MALAYSIAN JOURNAL OF SCIENCE

MJS

ISSN 1394-3065

Malaysian Journal of Science

Vol 45 | No 1 | March 2026

MJS in indexed in Scopus, Google Scholar, Chemical Abstracts Service Database, ASEAN Citation Index (ACI), & MyCite.

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 1-8 (March 2026) https://mjs.um.edu.my

Soil Preference and Burrow Characteristics of Two Theraphosidae Species in Penang Island, Malaysia

Irham Razak[1a], Mushahril Shukor[2b], Dzulhelmi Muhammad Nasir[3c], Ahmad Zafir Wahab[4d], Chai Min Hian[5e] and Amirrudin Ahmad[6a*]

Abstract: Tarantulas play a crucial role in maintaining ecological balance by regulating insect populations. However, little is known about the soil preferences and burrow structures of tarantulas in Malaysia. This study aims to determine the soil preference as well as the burrow structure of *Coremiocnemis cunciularia* and *Chilobrachys andersoni* from Penang Island. The soil characteristics of the soil samples collected around the burrows of *Coremiocnemis cunciularia* (n = 30) and *Chilobrachys andersoni* (n = 30) were determined using soil texture analysis. The measurements and burrow structures from adults and juveniles of *Coremiocnemis cunciularia* and *Chilobrachys andersoni* were determined. It was revealed that the moisture content and clay percentage in the soil samples around burrows of *Chilobrachys andersoni* and *Coremiocnemis cunicularia* were significantly different. Meanwhile, there is some variation in the structure and measurements of the burrows of the two tarantula species. The findings in this study could be useful for the conservation works and habitat management of tarantulas on Penang Island.

Keywords: *Arachnid, burrow structure, tarantula, soil characteristics.*

1. Introduction

Tarantulas, which belong to the Theraphosidae family, are known for their imposing size and distinctive appearance. Despite their formidable reputation, tarantulas play a crucial role in maintaining ecological balance by regulating insect populations. Furthermore, Theraphosidae can act as the sentinel or indicator species to determine the health and stability of the ecosystem (Wilson et al., 2012). The presence of these eight-legged marvels is scattered across diverse habitats worldwide. From dense rainforests to arid deserts, tarantulas have adapted to a wide range of environments, demonstrating remarkable resilience and versatility (Bertani, 2013).

Briefly, tarantulas are terrestrial spiders that burrow underground as a temporary or permanent home, providing shelter from adverse weather conditions, protection from predators, a place to ambush prey, and a breeding ground (Hils & Hembree, 2015). Additionally, fossorial tarantulas, such as Cyriopagopus lividus, often require an environment with a deeper layer of substrate when constructing a burrow (Marnell, 2016). Spiders dig their burrows by using both their fangs and pedipalps, which can efficiently create a hole inside the soil (Hils & Hembree, 2015). Furthermore, spiders will create webbing on the wall burrow, depending on the species (Pérez-Miles et al., 2005; Hils & Hembree, 2015). However, the structure of the burrow varies, depending on the species, size, and behaviours of the tarantula (Pérez-Miles et al., 2005; Machkour-M'rabet et al., 2007; Hils & Hembree, 2015).

Malaysia, blessed with diverse ecosystems ranging from dense rainforests to urban pockets of greenery, provides a habitat for several species of tarantulas. To date, three species of Theraphosidae have been previously found living on Penang Island, including Omothymus schioedtei, Coremiocnemis cunicularia, and *Chilobrachys* sp. (Thorell, 1891; Karsch, 1892; Simon, 1892). The *Omothymus schioedtei* is an arboreal living tarantula, while the other two species live terrestrially (Thorell, 1891). Interestingly, *Coremiocnemis cunicularia* is fossorial in nature, preferring its habitat on sloped ground with shaded areas at elevations above 600 m (West & Nunn, 2010). Unfortunately, there have been no in-depth studies on the soil preferences and burrow structures of Malaysian Theraphosidae. Therefore, the present study aims to determine the soil preferences and burrow structures of *Coremiocnemis cunicularia* and *Chilobrachys andersoni* found in Penang Island, Malaysia.

Authors information:
[a]Biology and Ecology Research (BERes), Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Kuala Nerus, MALAYSIA. E-mail: irhamrazak93@gmail.com[1], amirrudin@umt.edu.my[6]
[b]Pencinta, Kuala Pegang, MALAYSIA. E-mail: mushahril1985@gmail.com[2]
[c]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, MALAYSIA. E-mail: dzul_3my@yahoo.com[3]
[d]The Habitat Foundation, George Town, MALAYSIA. E-mail: ahmadzafir@habitatfoundation.org.my[4]
[e]Faculty of Veterinary Medicine, Universiti Sultan Zainal Abidin Kampus Besut, 22200 Besut, Terengganu, MALAYSIA. E-mail: amthonychai@gmail.com[5]
*Correspondence: ahmadzafir@habitatfoundation.org.my

Received: April, 2024
Accepted: June, 2024
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

2. Materials and Methods

Study Sites

This study was done at several selected locations in the Bukit Bendera Biosphere Reserve on Penang Island, Malaysia. The study areas include Bukit Bendera, Penang Hill, Penang National Park, Balik Pulau, Air Itam Dam, and Elvira Hill, as shown in Figure 1.

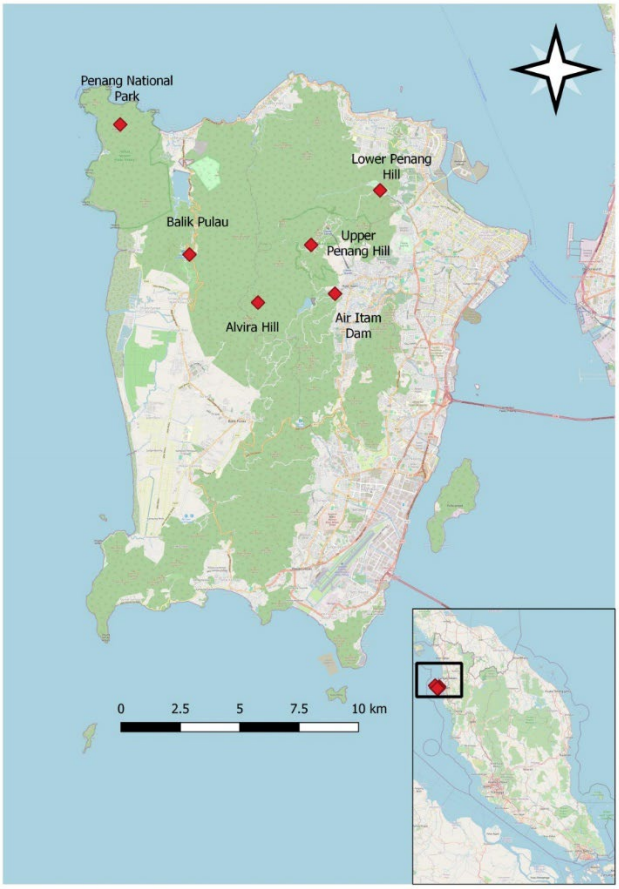


Figure 1. The study sites are on Penang Island.

Identification of Tarantula Species

In this study, various sampling sites inside Penang Island were selected based on the availability of the tarantula burrows. The sampling periods began in May 2022 and continued until September 2022. As the tarantulas are nocturnal animals that stay near their burrows during nighttime, all fieldwork was conducted at night to facilitate the identification of tarantulas (Fukushima et al., 2019). The species can be differentiated by their physical morphology. The *Coremiocnemis cunciularia* has finer hairs and shorter legs, but with stouter and black femurs, a cinnamon-colored carapace, and long hair on its hind legs. (West & Nunn, 2010). The Chilobrachys andersoni generally have long legs, a carapace that is slightly shorter than the protarsus number IV, and coarser hair, with a light brown body coloration (Pocock, 1900). Voucher specimens from the two species were deposited at Universiti Malaysia Terengganu (UMT). The burrow of the tarantula was detected by the presence of silk webbing, covering the burrow entrance and the burrow wall (Machkour-M'rabet et al., 2007).

Soil Characteristic Analysis

In this study, sixty soil samples were collected from the burrows of Chilobrachys andersoni (n = 30) and Coremiocnemis cunciularia (n = 30) to investigate the moisture content, pH value, type of soil, as well as the proportion of sand and clay (%) within the soil samples. The sampling was conducted at night and on dry days to minimize the impact of weather on the soil's moisture content. During sampling, 500 g of soil samples were weighed and collected around 50 cm from the burrow entry of *Chilobrachys andersoni* and *Coremiocnemis cunciularia*. The soil characteristics around the tarantula burrows were determined using a soil texture analysis (jar method), following the United States Department of Agriculture soil triangle, and applying the soil moisture content standard test method of the Australian Department of Sustainable Natural Resources (Canning et al., 2014). Before conducting the soil moisture content analysis, the pH values of the soil samples were determined using a pH meter. For soil moisture content analysis, each soil sample was weighed before and after drying in an oven at 100 ˚C. The differences in weight between the soil before and after drying were recorded as a percentage (%). After that, the soil samples were sieved to remove extraneous soil components, including organic matter, leaves, and stone particles. The sieved soil samples were then placed into a cylindrical jar. Then, the soil samples were mixed with water and a tablespoon of detergent. Then, the mixture of soil and water was thoroughly shaken and left to stand for 24 h. After 24 h, the soil samples were divided into different layers. The length of each layer was measured and compared to the total length of the soil samples to determine the percentages of clay, silt, and sand within the soil samples.

Burrow Characteristics Analysis

In the present study, the burrow characteristics of Chilobrachys andersoni and Coremiocnemis cunciularia were investigated to determine burrow diameter (BD), total burrow length (TBL), and total burrow depth (TBD). As the abundance of two tarantula species is relatively low in the sampling areas, only four burrows from *Chilobrachys andersoni* (n = 4) and *Coremiocnemis cunciularia* (n = 4) were selected to prevent disturbance to the tarantula population. Prior to conducting the experiments, the tarantula was carefully removed from its burrow by luring it out. The tarantula species was identified and released back into the wild. The burrow characteristic was determined by using Plaster of Paris (POP) to know the shape of the burrow (Chakraborty, 2017). First, the aqueous solution of Paris was poured into the burrows with the help of an air pump and syringe until the burrows were filled. It was then allowed to dry for 30 to 60 min. The casts were then carefully dug up with a spade and cleaned to remove any unwanted sediment from their surface. Each cast was then marked. The burrow casts were then brought to the laboratory for further analysis. The BD, TBL, and TBD of the burrows were then measured using the parameters, as shown in Figure 2 (Qureshi & Saher, 2012).

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

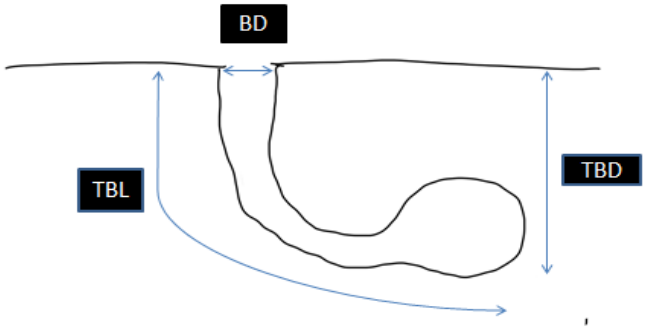


Figure 2. The measurement of the characteristics of the burrow. BD = Burrow diameter; TBL = Total length; TBD = Total burrow depth.

2.5 Data Analysis

The collected data on soil and burrow characteristics were analyzed using descriptive statistical methods. The t-test was used to compare the soil characteristics of the two tarantula species. PCA was applied to continuous data, including soil moisture content, pH value, as well as the sand, silt, and clay content, to determine the most significant parameters contributing to the highest variation in the burrows of *Coremiocnemis cunicularia* and *Chilobrachys andersoni*. The PCA was carried out based on the variance-covariance matrix. All statistical analyses were performed using PAST software version 4.03 (Kędzior & Kosewska, 2022; Mugot, 2021; Viana et al., 2021).

3. Results

Distribution of Tarantula Species

In this study, *Coremiocnemis cunicularia* was found at Bukit Bendara, Penang Hill (above 400 m above sea level), and Elvira Hill. Meanwhile, *Chilobrachi andersoni* was detected at Penang Hill (below 400 m above sea level), Penang National Park, Balik Pulau, and Air Itam Dam, as shown in Table 1.

Table 1. The presence of tarantula species at sampling sites.

No	Localities	GPS	*Coremiocnemis cunicularia*	*Chilobrachi andersoni*
1	Bukit Bendera	5°25'35.8"N, 100°15'08.0"E	Yes	No
2	Penang Hill > 400m a.s.l	5°25'22.0"N, 100°15'54.6"E	Yes	No
3	Penang Hill < 400m a.s.l	5°24'30.7"N, 100°16'37.3"E	No	Yes
4	Penang National Park	5°27'21.4"N, 100°11'50.0"E	No	Yes
5	Balik Pulau	5°25'36.1"N, 100°13'10.6"E	No	Yes
6	Air Itam Dam	5°23'57.2"N, 100°16'28.9"E	No	Yes
7	Elvira Hill	5°23'27.2"N, 100°14'26.6"E	Yes	No

* N = North; E = East; a.s.l = Above sea level.

