metric tons of pineapples in 2022, with Sabah contributing around 468 hectares of cultivated area and production of 7,587 metric tons (MPIB, 2022). Hence, the leaves produced from this pineapple cultivation can be developed for use as fiber in the pulp and paper-making industry.

In this research, the potential of pineapple leaf fiber (PALF) as a substitute for wood in paper production was explored. The use of PALF in the paper industry is still relatively new (Sibaly & Khadoo-Jeetah, 2017). The abundant availability and lower cost of lignocellulosic fibers like PALF in Malaysia provide a promising alternative source for pulp and papermaking (Dhanasekar *et al.,* 2023). Pineapple, scientifically known as *Ananas comosus*, belongs to the Bromeliaceae family. Pineapple leaf is primarily composed of cellulose (70 - 80%), lignin (4.8%), and hemicellulose (19%) (Amirul Azan *et al.*, 2020; Asim *et al*., 2015). The high cellulose content of pineapple leaf makes it a viable raw material for producing quality paper, as cellulose is critical to paper strength and durability (Laftah & Wan Abdul Rahman, 2016). PALF also boasts consistent fiber quality, favorable chemical composition, and good mechanical strength (Mahatme *et al*., 2018). Furthermore, PALF has a lower lignin content compared to other non-wood cellulosic fibers (Kumar, 2020).

This study focused on the characterization of paper produced from pineapple leaf fibers (PALF), addressing a critical gap in sustainable material alternatives for the paper industry. While PALF has shown potential as a non-wood lignocellulosic fiber, there is limited research on its application in environmentally friendly pulping processes and the quality performance of the resulting paper (Rodríguez *et al*., 2018). However, there remains a lack of comprehensive evaluation of PALF using greener pulping technologies, such as organosolv pulping, particularly in the context of Malaysian agricultural waste utilization (Saberikhah *et al*., 2011). To address this gap, the present research investigates the use of organosolv pulping, a chemical process that uses organic solvents to remove lignin as a more sustainable alternative. Despite its environmental advantages, organosolv pulping has not been extensively applied to PALF, and its effects on fiber integrity, chemical composition, and paper mechanical properties remain underexplored. Therefore, this study aimed to fill this knowledge gap by characterizing PALF-based paper through a combination of analytical techniques: Fourier-transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) for chemical and morphological assessment, and a Universal Testing Machine (UTM) for mechanical performance evaluation.

MATERIALS AND METHODS

Sample Collection

The pineapple leaves (*Ananas comosus*) were collected from a local pineapple plantation at Putatan, Sabah, Malaysia. The green fresh leaves were used in this study.

Pulping and Paper Making Process

The pineapple leaves were treated with varying concentrations of acetic acid, AcOH (16%, 20%, 24%, 28%, and 32%) for 9 hours at 100 °C, with 1 mL of concentrated HCl added as a catalyst. Following the delignification process, the treated leaves were filtered and rinsed with distilled water to remove any residual chemicals. The resulting pulps were bleached with 30% hydrogen peroxide (H_2O_2) for 4 hours at 60 °C. The bleached pulps were then used to prepare PALF paper sheets using a mold and deckle. The paper sheets were flattened and dried overnight in an oven set to 60 °C. Finally, the dried paper sheets were stored at room temperature for further characterization. The obtained paper sheet was labeled as shown in Table 1.

Fourier Transform Infrared (FTIR) Analysis

The paper samples were analyzed using Fourier Transform Infrared (FTIR) spectroscopy to identify the presence of cellulose and lignin functional groups in the PALF, both before and after pulp treatment.

The analysis was performed with a Perkin Elmer Spectrum 100 FTIR Spectrometer. The spectra were recorded in the wavelength range of 4000 to 450 cm^{-1}, with each measurement consisting of 4 scans.

Table 1. Pineapple leaf fibre paper (PALF-paper).

PALF-Paper	Acetic Acid Concentration (%)
PALFP16	16%
PALFP20	20%
PALFP24	24%
PALFP28	28%
PALFP32	32%

Surface Morphology Analysis

The paper samples were analyzed using a Scanning Electron Microscope (SEM) to examine the surface morphology of the PALF in each sheet. This analysis was conducted with a Carl Zeiss EVO MA 10 instrument. Images of the fibers were captured at magnifications of 100× and 500×, with an accelerating voltage of 15 kV (Soloi & Hou, 2019). The samples, being non-conductive, were coated with a sputter coating to enhance image quality and resolution.

Mechanical Properties

The tearing resistance, also known as the Elmendorf tear test, was measured to assess the internal tearing resistance of the PALF paper using a ZB-SLY1000B tearing machine. Test pieces were cut to dimensions of 6.5 cm × 7.5 cm. Additionally, the tensile strength of the samples was evaluated using a GOTECH Electronic Mechanical Testing Machine, with test pieces cut to dimensions of 10 cm × 1.5 cm.

RESULTS AND DISCUSSIONS

Fourier Transform Infrared Analysis

The effect of chemical treatment using the organosolv method on the removal of lignin from PALF was examined in this study. Lignin is typically regarded as an undesirable polyphenolic compound with an amorphous structure composed of three types of phenylpropane units: p-coumaryl, coniferyl, and sinapyl alcohols. The quality of paper is defined by its high cellulose and hemicellulose content, along with low lignin content, which contributes to its strength and bleachability. The FTIR spectra of untreated PALF and the PALF paper are shown in Figure 1. As observed in the spectrum in Figure 1, the strong absorption band occurs at 3300 cm^{-1} corresponds to the -OH stretching vibration (Obi Reddy *et al.,* 2014) in both untreated and treated PALF. These peak decreases in intensity and become narrower due to the formation of free -OH groups. Meanwhile, the absorption bands around 2918 cm^{-1}, which correspond to the asymmetric and symmetric stretching vibrations of methylene groups (C-H) in cellulose component, become prominent, reflecting that the removal of the lignin was successful (Reddy *et al.,* 2009). It is also notable that the peak around 1730 cm^{-1}, attributed to carbonyl stretching (C=O), has decreased in intensity, likely due to the removal of a substantial portion of uronic acid, a constituent of hemicellulose xylan, from the PALF (Reddy *et al.,* 2009). Furthermore, the intensity of the peaks around the 1630 cm^{-1} which are attributed to the C-H of aromatic lignin, also decreased and completely disappeared in the PALF treated with the highest concentration (32% AcOH). The absorption band near 1425 cm^{-1} showed a reduction in intensity due to the changes in the environment of the C6 atom, potentially related to the formation or breaking of the hydrogen bond at the O6 position in cellulose (Saha *et al.,* 1991). Furthermore, the band near the 1245 cm^{-1} region has also reduced in intensity, probably due to the disappearance of ester and acid carbonyl stretching vibrations in hemicellulose (Obi Reddy *et al.,* 2014). In addition, the peaks near 890 cm^{-1} are associated with the deformation and

stretching of C-O-C, C-C-H, and C-C-O in the β-glycosidic linkage of the glucose ring (Gea *et al*., 2018). The appearance of the 890 cm^{-1} peak in the treated fibers could be due to an increase in cellulose components (Sasikala & Umapathy, 2018). Overall, the organosolv pulping process resulted in effective lignin removal, as observed from the FTIR spectra, where lignin-related peaks have reduced in intensity as the concentration of AcOH increases.

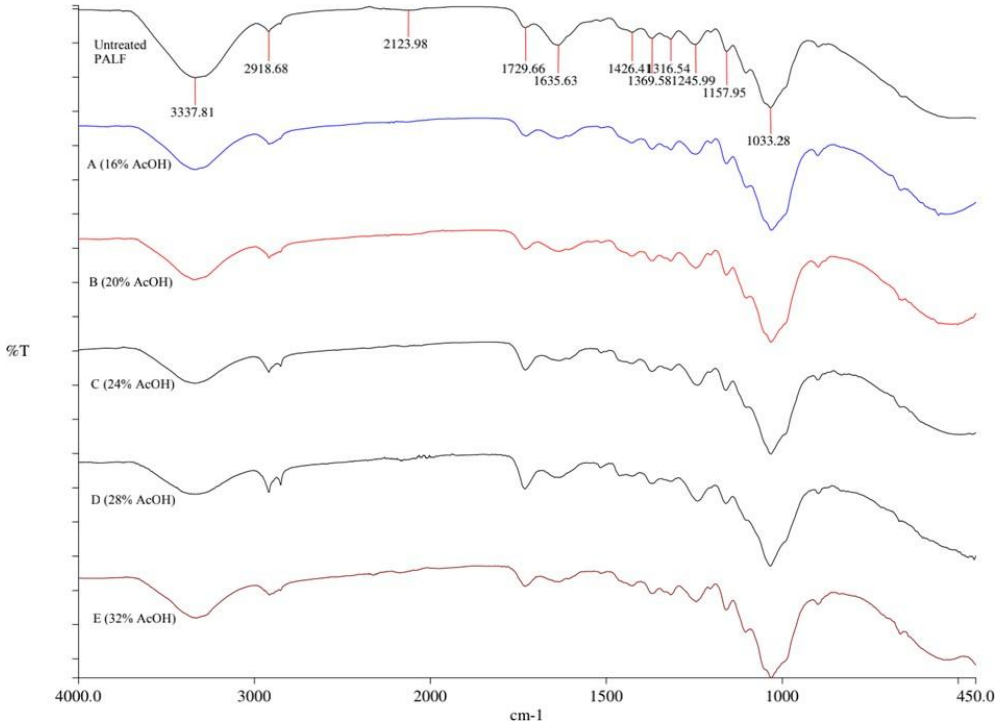


Figure 1. FTIR spectra of untreated PALF and treated PALF using different AcOH concentrations.

In organosolv pulping, the organic acid used principally acts on the impregnation of vegetal tissue and the solubilization of lignin fragments (Xu *et al*., 2006). The principal chemical reactions involved in this pulping are the cleavage of β-O-4 linkages of lignin, lignin condensation, ester hydrolysis, along with OH groups esterification (Li *et al*., 2016). At high temperature, several chemical reactions take place during the pulping process, which include the cleavage of β-O-4 and aromatic C-C linkages, new C-O bonds formation which is attributed to the lignin repolymerization (Li *et al.*, 2016). Acidic treatment of fiber also involved the hydrolyzation of hemicellulose and lignin through the breakdown of the polysaccharides to simple sugars, leading to the release of the cellulosic fibers. Meanwhile, the mechanism involved in the bleaching process is lignin oxidation, which results in the dissolution and degradation of lignin (Cherian *et al*., 2008). The main purpose of the bleaching process is to remove any excess lignin, apart from removing the phenolic compounds or molecules containing chromophore groups after the pulping. This is to increase the brightness of the produced pulp. Bleaching is also essential for the improvement of the physical properties of the fiber, which can be attributed to the better hydration of the pulp during the bleaching process (Jahan *et al.,* 2014). This is because for unbleached organic acid pulp, the hydroxyl groups of the cellulose were either acetylated or formylated, which causes the hydration of the pulp to be reduced (Jahan *et al*., 2014).

Scanning Electron Microscope (SEM) Analysis

The morphological changes in untreated PALF and pulp fibers treated with five different concentrations of acetic acid, as observed through SEM analysis, are shown in Figure 2. The morphological images of untreated PALF reveal the multicellular nature of the fiber. Generally, most natural cellulosic fibers have a multicellular structure, with cellulose bound together by lignin and hemicelluloses. This is evident in the image, where the fiber's surface is completely covered with lignin and other impurities,

making the cellulosic fibers indistinguishable. Meanwhile, in the treated fibers PALF16-PALF32, the emergence of a fibrillar structure, consisting of cellulosic microfibrils, can be observed. Fibrous cell of PALF consists of a vascular bundle system in the form of bunches (Asim *et al.,* 2015), as can be seen at 500× magnification at higher AcOH concentration. Additionally, the impurities on the fiber surface have disappeared. As the concentration of AcOH increases, more fibrils become visible, and the fiber bundles are oriented in various directions within the samples (Soloi & Hou, 2019). With higher concentrations of AcOH, the fiber bundles disintegrate into elementary fibers, which is noticeable through the more widespread distribution of these fibers. The rough surface of the fibers is attributed to increased adhesion between the fiber interfaces and the absorption of water during the papermaking process (Obi Reddy *et al*., 2014). The SEM image of this PALF paper, compared to hardwood paper for newsprint purposes, shows that the fibre oriented in a similar manner to hardwood paper reflects the potential of PALF as an alternative for pulp and paper production (Chinga-Carrasco, 2009).

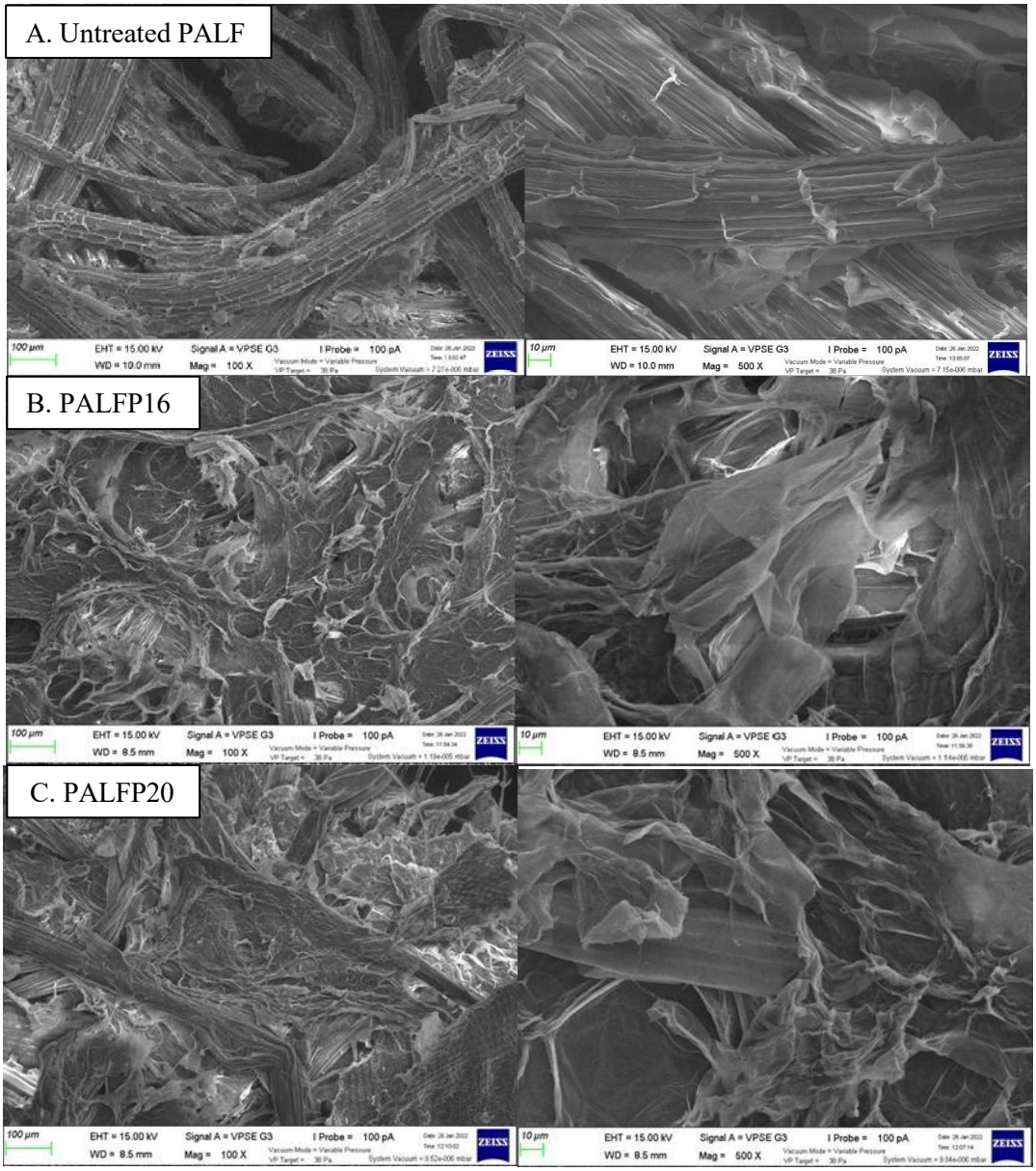
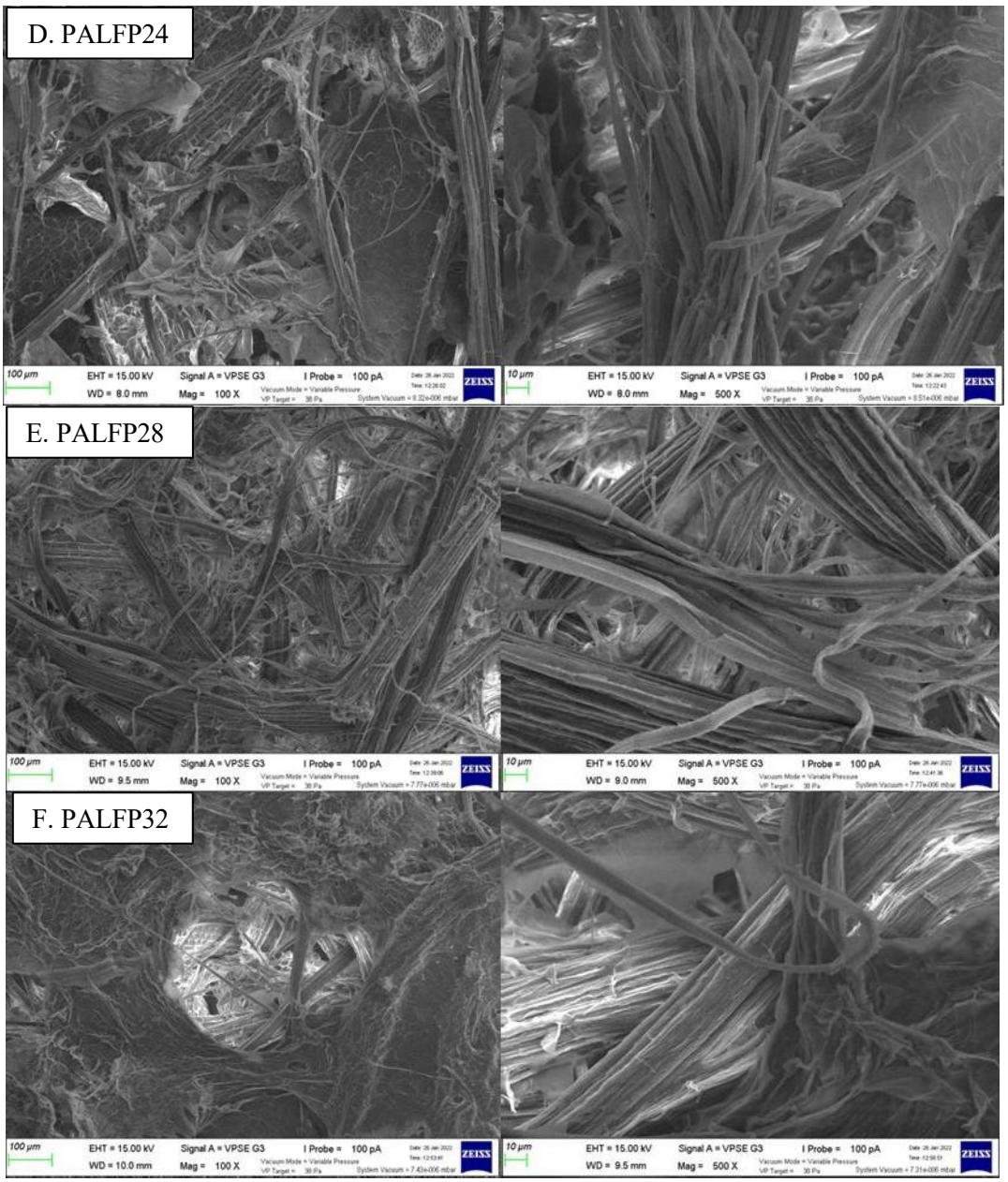


Figure 2. Surface morphology of PALF paper at 100× (left) and 500× (right) magnification: (a) Untreated PALF; (b) PALFP16; (c) PALFP20; (d) PALFP24; (e) PALFP28; and (f) PALFP32.