Soil Characteristics

The average moisture from soil samples close to burrows of *Chilobrachys andersoni* was 11.4%, whereas that of *Coremiocnemis cunicularia* was 22.2%. The average pH value of the soil samples near the burrows of Chilobrachys andersoni and Coremiocnemis cunicularia was 6.31 and 6.25, respectively. Meanwhile, the average percentages of sand, silt, and clay in the soil from Chilobrachys andersoni burrows were 75%, 12%, and 10%, respectively. As for the soil samples from the *Coremiocnemis cunicularia* burrow, the average percentages of sand, silt, and clay were 80%, 14%, and 6%, respectively. The types of soil around the burrows of *Chilobrachys andersoni* were mostly sandy loam (n = 23), followed by loamy sand (n = 6) and sand (n = 1). However, the soils around the burrow of *Coremiocnemis cunicularia* were mostly loamy sand (n = 24), followed by sandy loam (n = 6), as shown in Table 2.

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

Table 2. Loadings showing the association between soil characteristics and PC1 and PC2.

No.	Soil characteristics	PC 1	PC 2
1.	Moisture content (%)	0.138	**0.876**
2.	pH value	0.001	-0.004
3.	Sand content (%)	**0.958**	-0.064
4.	Silt content (%)	-0.236	0.363
5.	Clay content (%)	-0.085	-0.312

Boxplot analysis of the moisture content of the soil samples demonstrated that the boxplots of both species appear asymmetric and normally distributed, as shown in Figure 3(A). Nonetheless, both species have different ranges of moisture content in their soil. The value from the boxplot of *Chilobrachys andersoni* was between 10% and 15%, whereas Coremiocnemis cunicularia ranged between 20% and 25%. The pH values for both species were not significantly different from each other. The soil pH values of the soil around *Coremiocnemis cunicularia* burrows ranged from 6.1 to 6.4. Meanwhile, the pH values of the soil around the burrow of Chilobrachys andersoni ranged from 6.1 to 6.4, as shown in Figure 3(B). The soils around the burrow of *Chilobrachys andersoni* have a maximum pH value higher than that of *Coremiocnemis cunicularia*. In addition, the boxplot also revealed that the percentage of the sand range for *Chilobrachys andersoni* was lower compared to *Coremiocnemis cunicularia*, as shown in Figure 3(C). Moreover, the range of sand percentage of soil samples from the burrows of *Coremiocnemis cunicularia* was higher (9% to 17%) compared to soil samples from *Chilobrachys andersoni* burrow soil (7% to 14%), as shown in Figure 3 (D). Lastly, the percentage of silt was different between the two species. Based on Figure 3 (E), the percentage ranges of silt in soil samples surrounding the burrows of *Chilobrachys andersoni* (8% to 12%) were higher as compared to the soils around burrows of *Coremiocnemis cunicularia* (4%-6%).

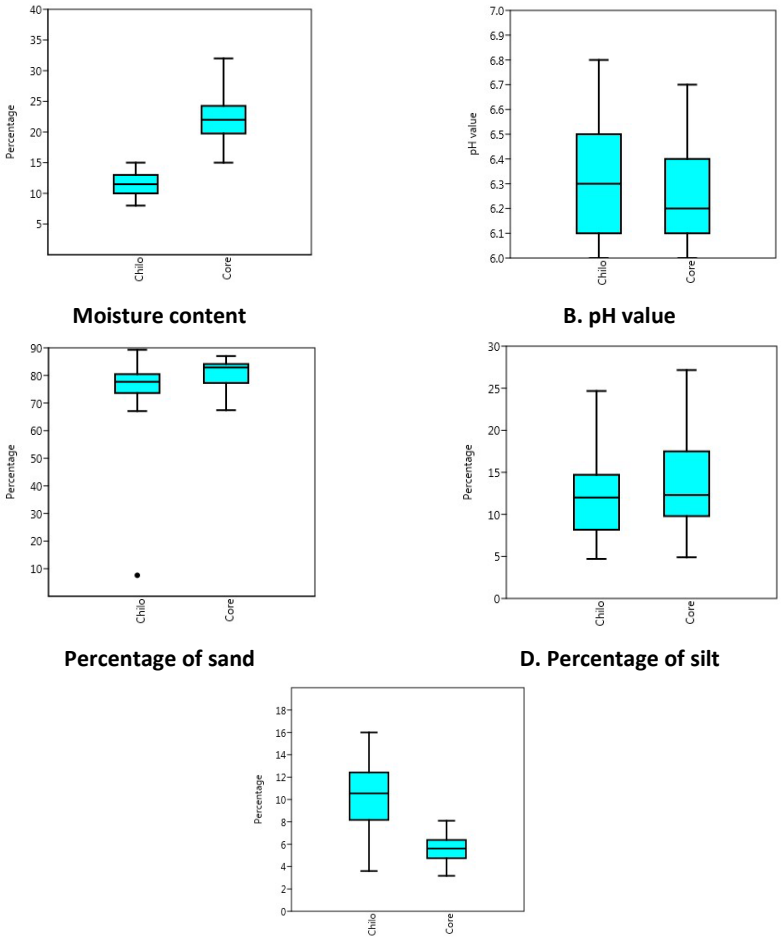


Figure 3. The boxplot for the (A) moisture content of the soil, (B) pH value of the soil, (C) Percentage of sand in the soil, (D) Percentage of silt in the soil, (E) Percentage of clay in the soil. Chilo represents *Chilobrachys andersoni*, while Core stands for *Coremiocnemis cunicularia.*

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

The T-test analysis demonstrated that the moisture content of the soil (*p*-value = 0.0001) and clay percentage in the soil samples (*p*-value = 0.0001) around burrows of *Chilobrachys andersoni* and *Coremiocnemis cunicularia* were significantly different. Nonetheless, the pH value (*p*-value = 0.26), sand percentage (*p*-value = 0.06), and silt percentage (*p*-value = 0.19) between the two species were not significantly different.

PCA Analysis of Soil Samples

Regarding the PCA test of the continuous microhabitat parameters, principal component 1 (PC1) and principal component 2 (PC2) explain 64.8% and 23.6% of the variance, respectively. It was found that PC1 is strongly associated with the percentage of sand within the soil samples. Hence, the sand content within the soil samples was the most significant parameter contributing to the variation observed in the soil samples of both species. Meanwhile, PC2 showed the strongest association with the moisture content of the soil samples, as shown in Table 2. A scatter plot based on PC1 and PC2 was generated, as shown in Figure 4. It was revealed that the percentages of sand in the soil samples of the species *Coremiocnemis cunicularia* and *Chilobrachys andersoni* showed variation. Furthermore, the moisture content of soil samples from the burrows of *Chilobrachys andersoni* appeared to be lower compared to *Coremiocnemis cunicularia*. Nonetheless, *Coremiocnemis cunicularia* and *Chilobrachys andersoni* can be clustered into main groups, illustrating the differences between these two species based on the soil characteristics around their burrows.

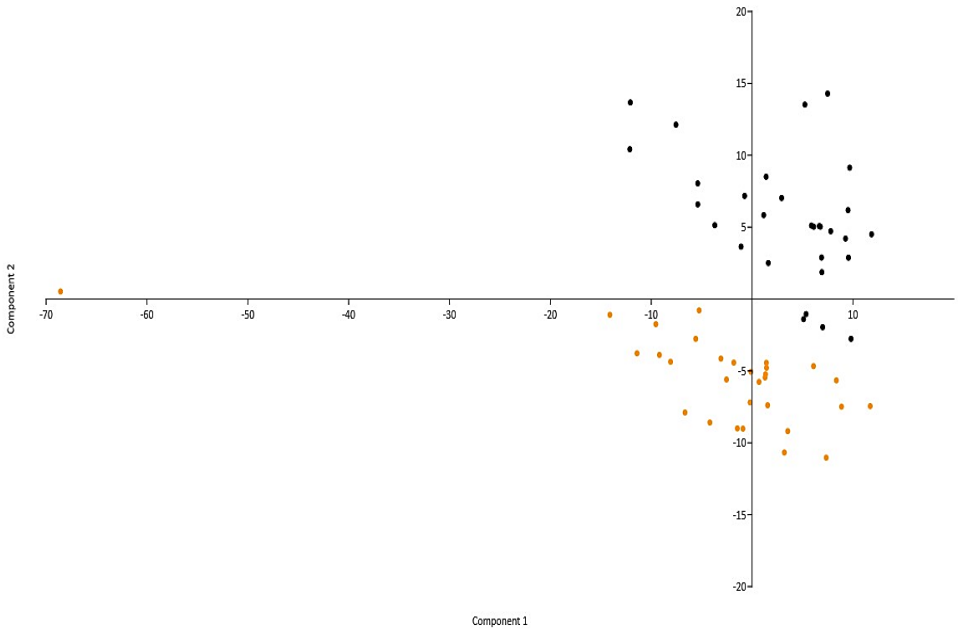


Figure 4. PCA scatter plot (PC1 vs PC2) for the soil characteristics of *Coremiocnemis cunicularia* (black colour dots) and *Chilobrachys andersoni* (orange colour dots).

Burrow Characteristics

In this study, a total of four samples of burrows (three burrows from juvenile and one burrow from adult tarantula) from each species were examined. The burrow of the adult *Coremiocnemis cunicularia* was 'U' shaped with a basal chamber near the end of the burrow as shown in Figures 5 (A) and 5 (B). The BD, TBL, and TBD of the burrow from adult *Coremiocnemis cunicularia* were 4.0, 51.2, and 39.6 cm, as shown in Table 3. The shaft of the burrow is quite long, with a uniform diameter up to the basal chamber. Meanwhile, the burrow of the adult *Chilobrachys andersoni* was 'C' shaped, as shown in Figure 5 (C). The BD, TBL, and TBD of the burrow of adult *Chilobrachys andersoni* were 4.7, 36.5, and 31.0 cm, respectively, as shown in Table 3. The burrow of adult *Coremiocnemis cunicularia* has a well-built, smooth surface. In contrast, the burrow of *Chilobrachys andersoni* is rough and has a small stone effect on its shaft and basal chamber.

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

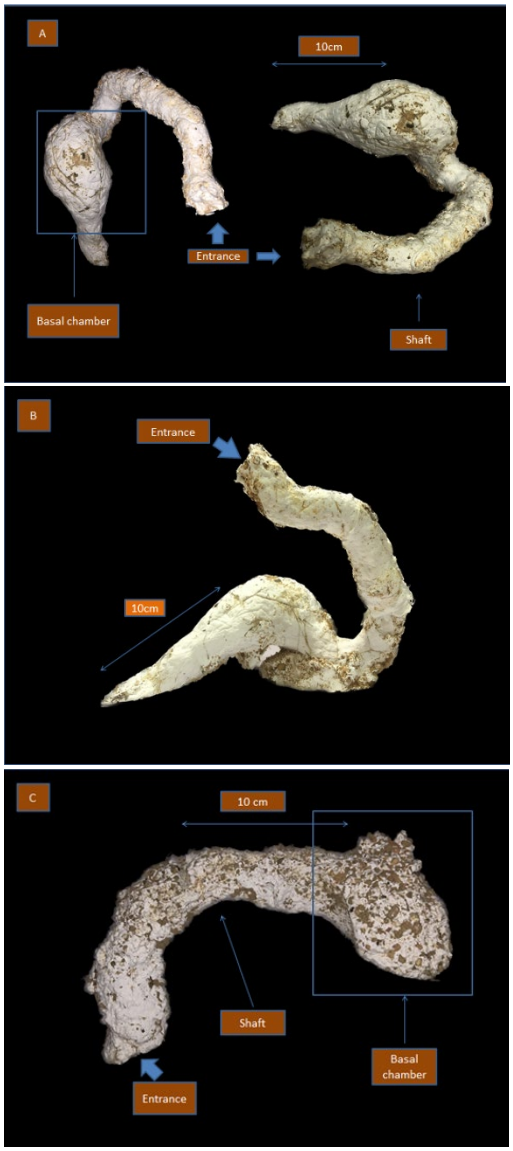


Figure 5. Plaster cast of burrows using POP. (A) and (B) are the burrow of adult *Coremiocnemis cunicularia,* whereas (C) is the burrow of adult *Chilobrachys andersoni*.

Table 3. The burrow diameter (BD), total burrow length (TBL), and total burrow depth (TBD) of the burrows for both *Coremiocnemis cunicularia* (n = 4) and *Chilobrachys andersoni* (n = 4).

	Species	Burrow measurement (cm)		
		BD	TBL	TBD
Adult	Core 1	4.0	51.2	39.6
	Chilo 1	4.7	36.5	31.0
Juvenile	Core 1	1.5	27.5	24.0
	Core 2	1.3	27.1	21.2
	Core 3	1.2	28.7	26.1
	Chilo 1	1.9	27.5	19.5
	Chilo 2	2.0	24.3	20.6
	Chilo 3	2.1	27.3	23.6

* Core = *Coremiocnemis cunicularia*; Chilo = *Chilobrachys andersoni.*

Regarding the structure of burrows from juveniles, the shape of the burrows of *Coremiocnemis cunicularia* was more defined and sophisticated. Moreover, the shaft of the burrows of *Coremiocnemis cunicularia* is better shaped compared to juveniles of *Chilobrachys andersoni,* as shown in Figures 6 and 7. Furthermore, the burrow basal chamber of juveniles *of Coremiocnemis cunicularia is smoother, and its* shape is more visible. Meanwhile, the burrows of juveniles of *Chilobrachys andersoni* have coarse walls, which are compensated for by crevices in the soil. Both species were observed to construct additional tunnels within their burrows, as shown in Figures 6(c) and 7(c). The BD, TBL, and TBD of burrows from juveniles of *Coremiocnemis cunicularia* ranged from 1.2 to 1.5 cm, 27.1 to 28.7 cm, and 24.0 to 26.1 cm, respectively (Table 2). The burrow diameter of juveniles of *Coremiocnemis cunicularia* is smaller than that of *Chilobrachys andersoni,* as shown in Table 2. However, the ranges for TBD and TBL between the juvenile species were not significantly different.