Continue **Figure 3.** Surface morphology of PALF paper at 100× (left) and 500× (right) magnification: (a) Untreated PALF; (b) PALFP16; (c) PALFP20; (d) PALFP24; (e) PALFP28; and (f) PALFP32.

The physical appearances of the PALF paper sheets are shown in Figure 3. Noticeably, the appearances of the PALF paper sheets are not very different from one another. PALFP16 – PALFP28 are quite alike in terms of their brightness and surface textures. Meanwhile, PALFP30 and PALFP32 have a rougher surface, which is most probably due to the fibrillation of the PALF at higher concentrations of AcOH. Furthermore, the coarseness of the papers' surfaces could also be attributed to the non-fibrous material, such as the outer skin of the leaves, which were caught in the mold along with the fiber during the molding step and not finely ground during the grounding process. But considering the deep green color and hard outer coating of the pineapple leaves, it can be said that the organosolv pulping has effectively delignified the PALF, even though the AcOH concentration used was relatively lower than that of other studies. Besides, the bleaching with H_2O_2 had helped in improving the appearance of the pulp.

Figure 4. Physical appearance of PALF paper using organosolv pulping.

Tensile Strength Analysis

The mean tensile strengths of the PALF paper samples are shown in Figure 4. It was found that the average tensile strength of the PALF paper varies with the varying concentration of AcOH. The tensile strength wane from PALFP16 to PALFP20 but then improved on PALFP24. This could be due to strong inter-bonding of the fiber in PALFP16, but weaker if compared to that of PALFP24. However, the tensile strength then reduced again from PALFP30 to PALFP32. As the cooking solvent's concentration increased, the tensile strength decreased except for PALFP24. The decreasing trend can probably be associated with the cellulosic fiber degradation at higher concentrations of AcOH (Soloi & Mohammad, 2023). This is because, although the lignin removal is enhanced at higher concentration, there is a possible risk of cellulose degradation and fiber rupture (Soloi & Mohammad, 2023). Moreover, the decrease in tensile strength could be related to the hydrophilic nature of the PALF (Asim *et al.,* 2015; Rajeshkumar *et al.,* 2020). The tensile strength of PALF observed in this study was slightly higher than that reported for NaOH-treated PALF in the study by Evelyn *et al.* (2019), suggesting that the current treatment method may offer improved reinforcement properties.

Tearing Resistance Analysis

The tearing resistance analysis of the PALF paper was tested using the Elmendorf tearing tester machine. Figure 5 shows the tearing resistance of the paper sheet. The tearing index of the PALF paper samples produced increases from PALFP16 to PALFP32. This suggests that the increasing concentration of the AcOH has improved the tearing resistance as more of the cellulose bunches were formed during the pulping. As can be seen in the SEM image, PALF has a long fibre bunch. The longer the fibre, the greater the resistance of the paper to tearing (Dwivedi *et al*., 2010). The tearing index of PALFP16 shows the lowest value, which could be due to the poor paper formation of the fibers, which resulted from the high amount of residual lignin and impurities. The tear index of PALF obtained in this study

exceeded that of NaOH-treated PALF reported in earlier research (Evelyn *et al*., 2019), suggesting improved fibre integrity and resistance to tearing under the current treatment method.

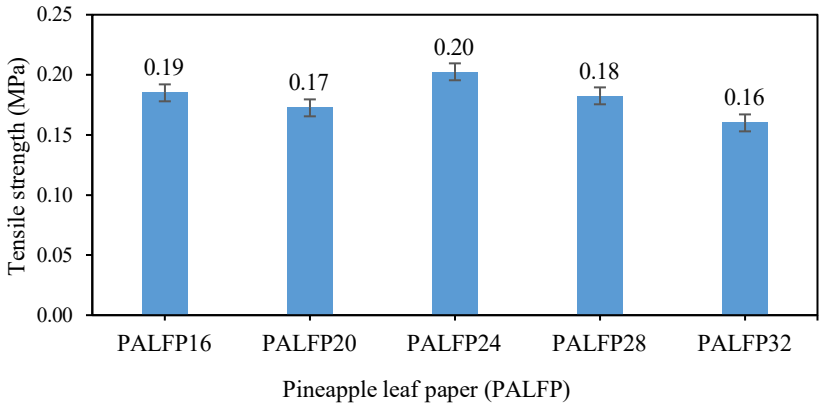


Figure 5. Tensile strength of PALF paper at different concentrations of acetic acid.

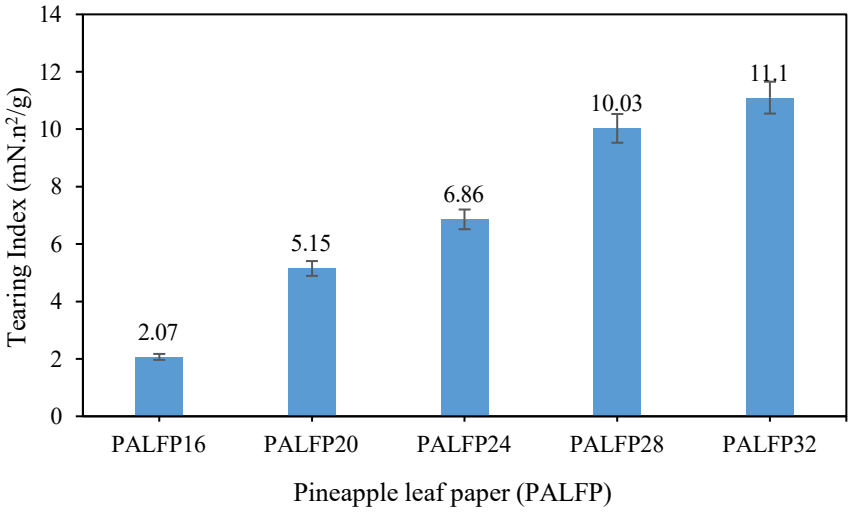


Figure 6. Tearing Index of PALF paper at different acetic acid concentrations.

CONCLUSION

In this study, sustainable paper was successfully produced from pineapple leaf fibre (PALF) without the addition of any external binder, as the resulting pulp was readily mouldable into paper sheets. Moreover, Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed effective delignification of PALF, as evidenced by the reduced intensity of lignin- and hemicellulose-associated peaks, along with the emergence of cellulose-characteristic peaks in the treated samples. Additionally, Scanning Electron Microscopy (SEM) revealed notable morphological differences between untreated and treated fibres. The treated PALF exhibited the emergence of exposed cellulosic fibrils, as well as the separation of fibre bundles into individual fibres. Furthermore, mechanical testing demonstrated variability in tensile strength across all paper samples, with the highest tensile strength recorded in paper produced from PALF treated with 25% acetic acid (AcOH). On the other hand, the tear resistance of the PALF paper showed a consistent increasing trend with higher concentrations of the pulping solvent, indicating a positive correlation between solvent strength and tearing performance. Above all, the ability to produce mouldable, binder-free paper with adequate mechanical strength suggests potential for small-scale paper production or eco-friendly packaging applications to support the pulp and paper industry in Malaysia. However, further optimisation of treatment conditions, scale-up trials, and durability testing under real-

use conditions are recommended to enhance the material's applicability and move toward industrial implementationPlease conclude your work incorporating your most important findings as well as future works.

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RESEARCH ARTICLE

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ORCHIDACEAE IN AYER HITAM UTARA, LAST REMAINING PEAT SWAMP FOREST IN JOHOR, MALAYSIA

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Ayer Hitam Utara Forest Reserve; Biodiversity; Conservation; Orchidaceae; Peat swamp forest.

ABSTRACT. *Ayer Hitam Utara Forest Reserve is the last remaining peat swamp forest in Johor, South Peninsular Malaysia, and represents a critical refuge for biodiversity, including the diversity of Orchidaceae. However, the peat swamp forest is exposed to various threats and pressures from land-use changes, forest fires, and drainage activities. Monitoring of biodiversity and conservation measures is urgently needed to preserve Johor's largest and only remaining peat swamp forest and, indirectly, its orchid diversity. This study aims to document the diversity of orchid species within the forest reserve and evaluate conservation aspects and concerns. Field surveys were conducted using purposive sampling methods, and species were identified according to morphological and taxonomic analyses. A total of 55 orchid species from 31 genera and four subfamilies were identified, including three species endemic to Malaysia and one species endemic to Peninsular Malaysia. Results highlight the significance of orchid diversity in Johor's peat swamp forests, contributing valuable insights into their ecological and habitat preferences. This study provides additional data to further understand regional biodiversity patterns, taxonomic significance, and highlights the importance of incorporating orchid conservation into peat swamp forest management strategies.*

INTRODUCTION

Malaysia, recognized as a megadiverse country and a biodiversity hotspot, is home to approximately 9,030 vascular plant species, encompassing 248 families and 1,651 genera (Yong *et al.,* 2021). In Peninsular Malaysia alone, 972 species belonging to 146 genera have been recorded, with 20% of these species classified as endemic and threatened (Ong *et al.,* 2017). According to Hernández-Mejía (2024), orchid species provide a variety of ecosystem services, including provisioning (food and medicine), regulating (pollination and water quality), and cultural services (ornamental use, traditional knowledge, and tourism). Despite Malaysia's rich diversity of orchid species, many are now under significant threat and require urgent conservation efforts (Gale *et al.,* 2018).

Southeast Asian tropical peat swamp forests are a unique and evolutionarily young type of vegetation characterized by highly acidic, waterlogged, and mineral-poor soils (Anamulai *et al.,* 2019; Helbert *et al.,* 2024). These peat swamps are recognized globally for their essential ecosystem services, including carbon storage and water regulation (Tonks *et al.,* 2017). The extreme conditions of these forests host a variety of specialized, rare, and threatened species (Giesen *et al.,* 2018; Posa *et al.,* 2011). However, most Southeast Asian tropical peat swamp forests have suffered severe degradation due to agricultural conversion (Koh *et al.,* 2011; Ledger *et al.,* 2023; Miettinen *et al.,* 2016). Malaysia no longer has any pristine peat swamps, and these ecosystems have been identified as the most threatened in the country (Wetlands International, 2010). The loss of peat swamp forests contributes to higher microclimate temperatures and increased soil acidity (Anamulai *et al.,* 2019) and raises serious environmental issues, such as elevated atmospheric carbon levels.

Johor is located in the southernmost part of Peninsular Malaysia. An inventory assessment of peatland distribution in the region indicates that Johor has approximately 143,974 hectares of peatland (Construction Research Institute of Malaysia, 2019). Unfortunately, much of this peatland has been degraded or lost, with Johor responsible for nearly one-third (34%) of the total area converted for oil palm plantations in Peninsular Malaysia (Wetlands International, 2010). The Ayer Hitam Utara Forest Reserve (AHUFR) is a permanent forest reserve that includes some of the last remaining intact sections of tropical peat swamp forest in southern Peninsular Malaysia, alongside lowland dipterocarp forests. Although several studies on biodiversity have been conducted in this reserve, the diversity of orchids has not been comprehensively evaluated. The forest receives an average annual rainfall of 2,215 mm and plays a vital role in the hydrological dynamics of the Ayer Hitam peatland (Mujilan *et al.,* 2023; Shamsuddin *et al.,* 2021). It helps regulate water flow within and outside its boundaries, serving as an essential water reservoir and playing a key role in flood mitigation, which indirectly supports community resilience and economic productivity. Acknowledging its ecological and socio-economic importance, the state government has allocated funding for infrastructure development and is promoting the forest reserve as an ecotourism destination (Jabatan Perhutanan Negeri Johor, 2022).

Given the peat swamp forest management plan aimed at achieving sustainable tourism (Jabatan Perhutanan Negeri Johor, 2022), it is crucial to document the orchid diversity in the forest reserve to ensure the integration of conservation measures. Thus, this study aims to create a checklist of orchid species to highlight the importance of biodiversity conservation in Johor's last and only peat swamp forest reserve.

MATERIALS AND METHODS

Study Area

The Ayer Hitam Utara Forest Reserve (2.057427°N, 102.806116°E) is situated in the Muar district of Johor, Malaysia. Spanning approximately 3,795.84 hectares, the reserve primarily comprises peat swamp, alongside some areas of freshwater swamp and lowland evergreen rainforest vegetation. Its ecological isolation is exacerbated by its proximity to human settlements and agricultural lands (Figure 1).

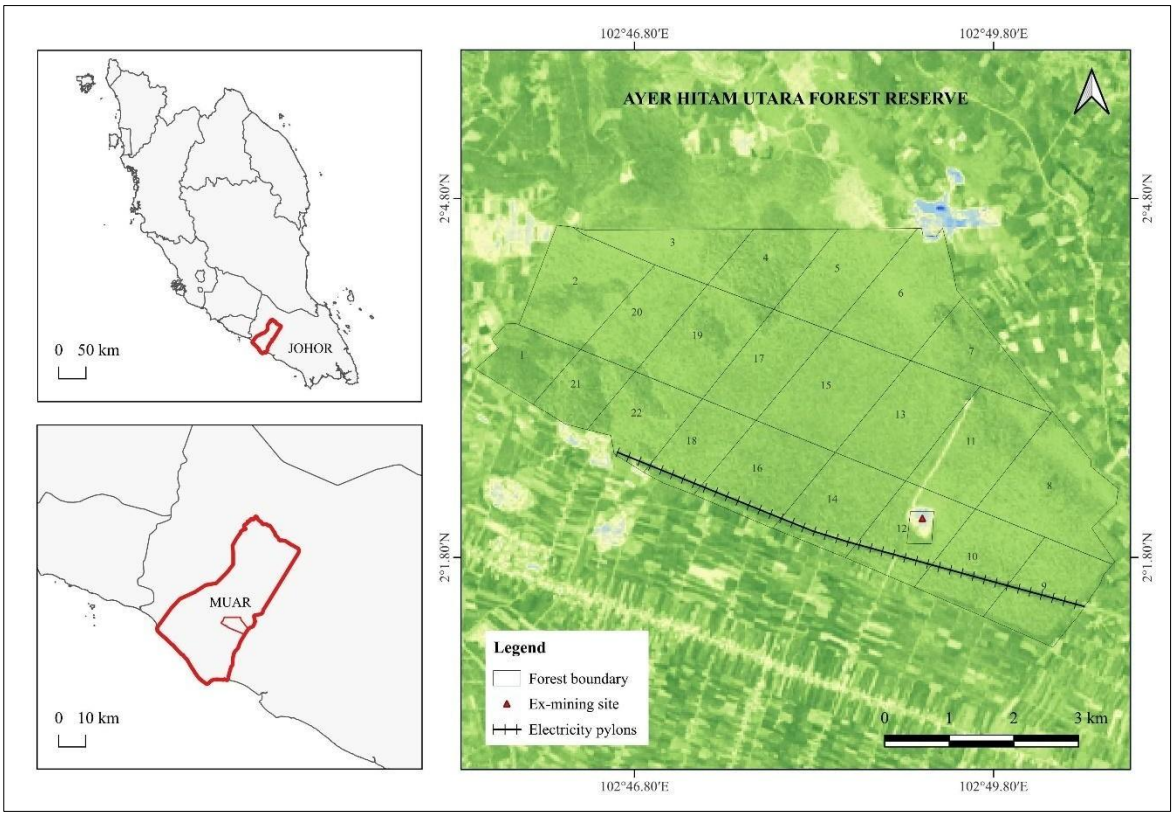

Figure 1. The location of Ayer Hitam Utara Forest Reserve, Johor.

Data Collection and Analysis

Field surveys were carried out in both established trails and off-trail areas across various sections of the forest reserve. Representative species were collected, photographed, preserved, and deposited in the herbaria at Universiti Tun Hussein Onn Malaysia, Pagoh Campus, and the Kim Ichthyologist Centre. The off-trail surveys provided access to less disturbed habitats, thereby increasing the chances of discovering rare or underrepresented species. Flowering materials were photographed, collected, and preserved as voucher specimens, while whenever feasible, non-flowering materials were gathered and cultivated in a greenhouse to aid in identification once they flowered.

Identification of species was performed through the examination of morphological characteristics both in the field and from herbarium vouchers, utilizing field guides, taxonomic keys, and consultations with experts. Growth habit observations were recorded during data collection and further corroborated with reliable taxonomic resources, such as Plants of the World Online (https://powo.science.kew.org). The ecosystems associated with each documented orchid species were also noted.

The conservation status of each species was obtained from the Malaysia Red List: Plants of Peninsular Malaysia via the Malaysia Biodiversity Information System (MyBIS) and the IUCN Red List of Threatened Species (www.iucnredlist.org). The species checklist was compared to historical flora records in Peninsular Malaysia by Ridley (1924), Corner (1978), and Turner (1995), with a focus on the documented orchid species in Johor. Distribution notes indicating widespread occurrences, specific locations in Johor, and other details were utilized in this analysis to identify overlapping species and compare differences.

RESULTS AND DISCUSSIONS

Species Composition

A total of 55 species were documented in the study (Figure 2), encompassing 31 genera and 4 subfamilies within the Orchidaceae family (Table 1). The three genera with the highest species richness were Bulbophyllum (11 species, accounting for 20% of the total), Dendrobium (7 species, 12.73%), and Thrixspermum (3 species, 5.45%). Together, these three genera comprised 41.82% of the total species diversity recorded. The remaining genera each contributed two species.

At the subfamily level, Epidendroideae was the most prevalent, representing 90.91% of the total species diversity, followed by Orchidoideae (5.55%), and both Apostasioideae and Vanilloideae (1.82% each). The taxonomic composition indicated a dominance of epiphytic species (78.18%) compared to terrestrial (30.91%) and lithophytic (10.91%) species, reflecting the habitat characteristics of the tropical peat swamp forest (Stephen *et al.,* 2022). The presence of epiphytic orchids serves as a key indicator of a well-functioning and healthy ecosystem (Sujalu *et al.,* 2021).

Further analysis examined the distribution of species across two ecological zones: lowland dipterocarp forest and peat swamp forest. The findings showed that 10.91% of the species were found in lowland dipterocarp forests, 25.45% in the transition area between lowland forests and peat swamp forests, and 63.64% in peat swamp forests. Additionally, two species (*Cystorchis javanica* and *Cystorchis variegata*) were exclusive to the waterlogged regions of the peat swamp forest (Lok *et al.,* 2011). Habitat preferences for 23 of the species were validated and documented in The Orchid of Peat Swamp Forests in Peninsular Malaysia (Go & Hamzah, 2008). The coexistence of peat swamp and lowland ecosystems facilitates diverse microhabitats and ecological niches, thereby supporting orchids with a wider range of environmental preferences (Zhang *et al.,* 2022).

Species Conservation Status

The study identified three species endemic to Malaysia and one species endemic to Peninsular Malaysia, specifically within Johor (Ong *et al.,* 2017; Turner, 1995). Although these species hold ecological significance, all have been classified as "Not Evaluated" on the Malaysia Red List. This lack of a formal conservation assessment underscores the urgent need for further research to establish their conservation status, especially given the ongoing threats to peat swamp forest ecosystems. The presence of endemic species highlights the critical importance of preserving these ecosystems to safeguard biodiversity in the region. This further emphasizes the role of the Ayer Hitam Utara Forest Reserve (AHUFR) as a vital conservation refuge for orchid species in Johor.

Comparison with Historical Records

To provide context for the findings, orchid records from historical studies in Johor by Ridley (1924), Corner (1978), and Turner (1995) were compared. Ten species identified in this study were not present in these earlier records, including the Malaysian endemic *Crepidium lowii* and the Johor endemic *Pinalia atrovinosa*. The absence of these species in older records may be attributed to several factors, such as limited survey coverage in past studies, shifts in species distributions over time, or the discovery of previously overlooked or unidentified species. The presence of these endemic species emphasizes the need for ongoing fieldwork and thorough surveys of unique and localized biodiversity areas.

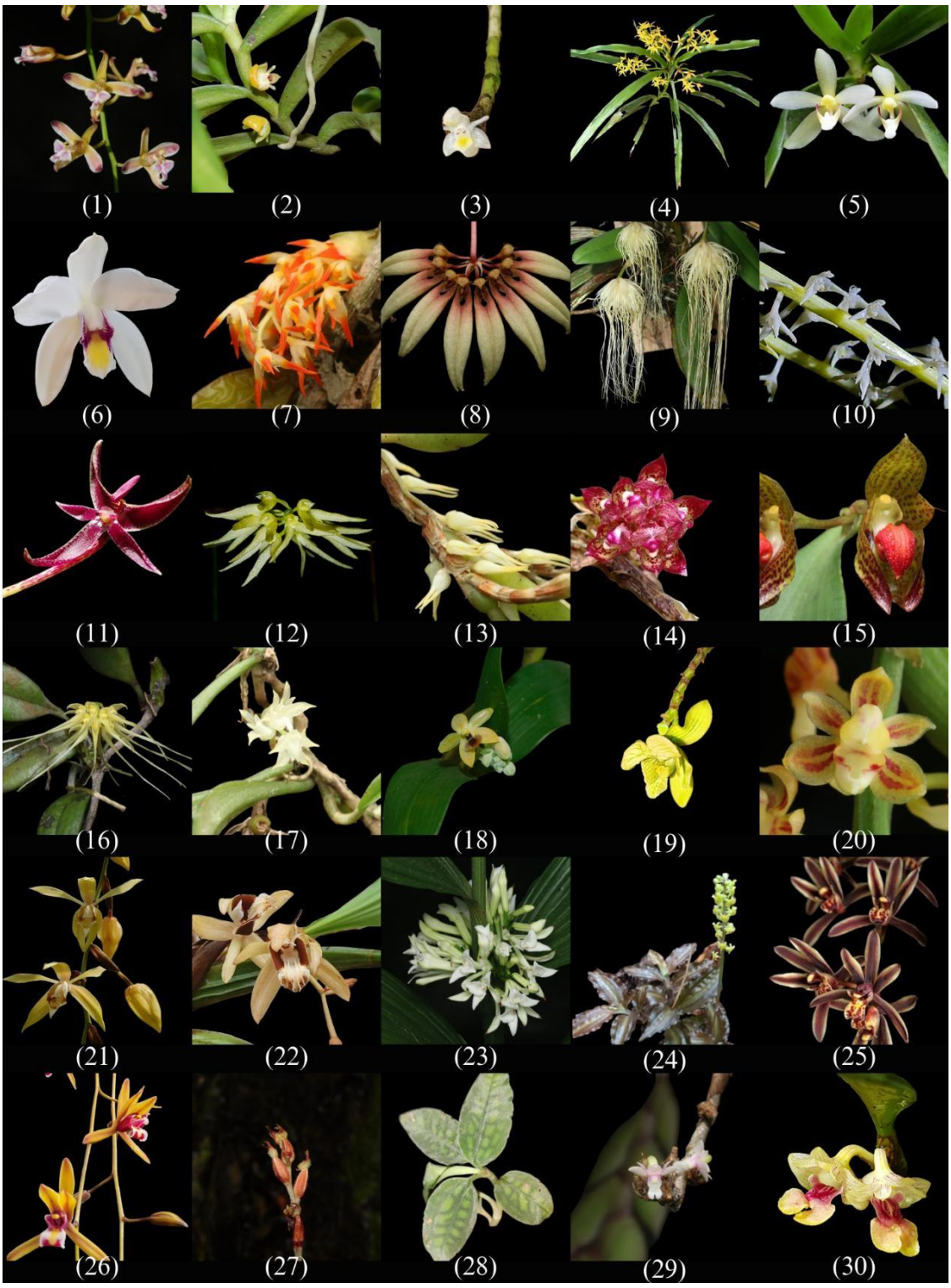

Figure 2. Orchidaceae species recorded in Ayer Hitam Utara Forest Reserve: 1) *Acriopsis liliifolia*; 2) *Adenoncos parviflora*; 3) *Agrostophyllum stipulatum*; 4) *Apostasia wallichii*; 5) *Brachypeza pallida*; 6) *Bromheadia finlaysoniana*; 7) *Bulbophyllum flammuliferum*; 8) *Bulbophyllum gusdorfii*; 9) *Bulbophyllum medusae*; 10) *Bulbophyllum odoratum*; 11) *Bulbophyllum patens*; 12) *Bulbophyllum purpurascens*; 13) *Bulbophyllum clandestinum*; 14) *Bulbophyllum singaporeanum*; 15) *Bulbophyllum subumbellatum*; 16) *Bulbophyllum vaginatum*, 17) *Bulbophyllum vermiculare*; 18) *Callostylis pulchella*; 19) *Claderia viridiflora*; 20) *Cleisostoma subulatum*; 21) *Coelogyne rochussenii*; 22) *Coelogyne testacea*; 23) *Corymborchis veratrifolia*; 24) *Crepidium lowii*; 25) *Cymbidium bicolor*; 26) *Cymbidium finlaysonianum*; 27) *Cystorchis javanica*; 28) *Cystorchis variegata*; 29) *Dendrobium aloifolium*; and 30) *Dendrobium angustifolium*.

Figure 2. (continued) 31) *Dendrobium crumenatum*; 32) *Dendrobium leonis*, 33) *Dendrobium linguella*; 34) *Dendrobium plicatile*; 35) *Dendrobium lampongense*; 36) *Dienia ophrydis*; 37) *Dipodium* cf. *paludosum*; 38) *Eulophia graminea*; 39) *Goodyera rubicunda*; 40) *Nephelaphyllum pulchrum*; 41) *Phalaenopsis deliciosa*; 42) *Phalaenopsis sumatrana*; 43) *Pinalia atrovinosa*; 44) *Plocoglottis javanica*; 45) *Plocoglottis lowii*; 46) *Podochilus microphyllus*; 47) *Pomatocalpa spicatum*; 48) *Pomatocalpa diffusum*, 49) *Renanthera elongata*; 50) *Robiquetia spathulata*; 51) *Thrixspermum amplexicaule*; 52) *Thrixspermum centipeda*; 53) *Thrixspermum trichoglottis*; 54) *Trichotosia velutina*; and 55) *Vanilla griffithii*.

Table 1. Species list: Comparison of orchid species recorded in AHUFR with Johor records by Ridley (1924), Corner (1978), and (Turner, 1995).

No.	Genus	Species	Ridley (1924)	Corner (1978)	Turner (1995)
1	*Acriopsis*	*Acriopsis liliifolia* Rolfe		√	√
2.	*Adenoncos*	*Adenoncos parviflora* Ridl.			
3.	*Agrostophyllum*	*Agrostophyllum stipulatum* (Griff.) Schltr.		√	
4.	*Apostasia*	*Apostasia wallichii* R.Br.			
5.	*Brachypeza*	*Brachypeza pallida* (Blume) Kocyan & Schuit.			√
6.	*Bromheadia*	*Bromheadia finlaysoniana* (Lindl.) Miq.		√	√
7.	*Bulbophyllum*	*Bulbophyllum flammuliferum* Ridl.			
8.		*Bulbophyllum gusdorfii* J.J.Sm.			√
9.		*Bulbophyllum medusae* (Lindl.) Rchb.f.	√		√
10.		*Bulbophyllum odoratum* (Blume) Lindl.			
11.		*Bulbophyllum patens* King ex Hook.f.	√		
12.		*Bulbophyllum purpurascens* Teijsm. & Binn.	√	√	
13.		*Bulbophyllum clandestinum* Lindl.	√	√	
14.		**Bulbophyllum singaporeanum* Schltr.		√	√
15.		**Bulbophyllum subumbellatum* Ridl.	√	√	√
16.		*Bulbophyllum vaginatum* (Lindl.) Rchb.f.	√	√	√
17.		*Bulbophyllum vermiculare* Hook.f.			√
18.	*Callostylis*	*Callostylis pulchella* (Lindl.) S.C.Chen & Z.H.Tsi	√	√	
19.	*Claderia*	*Claderia viridiflora* Hook.f.		√	√
20.	*Cleisostoma*	*Cleisostoma subulatum* Blume	√		√
21.	*Coelogyne*	*Coelogyne rochussenii* de Vriese	√	√	√
22.		*Coelogyne testacea* Lindl.		√	
23.	*Corymborkis*	*Corymborkis veratrifolia* (Reinw.) Blume			√
24.	*Crepidium*	**Crepidium lowii* (É.Morren) Szlach.			
25.	*Cymbidium*	*Cymbidium bicolor* Lindl.			√
26.		*Cymbidium finlaysonianum* Lindl.	√	√	√
27.	*Cystorchis*	*Cystorchis javanica* (Blume) Blume			
28.		*Cystorchis variegata* Blume	√		√
29.	*Dendrobium*	*Dendrobium aloifolium* (Blume) Rchb.f.		√	√
30.		*Dendrobium angustifolium* (Blume) Lindl.		√	
31.		*Dendrobium crumenatum* Sw.		√	√
32.		*Dendrobium leonis* (Lindl.) Rchb.f.	√	√	√
33.		*Dendrobium linguella* Rchb.f.			√
34.		*Dendrobium plicatile* Lindl.	√	√	
35.		*Dendrobium lampongense* J.J.Sm.			
36.	*Dienia*	*Dienia ophrydis* (J.Koenig) Seidenf.			

Table 1. (continued) Species list: Comparison of orchid species recorded in AHUFR with Johor records by Ridley (1924), Corner (1978), and (Turner, 1995).

No.	Genus	Species	Ridley (1924)	Corner (1978)	Turner (1995)
37.	*Dipodium*	*Dipodium* cf. *paludosum* (Griff.) Rchb.f.			
38.	*Eulophia*	*Eulophia graminea* Lindl.	√	√	√
39.	*Goodyera*	*Goodyera rubicunda* (Blume) Lindl.			√
40.	*Nephelaphyllum*	*Nephelaphyllum pulchrum* Blume			√
41.	*Phalaenopsis*	*Phalaenopsis deliciosa* Rchb.f.			
42.		*Phalaenopsis sumatrana* Korth. & Rchb.f.			√
43.	*Pinalia*	***Pinalia atrovinosa* (Carr) Schuit., Y.P.Ng & H.A.Pedersen			
44.	*Plocoglottis*	*Plocoglottis javanica* Blume	√	√	√
45.		*Plocoglottis lowii* Rchb.f.			√
46.	*Podochilus*	*Podochilus microphyllus* Lindl.	√	√	√
47.	*Pomatocalpa*	*Pomatocalpa spicatum* Breda			
48.		*Pomatocalpa diffusum* Breda		√	
49.	*Renanthera*	*Renanthera elongata* (Blume) Lindl.			√
50.	*Robiquetia*	*Robiquetia spathulata* (Blume) J.J.Sm.		√	√
51.	*Thrixspermum*	*Thrixspermum amplexicaule* (Blume) Rchb.f.	√	√	√
52.		*Thrixspermum centipeda* Lour.	√	√	√
53.		*Thrixspermum trichoglottis* (Hook.f.) Kuntze			√
54.	*Trichotosia*	*Trichotosia velutina* (Lodd. ex Lindl.) Kraenzl.		√	√
55.	*Vanilla*	*Vanilla griffithii* Rchb.f.	√	√	√
	Total		19	27	34

*Note: *Endemic to Malaysia, **Endemic to Peninsular Malaysia*

The results highlighted the diversity of Orchidaceae in the Ayer Hitam Utara Forest Reserve (AHUFR), with a significant number of species showing habitat tolerance and specificity to peat swamp forests. This taxonomic analysis emphasizes the high species richness and ecological importance of the study area, underscoring the necessity for targeted conservation strategies in the remaining sensitive forest reserves. The ecological significance of AHUFR was first acknowledged in 1995 through a rapid survey by Giesen *et al.* (1995), which identified its biodiversity potential; however, no orchid species were recorded, creating a gap in the understanding of the forest's overall floral diversity. Interest in the forest reserve was renewed following a scientific expedition in 2019, which led to more detailed biodiversity research. Subsequent studies have further enhanced knowledge of the forest's flora, including a comprehensive inventory of ferns conducted by Akomolafe *et al.* (2022) and an analysis of tree species diversity by Izwan *et al.* (2022).

Comparing the orchid species recorded in this study with those in historical publications underscores the ecological and floristic significance of the forest reserve. Corner (1978) shows a higher species similarity compared to Ridley (1924), as the study specifically examines freshwater peat swamps in Johor, a habitat type that the forest reserve shares (Figure 3). Turner (1995) demonstrates the highest similarity, despite his work being limited to species records from Johor.

This is likely due to its comprehensive nature, compiling floral literature and documentation from across Peninsular Malaysia, thereby covering a wider range of habitats and species.

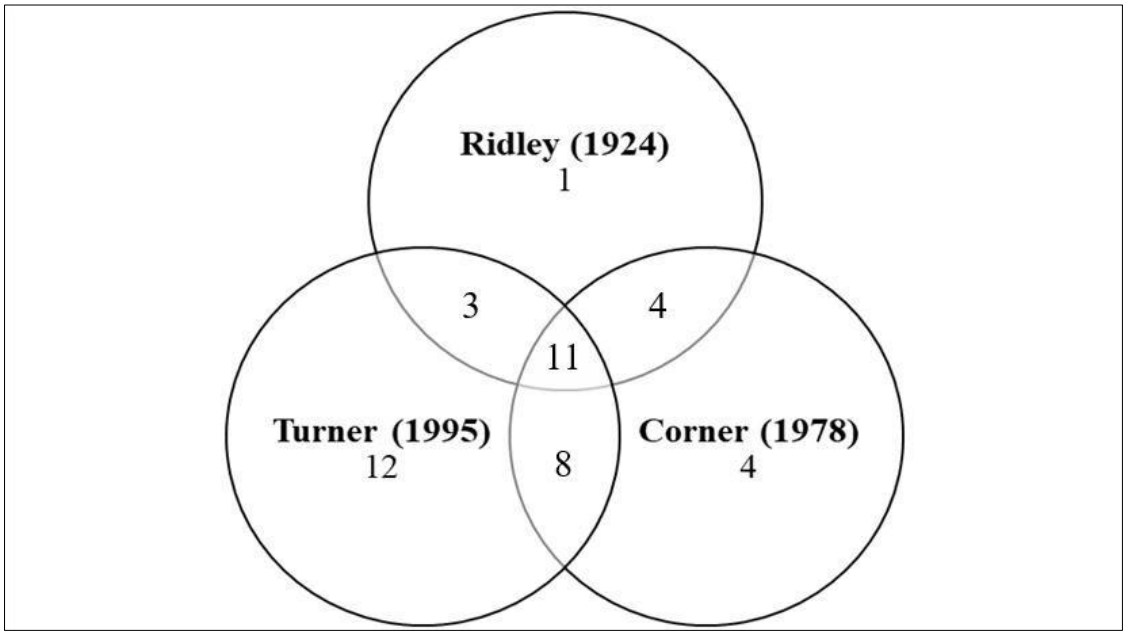

Figure 3. Comparison of orchid species records in Johor.

One of the major challenges facing biodiversity research in Malaysia is the lack of accessible and comprehensive records of flora organized by individual states. Although national - level inventories and regional assessments are available, detailed data specific to states are often fragmented or nonexistent. This limitation impairs the ability to thoroughly evaluate local biodiversity and identify conservation priorities at more localized levels. In the case of AHUFR, the absence of orchid records in previous studies illustrates this broader issue, highlighting the need for systematic floristic inventories at the state level. Strengthened efforts to document and digitize state-specific biodiversity data, including both historical and contemporary records, would create a more robust foundation for conservation planning. By integrating local knowledge, utilizing advanced technologies such as remote sensing and species distribution modeling, and fostering collaborative research initiatives, these gaps can be addressed, leading to more effective conservation strategies for Malaysia's unique ecosystems.

CONCLUSION

This study significantly advances our understanding of orchid diversity within the Ayer Hitam Utara Forest Reserve (AHUFR) by documenting 55 species across multiple genera and emphasizing the ecological importance of habitats such as peat swamp forests. The dominance of epiphytic species and the presence of endemic orchids highlight the urgent need for targeted conservation strategies to safeguard this biodiversity hotspot. These findings not only bridge gaps in historical records but also stress the vital need for ongoing research and systematic inventories to assess the conservation status of these species considering the ongoing threats to peat swamp ecosystems.

Furthermore, the comparison with historical studies reveals the fluid nature of species distributions and the necessity for enhanced recordkeeping at the state level. The absence of certain species in previous surveys suggests the possibility of discovering new records, reinforcing the importance of continued field research. By enhancing efforts to document and digitize biodiversity data and leveraging advanced technologies along with local expertise, we can promote effective conservation planning.

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SEMI-PARAMETRIC ANALYSIS OF CERVICAL CANCER DATA: A SINGLE-CENTRE STUDY

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ABSTRACT. *In women, cervical cancer ranks fourth in terms of mortality and is the third most prevalent disease. It remains a significant concern among clinicians in Malaysia; however, published works on the survival of cervical cancer patients are somewhat scarce. Thus, this study aims to identify the prognostic factors that significantly affect the risk of death in cervical cancer patients using semi-parametric Cox proportional hazards regression analysis. This study began with univariate and multivariate Cox proportional hazards regression analyses, followed by a proportional hazards assumption test for the preliminary final model. Data on cervical cancer patients treated at Hospital Universiti Sains Malaysia (HUSM) between 2013 and 2017 were utilized. In the univariate analysis, stage at diagnosis and primary treatment were found to be statistically significant at the 5% level. In the multivariate analysis, histologic type, stage at diagnosis, and distant metastasis were found to be statistically significant. The proportional hazards assumption for each variable in the preliminary final model is tested based on the scaled Schoenfeld residuals. Accordingly, this study showed that patients with stage III–IV adenocarcinoma-type cervical cancer treated at HUSM have the highest likelihood of death from the disease.*

INTRODUCTION

The disease's progression is often asymptomatic in its early stages, underscoring the importance of regular screenings for timely detection. A study carried out in the northern state of Malaysia revealed that 38.6% of women there had a Pap smear in the last five years, compared to 48.5% of women in the southern state who visited outpatient clinics and had a Pap smear in the previous three years (Chin *et al*., 2022; Mustafa *et al*., 2022; Norkhafizah & Norehan, 2019). The alarming statistics drive clinicians worldwide to develop and implement various preventive strategies through early screening and creating awareness about getting HPV vaccinations to improve women's overall health outcomes (Okunade, 2020; Small *et al*., 2017).