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

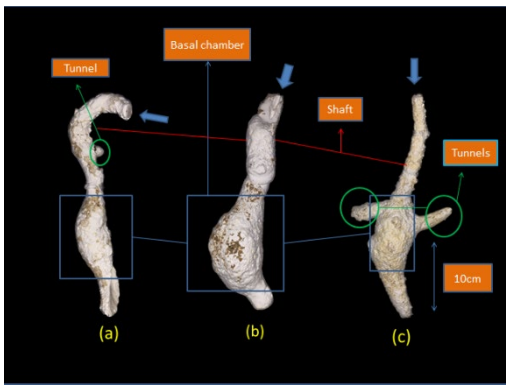


Figure 6. Plaster cast of three burrows of juvenile *Coremiocnemis cunicularia*.

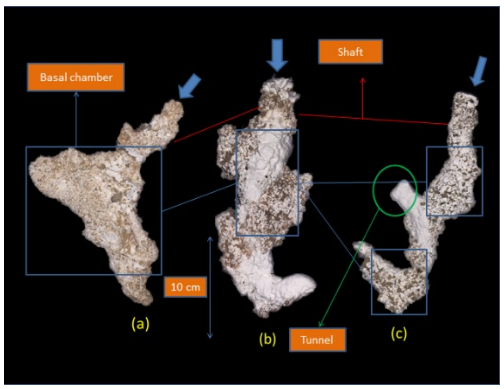


Figure 7. Plaster of cast burrow for juvenile samples of *Chilobrachys andersoni*.

4. Discussion

Soil type is considered one of the most important criteria when it comes to terrestrial Theraphosidae habitat selection. Due to the fossorial lifestyle of tarantulas, it is important to study the soil type preferred by these arachnids on Penang Island to better understand the possible habitat of the tarantula species (West & Nunn, 2010). From the soil samples, it was revealed that both species have different preferences for soil types. *Coremiocnemis cunicularia* prefers loamy sand, whereas Chilobrachys andersoni prefers sandy loam. In the past, several studies have linked soil characteristics with the habitat preference of certain Theraphosidae (Canning et al., 2014; Machkour-M'rabet et al., 2007; Yáñez & Floater, 2000). For example, *Nesiergus insulanus* prefers to build burrows in sandy loam-type soil, while *Brachypelma klaasi* prefers sandy soil (Yáñez & Floater, 2000; Canning et al., 2014). Meanwhile, *Brachypelma vagans* commonly build their burrows on soil with high clay content while avoiding soil with high stone density (Machkour-M'rabet et al., 2007). Furthermore, it was revealed that *Ami bladesi* favor clay soil (Lapinski et al., 2018).

The structure of the burrow is one of the most crucial factors for the tarantula's survival. However, the burrow structure of the tarantula on Penang Island is poorly understood. As the number of tarantulas in the sampling areas is low, only four samples of burrows from each species were taken to minimize the disturbance to the local tarantula population. In this study, the burrow structures of both species of tarantula differed, mainly in terms of the inner surface of the burrows. These differences in burrow structure may be due to different behaviour or stage of life of the tarantula species. In this study, the burrows of juveniles of *Chilobrachys andersoni* have coarse walls, possibly due to the preference of this tarantula species to build its burrows around crevices, without significantly altering the internal structure of the burrow. Another study by Marshall & West (2008) also showed that the burrow characteristics of tarantulas differ according to their stage of life. In addition, the height, depth, and structure of burrows can also vary between species within the same genus. For instance, a study by Machkour-M'rabet et al. (2007) demonstrated that *Tiltocatl vagans* can have four different types of burrow structures, where the adult female constructs a more complex burrow with more chambers. Another recent study showed that the burrows of *Nesiergus insulanus* can be J-shaped, V-shaped, and U-shaped (Canning et al., 2014).

Tarantulas, which belong to the Theraphosidae family, are known for their imposing size and distinctive appearance. Despite their formidable reputation, tarantulas play a crucial role in maintaining ecological balance by regulating insect populations. Furthermore, Theraphosidae can act as the sentinel or indicator species to determine the health and stability of the ecosystem (Wilson et al., 2012). The presence of these eight-legged marvels is scattered across diverse habitats worldwide. From dense rainforests to arid deserts, tarantulas have adapted to a wide range of environments, demonstrating remarkable resilience and versatility (Bertani, 2013).

5. Conclusion

In summary, this study reveals that both species have distinct soil requirements and habitat preferences. Thus, it is essential to conserve the natural habitat of spiders, particularly in hilly areas, to protect and maintain their populations in the wild. This information can serve as a reference for Bukit Bendera and The Habitat Foundation regarding the conservation efforts for tarantulas on Penang Island, Malaysia.

6. Acknowledgements

The authors would like to thank the Habitat Foundation Research Grant for funding this study. Besides, thank you to the Forestry Department of Peninsular Malaysia for approving this study and providing the research permit, JH/100 Jld. 34(70). We would also like to thank the Department of Wildlife and National Parks, Peninsular Malaysia (DWNP) (JPHLTN.600-6/1/4 JLD(53)) for the research permit.

7. References

Bertani R. (2013). A new species of Melloina (Araneae: Paratropididae) from Venezuela, *Zoologia (Curitiba)* 30: 101-106.

Canning G., Reilly B., Dippenaar-Schoeman A. (2014). Burrow structure and microhabitat characteristics of *Nesiergus*

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

insulanus (Araneae: Theraphosidae) from Frégate Island, Seychelles, *Journal of Arachnology* 42: 293-298.

Chakraborty S. K. (2017). Ecological services of intertidal benthic fauna and the sustenance of coastal wetlands along the Midnapore (East) Coast, West Bengal, India. In C. Finkl & C. Makowski (Eds), *Coastal Wetlands: Alteration and Remediation* (pp 777-866). Springer, Cham.

HILS J. M., Hembree D. (2015). Neoichnology of the burrowing spiders *Gorgyrella inermis* (Mygalomorphae: Idiopidae) and *Hogna lenta* (Araneomorphae: Lycosidae), *Palaeontologia Electronica* 18: 1-62.

Lapinski W., Gabriel R., Pérez-Miles F. (2018). Description of the female of *Ami bladesi* Pérez-Miles, Gabriel & Gallon, 2008 (Araneae: Theraphosidae), with notes on its distribution, ecology, and behaviour, *Arachnology* 17: 419-426.

Machkour-M'rabet S., Hénaut Y., Sepúlveda A., Rojo R., Calmé S., Geissen V. (2007). Soil preference and burrow structure of an endangered tarantula, *Brachypelma vagans* (Mygalomorphae: Theraphosidae), *Journal of Natural History* 41: 1025-1033.

Marnell C. (2016). Tarantula and hermit crab emergency care. *Veterinary Clinics of North America. Exotic Animal Practice* 19: 627–646.

Mugot D. A., Piloton Q. A., Ansigbat V. V., Ascaño II C. P. (2021). Inventory and habitat preference of pteridophytes in and around gold-mined areas in Gango, Libona, Bukidnon, Philippines, *Journal of Ecosystem Science and Eco-Governance* 3: 47-53.

Pérez-Miles F., Costa F. G., Toscano-Gadea C., Mignone A. (2005). Ecology and behaviour of the 'road tarantulas' *Eupalaestrus weijenberghi* and *Acanthoscurria suina* (Araneae, Theraphosidae) from Uruguay, *Journal of Natural History* 39: 483–498.

Pocock R. I. (1900). *Fauna of British India, including Ceylon and Burma. Arachnida*. Taylor and Francis.

Kędzior R., Kosewska A. (2022). Landscape heterogeneity determines the diversity and life history traits of ground beetles (Coleoptera: Carabidae), *Sustainability* 14: 13980.

Salman A., Jaafar M., Mohamad D. (2021). Ecotourism development in Penang Hill: A multi-stakeholder perspective towards achieving environmental sustainability, *Environmental Science and Pollution Research* 28: 42945–42958.

Simon E. (1903). *Histoire naturelle des araignées*. Paris.

Thorell T. (1891). Spindlar från Nikobarerna och andra delar af södra Asien, *Kongliga Svenska Vetenskaps-Akademiens Handlingar* 24: 1-149.

Viana T. A., Martins F. M., Lourenço A. P. (2021). The orchid bee fauna (Hymenoptera: Apidae: Euglossini) of a Neotropical Savanna: An efficient protocol to assess bee community and diversity along elevational and habitat complexity gradients, *Neotropical Entomology* 50: 748–758.

West R. C., Nunn S. C., Hogg S. (2012). A new tarantula genus, *Psednocnemis*, from west Malaysia (Araneae: Theraphosidae), with cladistic analysis and biogeography of Selenocosmiinae Simon 1889, *Zootaxa* 3299: 1-43.

Wilson J. S., Gunnell C. F., Wahl D. B., Pitts J. P. (2012). Testing the species limits of the tarantulas (Araneae: Theraphosidae) endemic to California's Southern Coast Ranges, USA, *Insect Conservation and Diversity* 6: 365-371.

Yáñez M., Floater G. (2000). Spatial distribution and habitat preference of the endangered tarantula, *Brachypelma klaasi* (Araneae: Theraphosidae) in Mexico, *Biodiversity & Conservation* 9: 795–810.

Qureshi N., Saher N. (2012). Burrow morphology of three species of fiddler crab (Uca) along the coast of Pakistan, *Belgian Journal of Zoology* 142: 114-126.

DOI:https//doi.org/10.22452/mjs.vol45no1.1
Malaysian Journal of Science 45(1): 1-8 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 9-16 (March 2026) https://mjs.um.edu.my

Utilizing Black Turtle Beans (*Phaseolus vulgaris*) for Enhanced Natto Production: A Promising Alternative to Soybeans

Huong Huynh-Lien Ly[1ab], Thanh Van Nguyen[2a]*

Abstract: This study aims to replace soybeans, which pose allergy risks, with black turtle beans as a fermentation substrate for natto production. *Bacillus subtilis* MS05, known for its production of the fibrinolytic enzyme nattokinase, was employed to determine the optimal fermentation conditions. This was achieved through a multifaceted approach, incorporating one-factor-at-a-time experiments, a Plackett-Burman experimental design, as well as a response surface methodology with central composite design. The study reported the highest fibrinolytic activity at 418.32 FU/mL, which is equivalent to the nattokinase enzyme activity found in 5g of natto. This is sufficient to meet the daily nattokinase requirement of an adult consuming 25g of natto. Key factors that significantly influenced the fermentation process were identified as a peptone concentration of 2.8%, a bacterial density of 10^4 CFU/g of *Bacillus subtilis*, an initial pH of 5.5, and a fermentation duration of 33 hours. Additionally, natto produced from black turtle beans exhibited remarkable antioxidant activity, as indicated by a DPPH activity of 81.21 µg/mL. These findings highlight the significant potential of black turtle bean-based natto in enhancing fibrinolytic activity and effectively combating free radicals. These attributes contribute to the treatment of atherosclerotic blood clots and hold promise for improving overall human health by nutritional interventions.

Keywords: *Bacillus subtilis, black turtle beans, fibrinolytic activity, nattokinase, soy allergies.*

1. Introduction

Natto, a traditional Japanese fermented product produced from soybeans and *Bacillus subtilis*, is renowned for its potent fibrinolytic activity, largely attributed to the presence of nattokinase. This enzyme is recognized for its important role in the prevention and treatment of cardiovascular diseases, which are a leading cause of death worldwide owing to blood clot formation (Roth et al., 2018; Wang et al., 2023). Despite these health benefits, soybeans are well-established allergens, posing challenges for individuals with soy allergy (Inomata et al., 2007).

To address this limitation, recent studies have investigated alternative legumes for natto production. Evidence suggests that some legumes, when fermented with *Bacillus subtilis*, can produce nattokinase with fibrinolytic activity comparable to that of conventional soy-based natto (Sumi et al., 2022). In Vietnam, black turtle beans (*Phaseolus vulgaris*) have emerged as a promising candidate to substitute for soybeans in natto production. These beans not only exhibit a protein profile similar to that of soybeans but also lack the major allergens Gly m4, Gly m5, and Gly m6, which are primarily responsible for allergic reactions to soy (Platteau et al., 2011; He et al., 2021). Therefore, black turtle beans represent a potential allergen-free substrate for natto fermentation while preserving the cardiovascular benefits of nattokinase.

This study aims to optimize the fermentation process of black turtle bean natto by identifying key fermentation parameters and applying Response Surface Methodology (RSM) to maximize nattokinase production. RSM has proven effective for optimizing medium composition for recombinant nattokinase production from *Bacillus subtilis*, resulting in increased enzyme yields (Tran et al., 2014). It has also been widely used to optimize fermentation conditions for nattokinase across different settings, highlighting its utility as an optimization technique (Nguyen et al., 2022). Additionally, given the role of oxidative stress in abnormal blood clot formation, this study will evaluate the antioxidant properties of crude nattokinase extracts from black turtle bean natto (Wang et al., 2020).

By optimizing the fermentation process and examining the biofunctional properties of black turtle bean natto, this research aims to provide a safe alternative for individuals with soy allergy while maintaining its cardiovascular health benefits.