Survival analysis is one of the most common statistical techniques used to estimate the time until an interesting event, such as death, disease relapse, the onset of an adverse reaction, or the emergence of a new disease entity. This method examines time-based data, starting from a specific origin time and ending when a specific endpoint event occurs (Rai *et al.*, 2021). This approach to time-

to-event series analysis was initially developed in the medical field and has since been extended to a range of other fields, such as event history analysis in the social sciences, reliability theory in engineering, and duration analysis in economics (Lánczky & Győrffy, 2021).

Most survival analyses in cancer journals use some or all of the survival analysis methods, such as Kaplan-Meier (KM) plots, log-rank tests, and Cox (proportional hazards) regression. The Cox proportional hazards regression analysis is a method that researchers frequently use to investigate the impacts that several variables may have on the time it takes for a specified event to occur (Tshewang *et al*., 2021). Previous studies showed that the authors applied the Cox proportional hazards model to investigate the impact of COVID-19 on the Mexican population, survival prediction in women newly diagnosed with cervical cancer, and the prognosis of survival rates among several factors in inpatient breast cancer cases in Indonesia (Salinas *et al*., 2020; Matsuo *et al*., 2019; Nadjib *et al*., 2018).

In Malaysia, there has been a scarcity of nationally representative published works on the survival of cervical cancer. Therefore, this study aims to identify the prognostic factors that significantly affect the risk of death in cervical cancer patients receiving treatments and clinically diagnosed at Hospital Universiti Sains Malaysia (HUSM) in Kelantan, Malaysia, using the semi-parametric Cox proportional hazards regression analysis.

MATERIALS AND METHODS

Source of Data

The cervical cancer data were obtained from Hospital Universiti Sains Malaysia (HUSM), located in Kubang Kerian, Kelantan, Malaysia. This hospital serves as a referral facility for Malaysia's East Coast. This study includes all patients who underwent at least one cervical cancer treatment at HUSM and were histopathologically and clinically diagnosed with the disease between 2013 and 2017. Those who passed away for various reasons were excluded. There are 132 patients in total; 70 (53%) of them are deceased, and 62 (47%) are still alive. Ethical approval was obtained from the research ethics committee.

Definition of Key Medical Terms

Histologic type refers to the microscopic classification of cancer cells, such as squamous cell carcinoma or adenocarcinoma, while distant metastasis describes the spread of cancer cells from the original tumor site to distant organs or tissues in the body (Chin *et al*., 2022).

Statistical Analysis

Cox Proportional Hazard Regression Model

The prognostic model for cervical cancer patients is initiated using the Cox proportional hazards regression model. The analysis is conducted to identify significant factors associated with the risk of death in these patients. The proportional hazards model was first introduced by Cox (1972) and is known as the Cox proportional hazards regression model. This model is the most commonly used model in the analysis of survival data. It is also referred to as a semi-parametric model because no specific form of the probability distribution is assumed for the survival time. Similarly, no assumptions have been made regarding the actual form of the baseline hazard function h0(t), making the model more flexible and applicable. The hazard function of the Cox proportional hazards regression model is given by equation 1.

$$h(t) = h_0(t)exp(\beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p) \quad (1)$$

where the hazard function $h(t)$ is dependent on a set of p covariates $(x_1, x_2 \ldots, x_p)$, where the impact is measured by the size of the respective coefficients $(\beta_1, \beta_2, \ldots, \beta_p)$.

In the Cox proportional hazards regression model, β −parameters are estimated by maximizing the partial log-likelihood function. Of the n observed survival times, suppose that there are D uncensored times. Let $t_1 < t_2 < \cdots < t_D$ denote the ordered D distinct event times (no ties between the event times). The partial likelihood function of the Cox proportional hazards model may be written in equation 2.

$$L(\beta) = \prod_{i=1}^{D} \frac{exp[\sum_{j=1}^{p} \beta_j x_{(i)j}]}{\sum_{l \in R(t_i)} exp[\sum_{j=1}^{p} \beta_j x_{(i)j}]} \quad (2)$$

where $X_{(i)j}$ be the jth covariate associated with the individual whose failure time is t_i. Meanwhile, $R(t_i)$ consists of all individuals whose survival times are at least t_i.

Schoenfeld Residual Tests

Based on the scaled Schoenfeld residuals, the proportional hazards assumption for each variable is examined. In the meantime, the overall model is evaluated using the global Schoenfeld residuals test. If the tests show statistical significance (p-value < 0.05), the proportional hazards assumption is violated. From Collet (2023), the Schoenfeld residual denotes r_{Pji} may be obtained in equation 3 – 5:

$$r_{Pji} = \delta_i \{x_{ji} - \hat{a}_{ji}\} \quad (3)$$

where x_{ji} is the value of the jth explanatory variable, $j = 1, \ldots, p$ for the ith subject in the study,

$$\hat{a}_{ji} = \frac{\sum_{l \in R(t_i)} x_{ji} exp(\beta' x_1)}{\sum_{l \in R(t_i)} exp(\beta' x_1)} \quad (4)$$

and $R(t_i)$ is the set of all individuals at risk at the time t. Meanwhile, the scaled Schoenfeld residuals r^*_{Pji} are the components of the vector

$$r^*_{Pji} = r\ var(\beta) r_{Pi} \quad (5)$$

where r is the number of deaths among the n individuals, $var(\beta)$ is the variance-covariance matrix of the parameter estimates, and $r_{Pi} = (r_{P_{1i}}, r_{P_{2i}}, \ldots, r_{P_{pi}})'$ is the vector of Schoenfeld residuals for the ith subject.

RESULTS AND DISCUSSIONS

In univariate analysis, each factor is analyzed using the Cox proportional hazards regression model to identify the association between each covariate and the outcome individually. This analysis may also provide a preliminary idea of which variables have possible prognostic importance. Table 1 shows that the stage at diagnosis and primary treatment are statistically significant at the 5% level of significance.

All variables are further analyzed using the multivariate Cox proportional hazards regression analysis. Independent variables that are significantly associated with the hazards of death of the cervical cancer patients under study are selected based on the stepwise selection method, which involves systematically adding or removing variables based on specific criteria. Forward selection begins with no variables and adds them one at a time based on statistical significance, while backward elimination starts with all candidate variables and removes the least significant ones. This selection method consists of forward selection followed by the backward elimination process, with a p-value < 0.05 for variable entry and a p-value > 0.10 for variable removal (Collet, 2023). At this step, the preliminary main effects model is obtained. In the multivariate analysis, histologic type, stage at diagnosis, and distant metastasis are found to be statistically significant.

Table 1. The univariate Cox proportional hazards regression model.

Variables	Coefficient	Crude HR (95% CI)	LR (df)	*p*-value
Ethnicity				
Non-Malay				
Malay	-0.1653	0.8476 (0.4312-1.6660)	0.22(1)	0.6382
Lymph node involvement				
Negative				
Positive	-0.08687	0.9168 (0.5218-1.6113)	0.09(1)	0.7609
Histologic type				
Squamous cell carcinoma				
Adeno cell carcinoma	0.3219	1.3797 (0.8013-2.3760)	1.28(1)	0.2577
Age at diagnosis				
<40				
40 – 59	-0.4092	0.6642 (0.3194-1.3813)		
≥60	-0.6155	0.5404 (0.2277-1.2832)	2.97(2)	0.3962
Stage at diagnosis				
I– II				
III – IV	1.0176	2.7665 (1.4272-5.3621)	9.6(1)	0.0080
Primary Treatment				
Surgery				
Non-surgery	0.6360	1.889 (1.077-3.3123)	5.37(1)	0.0205
Distant Metastasis				
No				
Yes	0.3412	1.4143 (0.8001-2.3214)	1.18(1)	0.2769

Note: HR = Hazard Ratio, CI = Confidence Interval, SE = Standard Error, LR = Likelihood Ratio, df = degrees of freedom

The interaction between the covariates in the preliminary main effects model is checked by adding the interaction term to the model. The interaction is not statistically significant at the 5% level of significance. The preliminary final model is obtained after checking for the interaction. The results are presented in Table 2. It has been found that the stage at diagnosis, histologic type, and distant metastasis are significant prognostic factors that influence the risk of dying for these patients.

Table 2. The multivariate Cox proportional hazards regression model.

Variables	Coefficient	SE	Adjusted HR (95% CI)	*p*-value
Histologic type				
Squamous cell carcinoma				
Adeno cell carcinoma	0.5946	0.2897	1.8123(1.027-3.198)	0.0401
Stage at diagnosis				
I-II				
III-IV	0.8643	0.2700	2.3734(1.398-4.029)	0.0014
Distant Metasis				
No				
Yes	0.7211	0.2636	2.056(1.227-3.448)	0.0062

Note: HR = Hazard Ratio, CI = Confidence Interval, SE = Standard Error

This study found that the prognosis of cervical cancer depends significantly on the stage at diagnosis. Patients diagnosed with advanced stages (stage III-IV) of cancer have a higher risk of death than those with early stages (stage I-II). This finding is also concurred with findings from other research (Seifu *et al*., 2022; Mebratie *et al*., 2022; Manzour *et al*., 2022; World Health Organization, 2020; Juhan *et al*., 2013). This study also found that histologic type significantly affects survival, as patients

diagnosed with adenocarcinoma are identified to have a higher risk of dying compared to squamous cell carcinoma, which is supported by previous findings from other research. For example, Liu *et al*. (2022) found that adenocarcinoma had a significant negative impact on the prognosis of cervical cancer patients studied.

Furthermore, Li *et al*. (2022) claimed that adenocarcinoma was associated with a worse prognosis because those diagnosed with this histologic type were detected later and at more advanced stages than those with squamous cell carcinoma. As for distant metastasis, the results indicated that cervical cancer patients with distant metastasis were twice as likely to die compared to those without it. This is in line with other studies, where cervical cancer patients with metastasis have a poor survival rate (Singh *et al.*, 2023; Zhou & Peng, 2020; Zhang *et al*., 2020).

The proportional hazards assumption for each variable in the preliminary final model is tested based on the scaled Schoenfeld residuals. The global Schoenfeld residuals test is used to assess the assumption for the overall model. These tests are performed using the function cox.zph from the survival package (Therneau, 2014) in R software. The result is tabulated in Table 3. The global test indicates that the proportional hazards assumption for the overall model is violated. Meanwhile, the scaled Schoenfeld residuals test shows that the hazard for the distant metastasis variable is not proportional (p-value < 0.05). Therefore, the stratified Cox model might be adopted in future studies. The stage at diagnosis and histologic type remain significant prognostic factors in the model. Meanwhile, distant metastasis can be considered a stratification factor in the future stratified Cox model study.

Table 3. The proportional hazards assumption test results for the preliminary final model.

Variables	**ρ (rho)**	2	***p*-value**
Histologic type	0.0712	0.373	0.5416
Stage	-0.1434	1.336	0.2477
Distant metastasis	0.2934	6.591	0.0102
Global	NA	7.956	0.0419

Note: ρ (rho) = Correlation between scaled Schoenfeld residuals and time, χ^2 = Chi-square statistic

CONCLUSION

In this study, prognostic factors significantly affecting the risk of death in cervical cancer patients treated at HUSM are identified using Cox proportional hazards regression analysis. The findings indicate that cervical cancer patients treated at HUSM with stage III-IV adeno cell carcinoma are at the greatest risk of death from cervical cancer. Since the proportional hazard assumption was violated for distant metastasis in the Schoenfeld residuals tests, a stratified Cox regression model should be considered for future study. The results of the analysis will provide a better understanding of the survivorship and hazards facing cervical cancer patients in Malaysia.

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STAKEHOLDER PREPAREDNESS IN NATURE-BASED TOURISM GOVERNANCE: A SYSTEMATIC REVIEW AND FREQUENCY ANALYSIS OF NATURAL DISASTER RESEARCH

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ABSTRACT. *Nature-based tourism and recreation destinations present substantial challenges to the safety, sustainability, and resilience of these ecosystems due to their inherent vulnerability to natural disasters. This systematic literature review (SLR) provides a descriptive overview of the current research landscape and identifies trends in NBT disaster preparedness. A systematic search of scholarly databases was conducted for studies published between 2006 and 2023 on disaster preparedness in nature-based tourism. A PSALSAR framework was utilized in the review process to identify, screen, and select the pertinent studies. The inclusion criteria were met by 37 peer-reviewed articles. Throughout the included publication span, our frequency analysis showed a trend of growing research attention on disaster preparedness in NBT. The predominant research approaches employed were qualitative in nature, emphasizing the comprehension of stakeholder viewpoints through interviews and focus groups. Most of the NBT scholars covered climate-related disasters, which overlooked non-climate disaster studies. Data availability and quality were identified as the predominant limitations of the studies reviewed. Regarding stakeholder focus, the literature primarily emphasized NBT operators in terms of their capacities, challenges, and disaster preparedness measures. This review identifies opportunities for future research to develop a more comprehensive and holistic approach, highlighting the growing significance of disaster preparedness in NBT.*

INTRODUCTION

Nature-based tourism (NBT), which frequently involves destinations such as national parks and wilderness areas, is the component of tourism that is expanding at the quickest rate. The sector now accounts for approximately 7% of global tourism spending, contributing to over US$600 billion in revenue (WTTC, 2022). Additionally, the demand for NBT has increased since COVID-19, with rural areas and protected parks showing the growth in visitation and development (Haukeland *et al.,* 2023). NBT is also adopted in many developing countries as a means of both preserving natural resources and promoting economic growth (Balmford *et al*., 2009; Cheng *et al*., 2022). Despite the popularity of this

tourism segment, nature-based tourism is a sensitive and susceptible economic sector due to its physical proximity to the natural environment and resources (Ma *et al*., 2021; Dogru *et al*., 2019; Hambira *et al*., 2020; Rutty & Scott, 2013; Verbos *et al*., 2018). Any changes in the destinations, whether they are man-made or natural, pose serious safety and economic risks to tourism activities and their stakeholders. Along with an upsurge in global warming, as well as the frequency and severity of extreme weather in recent decades, these potential risks are expected to increase (Hsu & Sharma, 2021). As the number of disaster occurrences is concerning, the tourism industry has been threatened by a series of natural disasters that resulted in a major disruption to the sector (Nguyen *et al*., 2022; Filimonau & de Coteau, 2019; Chan *et al*., 2020; Bird & Gísladóttir, 2020).

Natural disasters can be characterized as severe meteorological phenomena that frequently occur without sufficient forewarning, resulting in substantial devastation encompassing economic ramifications and loss of human life (Craig, 2019). The United Nations (2006) further classified natural disasters into three types: hydro-meteorological disasters (e.g., floods, storms, droughts, and extreme temperature-related disasters including wildfires), geophysical disasters (e.g., volcanic eruptions, earthquakes, and tsunamis), and biological disasters (e.g., epidemics and insect infestations). As concern over natural disasters grows, it is critical to ensure that NBT destinations have effective disaster preparedness strategies. The strategies should incorporate various levels of stakeholders, which encompass the governmental organizations, industry bodies, and nature-based tourism operators that are directly involved in the development, administration, and operation of nature-based tourist sites and activities (Chan *et al.,* 2020; Dunning, 2020; Dunning, 2021). Policymakers use rules and regulations to create the foundation for a safe, sustainable, and ethical NBT development (Fabeil *et al*., 2018; Hughey & Becken, 2016), whereas industry players and operators at the forefront of tourist engagement actively influence visitor experiences and implement sustainable practices (Mushawemhuka *et al*., 2018). By working together, both policymakers and industry players can ensure a sustainable NBT industry that protects natural environments, fosters positive visitor experiences, and contributes to local communities.

Despite its importance, it is still unclear how much academic attention has been given to this topic. This comprehensive and systematic literature review aims to provide insight into the trends and research landscape of NBT governance practiced by various stakeholders in addressing disaster preparedness. Using frequency analysis, we examine patterns in a variety of areas; (1) years of publication to monitor the development of research interest in NBT disaster preparedness, (2) the most commonly used research method to explore preparedness measures, (3) type of disasters focused on in the literature, (4) limitations of studies and, (5) the balance of studies between local agencies or policymakers and nature-based operators. Investigating the scientific trend of this topic may open new opportunities for future research related to natural disasters in NBT.

MATERIALS AND METHODS

The current review employs systematic search criteria to identify and select pertinent studies, thereby reducing the likelihood of reviewer bias and ensuring data consistency and replicability (Collins & Fauser, 2005). Adhering to established guidelines enhances methodological transparency and ensures alignment with best practices (Haddaway *et al*., 2018). To conduct a systematic literature review, this investigation followed the PSALSAR framework as established by Mengist *et al.* (2020) (Table 1). The research process begins with the development of a research protocol that outlines the scope of the study (Figure 1). This is followed by a search conducted using predetermined criteria for identifying and excluding literature, as well as assessing its quality. Synthesis occurs through the extraction and categorization of data. The analysis concludes with the presentation of a narrative of the findings. Lastly, the reporting phase entails describing the methodology utilized and disseminating the results.

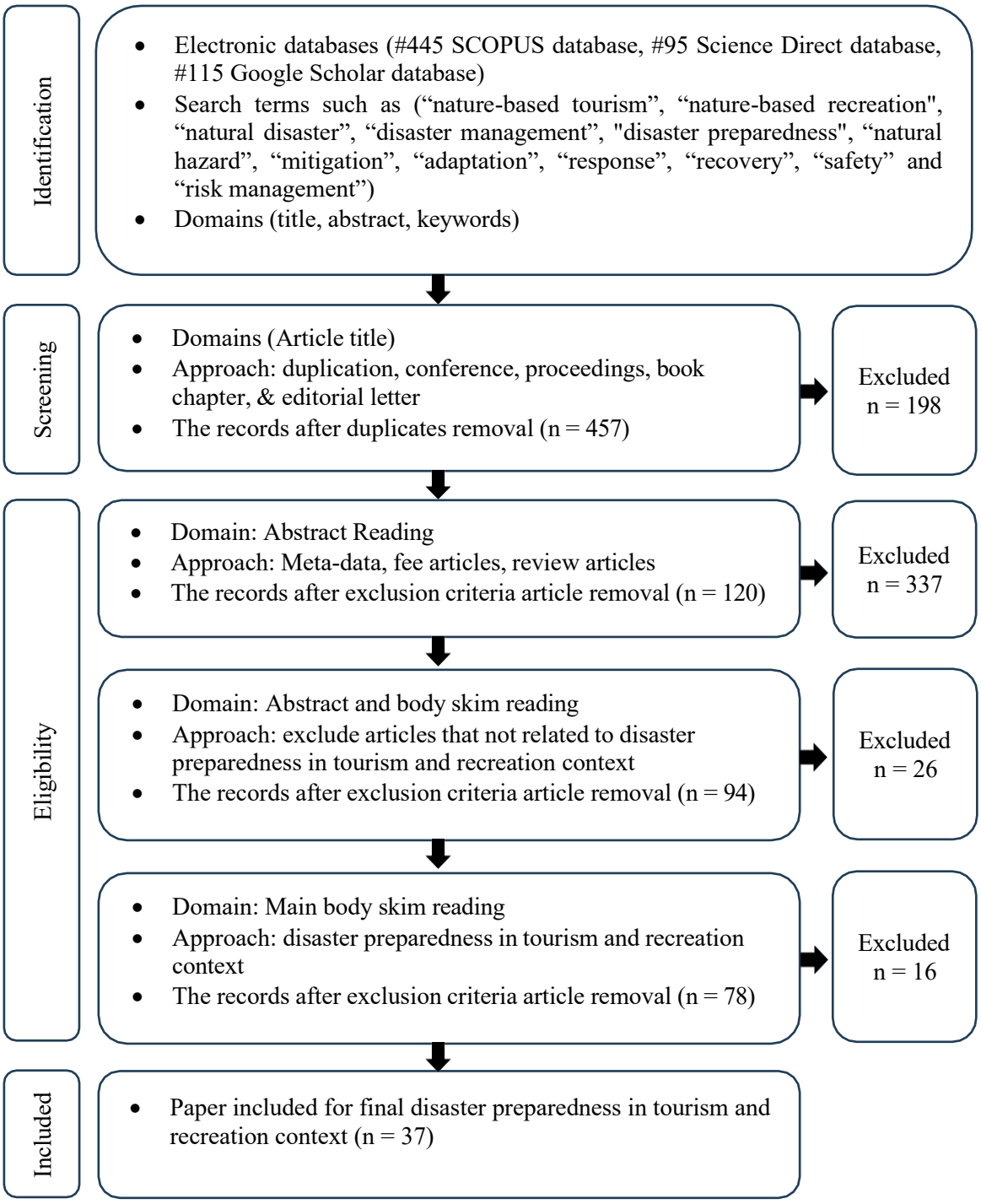

Figure 1. The flow diagram for database search of publications for systematic reviews (Source: Modified from Moher *et al*. (2010) and Mengist *et al*. (2020)).

Table 1. The framework of systematic analysis.

	Steps	Outcomes	Methods
PSALSAR Framework	Protocol	Defined study scope	
	Search	Define the study search strategy	Searching strings
	Appraisal	Selecting studies	Defining inclusion and exclusion criteria
	Synthesis	Extract data Categorize the data	Extraction template Categorize the data on the iterative definition and prepare it for further analysis work
	Analysis	Data analysis	Quantitative categories, description, and narrative analysis of the organized data
		Result and discussion	Based on the analysis, show the trends, identify the gap, and the result comparison
		Conclusion	Deriving conclusions and recommendations
	Report	Report Writing Journal Article production	PRISMA methodology Summarizing the report results for the larger public

Research Scopes

To ascertain the scope of the research, this literature review employed the Population, Interest, and Context (PICo) framework to formulate the research inquiries, as emphasized in Table 2. By focusing on the topic of natural disasters, the literature review considered stakeholders in the nature-based tourism industry as the target population. A distinct characteristic of this study is its focus on existing knowledge in disaster preparedness research. The SLR was used to examine the frequency of studies published on this topic across various governance stakeholder groups and research methods.

Table 2. SLR research scope based on the application of the PICo framework to the defined objectives.

Population	What are the characteristics of the population? What is the condition of interest?	Governance stakeholders of nature-based tourism
Interest	The phenomena of interest relate to a defined event, activity, experience, or process	Natural disasters
Context	The setting or distinct characteristics	Existing knowledge in disaster preparedness studies

Literature Searching Strategy

A comprehensive literature search was conducted as the second step in performing a systematic review. A search strategy refers to a systematic approach to gathering evidence for a review. This process requires the creation of search terms, selection of sources (including databases), and identification of relevant keywords (Petticrew & Roberts, 2006). For the present study, searches were conducted using electronic databases such as SCOPUS, ScienceDirect, and Google Scholar. These databases were selected for their reliability and frequent updates, making them highly

relevant for this review (Zhu *et al*., 2020; Xiao & Watson, 2019). Following the PICo framework, the specification of the search strings reflects the terminology aligned with the target population in the systematic literature review application. Specifically, Table 3 illustrates examples of the search string applied.

Table 3. Search string syntax.

Database	Search String Syntax
SCOPUS	TITLE-ABS-KEY (("nature-based tourism") OR ("nature-based recreation") OR ("nature based tourism") AND ("natural disaster") OR ("disaster management") OR ("disaster preparedness") OR ("natural hazard") OR ("hazard management") OR ("safety") OR ("risk management"))
Science Direct	nature-based and natural disaster
Google Scholar	(nature-based tourism OR recreation) AND (disaster management OR preparedness OR mitigation OR adaptation OR response OR resilience OR recovery)

The search terms were entered either individually or in limited combinations, depending on the limitations or requirements of each database used. Articles limited to abstracts that were not accessible in full text were excluded from the subsequent systematic review. The articles were published in peer-reviewed journals between 2006 and 2023, and the search was conducted from January to October 22, 2023.

Articles Assessment

The selected articles were assessed in alignment with the review's objectives during the appraisal phase. In this process, the titles, abstracts, and full texts of all articles were thoroughly examined to ensure they met the inclusion criteria and were appropriate for further analysis. The inclusion and exclusion criteria are explained in Table 4.

Extensive abstracts, keynote addresses, presentations, conceptual papers, review articles, and papers written in a language other than English were excluded. Inaccessible papers and duplicated documents were eliminated from the final selection. Figure 1 depicts the overall screening procedures and the sequential progression of selecting relevant literature. A total of 655 records were initially acquired; 115 were retrieved from Google Scholar, 95 from Science Direct, and 445 from Scopus. After excluding extended abstracts, presentations, keynotes, book chapters, non-English language papers, and inaccessible publications, the number of records retained for title screening was reduced to 457. Following the appraisal stage, 120 articles met the criteria for abstract screening. After carefully reviewing the abstracts, 94 articles were selected for full -text examination. Among these, 78 articles assessed disaster preparedness within the context of tourism and recreation. Further screening was conducted before downloading these articles. During the main body reading, duplicate papers and those lacking empirical data, not pertaining to nature-based tourism, or not addressing disaster preparedness, were manually removed. As a result, 37 publications met every inclusion criterion.

The 37 articles then underwent quality assessment based on three elements: (i) the inclusion and exclusion criteria were clearly defined and appropriate; (ii) the literature search likely captured all significant studies of the subject matter; and (iii) the studies were published in journals that use single-blind or double-blind peer review. All 37 publications satisfied the inclusion criteria, were peer-reviewed, and focused on disaster preparedness in nature-based tourism involving diverse stakeholders. These articles were then included in the final stage of analysis.

Table 4. Inclusion and exclusion criteria for the articles' assessment.

Criterion	Eligibility	Exclusion
Literature Type	Peer-reviewed empirical journal articles	Review articles, books, book chapters, conference proceedings, conceptual papers, abstracts, presentations, and keynotes
Language	Articles published in English	Articles published in languages other than English
Keyword Relevance	Articles that contain the specified keywords in the title, abstract, or keyword section	Articles that do not contain the specified keywords in any of the key searchable fields
Publication Type	Studies published in peer-reviewed scientific journals	Non-peer-reviewed publications
Topical Relevance	Studies that provide evidence on disaster management in nature-based tourism (NBT)	Studies that do not focus on disaster management or are unrelated to NBT
Stakeholder Focus	Articles that examine at least one NBT governance stakeholder group (e.g., policymakers, operators)	Articles that do not mention or analyze any governance stakeholders in NBT
Publication Date	Articles published from 2006 onward	Articles published before 2006
Originality	Original, primary research studies	Duplicate articles or secondary analyses
Literature Type	Peer-reviewed empirical journal articles	Review articles, books, book chapters, conference proceedings, conceptual papers, abstracts, presentations, and keynotes
Language	Articles published in English	Articles published in languages other than English

Synthesis

The synthesis phase involved systematically retrieving and classifying relevant information from the selected articles to derive conclusions and gain knowledge. Data from each selected paper was extracted and imported into Atlas.ti version 9 for processing. During the categorization phase, the extracted data were organized and classified in preparation for further analysis.

Analysis

Throughout the analysis phase, all relevant information was systematically examined. The process began with frequency statistics, providing an overview of the characteristics of the 37 articles. Evaluation criteria included publication year, research methodology, and stakeholder involvement. Building upon the preliminary frequency analysis, an additional examination was conducted to investigate the specific indicators employed in the studies and the rationales behind the interpretation of the data.

RESULTS AND DISCUSSIONS

This section presents the key findings from the systematic literature review of 37 articles that examined disaster preparedness practices in nature-based tourism (NBT) from the perspective of governance stakeholders. The data were consolidated according to publication year, research methods, and categories of governance stakeholders. Overall, the number of articles included in the review increased from 2006 to 2022 (Figure 2). The minimal number of publications in 2023 is attributed to the ongoing review and publication process. This trend suggests a growing academic interest in disaster preparedness and adaptation in nature-based tourism over the past two decades.

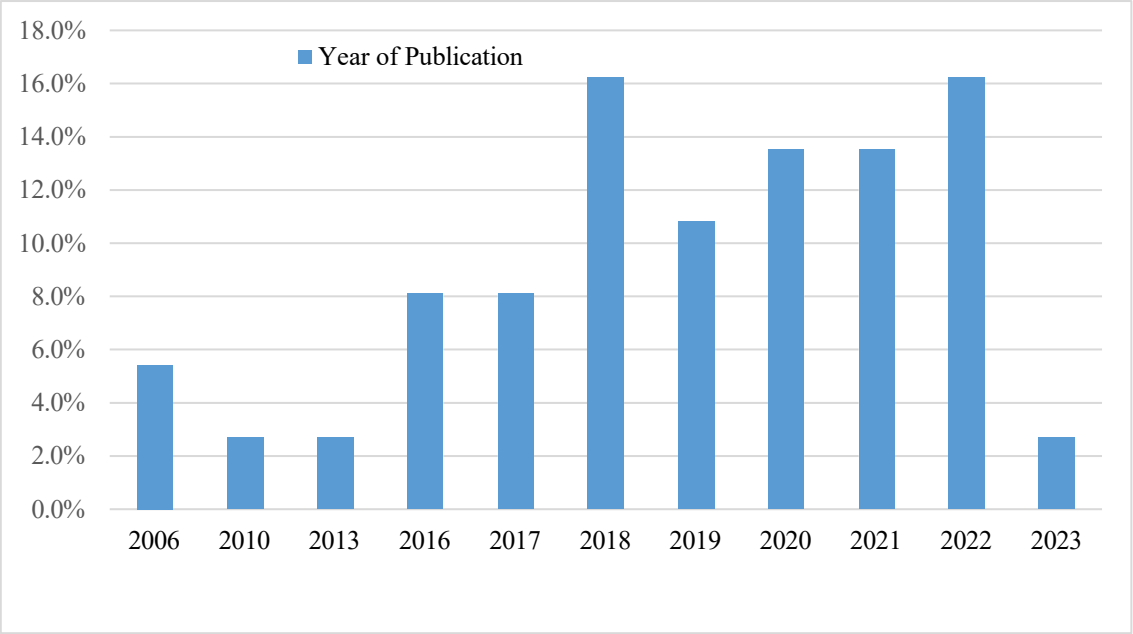

Figure 2. Number of publications by year.

This study found that the most employed research techniques to explore how nature-based tourism (NBT) can prepare for and adapt to disasters were qualitative methods, accounting for about 40%, followed by quantitative methods, with the remaining classified as mixed methods, as shown in Figure 3. The qualitative method was the most commonly used research approach (Chan *et al*., 2020; Dunning, 2020; Dunning, 2021; Van der Veeken *et al*., 2016; Jamaliah & Powell, 2017; Tervo-Kankare *et al*., 2020; Kutzner, 2019; Horne *et al*., 2018; Mushawemhuka *et al*., 2018; Murray & Watson, 2019; Fabeil *et al*., 2018; Whitworth & May, 2006; Fountain & Cradock-Henry, 2020; Jones, 2016; Antonsen *et al*., 2022). Qualitative research employs inductive designs to investigate subjects and gain insights into the processes by which individuals construct meaning and describe their experiences (Leavy, 2017).

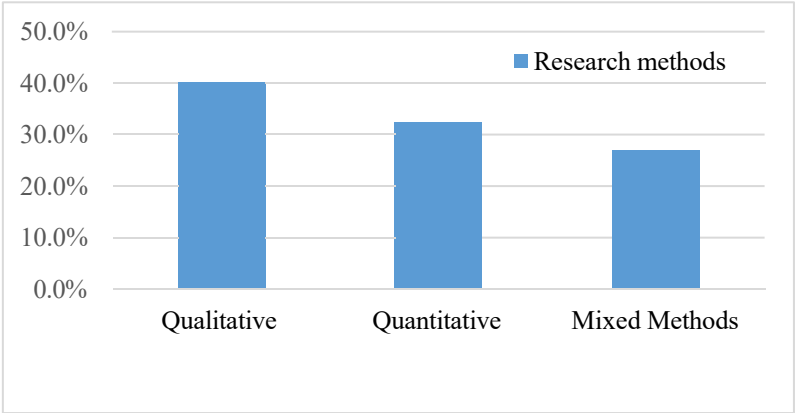

Figure 3. Frequency of research methods employed.

In this SLR, qualitative studies emphasized understanding the viewpoints, perceptions, and experiences of stakeholders engaged in disaster preparedness in NBT. Most studies employed individual interviews applying either semi-structured or structured formats (Chan *et al*., 2020; Dunning, 2020; Dunning, 2021; Van der Veeken *et al*., 2016; Jamaliah & Powell, 2017; Tervo-Kankare *et al*., 2020; Kutzner, 2019; Mushawemhuka *et al*., 2018; Murray & Watson, 2019; Fabeil *et al*., 2018; Fountain & Cradock-Henry, 2020; Jones, 2016; Antonsen *et al.,* 2022). Several studies also used focus group discussions to gain collective insights among stakeholder groups (Horne *et al*., 2018; Mushawemhuka *et al*., 2018; Van der Veeken *et al*., 2016; Whitworth & May, 2006;

Fountain & Cradock-Henry, 2020; Antonsen *et al*., 2022). Additionally, content analysis of governance-related documents such as reports, planning materials, directories, and official records was generally used to support interview data (Jones, 2016; Fountain & Cradock-Henry, 2020; Kutzner, 2019; Van der Veeken *et al*., 2016; Dunning, 2020). These studies relied on primary and secondary data to ensure a balanced and valid understanding of NBT disaster preparedness. Depending on the study objectives, interview-based studies involved between 9 and 80 participants, while focus group discussions included 9 to 19 participants.

On the other hand, quantitative research uses numerical data collection techniques to identify trends, measure impacts, and evaluate the effectiveness of preparedness strategies. Secondary data analysis was the most used approach, with studies drawing from existing datasets related to meteorological conditions, visitor statistics, or the economic impacts of disasters (Ma *et al*., 2020b; Ma *et al*., 2021; Craig, 2019; Coldrey & Turpie, 2020; Liu *et al*., 2021; Fitchett & Meyer, 2023; Ma *et al*., 2020a; Craig *et al.,* 2021). While sample size was not a direct determinant in this context, these analyses depended on the temporal coverage and quality of the available data. Surveys were another method used in quantitative studies, involving NBT operators and visitors. These studies typically utilized large numbers of participants, ranging from 250 to 2400 samples (Fountain & Cradock-Henry, 2020; Liu *et al*., 2021; Craig *et al*., 2021). Other studies implemented mixed-method research approaches, combining both quantitative and qualitative designs to integrate deductive and inductive perspectives (Leavy, 2017). These approaches provided a more holistic understanding of disaster preparedness in NBT contexts (Dube & Nhamo, 2020a; Ngxongo, 2021; Bitsura-Meszaros *et al*., 2019; Bird & Gísladóttir, 2020; Dube & Nhamo, 2020b; Jedd *et al*., 2017; Mushawemhuka *et al*., 2022; Pyke *et al*., 2016; Cuirong, 2016; Dahan *et al*., 2010). These studies often triangulated interview data with surveys and secondary sources such as weather data, geographic information systems (GIS), and economic indicators.

The impact of natural disasters on NBT has been identified in the reviewed articles, with most of the studies, 67.6%, focused on climate-related disasters (drought, flood, wildfire, hurricane, and extreme temperature) (Figure 4). In contrast, 32.4% of studies on non-climate disasters (earthquake, forest fire, landslide, and volcanic eruption) related to NBT cover the remainder of the investigation from the literature. Based on the statistics, most of the research on NBT placed a strong emphasis on climate-related disasters, such as heat waves, droughts, floods, wildfires, and hurricanes, while overlooking studies on non-climate disasters.

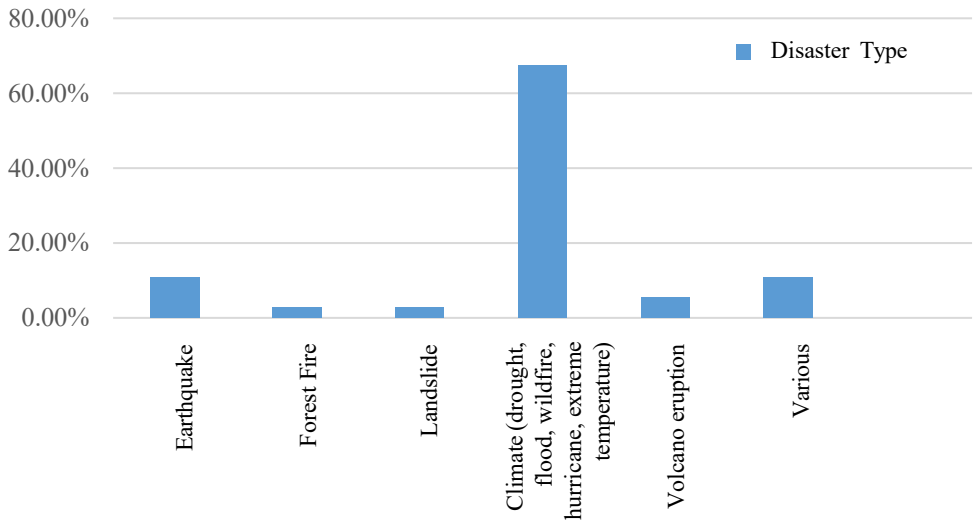

Figure 4. Statistics by type of disaster.

These findings support the evidence from the Global Risk Landscape map (World Economic Forum, 2024), which emphasized the critical threats of climate-related disasters, especially extreme weather events with high cascading effects. Non-weather-related natural disasters demonstrate a lower perceived influence compared to climate-related hazards. This trend supports current scholarly arguments that non-climate disasters are underrepresented in global disaster preparedness and tourism research, partly due to their geographical specificity and reduced perceived generalizability (Overland & Sovacool, 2020; Rossello *et al*., 2020). Accordingly, research on climate-related disasters has been emphasized in recent years, largely to address the global escalation of extreme weather events and thus demand urgent scholarly and policy attention.

Another key finding from the review focuses on the research limitations identified by the studies. Most articles highlighted common challenges in conducting research on disaster preparedness in nature-based tourism (Figure 5). Among these, data availability and quality appeared as a central concern, especially in studies on climate and weather-related disasters. This limitation is widely emphasized because accurate and complex data are essential for reliable analysis in this field. A significant portion of these challenges stems from the lack of access to and the quality of secondary data focused on meteorological and operational records. Researchers do not have enough capacity to retrieve extensive and available meteorological data, and most agencies do not consistently apply practices to record or share their operational data. It is crucial to strategize data retrieval that can enhance the quality of data analysis. For example, Coldrey and Turpie (2020) opted to use Climate Research Unit (CRU) data compared to the South African Weather Service (SAWS) due to its advantages in accessibility, consistency, quality, and wide-ranging coverage, which contribute to conducting in-depth and reliable research. Consequently, this limitation also impacts generalizability and transferability, as underscored by most of the literature. Similarly, the specific scope of geographical focus and context-specific nature contribute significantly to impacting generalizability and transferability. To improve generalizability, future research should aim to include a broad range of regions, climates, and sectors. Cross-regional and cross-sectoral research would provide more comprehensive and universally applicable findings.