2. Materials and Methods

Screening of Suitable *Bacillus sp.* For Black Turtle Bean Natto Fermentation Through Evaluation of Extracellular Fibrinolytic Enzyme Production Capability

Numerous prior studies have consistently demonstrated that *Bacillus subtilis*, during the fermentation process of natto, produces the nattokinase enzyme, which effectively dissolves blood clots (Hmood et al., 2016; Yanagisawa et al., 2010; Hmood & Aziz, 2016). Consequently, the primary objective of this study is to evaluate the fibrinolytic activity of four indigenous strains of *Bacillus* sp., namely CO18, MO39, TO46, and MS05. These strains, derived from the strain collection at the Institute of Food and Biotechnology, Can Tho University, were carefully selected and subjected to in vitro assessments to determine their fibrinolytic potential. The ultimate aim is to identify the most suitable *Bacillus*

Authors information:
[a]Institute of Food and Biotechnology, Can Tho University, Can Tho City 900000, VIETNAM. E-mail: lhlhuong@ctump.edu.vn[1], E-mail: nvthanh@ctu.edu.vn[2]
[b]Can Tho University of Medicine and Pharmacy, Can Tho City 900000, VIETNAM. E-mail: lhlhuong@ctump.edu.vn[1]
*Correspondence: lhlhuong@ctump.edu.vn

Received: July, 2024
Accepted: March, 2025
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

strain for natto fermentation on a black turtle bean substrate, a prevalent bean variety in the Mekong Delta region of Vietnam.

The fermentation time for producing natto using *Bacillus* sp. was examined within a range of 6 to 30 hours to determine the optimal fermentation duration for further research.

In the natto fermentation process, black turtle beans were soaked in water at a ratio of 1:5 (g/mL) for 12 hours. The soaked beans were then steamed and sterilized at 121°C and 1 atm pressure for 15 minutes using an autoclave (Hirayama HVE–50, Japan) (Hui et al., 2004). Natto fermentation was carried out for 30 hours at 30°C and pH 8.0. Additionally, 0.25% (w/v) glucose and 0.3% (w/v) fungal extract were added to the fermentation mixture (Suwanmanon & Hsieh, 2014).

The fibrinolytic activity of the nattokinase enzyme (FU/mL) was defined as the quantity of enzyme necessary to elicit a 0.01 increase in absorbance at 275 nm within 1 minute, under specific reaction conditions. The evaluation of fibrinolytic activity on the thrombin-fibrinogen model was performed using the method described as "Degradation of artificial thrombus by nattokinase" (Suwanmanon & Hsieh, 2014).

Investigating The Media Components

This investigation sought to determine the most effective carbon and nitrogen sources for maximizing the fibrinolytic activity of natto during fermentation by *Bacillus* strains. To assess the effect of medium composition on fibrinolytic activity, several common sugar sources were investigated. Fermentation experiments were conducted using glucose, maltose, sucrose, and lactose were carried out varying from 2 to 6% of concentration to investigate the influence of carbon sources on natto fermentation (Suwanmanon & Hsieh, 2014). The effect of different nitrogen sources on enzyme activity was also examined. Peptone, yeast extract, and whey protein were utilized as nitrogen sources at concentrations ranging from 1.5% to 3.5% (Suwanmanon & Hsieh, 2014).

Identification of Fibrinolytic Enzyme in Black Turtle Bean Natto

The bacterial strain with the highest fibrinolytic activity was identified using a specific primer pair designed to amplify a segment of the *Bacillus subtilis* 16S rRNA gene. The primers Bsub5F (5'-AAGTCGAGCGGACAGATGG-3') and Bsub3R (5'-CCAGTTTCCAATGACCCTCCCC-3') (Bharose et al., 2017) were used for PCR amplification. The resulting amplicons were purified using the Isolate II PCR and Gel Kit (Bioline) prior to Sanger sequencing at Macrogen (South Korea). The sequences were processed and analyzed using BioEdit software, and BLAST searches against the NCBI gene database were conducted to confirm species identity.

The specific gene segment was amplified using the polymerase chain reaction (PCR) protocol described by Sambrook & Russell (2001). The amplified *Bacillus subtilis*-specific gene segment, approximately 600 bp in size, was analyzed by agarose gel electrophoresis (Bharose et al., 2017).

Extracellular enzymes exhibiting the highest fibrinolytic activity, obtained after fermentation of black turtle bean natto by *Bacillus* sp. strains, were purified and characterized using two protein precipitation steps: step 1, 60% ammonium sulfate precipitation, followed by step 2, 80% ethanol precipitation (Yanagisawa et al., 2010). The protein fraction obtained after precipitation was separated, and the nattokinase enzyme was identified using ion exchange chromatography based on the enzyme's isoelectric point (pI) of 8.6 (Hmood & Aziz, 2016). Finally, the purified enzyme was analyzed by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Yanagisawa et al., 2010).

Evaluation of The Antioxidant Capacity of Nattokinase

The free radical scavenging activity was evaluated using the DPPH* method (Barriada-Bernal et al., 2014). Nattokinase was diluted to various concentrations ranging from 10 to 60 µg/mL in separate Eppendorf tubes, maintaining a DPPH (2,2-diphenyl-1-picrylhydrazyl) concentration of 40 mg/L and a final volume of 1 mL in each tube. The reaction mixture comprised 630 µL of DPPH solution (4.0 mg/mL in methanol), 100 µL of samples at different concentrations of the fermented solution, and 270 µL of MeOH. The mixture was incubated in the dark at 25°C for 30 minutes, and the absorbance was measured at 517 nm using a spectrophotometer. A control sample comprising 630 µL of DPPH solution (4.0 mg/mL) and 370 µL of methanol was also measured under the same conditions. Double-distilled water was utilized as the negative control, and ascorbic acid was employed as the positive control. The IC_{50} value of the sample, defined as the concentration at which 50% of DPPH free radicals are scavenged, was calculated from a plot of sample concentration versus percentage free radical scavenging activity.

Improvement of Fibrinolytic Activity of Enzyme in Black Turtle Bean Natto

Screening of Significant Components

A screening Blackett-Burman matrix design was conducted to investigate the influence of key parameters that affect nattokinase enzyme activity. The experimental design consisted of 13 runs with five levels for each parameter. Six factors were investigated, including concentration of carbon source, concentration of nitrogen source, Bacillus sp. initial density, fermentation temperature, substrate pH, and fermentation time (Deepak et al., 2008). The design was created using Stagraphics Centurion software, version XV, with nattokinase enzyme activity as the monitored response. Nattokinase production was recorded for each experiment. Analysis of variance (ANOVA) was performed to determine the significance of each factor, and significant factors were further optimized using response surface methodology (RSM).

Optimization of Conditions for Natto Fermentation Using RSM

In evaluating the clot-dissolving potential of black turtle bean natto, the combined effects of fermentation factors were found to influence nattokinase enzyme activity. To examine this multivariable relationship, response surface methodology (RSM) was employed as an experimental statistical modeling technique. A central composite design (CCD) with seven factors and five levels was used to determine the optimal process parameters for

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

maximizing nattokinase production. The experimental design was generated using Statgraphics Centurion software, version XV. The relationship between the independent variables and the response variable was described using a second-order polynomial model. The coefficients of this polynomial equation were estimated using statistical methods, and response surface plots were generated to illustrate the relationships among the variables and the response (Deepak et al., 2008). This approach enabled the researchers to identify optimal conditions for maximizing the clot-dissolving capacity of nattokinase in the black turtle bean natto fermentation process.

Statistical Analysis

The results are presented as mean values with associated standard deviations, calculated from at least three independent measurements. Statistical analysis was conducted using the Stagraphics Centurion software version XV and Microsoft Excel 2016. Analysis of variance (ANOVA) and the least significant difference test (LSD) were utilized to assess the statistical significance of any differences observed, with a significance threshold set at $p<0.05$.

3. Results and Discussion

Suitable *Bacillus sp.* For Black Turtle Bean Natto Fermentation

The fibrinolytic effect was assessed by examining the ability of the crude extracellular enzyme derived from natto fermentation to dissolve artificial blood clots composed of thrombin and fibrinogen. The results of these evaluations are presented in Figure 1. The relationship between independent factors associated with natto fermentation time and different strains of *Bacillus*, in terms of fibrinolytic activity, was analyzed using a two-way ANOVA. The findings depicted in Figure 1 indicate a strong association between the bacterial strains used in fermentation and the enzymatic activity generated during the process. The bacterial strains utilized in natto fermentation exhibit varying capacities to dissolve fibrin, with the ascending order of activity being CO18, MO39, MS05, and TO46. Among these, both TO46 and MS05 exhibited similar and significant effectiveness in dissolving blood clots, with a confidence level of $p<0.05$.

The strong association, with a p-value below 0.05, highlights the critical role of time and specific *Bacillus* species in the fermentation process. The statistical findings provide evidence that natto fermented for 30 hours using the *Bacillus* sp. TO46 strain exhibited the highest fibrinolytic activity at 42.36 FU/mL. Following closely, natto fermented for 24 hours with the *Bacillus* sp. MS05 strain showed an enzyme activity of 42.23 FU/mL. These two bacterial strains have been selected for further investigation into suitable nutritional supplementation for natto fermentation.

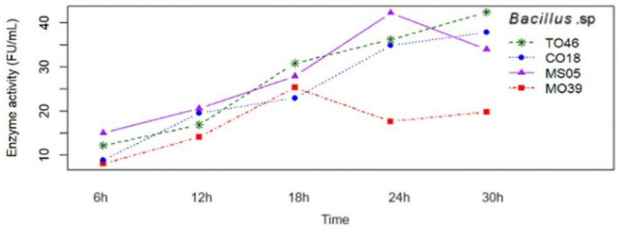


Figure 1. Coefficients of *Bacillus* sp. strains and fibrinolytic activity.

Figure 1 also suggests that the optimal fermentation time for black turtle bean natto is 24 hours. The difference in fibrinolytic activity between 24 hours and 30 hours among most bacterial strains is not statistically significant at the 95% confidence level. However, the fibrinolytic activity of natto fermented with *Bacillus* sp. MS05 tends to decrease when fermentation time exceeds 24 hours.

Effect of Carbon and Nitrogen Sources on Nattokinase Enzyme Activity

The study examined various sugar sources, including glucose, lactose, maltose, and sucrose, as well as different nitrogen sources, consisting of peptone, yeast extract, and whey protein. Figure 2a illustrates that, among the tested carbon sources, glucose supplementation at a concentration of 5% yielded the highest nattokinase activity, reaching 278.38±1.11 FU/mL during fermentation with *Bacillus* sp. MS05. Lactose at 4% and maltose at 2% also showed substantial enzyme activity when fermented with *Bacillus* sp. MS05. In contrast, sucrose at 2% yielded the lowest nattokinase activity relative to the other carbon sources. Additionally, sucrose at 3% and 4%, together with maltose at 5%, showed comparatively lower enzyme activity. These findings suggest that sucrose is not a suitable carbon source for the growth of *Bacillus* subtilis during black turtle bean natto fermentation, thereby reducing nattokinase production. This observation aligns with previous studies conducted on soybean natto (Suwanmanon & Hsieh, 2014; Deepak et al., 2008).

Among the nitrogen sources examined in Figure 2b, peptone at a concentration of 2.5% exerted the strongest effect on nattokinase activity, reaching 277.15±1.2 FU/mL. In addition, peptone at 2% produced significantly higher enzyme activity than other organic nitrogen sources, such as yeast extract and whey protein, which yielded enzyme activity 10-20% lower than the maximum observed activity. These findings are consistent with previous studies on nitrogen sources for natto fermentation (Suwanmanon & Hsieh, 2014; Deepak et al., 2008).

It is worth noting that the glucose concentration used in this study was higher than in previous reports, whereas the peptone concentration was higher or lower depending on the study. These variations may be attributed to differences in source materials, as black turtle beans contain less glycine compared to soybeans.

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

Therefore, a higher supplementary nutritional requirement may be necessary for the synthesis of nattokinase with high activity in black turtle bean natto fermentation (Pradhananga, 2019; Amin et al., 2020). The results shown in Figure 2 also indicate that *Bacillus* sp. strain MS05 exhibits higher fibrinolytic enzyme activity than that produced by *Bacillus* sp. strain TO46, and this difference is statistically significant. Therefore, *Bacillus* sp. MS05 was selected for use in the fermentation process of black turtle bean natto.

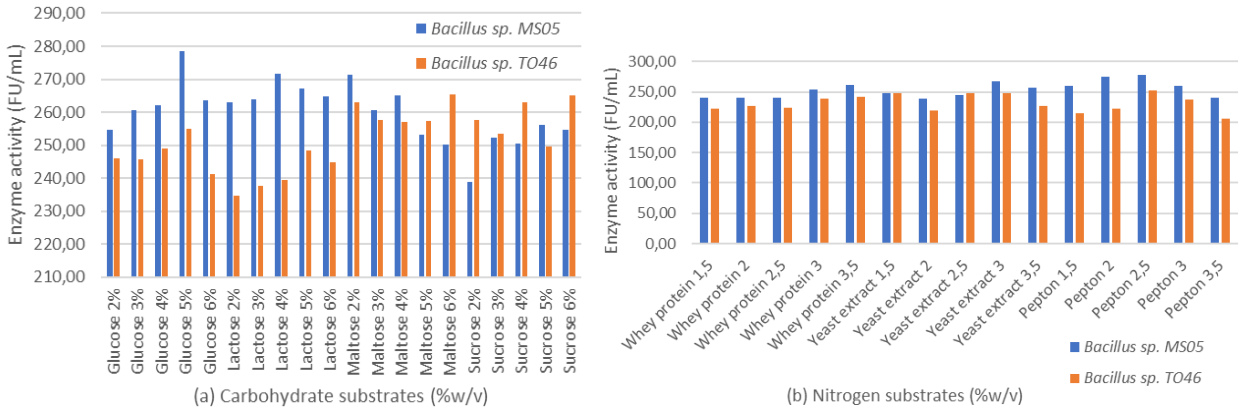


Figure 2. The influence of different nutrient sources on enzyme activity.
(a) Carbohydrate sources; (b) Nitrogen sources

Identification of Nattokinase from Black Turtle Bean Natto

The sequencing of the 16S rRNA gene after PCR using the specific primer pair Bsub5F and Bsub3R revealed a 602 bp gene segment specific to *Bacillus subtilis* in the *Bacillus* sp. MS05 bacterial strain (Figure 3). The 16S rRNA gene sequence of this strain was compared with 16S rRNA sequences of bacteria in the NCBI database using the BLAST tool. The sequencing results for the isolated strain showed 100% coverage and 99.5% similarity to the 16S rRNA sequence of *Bacillus subtilis* var *subtilis*, with accession number MN888748.1 in the NCBI database (Table 1).