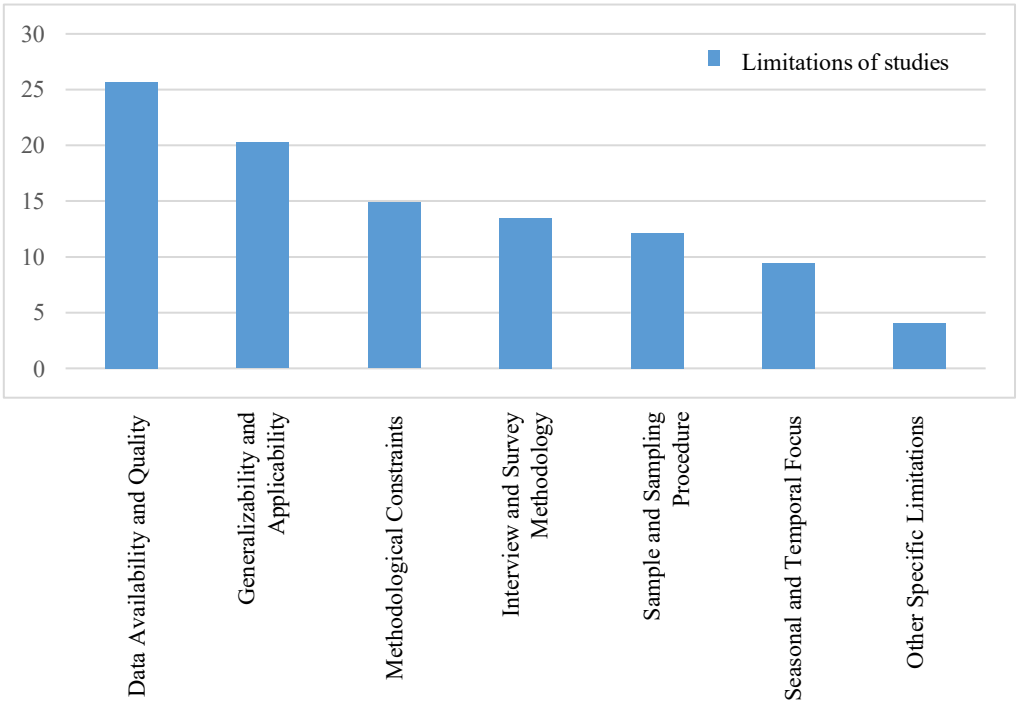

Figure 5. Statistics by type of limitations in NBT and disaster studies.

Other common problems in conducting the studies are identified in the literature, such as limited sample and sampling procedure (Murray & Watson, 2019; Ngxongo, 2016; Fountain & Cradock-Henry, 2020; Bitsura-Meszaros *et al*., 2019; Chan *et al*., 2020), methodological constraints (Ngxongo, 2016; Chan *et al*., 2020; Coldrey & Turpie, 2020; Jamaliah &Powell, 2017), and interview and survey methodology (Craig *et al*., 2021; Fabeil *et al*., 2021; de Uriost e-Stone *et al*., 2015; Chan *et al*., 2020; Bird & Gísladóttir, 2020; Jamaliah & Powell, 2017; Antonsen *et al*., 2022; Fountain & Cradock-Henry, 2020; Dahan *et al*., 2010), which underscores the importance of methodological innovation. Seasonal and temporal limitations were identified, especially in studies with short or fixed data collection periods that do not account for seasonal climate variability (de Urioste-Stone *et al*., 2015; Tervo-Kankare *et al*., 2017; Craig *et al*., 2021; Antonsen *et al*., 2022; Jedd *et al*., 2017; Craig & Feng, 2018)

Governance stakeholders, including policymakers, governmental agencies, industry institutions, and NBT businesses, play a critical role in tourism disaster management (Figure 6). The role of governmental agencies is emphasized through the implementation of policies and coordination of disaster response initiatives, while policymakers are responsible for establishing regulatory frameworks that guide disaster management efforts (Ritchie, 2004; Becken & Hughey, 2013; Dunning, 2020; Dunning, 2021). The findings elaborate on the types of NBT governance stakeholders studied in disaster preparedness. NBT operators receive more attention in the literature compared to policymakers. The critical role of NBT operators is highlighted in disaster preparation, response, and recovery, as they are directly affected by disasters and are crucial to both immediate and long-term recovery. During disasters, operators provide emergency accommodations, communication, and information (Chan *et al*., 2020; Bird & Gísladóttir, 2020; Whitworth & May, 2006), especially to ensure the safety of visitors (Fountain & Cradock-Henry, 2020; Craig *et al*., 2021; Ngxongo, 2021). During the long-term recovery and resolution phases of disaster management, the tourism industry's contributions are more fundamental, as they differ across various phases (Chan *et al*., 2020). In conclusion, nature-based tourism studies concentrate on operators due to their direct impact on disasters, their responsibility for instituting preparedness measures, and their critical role in the immediate response and long-term recovery of the tourism sector. Their direct experiences, operational data, and practical insights provide a comprehensive understanding of disaster impacts and management, making them essential for effective disaste r preparedness research.

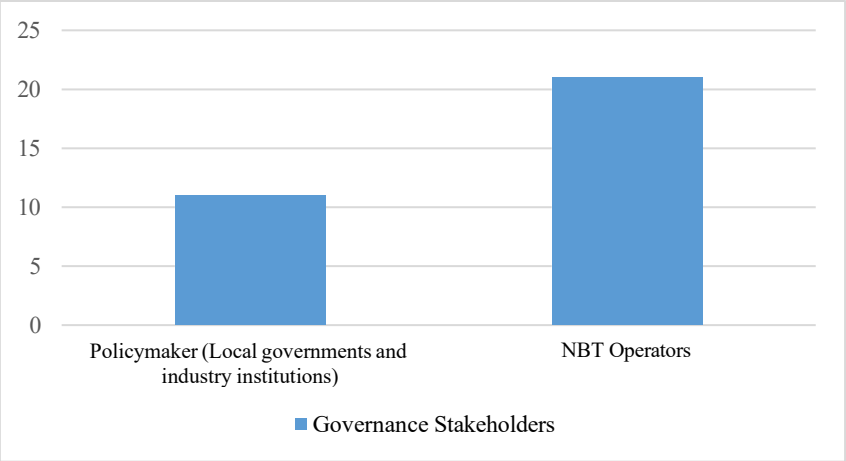

Figure 6. Statistics by category of governance stakeholders

A few determining factors are emphasized regarding the preparedness level of NBT operators, as noted in the literature. Most studies confirm that organizational and background profiles are important factors in preparedness measures. For example, the size of organizations

(Hystad & Keller, 2017; Kutzner, 2019; Murray & Watson, 2019; Fabeil *et al*., 2018), organizational income (Orchiston, 2013; Murray & Watson, 2019; Fabeil *et al*., 2018), and experience with disaster occurrences (Orchiston, 2013; Kutzner, 2019) contribute to disaster preparedness capabilities. These studies highlight how larger organizations, higher income, and experience with disasters lead to better preparedness measures. However, other determining factors should be evaluated to better explore the capability and capacity of disaster preparedness, such as the degree of an organization's exposure to disaster risks and the availability of resources for preparedness efforts.

Findings on NBT operators also reveal discrepancies between the perceived significance and actual performance of disaster management attributes. This is due to insufficient remedial actions (Mushawemhuka *et al*., 2018; Hystad & Keller, 2006; Fabeil *et al*., 2018; Orchiston, 2013; Kutzner, 2019), low self-efficacy (Horne *et al*., 2018), optimism bias (Tervo-Kankare *et al*., 2017), lack of awareness (Jamaliah & Powell, 2017), and a reactive mindset (Dahan *et al*., 2010; Hystad & Keller, 2006). Researchers recommend implementing more comprehensive, systematic strategies for tourism disaster management, such as community-led planning initiatives and incorporating knowledge from various fields to overcome these obstacles (Ritchie, 2008; Orchiston, 2013).

Studies on policymakers (i.e., local government and industry institutions) show an increasing awareness of their role in influencing disaster preparedness within NBT, even though they have received less focus in most studies. Most studies concentrate on risk perceptions and actions taken to reduce the impact of disasters at destinations as a responsibility of local governments and industry institutions (Hystad & Keller, 2006; Chan *et al*., 2020; Dube & Nhamo, 2020a; Antonsen *et al*., 2022; Ngxongo, 2021; Bitsura-Meszaros *et al*., 2019; Bird & Gísladóttir, 2020; Fountain & Cradock-Henry, 2020; Jones, 2016). This distribution highlights the potential for improvement in the gaps between practice and policy. The role of policymakers in NBT disaster preparedness is growing, but studies focusing on policy effectiveness are scarce concerning how their frameworks affect NBT operators' preparedness measures. For example, Dunning (2020), Dunning (2021), Van der Veeken *et al*. (2016), and Mushawemhuka *et al*. (2018) noted the burdensome nature of policies affecting operators' preparedness measures for disasters, which should be explored further in future research. Further studies may examine ways to better customize policy so that operators have the resources and tools needed to successfully reduce disaster risk. This could include ensuring that policies are understandable and implementable and that NBT operators have access to funding or training courses. By promoting closer collaboration between NBT operators and policymakers, the NBT industry can effectively bridge this gap and develop a more comprehensive approach to disaster preparedness.

CONCLUSION

This systematic research examined the trends and research landscape of governance stakeholders in nature-based tourism (NBT) preparedness for disasters. This study found that during the past two decades, there has been a notable increase in publications, indicating a growing interest in this field. The study emphasizes the importance of understanding stakeholder perspectives on preparedness by using various research methods, including qualitative techniques such as focus groups and interviews, as well as quantitative and mixed methods approaches, which yield useful data for identifying trends and quantifying impacts. Most research focused on NBT has emphasized climate-related disasters, such as heat waves, droughts, floods, wildfires, and hurricanes, while studies on non-climate disasters have been underrepresented. This is significantly evidenced by the World Economic Forum (2024) regarding the major focus on global critical threats, particularly extreme weather events, while non-weather-related natural disaster risks receive less emphasis, partly due to geographical specificity and perceived reduced generalizability. Therefore, it is not surprising that most existing research and policy discussions have drawn significant attention to climate-related disasters, which have heightened visibility and perceived urgency, thus underrepresenting non-climate disasters in nature-based tourism and disaster preparedness literature. This gap, driven

by regional specificity and reduced generalizability, leads to a need for more inclusive research and preparedness strategies among governance stakeholders in nature-based tourism contexts.

In the study's limitations, most literature highlighted data availability and quality as the primary limitation, mainly challenging the retrieval of secondary data such as meteorological and operational data. Common limitations noted in the studies include generalizability and applicability, limited sample size and sampling procedures, methodological constraints, interview and survey methodology, as well as seasonal, temporal, and specific limitations to certain studies. Most research examines how NBT operators operate, emphasizing the importance of comprehending their unique direct experiences, operational data, and practical insights in providing a comprehensive understanding of disaster impacts and management, making them essential for effective disaster preparedness research.

Studies involving policymakers are becoming less frequently reported, but they reflect a growing understanding of their importance in creating a framework that supports NBT disaster preparedness. This trend raises the possibility of improvement between practice and policy. Future studies should explore how to better tailor policy frameworks so that NBT operators have the necessary resources and tools to effectively reduce disaster risks. In summary, the systematic literature review findings establish a strong foundation for enhancing the quality, scope, and impact of future research on NBT disaster preparedness among governance stakeholders. Future research can contribute to a more comprehensive understanding of disaster preparedness in the context of nature-based tourism by addressing identified gaps and limitations in studies and focusing on underrepresented disaster types and stakeholders.

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RESEARCH ARTICLE

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IN SILICO ANALYSIS AND STRUCTURE MODELLING OF GAHP2, A CONSERVED HYPOTHETICAL PROTEINS RELATED TO THERMAL STRESS RESPONSE IN *GLACIOZYMA ANTARCTICA* PI12

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ABSTRACT. *The genomic data of the native Antarctic yeast, Glaciozyma antarctica PI12, has garnered attention due to its distinctive thermal adaptation. Nonetheless, a significant percentage of the proteins associated with thermal stress adaptation were identified as conserved hypothetical proteins (HPs), suggesting that these proteins remain experimentally uncharacterized. Consequently, this study aims to determine the structural characteristics of GaHP2, an uncharacterized conserved hypothetical protein believed to play a significant role in the thermal stress response. The gene was subjected to an extensive analysis utilizing computational tools to explore its function, physicochemical properties, and three-dimensional structure. Functional annotation was executed using NCBI BLAST and InterProScan; physicochemical properties were evaluated with ExPASy's ProtParam; homology modeling was performed using Phyre2 and AlphaFold2, while structure validation, refinement, and superimposition were implemented with ModRefiner and UCSF Chimera. The results indicated that the homology modeling approach effectively generated reliable 3D models of GaHP2. The high confidence score (PROCHECK), stereochemical quality (VERIFY3D), energy of the protein chain (ANOLEA), and RMSD of 0.540 Å indicate that the proposed model closely reflects the actual protein conformation. One interesting finding of the study was the correlation between the presence of aromatic clusters in GaHP2 and its stability at higher temperatures, a finding not previously documented in cold-adapted Antarctic proteins. The GaHP2 protein was also found to contain domains that encoded oxygen-binding and/or oxygen-transporting globins, as indicated by functional analysis, suggesting a role in cold adaptation under low oxygen conditions. This research illustrates that thermal stress proteins may possess distinctive structural flexibility and stability that enable them to function under thermal stress, thereby safeguarding host organisms from heat aggregation and cold denaturation.*

INTRODUCTION

Glaciozyma antarctica is a native Antarctic yeast that has generated research interest due to its exceptional capacity to thrive in a diverse range of climatic extremes (Convey & Peck, 2019). Researchers have observed that *G. antarctica* can tolerate temperatures ranging from -12°C to 20°C (Boo *et al*., 2013; Soon *et al*., 2018). As a means of surviving and adapting to the Antarctic climate,

these extremophiles produce a wide range of biologically important proteins, particularly those involved in the thermal stress response (Song *et al*., 2017; Yusof *et al*., 2021). Genomic analysis of *G. antarctica* revealed several genes encoding proteins crucial to the capacity of the cell to adapt to cold settings (Firdaus-Raih *et al*., 2018). However, a considerable proportion of these proteins remain uncharacterized, particularly the conserved hypothetical proteins (HPs) (Soon *et al*., 2018).

Conserved HPs are proteins found in organisms from several phylogenetic lineages but have not been functionally characterized (Ijaq *et al*., 2019). Determining the structure and function of proteins shared by numerous organisms can provide insights into their evolutionary changes, operational mechanisms, and molecular functions (Hauri *et al*., 2019; Morris *et al*., 2022; Zhao *et al*., 2021). These functionally unknown proteins may be involved in significant aspects of this microorganism's biological function. For instance, the evaluation of the immune protective effects of EtCHP18905, a conserved hypothetical protein of the obligate intracellular parasite *Eimeria tenella*, revealed that it could be an effective candidate for the development of new vaccines (Zhao *et al*., 2021). Previous research has demonstrated that a set of proteins with unknown functions is vital in the physiological regulation and cold adaptation of psychrophilic microorganisms (Teoh *et al*., 2021). Another study suggests that *G. antarctica*'s HPs play a crucial role in the early stages of cold and freeze stress, although validation of these findings is still pending (Wong *et al*., 2019). This offers a chance to uncover new insights into the unique characteristics of their adaptation mechanisms, specifically regarding protein flexibility, structural stability, and residue interactions that facilitate functionality in cold and thermally fluctuating conditions.

The main problem in the structural determination of uncharacterized proteins is the difficulty of obtaining soluble proteins for downstream processing (Ahmad *et al*., 2018; Kielkopf *et al*., 2021). To understand biological processes at the system level, we must evaluate the three-dimensional (3D) protein structures that mediate biochemical interactions (Hauri *et al*., 2019). Structure determination experiments are also labor-intensive and complicated processes (Rigden, 2017). Comparative homology modeling, in the absence of an experimentally determined structure, can generate a useful 3D model of a protein that links to at least one known protein structure. The rapid improvements in omics technologies allow researchers to complement the expensive and time-consuming structural determination experiments. This has allowed the theoretical assessment of several physicochemical parameters to reveal the function and makeup of proteins about which we previously lacked information (Jumper *et al*., 2021).

Thus, by combining structural knowledge of proteins with functional annotation tools, previously uncharacterized proteins can be elucidated (Jez, 2017). Therefore, this study uses in silico analysis to investigate the structure–function relationship of heat shock proteins (HPs) involved in *G. antarctica*'s thermal stress response. The current study aims to evaluate the conserved HPs associated with thermal stress responses in *G. antarctica* to establish a better understanding of their adaptation mechanisms.

MATERIALS AND METHODS

Sequence Analysis

The *G. antarctica* PI12 genome transcriptome data (PRJNA41257) were examined for conserved thermal stress response heat shock proteins. Proteins suitable for protein structure determination were identified based on a reliable level of expression (1.5-fold, p-value < 10^{-5}) (Firdaus-Raih *et al*., 2018) and a protein size range of 18 to 55 kDa (Jez, 2017; Klebe, 2013). Normalized counts underwent differential expression analysis using DESeq2. Genes were deemed significantly differentially expressed if they exhibited an adjusted p-value of less than 0.05 and a log2 fold change of greater than or equal to ±1. The selected heat shock protein's amino acid FASTA sequence was translated from the DNA sequence through the ExPASy Translate Tool (https://web.expasy.org/translate/) and utilized for functional annotation, physicochemical analysis, and homology modeling. The sequence quality was

confirmed through sequence alignment and validation using the EMBL-EBI Clustal Omega Tool (Madeira *et al*., 2024).

Physicochemical Analysis

Physicochemical characteristics of HPs in raw sequence format were assessed using ExPASy's ProtParam tool (http://web.expasy.org/protparam/) (Gasteiger *et al*., 2005). The computed isoelectric point (pI) is valuable for protein characterization since it indicates that the surface of the protein is charged, but the overall charge of the protein is zero, rendering it stable and compact (Tokmakov *et al*., 2021).

Functional Annotation

An analysis was conducted for functional annotation, utilizing the Basic Local Alignment Search Tool (BLAST) to search for similar proteins in the NCBI non-redundant (nr) database. The purpose was to identify homologous proteins from related organisms that are likely to have the same function as the query protein. The BLAST search utilized an E-value threshold of 1e-5 and a maximum of 100 target sequences to ensure relevant and statistically significant matches. The presence of domains and important sites in functional protein families was predicted using InterProScan (https://www.ebi.ac.uk/interpro/about/interproscan), as described by Mitchell *et al*. (2019).

Comparative Homology Modelling and Structure Assessment

Structure prediction

Homology modeling was performed using both the Phyre2 server (www.sbg.bio.ic.ac.uk/phyre2) (Kelley *et al*., 2015) and the AlphaFold server (https://alphafoldserver.com/) (Abramson *et al*., 2024; Jumper *et al*., 2021). The 3D models generated were refined using the ModRefiner web server (https://zhanggroup.org/ModRefiner/). Refining the structure is an essential process in bringing a starting structure closer to its native state and achieving accuracy that is comparable to experimental results (Bhattacharya, 2019).

Structure validation

The best homology models were assessed for their stereochemical quality using the Ramachandran plot (Lovell *et al*., 2003) and VERIFY3D (https://servicesn.mbi.ucla.edu/Verify3D) (Eisenberg *et al*., 1997). The energy of the protein chain was calculated using the atomic empirical mean force potential ANOLEA (www.fundp.ac.be/pub/ANOLEA.html), as described by Melo *et al*. (1997). High-energy zones identified by ANOLEA denote regions of the protein exhibiting unfavorable atomic interactions, potentially corresponding to structurally strained, flexible, or poorly folded areas. These zones may indicate potential sites of instability or conformational variability, and in this study, they were interpreted as regions that could contribute to the dynamic nature or functional flexibility of the cold-adapted protein.

Once the 3D model was generated, the PDB files containing the constructed 3D models of *G. antarctica* HP were submitted to the DALI server for template search (Holm, 2020). Selected templates were incorporated with the 3D model of interest for comparative analysis using UCSF Chimera (Pettersen *et al*., 2004).

Protein-protein interactions were analyzed using the Protein Interactions Calculator (PIC) server (http://pic.mbu.iisc.ernet.in/), as described by Tina *et al*. (2007). Using the coordinate set of a protein or assembly, the PIC server calculates a range of interactions within the protein or complex. These include disulfide bonds, interactions between hydrophobic residues, ionic interactions, hydrogen bonds, aromatic–aromatic interactions, aromatic–sulfur interactions, and cation-π interactions.

RESULTS AND DISCUSSIONS

GaHP2 Sequence Analysis

The transcriptomic analysis of the *G. antarctica* genome revealed GaHP2, a 765 bp gene coding for a conserved hypothetical protein related to thermal stress response (Table 1). The gene exhibited a level of expression at low temperatures (below 37°C) greater than 1.5 (significant p-value 10^{-5}). The temperature threshold is significant as cold-adapted organisms, like *G. antarctica*, thrive in environments significantly below mesophilic conditions. Increased gene expression at temperatures below 37°C suggests a potential role in cold adaptation and survival mechanisms (Teoh *et al*., 2021). The verified sequences of the genes coding for GaHP2 were converted into their amino acid sequences via Expasy Translate Tools. Functional annotation and physicochemical analysis of the protein were conducted using the amino acid sequences arranged in GenBank/GB format. Functional annotation employs peptide sequences, considering that protein amino acid sequences are more conserved than gene nucleotide sequences (Kapli *et al*., 2023).

Table 1. Sequence analysis of *G. antarctica* transcriptomic data for conserved hypothetical proteins related to thermal stress response.

Transcript	Length (bp)	Protein ID	Description
GaHP2	765	GaHP2	Predicted uncharacterized protein, conserved

Physicochemical Analysis

ExPASy's ProtParam tool revealed the molecular weight of GaHP2 at 29 kDa and isoelectric point (pI) values of 5.46, indicating that the protein is acidic and negatively charged. Proteins exhibit a wide range of physicochemical properties that significantly impact their activity, structure, and consequently, their biological function (Yuan *et al*., 2020). Understanding the function and composition of HPs can be enhanced by computing and predicting their physicochemical properties (Moldoveanu & David, 2022; Naqvi *et al*., 2015). Understanding the pI value is crucial in protein purification as it reveals the pH value at which solubility tends to be at its lowest.

The protein pI represents the specific pH value at which the protein's movement comes to a halt in an electro-focusing device. This pH value also indicates the point at which the protein will be eluted (Novák & Havlíček, 2016). In addition, the molecular weight and pI parameters can be used to analyze two-dimensional electrophoresis gels, which helps in the experimental analysis of proteins (Molina-Mora *et al*., 2020; Sahab *et al*., 2005). Biologists have previously utilized the theoretical and experimental determination of the isoelectric point of peptides to assist in identifying peptides in complex mixtures (Kozlowski, 2021). With the help of the computed pI, protein purification protocols and crystal screening strategies can be developed.

Functional Annotation

After conducting a Protein BLAST search against the NCBI non-redundant (nr) database, it was found that GaHP2 has a similarity of less than 57% to the protein in the database (Table 2). Sequences were considered homologous only if they exhibited a 90% identity in a BLAST search against the NCBI non-redundant (nr) database (Altschul *et al*., 1997; Gazi *et al*., 2020). Based on the findings, it appears that the protein's characterization is still incomplete.

Table 2. The BLAST search results for GaHP2, a conserved hypothetical protein from *G. antarctica,* against the NCBI non-redundant (nr) database.

Protein ID	Sequence identity	e value	Description
GaHP2	57%	7.00E-86	ORY79343.1 Protoglobin-domain-containing protein [*Leucosporidium creatinivorum*]

InterProScan also provided limited information on known proteins for predicting homologous protein families and functional domains (Table 3). The GaHP2 protein appeared to be associated with biological processes related to oxygen and heme binding. However, no specific functional domain could be predicted for this protein. It is evident that there is still much to learn about GaHP2, allowing for potential novel discoveries. Described as part of the Globin or protoglobin homologous superfamily, GaHP2 also exhibits two GO terms for biological processes: heme binding and oxygen binding. It appears that this protein plays a role in the transportation of oxygen, similar to how haemoglobin functions in humans. Previously, scientists found that *G. antarctica* PI12 displayed remarkable adaptability in low oxygen conditions. By utilizing nitrite as an alternative electron terminal acceptor, along with other common mechanisms, *G. antarctica* PI12 was able to thrive in the cold environment (Wong *et al*., 2019).

Table 3. Functional annotation of GaHP2, a conserved hypothetical protein from *G. antarctica,* using InterProScan tool.

Protein ID	Homologous Superfamily	Domains	Biological process	Molecular function	Cellular component
GaHP2	Globin/Proto (IPR012292)	None predicted	heme binding (GO:0020037) oxygen binding (GO:0019825)	None predicted	None predicted

3D Model Development

The 3D structures of the GaHP2 protein exhibit a prominent pattern of α-helices, along with a significant number of random loops in the region between the α-helices and β-sheet (Figure 1). Based on the abundance of looped regions, it can be inferred that the protein structure is remarkably conserved (Neelamathi *et al*., 2009). The presence of numerous looped regions suggests that, despite a low overall sequence identity (< 30%), the tertiary structure topology, especially the fold and loop-rich architecture, might be conserved. This indicates that structural motifs or functional topologies, rather than primary sequences, may be retained to ensure stability or function in col d-adapted environments (Hamid *et al*., 2022; Michetti *et al*., 2017).

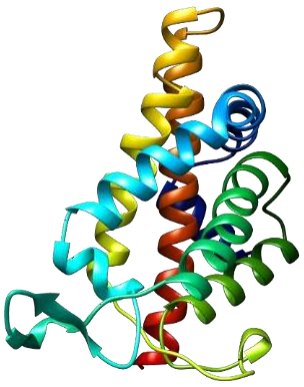


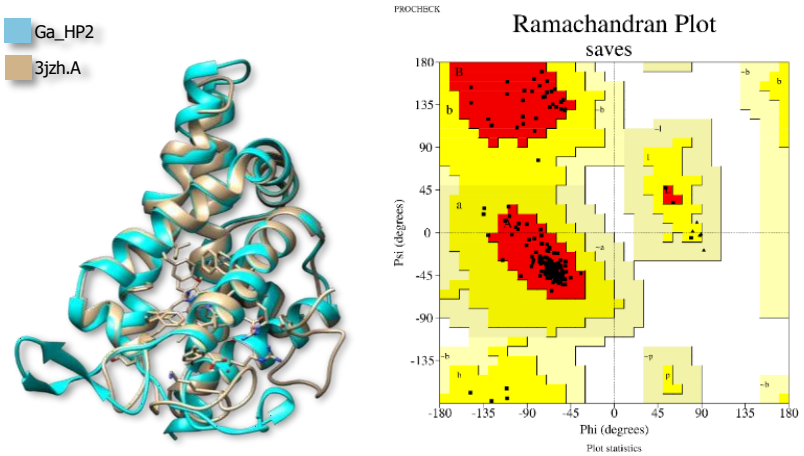
Figure 1. The three-dimensional (3D) structure of the GaHP2 protein constructed using Phyre2 Server.

The templates used by Phyre2 server for the 3D structure construction were Protoglobin (c2veeC_) from *Methanosarcina acetivorans* with 15% sequence identity and 100% confidence score. Confidence score represents the probability (from 0 to 100) that the match between our sequence and its template is a true homology. The score of > 90% indicates protein adopts the overall fold shown and that the core of the protein is modelled at high accuracy (Kelley *et al.,* 2015). The confidence score indicated that GaHP2 models fulfilled the structural validation score requirement despite a sequence identity of less than 30% similar to PDB structures. A high degree of confidence (> 90%) and a low degree of sequence identity (< 30%) suggest that the fold is likely right, accurate in the core (2 - 4Å), but may exhibit significant variations in loops and noncore areas. Phyre2 combines PSI-BLAST and the Hidden Markov Model (HMM) database to provide an extremely strong protein structure prediction algorithm capable of reliably identifying very distant homology and creating accurate models even when sequence identity is less than 15% (Kelley *et al.,* 2015). Additionally, structure refinement through ModRefiner can minimise template structure errors related to sequence identity below 25% (Adiyaman & McGuffin, 2019).

Comparative structural modelling is reliable and can predict protein structures with atomic precision (Jumper *et al*., 2021). The 3D structure was also compared with the latest AlphaFold2 (Abramson *et al*., 2024; Jumper *et al*., 2021) and RoseTTAfold (Baek *et al*., 2021) programs and results in less than 1.4 Å. The computational method for protein structure determination has advanced to the point that it can predict protein structures with atomic precision regularly, even in cases where no similar structure exists (Jumper *et al*., 2021). This provides a reliable and cost-effective alternative to the months to years of laborious effort required to determine the structure of a single protein.

Model Validation and Assessment

The structural comparisons of the constructed 3D model with its homologs, as well as the corresponding Ramachandran plot statistics, were presented in Figure 2. The PROCHECK through Ramachandran plot analysis revealed that GaHP2 model had a good quality score, with no residues in disallowed regions being found. DALI server was able to retrieve compatible homologs for GaHP2 for comparative structure analysis. Using the UCSF Chimera programme, structural comparisons of GaHP2 3D models resulted in a 14% overlap. The summary of structural quality evaluation shown in Table 4 confirmed that GaHP2 model was acceptable, with an RMSD value of 0.540 Å indicating very little variation from homologs.



(a) (b)

Figure 2. GaHP2 structural comparison with templates obtained from the DALI server: (a) Superimposition of GaHP2 predicted structure (turquoise) and hEED (PDB ID: 3jzh) (tan) with RMSD value 0.5 Å; and (b) Ramachandran plot provided by the PROCHECK program for the superimposed GaHP2 model with a comparison with 3jzh.

Table 4. Summary of structural quality assessment results of the superimposed 3D Structure of GaHP2 with templates retrieved from DALI server.

3D Model	% Sequence identity	PROCHECK V.3.5 (expected > 90% in favored region)	Verify3D (expected > 80%)	ANOLEA (expected < 35%)	RMSD
GaHP2	14% 3jzh.B Protoglobin *Methanosarcina acetivorans*	100.0% allowed 0.0% disallowed	91.62%	36.84% Z-score: 4.43	0.540 Å

Additionally, the 3D model achieved an excellent Verify3D score of 91.62%. Verify3D evaluates protein structures using 3D profiles. This software determines if an atomic model (3D) is compatible with its amino acid sequence (1D) (Eisenberg *et al*., 1997). The 3D-1D scoring matrix is a 20 × 18 matrix that reflects the environmental compatibility of each residue. The higher the value, the better the entire model's compatibility (Matsuo *et al*., 1995). For ANOLEA's energy calculations, it is noted that the GaHP2 built 3D model scored 36.84% in the high energy zone (HEz) in the protein profile, slightly higher than the acceptable score of 35%. However, a closer observation revealed that a significant portion of the amino acids with a high energy content were in the non-superimposed loop (Figure 3). A previous study showed that ANOLEA energy assessment of a few loop regions was high with positive values even after undergoing loop refinement (Pan *et al*., 2021; Singh *et al*., 2012). This is corroborated by ANOLEA's low Z-score of 4.43 for the GaHP2 model. The Z-score is calculated using the pseudo energies of target protein sequences, with a lower Z-score indicating more reliability (Melo & Feytmans, 1998). This is verified by the fact that the HEz value of 36.95% obtained from the 3D model of Arabidopsis thaliana HAC1 protein profile correlates with structural defects, while the lower Z-score of 3.51 suggests a high-quality 3D model (Ćemanović *et al*., 2014).

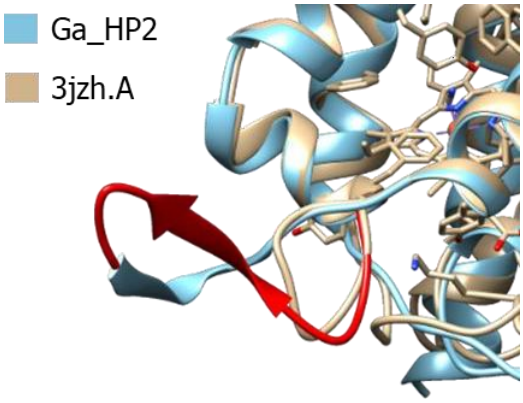


Figure 3. The amino acids in GaHP2's non-superimposed loop with high energy content are highlighted in red.

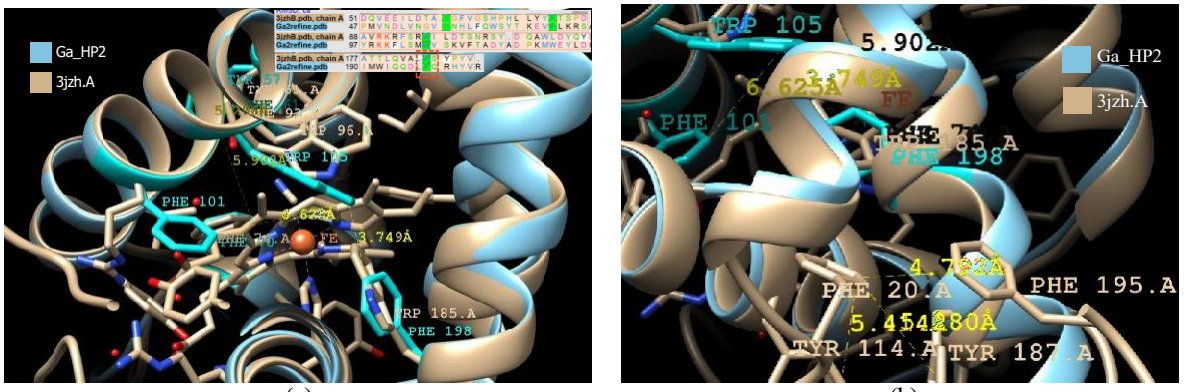
Analysis of the intra-protein interaction of the constructed 3D *G. antarctica* model with its respective homologs is shown in Table 5. Generally, GaHP2 3D models possessed lower hydrophobic interactions compared to their homologs. This demonstrates their structural flexibility, which enables them to function at low temperatures (Hamid *et al*., 2022). Increased structural flexibility of specific residues, mainly in the active region, or the entire protein structure, enables adaptation of psychrophilic enzymes by lowering the activation energy required for metabolism (Fields, 2001). The findings are corroborated by a study of a cold-adapted protein, chitobiase from *Arthrobacter sp.* TAD20, in which increased structural flexibility resulted in increased activity at low temperatures, particularly those that significantly impede molecular motions (Hamid *et al.,* 2022). A previous study on cold-adapted β-d-Galactosidases from *Arthrobacter sp.* 32cB demonstrated that the environment's lack of free energy, caused by low temperature and high

viscosity of water, can be compensated for by a higher efficiency of energy gain due to their high structural flexibility (Rutkiewicz *et al.,* 2019).

Table 5. Comparative analysis of intra- and inter-protein interaction between GaHP2 and its homologs, protoglobin (PDB ID: 3zjh chain B) from the mesophilic *Methanosarcina acetivorans*, the globin domain of a globin-coupled sensor protein (PDB ID: 2w31 chain A) from *Geobacter sulfurreducens*, and the heme-based aerotactic transducer (HemAT) sensor domain (PDB ID: 1or4 chain A) from *Bacillus subtilis*.

Protein Interaction	GaHP2	3zjh-B	2w31-A	1or4-A
Hydrophobic Interaction within 5 Å	135	166	145	145
Ionic Interactions within 6 Å	18	18	19	19
Aromatic-Aromatic Interactions within 4.5 and 7 Angstroms	7	20	2	2

In contrast, the higher aromatic-aromatic interaction in GaHP2 as compared to the two mesophilic homologs, the globin domain (2w31) from *G. sulfurreducens* and the HemAT sensor domain (1or4) from *B. subtilis,* indicated unusual rigidity for a psychrophilic protein. However, further inspection showed that the aromatic-aromatic interaction in GaHP2 was located at the heme-binding site (Figure 4a). This indicates that the aromatic-aromatic interaction may contribute to the high binding site efficiency. Some protein regions that are not involved in catalysis may become even more rigid than their mesophilic counterparts, as previously described in the comparison of psychrophilic and mesophilic DNA ligases' 3D models (Georlette *et al*., 2003). This correlates with the presence of a significant aromatic-aromatic interaction distant to the heme-binding site of the protoglobin (3zjh) homolog from the obligate anaerobic Archaea, *Methanosarcina acetivorans*, therefore contributing to the rigidity of the protein (Klinger *et al.,* 2003; Mitra & Das Mohapatra, 2021). In contrast, the protoglobin (3zjh) homolog was also detected with additional aromatic-aromatic interaction away from the heme-binding site (Figure 4b), therefore contributing to their overall structural rigidity. This was correlated with a previous report indicating that the obligate anaerobic Archaea, *M. acetivorans,* may be thermophilic facultatively (Klinger *et al*., 2003).



(a) (b)

Figure 4. The aromatic-aromatic interaction takes place at heme binding sites in GaHP2 and its protoglobin (3zjh) homolog: (a) The aromatic residues are generally conserved except for some substitution of TRP185 in 3zjh homolog to PHE198 in GaHP2; and (b) Strong aromatic-aromatic interaction between PHE20 to TYR114, PHE195, and TYR187 in the protoglobin (3zjh) homolog from *Methanosarcina acetivorans*.

This finding, in conjunction with a previous study comparing the cold-adapted citrate synthase from an *Arthrobacter* strain to the homologous enzyme from the hyperthermophile *Pyrococcus furiosus*, indicates that part of the cold-adapted enzyme's adaptation to low temperature may be due to increased accessibility of the active site (Gerike *et al*., 2001; Liu *et al*., 2023). Due to the high degree of

conservation and relative rigidity of the catalytic site across homologous enzymes, the GaHP2 binding efficiency is consistent with the capacity of the *M. acetivorans* protoglobin (3zjh) homolog to reshape its haem distal site structure. By changing the conformation of another aromatic residue, Trp60, the aromatic residues Phe93 operate as ligand sensors and control access to the haem through the tunnel system (Klinger *et al*., 2003). In addition, the decreased solubility of oxygen as temperatures rise requires the active sites to be more efficient at a higher temperature than their optimal growth temperature (Rafiq *et al*., 2019). Finally, the reversibility of some cold-adapted enzymes' thermal unfolding has been attributed to the fact that when they are unfolded, a smaller number of hydrophobic groups are exposed to the aqueous solvent, thereby preventing or limiting the irreversible aggregation process typical of more stable proteins (Nowak & Otzen, 2024). The structural insights derived from this study may be utilized to design enzymes with specific characteristics for biotechnological applications. Furthermore, expanding comparative studies to include a wider range of psychrophilic, mesophilic, and thermophilic organisms may uncover additional evolutionary adaptations that optimize thermostability or flexibility in proteins similar to GaHP2.