Numerous studies have shown that the nattokinase enzyme, which dissolves blood clots, is produced by *Bacillus subtilis* during natto fermentation (Hmood et al., 2016; Yanagisawa et al., 2010; Hmood & Aziz, 2016). Enzymes extracted from black turtle bean natto fermented by *Bacillus* sp. MS05 were subsequently purified to determine the presence of nattokinase. SDS-PAGE analysis demonstrated that the precipitation steps effectively removed specific extracellular proteins. The ion exchange chromatography results for the purified enzyme (PE) revealed a protein band of approximately 27 kDa (Figure 4). Previous studies have indicated that nattokinase is a single polypeptide chain consisting of 275 amino acids, with a molecular weight of 27,724 Da (Yanagisawa et al., 2010). Hence, the clot-dissolving enzyme in this study, with a molecular weight of 27 kDa, was identified as nattokinase.

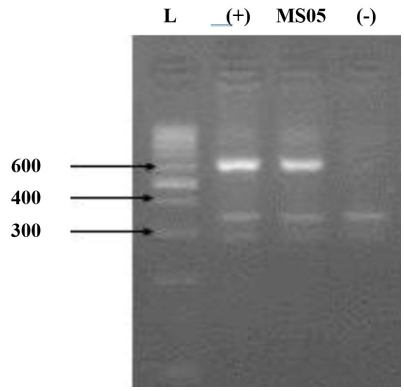


Figure 3. Electrophoresis to assess *B. subtilis*.
(L) Ladder 100bp, (+) positive control, (MS05) *Bacillus* sp. MS05, (-) Negative control

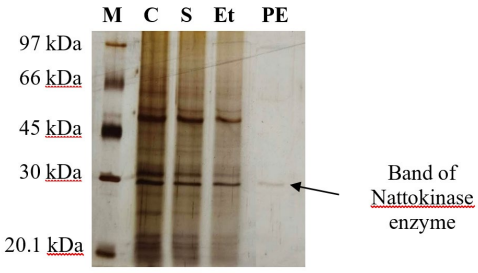


Figure 4. SDS-PAGE electrophoresis to assess nattokinase.
(M) Prestained protein LMW marker, (C) Crude enzyme, (S) Salt precipitated enzyme, (Et) Ethanol precipitated enzyme, (PE) Purified enzyme.

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

Sequencing the *Bacillus sp.* Using in Black Turtle Bean Natto Fermentation

The MS05 bacterial strain was sequenced using the Bsub5F and Bsub3R primers, which were designed to target a conserved region of the bacterial 16S rRNA gene, with the aim of amplifying a 600 bp segment. This region is highly conserved and is suitable for the identification of *Bacillus subtilis*. The obtained sequence was compared with bacterial 16S rRNA sequences in the NCBI database using the BLAST tool. The sequencing results for the isolated strain showed 100% coverage and 99.5% similarity to the 16S rRNA sequence of *Bacillus subtilis* var *subtilis*, accession number MN888748.1, in the NCBI database (Table 1).

Table 1. The similarity between the isolated strain MS05 and the sequence in NCBI database

Name of bacteria	Nomenclature	Size of 16S rRNA sequence	Coverage	Similarity	Accession number
Bacillus subtilis strain NTB-107 16S ribosomal RNA gene, partial sequence	*Bacillus subtilis* var *subtilis*	1512 bp	100%	99,5%	MN888748.1

Antioxidant Property of Fibrinolytic Enzyme

The antioxidant properties of the crude enzyme found in natto were evaluated using the DPPH free radical scavenging activity assay. The absorption peak observed at 517 nm signifies the interaction between the DPPH free radical and an unpaired electron. Determination of the IC_{50} value is a widely adopted method for assessing the free radical scavenging capacity of bioactive substances. In this study, the IC_{50} value, defined as the concentration of nattokinase required to convert 50% of the DPPH free radicals into their reduced form (DPPHH), was used to assess the extent of free radical scavenging activity. A lower IC_{50} value indicates a higher antioxidant activity.

The IC_{50} values are presented in Table 2. The standard curve equation, derived from regression analysis with an R^2 value of 0.9794, confirms the suitability of the linear regression model. A coefficient above 0.75 indicates a satisfactory fit; in this case, the model explains 97.94% of the variability in the response.

Table 2. The similarity between the isolated strain MS05 and the sequence in NCBI database

Sample	IC_{50} (µg/mL)
Negative control	-
Fibrinolytic enzyme	81.21
Positive control (acid ascorbic)	19.36
The regression equation: y = 0.0016x + 0.0391	
R^2= 0.9794	

The IC_{50} value of nattokinase was determined to be 81.21 µg/mL, indicating a relatively weaker free radical scavenging capacity compared with the reference standard, ascorbic acid (19.36 µg/mL). However, it is important to note that the IC_{50} value of the crude enzyme from black turtle bean natto in this study is lower than the IC_{50} value reported for crude nattokinase, which was 380 µg/mL (Amin et al., 2020). This finding suggests a higher potential free radical scavenging activity of the fibrinolytic enzyme in black turtle bean natto than that of the previously examined nattokinase enzyme. Overall, these findings indicate substantial antioxidant activity of the fibrinolytic enzyme in black turtle bean natto.

Nattokinase Activity Improvement by Optimization of Conditions for Natto Fermentation

Screening the Factors That Affect the Fermentation Process

By employing a one-factor-at-a-time approach based on existing literature reports, the fermentation process was performed with *Bacillus subtilis* MS05 using 5% glucose, 2% peptone, and the specified culture conditions, including initial bacterial density, temperature, and fermentation time (Table 3). The optimal conditions for achieving the highest nattokinase activity included a bacterial density of 10^4 CFU/g, a fermentation temperature of 30°C, an initial pH of 6.0, and a fermentation time of 36 hours. The corresponding activities at a confidence level of $p<0.05$ for each surveyed factor were 226.4±1.37 FU/mL, 223.8±6.5 FU/mL, 254.6±4 FU/mL, and 248.4±16.5 FU/mL, respectively.

It is important to note that the activity observed under these optimal conditions may vary compared to previous experiments; this highlights the role of fermentation factors in either promoting or hindering nattokinase production. During the fermentation of *Bacillus subtilis* in natto production, both positive and negative influencing factors have optimal thresholds. Exceeding these thresholds inhibits bacterial growth and subsequently reduces nattokinase activity (Yang et al., 2021). Therefore, a systematic examination of nattokinase activity variations under different fermentation conditions is crucial for improving overall activity.

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

Table 3. Nattokinase enzyme activity under single-factor surveys.

Run	Starter Density (CFU/ g)	Enzyme Activity (FU/mL)	Tempera-ture (°C)	Enzyme Activity (FU/mL)	pH	Enzyme Activity (FU/mL)	Time (Hrs)	Enzyme Activity (FU/mL)
1	10^3	219.4±0.97$^{a\ b}$	25	202.8±7.1^{b-f}	5.0	254.6±4^{a}	12	210.1±1.1^{b}
2	10^4	226.4±1.37^{a}	30	223.8±6.5^{a}	6.0	248.6±2.5$^{a\ b}$	24	235.8±12$^{a\ b}$
3	10^5	193.3±1.57^{b-f}	35	203.9±3.9^{b-f}	7.0	221.4±11.3^{b-f}	36	248.4±16.5^{a}
4	10^6	180.5±1.5^{c}	40	191.4±2.7^{f}	8.0	236.7±6.5$^{a\ b-f}$	48	236.3±8.9$^{a\ b}$
5	10^7	214.5±1.69$^{a\ b}$	45	196±5.4^{c}	9.0	222.6±17.5^{b-f}	60	228.3±5.8$^{a\ b}$
	CV%= 4.63		CV%= 8.04		CV%= 6.87		CV%= 8.65	

Note: Results of a 95% Least Significant Difference (LSD) test on the fibrinolytic activity. Letters in homogenous groups column denote significantly different groups.

To gain a comprehensive understanding of the combined effects and interactions among these factors, a screening process was conducted using an enzyme activity based approach. The Blackett-Burman matrix was used to evaluate the independent effects and interactions of the selected factors, which encompassed a range of values, including glucose concentration, peptone concentration, bacterial density, fermentation temperature, pH, and fermentation time. Factors exceeding the standardized effect line were deemed to have a significant impact on nattokinase activity, whereas those below the line were considered insignificant ($p>0.05$). The results illustrated in Figure 5 indicated that the initial pH of the substrate exerted the strongest influence on nattokinase activity. The subsequent factors, ranked in terms of their impact, were fermentation time, peptone concentration, initial bacterial density, glucose concentration, and fermentation temperature. Notably, an increase in fermentation time resulted in a decrease in nattokinase activity, whereas the other factors had positive effects, indicating that increases in these variables would enhance enzyme activity. The study also showed that glucose concentration and fermentation temperature had negligible effects on enzyme activity in black turtle bean natto, suggesting differences in fermentation conditions compared with soybean natto. This finding aligns with previous studies that also reported an insignificant influence of fermentation temperature and initial glucose concentration on enzyme activity (Suwanmanon & Hsieh, 2014; Wang et al., 2009).

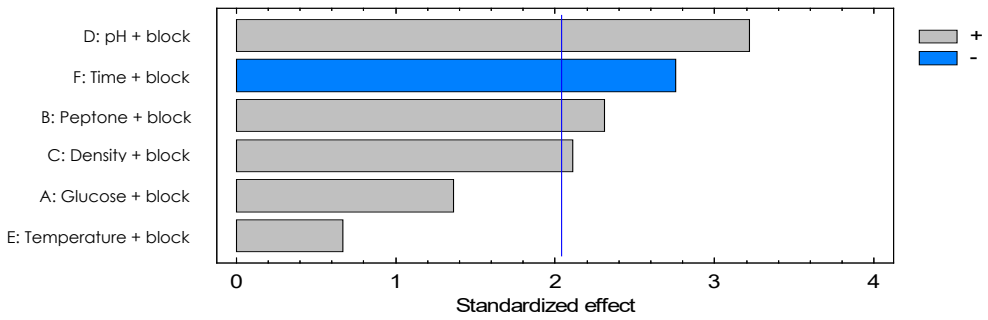


Figure 5. Influence of factors on nattokinase enzyme activity

Optimization of Conditions for Black Turtle Bean Natto Fermentation Using RSM

Based on the above screening results, only four factors, including X_1, initial pH of the substrate (5.0 to 9.0); X_2, fermentation time (12 to 60 hours); X_3, peptone concentration (1.5 to 3.5%); and X_4, initial bacterial density (10^3 to 10^7 CFU/mL), were identified as significant contributors to nattokinase activity. Therefore, these factors were selected for optimization. The experimental ranges for these factors were established, and a 26-experiment RSM-CCD model was designed using Statgraphics Centurion optimization software.

The influence of each factor on nattokinase enzyme activity was analyzed, with pH found to have the strongest effect, followed by peptone concentration, initial bacterial density, and fermentation time. The overall second-degree polynomial equation for nattokinase enzyme activity, Y (FU/mL) = $-1178.07 + 275.355*X_1 + 19.7741*X_2 + 220.015*X_3 + 104.913*X_4 - 26.7765*X_1^2 - 1.32823*X_1*X_2 + 10.7808*X_1*X_3 + 9.76781*X_1*X_4 - 0.122106*X_2^2 - 0.514241*X_2*X_3 - 0.784647*X_2*X_4 - 41.0529*X_3^2 - 6.33031*X_3*X_4 - 15.1853*X_4^2$, with an R-squared value of 0.6575, indicates that 65.75% of the variation in nattokinase enzyme activity can be explained by the fermentation conditions. Regression analysis showed that the two most influential factors, pH and peptone concentration, had positive coefficients, whereas the constant term was -1178.07, indicating a negative intercept. This finding suggests the presence of additional unexamined factors that could affect the production of nattokinase and its blood clot-dissolving capability. According to previous studies, many other factors can influence the natto fermentation process beyond those investigated (Suwanmanon & Hsieh, 2014; Deepak et al.,

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

2008). Therefore, further research is required to examine factors affecting nattokinase enzyme activity during the fermentation of black turtle bean natto to enhance its blood clot-dissolving activity.

The model predicted that the optimal fermentation conditions would include a peptone concentration of 2.8%, bacterial density of 10^4 CFU/g, an initial pH of 5.5, and a fermentation time of 33 hours. These conditions were expected to yield a nattokinase enzyme activity (Ymax) of 436.212 FU/mL. Experimental verification under these optimized conditions resulted in an observed enzyme activity (Y'max) of 418.32 FU/mL. The accuracy of the optimized model was confirmed by the Y'max/Ymax coefficient of 0.9760, indicating high agreement between predicted and observed values (approximately 97.6%). These results support the applicability of the RSM-CCD experimental matrix model for optimization studies.

Compared with previous studies on soybean natto, the optimized black turtle bean natto exhibited higher nattokinase enzyme activity (Suwanmanon & Hsieh, 2014; Liu et al., 2005). This finding indicates the potential of black turtle bean natto to promote blood clot dissolution. However, further optimization of fermentation conditions remains necessary to increase enzyme activity.

4. Conclusion

This study demonstrated the feasibility of utilizing black turtle beans as an alternative fermentation substrate for natto production, thereby addressing concerns related to soybean allergies. Using a systematic experimental approach to optimize the fermentation conditions for *Bacillus subtilis* MS05, a strain known for producing the fibrinolytic enzyme nattokinase, we identified key factors that significantly influenced the fermentation process. A peptone concentration of 2.8%, a bacterial density of 10^4 CFU/g of *Bacillus subtilis*, an initial pH of 5.5, and a fermentation duration of 33 hours were found to be the optimal conditions for maximizing fibrinolytic activity with 418.32 FU/mL of nattokinase activity.

Additionally, notable antioxidant activity of black turtle beans natto was indicated by a DPPH activity of 81.21 µg/mL. This highlights the potential of black turtle bean-based natto to enhance fibrinolytic activity and effectively eliminate free radicals. These properties have implications for the treatment of atherosclerotic blood clots and offer opportunities to improve overall human health through appropriate nutritional interventions.

5. Acknowledgement

This study was facilitated and supported by Can Tho University and Can Tho University of Medicine and Pharmacy, Vietnam, in terms of infrastructure and research conditions. The authors sincerely appreciate their invaluable assistance.

7. References

Amin, K., Zeng, X., You, Y., Hu, Y., Sun, H., Lyu, B., ... & Yu, H. (2020). Enhanced thermostability and antioxidant activity of Nattokinase by biogenic enrichment of selenium. *Journal of Food Measurement and Characterization*, *14*(4), 2145-2154.

Barriada-Bernal, L. G., Almaraz-Abarca, N., Delgado-Alvarado, E. A., Gallardo-Velázquez, T., Ávila-Reyes, J. A., Torres-Morán, M. I., ... & Herrera-Arrieta, Y. (2014). Flavonoid composition and antioxidant capacity of the edible flowers of Agave durangensis (Agavaceae). *CyTA-Journal of Food*, *12*(2), 105-114.

Bharose, A. A., Gajera, H. P., Hirpara, D. G., Kachhadia, V. H., & Golakiya, B. A. (2017). Molecular identification and characterization of bacillus antagonist to inhibit aflatoxigenic Aspergillus flavus. *Int. J. Curr. Miocrobiol. Appl. Sci*, *6*(3), 2466-2484.

Deepak, V., Kalishwaralal, K., Ramkumarpandian, S., Babu, S. V., Senthilkumar, S. R., & Sangiliyandi, G. (2008). Optimization of media composition for Nattokinase production by Bacillus subtilis using response surface methodology. *Bioresource Technology*, *99*(17), 8170-8174.

He, S., Zhao, J., Zhang, Y., Zhu, Y., Li, X., Cao, X., ... & Sun, H. (2021). Effects of low-pH treatment on the allergenicity reduction of black turtle bean (Phaseolus vulgaris L.) lectin and its mechanism. *Journal of agricultural and food chemistry*, *69*(4), 1379-1390.

Hmood, S. A., & Aziz, G. M. (2016). Optimum conditions for fibrinolytic enzyme (Nattokinase) production by Bacillus sp. B24 using solid state fermentation. *Iraqi Journal of Science*, 1391-1401.

Hmood, S. A., & Aziz, G. M. (2016). Purification and characterization of nattokinase produced by local isolate of Bacillus sp. B24. *Iraqi journal of Biotechnology*, *15*(2).

Hui, Y. H., Meunier-Goddik, L., Josephsen, J., Nip, W. K., & Stanfield, P. S. (Eds.). (2004). *Handbook of food and beverage fermentation technology* (Vol. 134). CRC Press.

Inomata, N., Osuna, H., Kawano, K., Yamaguchi, J., Yanagimachi, M., Matsukura, S., & Ikezawa, Z. (2007). Late-onset anaphylaxis after ingestion of Bacillus Subtilis-fermented soybeans (Natto): clinical review of 7 patients. *Allergology international*, *56*(3), 257-261.

Liu, J., Xing, J., Chang, T., Ma, Z., & Liu, H. (2005). Optimization of nutritional conditions for nattokinase production by Bacillus natto NLSSE using statistical experimental methods. *Process Biochemistry*, *40*(8), 2757-2762.

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

Nguyen, V. T., Tran, L. H., & Le, Q. H. (2022). Optimization of Nattokinase Production from Bacillus subtilis Using Response Surface Methodology. *International Journal of Science, Engineering and Applied Science*, 8(8), 1-10.

Platteau, C., Cucu, T., De Meulenaer, B., Devreese, B., De Loose, M., & Taverniers, I. (2011). Effect of protein glycation in the presence or absence of wheat proteins on detection of soybean proteins by commercial ELISA. *Food Additives and Contaminants*, *28*(2), 127-135.

Pradhananga, M. (2019). Effect of processing and soybean cultivar on natto quality using response surface methodology. *Food Science & Nutrition*, *7*(1), 173-182.

Roth, G. A., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., ... & Borschmann, R. (2018). Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The lancet*, *392*(10159), 1736-1788.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual 3rd ed. Chapter 6. Isolation of DNA Fragments from Polyacrylamide Gels by the Crush and Soak Method.

Sumi, H., Naito, S., Yatagai, C., Mitsuo, T (2022). Strong Fibrinolysis with New Fermented Foods (Mung Bean Natto and Natto Miso). *The International Society on Thrombosis and Haemostasis 2022 Congress in London, England, U.K*. Abstract number: VPB0639.

Suwanmanon, K., & Hsieh, P. C. (2014). Isolating Bacillus subtilis and optimizing its fermentative medium for GABA and nattokinase production. *CyTA-Journal of Food*, *12*(3), 282-290.

Tran, Q. T., Nguyen, T. T. K., Le, T. T. A., Dinh, M. H., & Tran, C. D. (2014). Optimization of medium composition for recombinant nattokinase production from Bacillus subtilis by using response surface methodology. *Academia Journal of Biology*, 36(1se), 130-137.

Wang, C., Chen, J., Tian, W., Han, Y., Xu, X., Ren, T., ... & Chen, C. (2023). Natto: A medicinal and edible food with health function. *Chinese Herbal Medicines*.

Wang, J. K., Chiu, H. H., & Hsieh, C. S. (2009). Optimization of the medium components by statistical experimental methods to enhance nattokinase activity. *Fooyin Journal of Health Sciences*, *1*(1), 21-27.

Wang, Q., & Zennadi, R. (2020). Oxidative stress and thrombosis during aging: the roles of oxidative stress in RBCs in venous thrombosis. International journal of molecular sciences, 21(12), 4259.

Yanagisawa, Y., Chatake, T., Chiba-Kamoshida, K., Naito, S., Ohsugi, T., Sumi, H., ... & Morimoto, Y. (2010). Purification, crystallization and preliminary X-ray diffraction experiment of nattokinase from Bacillus subtilis natto. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, *66*(12), 1670-1673.

Yang, Y., Lan, G., Tian, X., He, L., Li, C., Zeng, X., & Wang, X. (2021). Effect of fermentation parameters on natto and its thrombolytic property. *Foods*, *10*(11), 2547.

DOI:https//doi.org/10.22452/mjs.vol45no1.2
Malaysian Journal of Science 45(1): 9-16 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 17-26 (March 2026) https://mjs.um.edu.my

Diversity of Phytoplankton in The Mangrove Area of Kerteh River: A Preliminary Study

Nurul Nadhirah Zulkafli[1a], Nurul Shahida Redzuan[2a]*, Monaliza Mohd-Din[3a] and Nadiah Wan Rasdi[4bc]

Abstract: Mangroves, which are among the world's most productive ecosystems, harbour thousands of species of phytoplankton. Phytoplankton are microorganisms that provide essential ecosystem services within mangrove ecosystems. This ecosystem is currently under threat for various reasons. A study was conducted in a mangrove ecosystem along the Kerteh River, Terengganu, which is experiencing metal pollution due to rapid industrialisation. This pollution has a strong potential to directly affect the species composition, occurrence, and abundance of phytoplankton. Therefore, this study aimed to determine the spatial diversity of phytoplankton species at three stations along the Kerteh River, selected for their exposure to anthropogenic activities. A single sampling event was conducted for two days in August 2019. The study recorded 56 phytoplankton species from four divisions: Bacillariophyta (45 species), Chlorophyta (4 species), Dinophyta (6 species), and Cyanophyta (1 species). Phytoplankton diversity, measured by the Shannon-Weiner index (H'), ranged from 2.60 to 3.0, and equitability (J'), measured by Pielou's evenness index, ranged from 0.75 to 0.80. A one-way analysis of variance (ANOVA) indicated a significant difference in phytoplankton diversity among stations ($p < 0.05$). *Chaetoceros curvisetus* had the highest relative abundance at Stations 1 and 3, while *Frustulia vulgaris* was most abundant at Station 2. Five Dinoflagellate species were recorded at the study site: *Ceratium* sp., *Dinophysis* sp., *Protoperidinium pallidum*, and *Protoperidinium* sp. 1 and sp. 2. This preliminary record of phytoplankton species in the Kerteh River sheds light on the impact of the shift caused by current and emerging anthropogenic activities in the area.

Keywords: *Phytoplankton, mangroves, diversity, Kerteh River.*

1. Introduction

Mangroves are a conducive habitat for phytoplankton, the underrated phylogenetically diverse producers. These microscopic producers are mostly diatoms of the division Bacillariophyta, green algae of the division Chlorophyta, and Cyanobacteria. Ecologically, phytoplankton can be sampled in the water column, mediating numerous ecosystem functions, such as contributing to overall ecosystem productivity (Janousek, 2005). The mangrove ecosystem is known to accommodate higher phytoplankton than the estuarine system (Rajkumar et al., 2009). In turn, phytoplankton aid mangroves in playing their vital roles in servicing the ecosystem.

Phytoplankton inhabiting the mangrove system can be considered extremophiles, as they withstand the ecosystem's unique conditions. These microscopic extremophiles are not only able to adapt in the low dissolved oxygen of the ecosystem but also able to tolerate exposure to tidal cycles, limited sunlight (Graham & Wilcox, 2000), and variable salinity levels (Owen et al., 2004; Stanković et al., 2024).

Frequently, studies on mangrove ecosystems focus on the overall groups of microalgae, including benthic (Jeslin et al., 2021), epiphytic (Chen et al., 2010), and suspended microalgae (also known as phytoplankton) (Saravanakumar et al., 2008; Hilaluddin et al., 2020). The mangrove ecosystem receives notable nutrients from terrestrial runoff, which may have led to greater attention to the relationship between phytoplankton occurrence in the system and physico-chemical parameters (Tanaka & Choo, 2000). The terrestrial runoff that intensifies domestic discharge or anthropogenic influence (Saravanakumar et al., 2008; Manna et al., 2010) consequently channels into the ecosystem. The additional nutrients will concentrate the already highly nutrient-enriched ecosystem originating from decomposing mangrove litter fall, which is consequently at high risk of causing eutrophication (Tanaka & Choo, 2000; Perumal et al., 2009; Xu et al., 2022).

Most research carried out on phytoplankton in mangrove areas has reported diatoms (Bacillariophyta) as the most abundant division in the ecosystem. Kamal et al., (2022) recorded four phytoplankton divisions in the tropical mangrove estuarine of the South China Sea, which comprised the Bacillariophyta, Cyanophyta (Cyanobacteria), Chlorophyta, and Chrysophyta. *Coscinodiscus*, the pennate diatom of Bacillariophyta, was with the highest total density at the research area. The species was the primary phytoplankton recorded in both wet and intermediate seasons. There was, however, a notable difference in species composition between the three seasons. Centric diatoms, *Pleurosigma normanii* recorded to be was the dominant species in wet season. On the other hand, the intermediate season showed a high abundance of Coscinodiscus lineatus, which is classified in the centric diatom group. However, phytoplankton of the division Bacillariophyta were commonly reported as the highest species richness across the three seasons, while cells of

Authors information:
[a]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, MALAYSIA. E-mail: nadiyzz19@gmail.com[1]; nurulshahida@umt.edu.my[2]; monalizamd@umt.edu.my[3]
[b]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, MALAYSIA. E-mail: nadiah.rasdi@umt.edu.my[4]
[c]Institute of Tropical Biodiversity and Sustainable development, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, MALAYSIA. E-mail: nadiah.rasdi@umt.edu.my[4]
*Correspondence: nurulshahida@umt.edu.my

Received: November, 2024
Accepted: January, 2025
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

Dinophysis caudata of the Dinoflagellate were recorded with the highest density in the dry season.