CONCLUSION

This work presents the identification and in silico analysis of GaHP2, a conserved hypothetical protein that was involved in the thermal stress response in *G. antarctica*. The evaluation of physicochemical properties aided in understanding the unique characteristics of the annotated proteins, while functional annotation highlighted the role of the proteins in the biological processes of heme binding and oxygen binding. Comparative structural analysis of GaHP2 indicated cold-adapted traits, most notably increased flexibility in comparison to their mesophilic or thermophilic counterparts. This mostly results from their reduced hydrophobic, ionic, and aromatic interactions. Furthermore, the HPs include multiple additional loops and glycine residues, which have been associated with enhanced flexibility for activities suited to low temperatures. However, the presence of aromatic clusters in GaHP2 has been linked to the psychrophilic protein's unusually high thermostability. Therefore, this protein was hypothesized to preserve an ideal balance between molecular stability and structural adaptability in order to optimize its functional roles in oxygen transportation under thermal stress conditions. Further research should focus on experimental validation via in vitro enzyme assays, site-directed mutagenesis, and protein crystallography to verify the specific function of GaHP2 in the adaptation mechanism of *G. antarctica*.

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THE ROLE OF INSECTS AS SUSTAINABLE RESOURCES IN FOOD AND TOURISM: A COMPARATIVE GLOBAL-LOCAL ANALYSIS

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ABSTRACT. *This review paper explores insects as valuable natural resources for food and their role in the nature tourism industry. Utilizing a Systematic Literature Review (SLR), the study assesses existing research on edible insects and entomotourism from a global and local perspective. Edible insects offer a sustainable and nutritious alternative to traditional livestock, with over 2000 species recognized, especially in Africa, America, and Asia. Given the growing global population and shrinking agricultural land, reliance on conventional animal protein is unsustainable. Insects can be raised in large numbers with minimal resources, presenting a viable nutritional solution. Furthermore, certain insects, like fireflies and butterflies, significantly enhance nature tourism by attracting visitors and benefiting local economies. The rise of entomotourism, particularly in Sabah, Malaysia, illustrates the revenue potential of activities such as firefly-watching. This study emphasizes insects as both a sustainable food source and a driver of economic growth in tourism, addressing food security challenges. Integrating insect-based solutions into food systems and tourism can promote ecological sustainability and nutritional security. Continuous research and policy support are essential for recognizing insects as vital components of a sustainable future. The importance of insects in ensuring food security and enhancing nature tourism cannot be overstated.*

INTRODUCTION

Importance of Insects

Insects are an incredibly valuable resource to our world. With over 1.5 million named species and an estimated 5.5 million species in total, only 1% of them can be truly considered pests (Stork, 2018; Pedigo *et al*., 2021). The rest play important roles, whether their functions are known or not. Beneficial insects such as bees, silk moths, ladybirds, and parasitic wasps provide humans with valuable resources like honey, beeswax, silk, lacquer, shellac, and cochineal dye. Additionally, insects contribute to natural ecosystems by serving as pollinators, weed killers, natural biological control agents, aiding in decomposition and nutrient cycling, and providing food for wildlife (Getanjaly, 2015). Throughout history, people from all over the world have utilized insects as valuable resources. This paper will delve into the contemporary applications of insects, specifically their role as a food source and in the tourism industry. This research utilized a Systematic Literature Review (SLR) method to thoroughly examine

existing studies on the subject by searching for pertinent research articles in Google Scholar using the specified keywords. Following an introductory evaluation of titles and abstracts, qualifying studies were chosen according to stringent inclusion and exclusion criteria, which were then followed by a detailed review of the full texts. The SLR approach improves transparency, reduces bias, and lays a structured groundwork for recognizing trends, inconsistencies, and potential research avenues in the area (Snyder, 2019).

Insects as Food

The practice of consuming insects as food and insect products such as honey, known as entomophagy, has a rich history and is enjoyed by people across Asia, Australia, Africa, and the Americas. The word entomophagy comes from two Greek words, "entomos," meaning insect, and "phagein," meaning eating (Yen, 2015). Insects can be eaten at all stages of their lives, including eggs, larvae, pupae, nymphs, or adults (Costa-Neto, 2015). They are consumed in their original form or processed and mixed with other ingredients. According to Jongema (2017), there are approximately 2,037 edible insects, including beetles, caterpillars, ants, bees, wasps, bugs, termites, dragonflies, and cockroaches. Edible insects are recognized for their potential to contribute to global food security (Nowak *et al*., 2016). Entomophagy has been viewed from two perspectives globally: first, as a vital protein source in nations facing food scarcity, and second, as an alternative protein source in developed countries, adhering to sustainable development initiatives (Skotnicka *et al*., 2021). Comparatively, ants and crickets can contain between 9% and 77% dry-weight protein, while beef contains 25% to 28% (Abril *et al*., 2022). In 2013, the United Nations Food and Agriculture Organization (FAO) emphasized this potential in a report titled "Edible Insects: Future Prospects for Food and Feed Security," highlighting the nutritional and environmentally sustainable advantages of insects as a future food source (Huis *et al*., 2013).

Various insects are consumed around the world, such as the bamboo caterpillar (*Omphisa fuscidentalis*), house crickets (*Acheta domesticus*), and giant water bugs (*Lethocerus indicus*), which are primarily consumed in Thailand (Hanboongsong *et al*., 2013). In Africa, termites (*Macrotermes* sp., Odontotermes sp.), locusts (Ornithacris magnifica, Cyrtacanthacris tatarica), and African silk moth caterpillars (*Anaphe panda*) are commonly consumed (Morris, 2004). In Borneo, people consume the larvae of the palm weevil (*Rhynchophorus ferrugineus*), mole cricket (*Gryllotalpa longipennis*), and honeybee brood (*Apis dorsata*) (Chung, 2010).

Insects are recognized as valuable sources of essential nutrients for the human body, providing energy, protein, vital amino acids, and a variety of micronutrients (Oonincx and Finke, 2021). A thorough nutritional analysis of adult crickets (*Gryllodes sigillatus*), mealworm larvae (*Tenebrio molitor*), and adult locusts (*Schistocerca gregaria*) revealed a well-balanced nutrient profile, meeting human amino acid requirements and containing significant amounts of monounsaturated and polyunsaturated fatty acids, along with specific micronutrients (Zielińska *et al*., 2015). However, their nutrient composition can vary based on species and developmental stages, as well as location, season, feed, and gut content (Nowak *et al*., 2016). Certain species, such as termites and palm weevils, have high levels of saturated fat, making them unsuitable for inclusion in the diet of populations with a high incidence of cardiovascular disease and diets centered on refined carbohydrates (Payne *et al*., 2016). Despite the wealth of literature on edible insects, significant gaps remain in available data concerning the nutritional content of major edible insect species (Payne *et al*., 2016).

The consumption of insects extends beyond developing countries in South America, Asia, and Africa; it is also practiced in developed countries in Europe and North America. In the past, entomophagy was considered normal in many European contexts (Olivadese and Dindo, 2023). However, the tradition of consuming insects in Europe has largely been avoided until recently due to the perception of insects as a threat and health risk rather than food (Mancini *et al*., 2022; Moruzzo *et al.*, 2021). With a growing population and the need for substantial areas to produce livestock, the tradition of entomophagy has started to regain attention in Europe. In recent years, interest in entomophagy among European society has significantly increased. Some recent studies suggest that consumers are beginning to recognize the benefits of entomophagy (Kasza *et al*., 2023). To promote the

acceptance of entomophagy among the population in Europe, it is essential for authorities to comprehensively assess microbial contamination, toxicological hazards, and allergenic reactions (Raheem *et al*., 2018b).

The current consumption of insects has shifted from traditional practices to processing them into various forms and including them in food products to increase public acceptance (Olivades and Dindo, 2023). Studies have shown potential acceptance of entomophagy in European populations, with interest and willingness to consume insects observed in Belgium, the Czech Republic, Hungary, and Italy (Megido *et al.*, 2013; Bednářová *et al.*, 2013; Kasza *et al.*, 2023; Sogari *et al.*, 2015). However, psychological motivation and appropriate commercialization strategies are needed to mitigate the phobia of entomophagy in Italy (Toti *et al.*, 2020; Moruzzo *et al.*, 2021).

In Poland, many students are hesitant to switch from eating meat to insects due to psychological barriers such as fear and disgust (Kostecka *et al*., 2017; Orkusz *et al.*, 2020). In Switzerland, some are interested in trying insects, while others are repelled by the idea (Penedo *et al*., 2022). Although the Native people of North America, including the Inuit people of the Arctic, have a history of practicing entomophagy (Morris, 2004; Lesnik, 2019; Ferreira *et al.*, 2018), early Western settlers, much like present-day Europeans, were not prepared to adopt insects as a primary protein source. However, in recent years, there has been a growing willingness within the North American community to embrace entomophagy as a means of obtaining a more affordable protein source (Schrader *et al*., 2016).

Like the natives of North America, the aborigines of Australia also consume insects (Yen, 2005). Some insects and insect products are still enjoyed by the aborigines, such as witchetty grubs, leps from psyllid beetles, honey, and honey ants (Si and Turpin, 2015). However, surveys in Australia showed low acceptance of entomophagy by Australians of European origin due to factors such as neophobia, disgust for insects, and perceptions of masculinity (Sogari *et al*., 2019). Other influencing factors include appearance, taste, quality, and safety (Wilkinson *et al*., 2018). Nonetheless, entomophagy is expected to gain popularity in Western society, and education about it should be disseminated more widely to increase acceptance (Olivades and Dindo, 2023; Lensvelt and Steenbekkers, 2014). Unlike in Europe, Australia, and North America, the people of Asia, Africa, and South America are more inclined toward entomophagy, although many people in these areas still do not eat insects due to disgust. Historically, entomophagy has been established for a long time in these areas because insects have traditionally been food for generations (Durst and Shono, 2010).

The traditional use of insects for nutrition in Africa is significant, with insects consumed as a delicious delicacy, for emergency sustenance, or as a staple food (Hlongwane *et al.*, 2021). Entomophagy is prevalent in both urban and rural communities, with insects readily available in the environment, such as alates and termites, serving as a cost-effective source of protein. Notably, edible insects and their products are widely available in African markets (Das, 2020). Termites and alates are particularly important as a food source for Africans due to their abundance and accessibility, with villagers often collecting them for free as their primary source of protein (Morris, 2004). These insects can be consumed raw or cooked. Furthermore, a survey conducted in Côte d'Ivoire, West Africa, revealed that over half of the population practiced entomophagy, with nine common insect species being consumed, particularly saturniid moth larvae and termites, which are widely available in markets, with palm tree caterpillars preferred by 40% of respondents (Ehounou et al, 2018). Similarly, a survey in South Africa indicated that 95% of respondents occasionally consume insects, with primary reasons being nutritional benefits and cultural traditions (Hlongwane *et al*., 2021). Overall, insects are not only consumed as food in rural Africa but also serve as a livelihood, as they can be traded in other regions as a source of sustenance (Muvatsi *et al*., 2021).

The practice of entomophagy is widespread in Latin America, particularly in countries like Mexico, Peru, Brazil, Venezuela, Colombia, and Ecuador due to their rich sociocultural heritage. Mexico stands out in the region, with 450 documented species, making it the country with the highest number of recorded edible insects in Latin America. In total, there are 735 species of edible insects identified in Latin America. Additionally, Latin America is the world's second-largest market for edible

insects after the Asia-Pacific region. The larvae of beetles, butterflies, and wasps are the preferred stage for consumption due to their flatter body structure compared to adult insects with an exoskeleton. Given the considerable potential and interest in sustainable protein sources, there are ongoing efforts to further develop the edible insect food industry in Latin America, leveraging the region's rich culinary traditions and biodiversity to attract both domestic and international consumers.

As mentioned above, Asia-Pacific is the largest area for the edible insect market, with 932 species of edible insects recorded in Asia alone. In Asia, people eat a variety of bugs. In Yunnan Province, China, people eat common wasps, teak caterpillars in Java, edible wasps in Japan, edible bugs in Laos, edible bugs in the Philippines, Sri Lanka's potential as a gene pool of edible bugs, and insect nutrition in Thailand. In contrast to Europeans, Asians typically consume insects without transforming them into other forms, often eating the insects whole or with visible body parts. Consequently, it is common to find cooked insects being sold in markets.

In Asia-Pacific, most edible insects are sourced from three main outlets: wild harvesting, semi-domestication of wild insects, and farming. Harvesting insects from their natural habitats is the primary method of sourcing them for consumption in the Asia-Pacific and Africa, but this practice puts pressure on insect populations in their native habitats when insects are collected for commercial use. Establishing small- to medium-scale insect farms in residential areas is a viable way to produce edible food sources in many Asian and African countries. Insects can be reared in areas not suitable for other uses and can be fed with human food waste. The growth of the insect food market in Thailand presents an accessible opportunity for innovative entrepreneurs and small companies to offer affordable and more readily available insect-based food products, which could potentially be marketed as alternatives to meat, benefiting underprivileged communities.

Recently, the Singapore Food Agency has approved 16 species of insects for human consumption after a thorough scientific assessment. The approval follows a comprehensive scientific assessment that considered the practices of other countries and regions, such as the European Union, Australia, New Zealand, South Korea, and Thailand, where certain insects are already consumed as food, allowing the import of certain insect species and products with low regulatory concerns. As a country well-known as a global financial hub and highly developed economy, this recent development in Singapore will enhance the trade of edible insects in this region.

In a 2010 study, it was found that there are at least 80 species of edible insects consumed in Borneo, with over 60 species recorded in Sabah. These insects include sago grubs, grasshoppers, honeybee broods, crickets, termites, cicadas, rice bugs, beetles, and weaver ants. In the context of species diversity, beetles are notably the most consumed insects by the community in Sabah. There have been records of 9 beetle species consumed as edible insects in this region. Notably, the larvae of sago grubs, known as Butod in Sabah and Si'et in Sarawak, are popular in these regions. There are various methods for preparing these edible insects, ranging from simple boiling to frying with local spices and herbs. In Sarawak, the Lelamas, an edible leaf caterpillar, is highly valued, particularly among the Melanau community. This exotic food is only available from January to August and can fetch a price ranging from RM300 to RM600 per kilogram, providing additional income to the local residents. Furthermore, in Kalimantan, over 25 species of edible insects have been documented.

Regrettably, there is a noticeable deficiency in entomophagy research conducted in Borneo, particularly regarding the local community's willingness to engage in entomophagy and the sustainable harvesting of edible insects from natural habitats. Most of the available information on entomophagy in Borneo is limited to newspaper reports. Additionally, edible insects are solely sourced from the wild environment, and there is currently no established edible insect farming industry in Borneo.

Insects in Tourism Industry

The concept of entomological ecotourism traces its origins to the expeditions of renowned naturalists such as Charles Darwin and Alfred Russel Wallace, whose travels to uncharted territories provided them with the opportunity to gather, investigate, and marvel at insects from diverse global regions. While the term "entomotourism" appears to have entered formal discourse relatively recently, this niche form of tourism, which centers around insects, has captivated travelers with a penchant for observing, studying, or engaging with insects in their native habitats. Evolving as a distinctive and valuable facet of eco-tourism, entomotourism represents a burgeoning field focused on the observation and study of insects, offering educational, conservation, and experiential benefits (Fiffy *et al*., 2023).

By raising awareness regarding the significance of insects and their ecosystems, entomotourism, through sustainable practices, holds promise for the preservation of insect biodiversity while nurturing a deeper connection with the natural world. Its allure lies in the opportunity it affords to explore the often-overlooked realm of insects, fostering both appreciation and understanding of these vital yet frequently underestimated organisms. In essence, entomotourism emerges as an expanding domain offering significant educational, conservation, and experiential advantages; thereby, it assumes a pivotal role in promoting awareness about the importance of insects and their habitats, supporting conservation endeavors, and delivering distinctive and enriching travel experiences. Through the advocacy of sustainable practices and responsible tourism, entomotourism stands poised to contribute to the safeguarding of insect biodiversity while fortifying the profound connection between individuals and the natural world.

In Western countries such as Europe and North America, and now in Asia, butterfly and insect pavilions in museums and zoos have become international tourist attractions. In the past, people visited museums to see preserved insect collections. Insect collecting, especially butterflies, is a leisure activity in most countries, with collectors primarily being naturalists from the West (Veltman, 2013). Today, live butterfly exhibits have become attractions in North America, Europe, and Asia, leading to the importation of live specimens from Central and South America, Africa, and Asia (Veltman, 2013). Apart from live butterflies, various types of live insects are also exhibited, such as beetles, stick insects, and leaf insects.

Recently, there has been a growing interest in observing insects in their natural habitats, attracting international tourists to engage in entomotourism activities. Notable examples include butterfly watching in various locations such as Turkey, the United States, and Costa Rica (Çelik and Topsakal, 2017; Quinn and Klym, 2009; Henderson, 2002), monarch butterfly (Danaus plexippus) watching tourism in Mexico (Monterrubio *et al*., 2013; Solis-Sosa *et al*., 2019), firefly watching in Thailand, Malaysia, and the United States (Nurancha et al., 2013; Syazlina *et al*., 2016; Lewis *et al*., 2021), glow-worm tourism in Australia and New Zealand (Hall, 2013), and insect festivals in Europe (Hvenegaard *et al*., 2013). Entomotourism is often associated with entomophagy, where tourists visit countries known for their edible insects, such as Thailand, to indulge in insect delicacies.

The Malaysian rainforest is home to a diverse range of insects, offering significant potential for entomotourism. Travelers are attracted by the opportunity to observe and study the behavior and morphology of these insects. For example, the enchanting spectacle of fireflies in Kuala Selangor serves as a major attraction for visitors, thereby contributing to the economic growth of local tourism operators (Nadirah and Wan Norhidayah, 2020; Nadirah *et al*., 2021; Li and Nitanan, 2022). This firefly viewing tourism activity is not only able to provide additional income to the locals but also indirectly helps conserve the mangrove swamp area that is the habitat of the fireflies (Mahadimenakbar *et al*., 2009). In Sabah, there are also numerous tourist areas that offer firefly-watching activities, such as Weston, Sungai Garama, Sungai Klias, Kota Belud, and Lower Kinabatangan (Mahadimenakbar *et al*., 2003; Mahadimenakbar *et al*., 2007; Mahadimenakbar and Fiffy, 2016). Additionally, butterflies have captured substantial attention from tourists in various regions of Malaysia, including Kuala Lumpur, Penang, Malacca, Cameron Highland, and Sabah. Over recent years, entomotourism has emerged as a family-friendly recreational tourism option in Malaysia, often categorized under nature or ecotourism, thus playing a crucial role in raising public awareness for insect conservation (Fiffy *et al*., 2023). There are several notable butterfly farms that merit exploration. These include the Kuala Lumpur Butterfly

Park in the capital city of Kuala Lumpur, Entopia by Penang Butterfly Farm situated in Penang, the Melaka Butterfly and Reptile Sanctuary in Malacca, as well as the Poring Butterfly Garden and Kipandi Butterfly Park in Sabah. These establishments not only house various species of butterflies but also feature an array of unique insects and small creatures, including reptiles, for public viewing and study.

CONCLUSION

The practice of entomophagy is gaining increasing attention in developed countries like Europe and North America due to rising awareness of environmental sustainability. Various studies assessing the acceptance of edible insects among residents have shown a positive response, especially when the insects are processed before consumption. Additionally, authorities in developed countries have issued specific guidelines that must be followed before a species of insect can be commercially utilized, underscoring the importance of ensuring consumer safety. In contrast, there is a significant lack of research on entomophagy in Malaysia, including studies that evaluate consumer willingness to adopt entomophagy practices. Furthermore, the commercial conservation and production of edible insects in Malaysia remain limited.

Entomotourism, however, has considerable potential for promoting conservation efforts in local communities and attracting international visitors. By embracing entomotourism, developing countries have acknowledged the positive outcomes resulting from increased tourist engagement. This trend is particularly advantageous for environmental conservation efforts.

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