Mangrove sediments are responsible for holding nutrients and for significant nutrient cycling pathways, which promote coastal development along mangrove ecosystems (Saifullah et al., 2016). In addition, refractive materials such as humic acid, which abound in mangroves, promote the growth of phytoplankton (Xu et al., 2022). Tanaka & Choo (2000) conducted a study of mangrove phytoplankton in Matang Mangrove Estuary in Malaysia. The study, which primarily focused on the influence of nutrient availability and tidal variations, also documented monospecies blooms driven by increased nutrient availability during spring tides. Nutrients containing concentrated phosphate were shown to influence Dinoflagellates, such as *Ceratium kofoidii*, to dominate the Matang mangrove ecosystem. In a more recent study in the Matang Mangrove ecosystem, Hilaluddin et al. (2020) reported the alterations in phytoplankton species composition structures attributed to reduced mangrove vegetation, aquaculture, and human settlements, which directly shift mainly the chemical parameters of the mangrove ecosystem.

Studies on phytoplankton in the mangrove environment generally examine the effects of monsoon-related nutrient inputs. Some research discussed that phytoplankton abundance decreased during monsoon months because the mangrove's water column is highly turbid, largely stratified, with variedly lower salinity temperature, and pH (Rajkumar et al., 2009; Redzuan & Milow, 2021). A study that was done on phytoplankton at Pahang Estuary by Chowdhury et al. (2011) indicated that the phytoplankton community during the monsoon was quite diverse and dominant during the non-monsoon season.

The Kerteh Mangrove Forest is described as a mangrove estuary as it receives inflow of seawater from the South China Sea as well as fresh water from the Kerteh River. Nutrients in mangrove estuaries provide ideal conditions for phytoplankton productivity. These nutrients, together with environmental factors altered by pollution, urbanization, industrialization, anthropogenic activities, and climate change, largely affected the species composition, distribution, and also the abundance of phytoplankton (Ajibare et al., 2019). In 2014, Kerteh and Paka River were strongly impacted by major effluent from municipal and industrial outflows post-flood (Azaman et al., 2017), which potentially contributed to the active development of chemical manufacturing industries, such as the oil and gas industries. In addition, petroleum plants, agricultural areas, fishery areas, and residential areas near the Kerteh River have high potential to contribute to heavy metal pollution in the river (Yaakob et al., 2017). According to Azaman et al. (2017), the Kerteh River had significant concentrations of metals such as Cadmium, Copper, Zinc, Cobalt, Nickel, Arsenic, Chromium, and Lead. Thus, metal pollution significantly affected the structure of marine and freshwater ecosystems, particularly phytoplankton, thereby reducing community diversity and species richness (Utami et al., 2019).

With regards that mangrove ecosystem of Kerteh River is facing threat due to rapid industrial growth and development of adjacent terrestrial areas, and the fact that the ecosystem is also exposed to natural shift due to climate change, frequent storm and rise of sea level, this present study was set to create a checklist on the species composition of phytoplankton at selected areas of Kerteh River mangrove ecosystem. Although the collected and presented data are from a one-off sampling occasion, this preliminary work can hopefully serve as an initial step toward more comprehensive biomonitoring in the future. Related data on the distribution, with integration of abundance, species richness, and phytoplankton composition, with respect to any patterns, are crucial for determining the condition of the ecological structure and functioning of the Kerteh River mangrove. Findings of this study, even preliminary, can also aid future management of the Kerteh River and conservation efforts for the river's mangrove area and its associated organisms.

2. Methodology

Study Area

Kerteh River, which lies on the grid of '4°31'37.4" N 103°26'40.0"E', was the study area for this preliminary study, which lies in the district of Kemaman, Terengganu, Malaysia (Figure 1). Kerteh is part of the Kemaman watershed with highly varied land use. According to the United Nations Department of Economic and Social Affairs (2019) in World Population Review, the estimated population of Kerteh is 24,401, with a total area of 256 km². Kerteh is often referred to as a district in Malaysia that is a complex of oil and gas-related industries and landing facilities. Being one of the main outlets of the Kemaman watershed, the Kerteh River receives downstream flows from the Batu Putih and Rangon Rivers. There are more than 30 species of mangrove in the Kerteh River. Based on the EcoCare Environmental Education Centre website, the common species that can be observed there are *Rhizophora apiculata, R. mucronata, Sonneratia alba, Bruguiera parviflora, Avicennia alba,* and *Nypa fruticans.* Therefore, it is worth noting that the Kertih River is also important in mitigating the effects of coastal erosion initiated by the annual monsoon and the rise in sea level. In October 2005, the Kerteh River Mangrove Rehabilitation Project was launched by the Malaysian Nature Society (MNS) through the ecoCare Environmental Education Centre (ECC). This project aims to rehabilitate and replant the mangrove ecosystem along the Kerteh River. EcoCare is the first environmental education centre in the East of Peninsular Malaysia that serves as a resource centre with facilities to promote awareness and conservation among communities.

In relation to the aim of the project, three station points were selected near ecoCare, in the vicinity of the Kerteh River mangrove, based on their exposure to potential pollutants. The sites were ST1, which receives anthropogenic discharges from the adjacent village of Gelugor via a non-centralized drainage system. ST2, a station located at the site of replanted mangrove trees (Figure 1). Finally, ST3 is located adjacent to a boat parking area. All stations are also exposed to runoff or discharge from Kerteh, however, at an unknown rate.

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

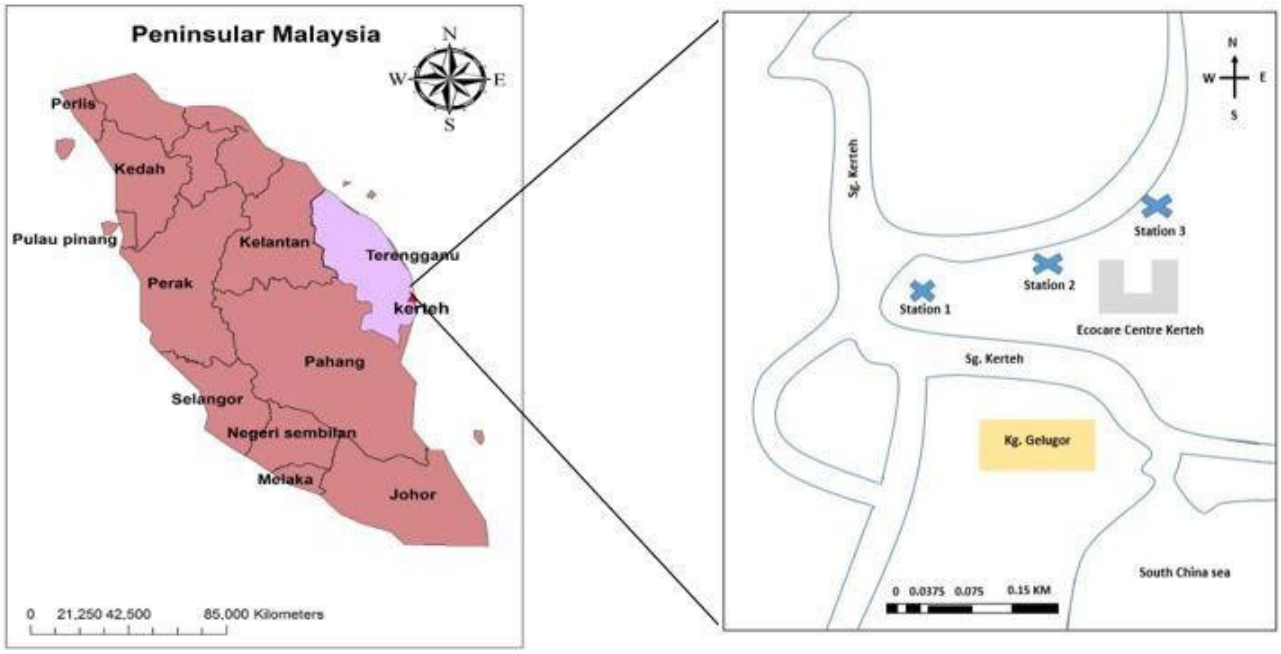


Figure 1. Location of sampling stations adjacent to the ecoCare Centre Kerteh: ST1 (4.525494°N, 103.441899°E), S2 (4.526935°N, 103.443382°E), and ST3 (4.527664°N, 103.444784°E).

Water temperature, DO, and pH at station 1 were recorded to be significantly lower than at stations 2 and 3 at $p < 0.05$. Station 2 showed a lower level of salinity than the other stations, with a salinity reading of 25.66±2.38 ppt ($p < 0.01$). The low salinity was concurrent with a low TDS at station 2. Physical parameter data were obtained in this study solely for the site characteristics record. No correlation analysis was carried out in this preliminary study to investigate the relationship between phytoplankton species diversity and the physical parameters.

Table 1. Physical water parameters at three different stations along the study site at Kerteh River. Also included the ANOVA scores of the spatial variability (n = 9).

Physical Water Parameters	Station			ANOVA SCORE
	1	2	3	
Temperature (°C)	28.90±0.01	29.30±0.18	29.68±0.54	$F_{2,9}$=4.83, p <0.05
Total dissolved solids (TDS) (g L^{-1})	28.61±1.99	26.20±2.19	28.25±2.83	$F_{2,9}$=20.41, p <0.01
Salinity (ppt)	28.31±2.18	25.66±2.38	28.18±2.76	$F_{2,9}$=16.67, p <0.01
Dissolved oxygen (DO) (mg L^{-1})	2.60±1.35	3.30±0.37	3.94±0.61	$F_{2,9}$=4.06, p <0.05
pH	5.53±0.17	5.90±0.02	6.04±0.03	$F_{2,9}$=5.94, p <0.05

Water Sampling and Physical Parameters Measurement for Site Characteristics

A one-off sampling was carried out in August 2019 for two consecutive days. Triplicate water samples were collected using a plankton net with a wire mesh of 30 µm during the high-tide period. The plankton net was towed for 10 m at approximately 10–20 cm below the water surface. Samples of filtered water containing phytoplankton were immediately transferred to 15 mL tubes, which were then preserved with 5 % formalin. The tubes were then fully wrapped in aluminium foil.

Abiotic physical water parameters, including water temperature, total dissolved solids (TDS), salinity, dissolved oxygen (DO), and pH, were measured in situ using a handheld YSI Multi-probe system (MPS) Model 556.

Phytoplankton Cells Extraction

Extraction of phytoplankton cells from water samples was done by separating the cells from water (with formalin) and suspended particles by means of centrifugation at 2300 rpm for 15 minutes. Excess formalin and distilled water, which were the supernatant, were siphoned off and discarded. Distilled water was then added to the samples (up to 0.5 cm). Phytoplankton samples in the water were concentrated and underwent frustule cleaning as the samples were centrifuged another five times at 2300 rpm for 15 minutes. The concentrated samples were retained in distilled water (up to 5 ml in the centrifuge tube) at room temperature.

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

Sedimentation Slides Preparation: Phytoplankton Cells Enumeration and Identification

One mL of sample was carefully pipetted into utermöhl sedimentation tube. The step was followed by the addition of 1 drop of Lugol's iodine. Sedimentation slides were prepared by following the preparation steps. by Bellinger & Sigee (2010). The abundance of phytoplankton was enumerated under a compound microscope by dividing the slides into 4 divisions. The calculation was carried out from one division to another (Evans, 1972). Only 200 phytoplankton cells were counted to represent the assemblage composition, which was expressed as relative abundance (%). Phytoplankton were identified to the lowest possible taxonomic level, either the genus or species level. The identification process was carried out by referring to taxonomic work by Redzuan (2012), Tomas (2007) and Salleh and Tajuddin (2006), by means of the phytoplankton cells' morphology, as guided in the mentioned taxonomic works.

Data Analyses

ANOVA was carried out to determine the variation in the means of the measured physical parameters between the 3 stations, using IBM SPSS Statistics 25. Species diversity of phytoplankton was analysed between stations and was investigated using the number of species or taxa S (S), Shannon Diversity Index (H'), and Pielou Evenness Index (J). The mentioned diversity indices were calculated using the Paleontological Statistics Software Package (PAST) version 4.16 (Hammer et al., 2001). Species data were pooled (n=3) to conduct the diversity analyses in PAST 4.16.

3. Results and Discussion

Phytoplankton Species Composition

Overall, a total of 56 phytoplankton species from four divisions were recorded in this one-off sampling survey. Division Bacillariophyta or the diatoms, had the highest recorded species with a total of 46 species, followed by 5 species of Dinophyta, 4 species of Chlorophyta, and finally, the Cyanophyta with a recorded S of only 1 species (Table 2). High Bacillariophyta taxa recorded in the mangrove ecosystem are well documented in most studies, such as reported by Canini et al. (2013), Saifullah et al. (2014), Fang & Sommer (2017) and Redzuan & Milow (2021).

Chaetoceros curvisetus (Figure 1a) of Bacillariophyta showed the highest abundance at Station 1 (27.08 ± 5.07) and Station 3 (8.80 ± 3.59), while at Station 2, it was *Frustulia vulgaris* with a relative abundance of 24.92 ± 3.44 (Table 2). Genus *Chaetoceros* is commonly reported to inhabit both oceanic and brackish ecosystems in the tropics. While in the temperate countries, *Chaetoceros* is reported to cause a spring bloom before the onset of summer heat. (Flynn et al., 2023; Onitsuka et al., 2018). Onset of *Chaetoceros* multi-species blooms proved to be significantly correlated to nitrate, phosphate, and temperature (Razali et al., 2015; Bosak et al., 2016; Redzuan & Milow, 2021; Mohd-Din et al., 2022). It is also reported that often, the blooms initiate as the genus increases its growth by proliferating rapidly and consequently increasing the cell numbers in a chain. (Begum et al., 2015; Shao et al., 2023).

C. curvisetus is also frequently disclosed as one of the species that causes a multispecies bloom of *Chaetoceros* spp (Fang & Sommer, 2017; Murty et al., 2017; Redzuan & Milow, 2021; Shao et al., 2023) worldwide. Interestingly, even in the multispecies blooms, numerous studies, including the present study, proved that the *C. curvisetus* was the dominant species in the blooms. (Fang & Sommer, 2017; Murty et al., 2017; Somsap et al., 2015). A number of studies reported a significant positive relationship between *C. curvisetus* and nitrate. (Begum et al., 2015) and temperature (Redzuan & Milow, 2021).

At Station 2, the significantly lower salinity and TDS of the newly replanted mangrove system, which characterized the station, possibly explained the different highest-abundance species, which was *Frustulia vulgaris* (Figure 1b). *Frustulia vulgaris,* although normally reported to inhabit the freshwater ecosystem (Odido et al., 2024), its occurrence in ocean-connected ecosystems, such as coastal rivers (Spaulding et al., 2021) and mangrove system (Redzuan & Milow, 2021) It is also well known. The species, however, has never been reported as either the dominant species or the species responsible for causing mono or multi-specific bloom in any ecosystem. Therefore, no comprehensive ecological study on this species has ever been reported.

Genus *Guinardia* showed the highest occurrence frequency, with all species of the genus present at all stations. *Guinardia* (Figure 1c) is known to be cosmopolitan and frequently reported as a dominant species in both temperate (Arsenieff et al., 2019) and tropical regions (Haridevi et al., 2022; Murty et al., 2017). Razali et al. (2022) reported that *Guinardia* sp. was the dominant species, as the species constituted 65–80% of the phytoplankton composition during a high biomass multi-species algal bloom in active aquaculture areas of the Johor Strait. *G. delicatula* blooms were reported in countries such as India, and most of the bloom events were the first report at the sites (Haridevi et al., 2022; Murty et al., 2017). Events of chain-forming species blooms in the Sub-tropical regions recorded multiple species of *Guinardia* blooms that dominated by 70 – 80 % of *Guinardia striata* (Taucher et al., 2018). Those multispecies *Guinardia* blooms, proven by Taucher et al. (2018) to closely relate to ocean acidification phenomena in the region.

Occurrence of five species of three genera from the division Dinophyta was also the highlight of this present study (Table 3) (Figure 2). Three of the five species, Ceratium sp. (Figure 2a), *Protoperidinium depressum* (Figure 2b), and *Alexandrium* sp. (Figure 2d), were present at all stations, indicating high occurrence frequency. The occurrence and distribution of harmful blooms by Dinophyta in both oceanic and estuarine ecosystems in Malaysia have been well reported by Usup et al. (2002), Lim et al. (2012), Lim et al. (2014) and Mohd-Razali et al. (2022). For example, harmful blooms involved shellfish toxin-producers *Alexandrium* species; *A. tamiyavanichii* (Usup et al., 2002; Mohammad-Noor et al., 2018; Liow et al., 2019) and *A. minutum* (Lim et al., 2012; Lau et al., 2017; Law et al., 2023); while fish killing-species caused high biomass blooms: *Margalefidinium polykrikoides*, *Noctiluca scintillans*, and *Karlodinium australe* (Lim

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

et al., 2012; Lim et al., 2014; Yñiquez et al., 2021; Mohd Razali et al., 2022) caused significant losses in the aquaculture industries in Malaysia. Global warming and increasing nutrient concentrations in oceanic ecosystems have been shown to stimulate the onset of harmful blooms. The three recorded species of Dinoflagellate recorded in this study were of the three genera listed as harmful taxon by Razali et al. (2015) that can either cause high biomass bloom forming water discoloration (Alvarez Dalinger et al., 2024) or shellfish toxin- producer (Ettoubi et al., 2020; Yñiguez et al., 2021).

Table 3. Abundance (in relative terms) of phytoplankton species recorded at Station 2, Station 3, and Station 4 in the chosen area of Kerteh River. The data were obtained through a one-off sampling occasion.

	Taxa	**Stations**		
	Division Bacillariophyta	**Station 1**	**Station 2**	**Station 3**
1	*Actinoptychus undulatus*	0.25 ± 0.29	*	1.02 ± 0.42
2	*Amphora* sp	*	*	0.42 ± 0.17
3	*Asterolampra* sp	*	*	0.21 ± 0.09
4	*Bacteriastrum delicatulum*	0.08 ± 0.02	*	*
5	*Bacteriastrum furcatum*	0.08 ± 0.02	*	*
6	*Bacteriastrum* sp. 1	6.67 ± 2.70	5.75 ± 3.59	2.72 ± 1.11
7	*Bacteriastrum* sp2	*	0.17 ± 0.26	*
8	*Chaetoceros constrictus*	*	0.08 ± 0.01	5.81 ± 2.37
9	*Chaetoceros curvisetus*	27.08 ± 5.07	7.50 ± 5.16	8.80 ± 3.59
10	*Chaetoceros* sp2	5.33 ± 3.75	*	2.42 ± 0.99
11	*Climacodium frauenfeldianum*	2.50 ± 0.63	0.92 ± 1.11	1.28 ± 0.52
12	*Coscinodiscus radiatus*	*	*	0.58 ± 0.24
13	*Coscinodiscus* sp1	1.33 ± 0.17	1.58 ± 1.11	1.21 ± 0.49
14	*Coscinodiscus* sp2	0.17 ± 0.10	0.25 ± 0.61	0.82 ± 0.33
15	*Cossoneis placentula*	0.08 ± 0.10	*	*
16	*Cyclotella meneghiniana*	0.08 ± 0.10	0.08 ± 0.20	0.20 ± 0.08
17	*Cymbella* sp	0.50 ± 0.20	0.33 ± 0.61	0.61 v 0.25
18	*Diploneis* sp	0.25 ± 0.17	*	0.41 ± 0.17
19	*Ditylum brightwellii*	0.08 ± 0.10	*	0.21 v 0.09
Continuation of Table 3				
20	*Eucampia zodiacus*	*	0.33 ± 0.82	*
21	*Eunotia valida*	0.92 ± 0.48	*	*
22	*Frustulia vulgaris*	15.17 ± 1.12	24.92 ± 3.44	3.13 ± 1.28
23	*Fragilaria* sp.	0	1.25 ± 1.75	3.90 ± 1.59
24	*Guinardia delicatula*	7.17 ± 3.15	1.92 ± 3.44	5.57 ± 2.27
25	*Guinardia flaccida*	4.50 ± 0.93	2.92 ± 2.69	4.83 ± 1.97
26	*Guinardia striata*	1.08 ± 1.27	3.67 ± 6.91	4.89 ± 1.99
27	*Gyrosigma scalproides*	*	0.17 ± 0.41	0.20 ± 0.08
28	*Gyrosigma sp.*	*	*	0.87 ± 0.27
29	*Navicula radiosa*	*	0.25 ± 0.03	*
30	*Navicula peticolasii*	*	0.08 ± 0.03	*
31	*Nitzshia longissima*	*	0.08 ± 0.02	0.20 ± 0.08
32	*Nitzschia epithemoides*	0.75 ± 0.12	0.17 ± 0.41	*
33	*Odontella sinensis*	*	*	0.42 ± 0.17
34	*Pinnularia* sp1	0.50 ± 0.10	1.00 ± 0.88	*

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

35	*Pinnularia* sp2	1.08 ± 0.88	*	*
36	*Pleurosigma directum*	0.08 ± 0.10	0.08 ± 0.03	*
37	*Pleurosigma elongatum*	*	0.33 ± 0.16	*
38	*Pleurosigma* sp	*	*	1.21 ± 0.49
39	*Rhizosolenia alata*	*	*	1.02 ± 0.42
40	*Rhizosolenia imrbicata*	0.83 ± 0.48	*	1.87 ± 0.27
41	*Rhizosolenia striata*	5.08 ± 0.40	1.08 ± 0.08	0.66 ± 0.27
42	*Skeletonema* sp	0.33 ± 0.10	*	0.66 ± 0.27
43	*Strauroneis producta*	*	*	0.49 ± 0.20
44	*Synedra ulna*	*	6.75 ± 1.72	2.09 ± 0.85
45	*Triceratium favus*	0.58 ± 0.58	0.08 ± 0.01	0.42 ± 0.17
46	*Zygoceros atlanticus*	0.75 ± 0.15	*	*
	Division Chlorophyta			
47	*Closterium* sp	*	*	0.20 ± 0.08
48	*Mougetia* sp	7.50 ± 1.34	10.00 ± 2.61	5.21 ± 2.13
48	*Pleurococcus miniatus*	7.83 ± 3.11	17.00 ± 3.12	4.77 ± 1.95
50	*Ulothrix* sp	0.50 ± 0.10	5.42 ± 1.30	4.46 ± 1.82
	Division Dinophyta			
51	*Ceratium* sp	0.08 ± 0.02	0.33 ± 0.10	0.41 ± 0.17
52	*Dinophysis caudata*	0.08 ± 0.10	*	0.41 ± 0.17
53	*Protoperidinium depressum*	0.42 ± 0.11	0.08 ± 0.01	0.80 ± 0.33
54	*Protoperidium* sp1	0.08 ± 0.02	*	0.42 ± 0.17
55	*Alexandrium* sp2	0.17 ± 0.20	0.25 ± 0.02	0.52 ± 0.21
	Division Cyanophyta			
56	*Oscillatoria tenuis*	*	4.83 ± 0.51	2.80 ± 1.14
		* indicates not recorded		

Figure 1. Light microscopy plate of; a. Eight cells of *Chaetoceros curvisetus* in a chain, b. *Frustulia vulgaris* and; c. Single cell of genus *Guinardia*, the *Guinardia striata.*

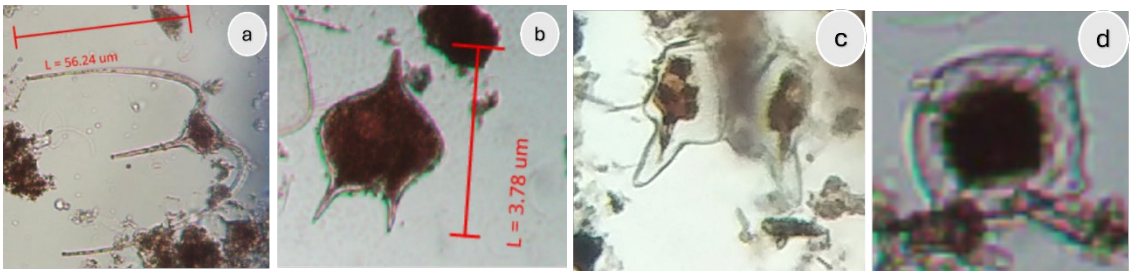


Figure 2. a. *Ceratium* sp., b. *Protoperidinium depressum*, c. *Dinophysis caudata* and d. *Alexandrium* sp. Four out of the five Dinoflagellate species recorded at chosen sites of Kerteh River.

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

Spatial Variability of Phytoplankton Diversity

Diversity-wise, Station 3, located further away from the main outlet to the South China Sea, had the highest species richness (S). There were 42 species recorded at Station 3, followed by Station 1 and Station 2, with 36 and 34 species, respectively. The high species richness at Station 3 potentially contributed to its relatively higher diversity value (H') (2.96)(Magurran, 2004; Supriatna, 2018) than the other two stations, Station 1 (2.697) and Station 2 (2.669), in addition, the H' value of Station 3 was also at high potential to be attributed by the station's small species relative abundance range of 0.2 – 8.80 % (Table 3). The range was the lowest range between the 3 stations and further confirmed by Station 3's Equitability (J) index score of 0.792, followed by Station 2 and 1 with the values of 0.7569 and 0.7526, respectively (Table 3). A positive relationship between the diversity indices scores and both the species richness and species equal abundance (evenness) was confirmed in a comprehensive analysis on measuring biodiversity by Magurran (2004).

Table 2. Diversity scores based on phytoplankton species composition and abundance at the three stations.

	Station 2	Station 3	Station 4
Taxa (S)	36	34	42
Shannon (H')	2.697	2.669	2.96
Equitability (J)	0.7526	0.7569	0.792

4. Conclusion

Although the Kerteh River is facing rapid urbanization from adjacent terrestrial areas, the abundance of the phytoplankton species suggested that the ecosystem is still in a 'not alarming ecosystem' condition. However, preliminary results of this study indicated that the phytoplankton species *Chaetoceros curvisetus* is the species that needs to be monitored in future biomonitoring projects. The presence of some species of bloom-forming and harmful dinoflagellates is also an important concern that needs attention. Biomonitoring or comprehensive temporal studies on phytoplankton abundance and diversity should be initiated immediately as a precaution to ensure no threat to phytoplankton diversity imbalance in the Kerteh River. Phytoplankton, although small, collectively, if in bloom, could potentially lead to the collapse of ecosystems, causing economic loss as well as biodiversity loss.

5. Acknowledgement

The authors would like to express their sincere gratitude to the Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, and Eco-Care, MNS for providing funding, facilities, and continuous support.

7. References

Ajibare, A. O., P. O. Ayeku, J. O. Akinola, and A. H. Adewale. (2019). Plankton Composition in Relation to Water Quality in the Coastal Waters of Nigeria. *Asian Journal of Fisheries and Aquatic Research* 5 (2):1-9. https://doi.org/10.9734/ajfar/2019/v5i230070.

Alvarez Dalinger, F. S., Claudia Nidia, B., Lozano, V. L., Moraña, L. B., & Salusso, M. M. (2024). Bloom-forming cyanobacteria and dinoflagellates in five Argentinian reservoirs: Multi-year sampling. *Water Biology and Security*, 3(1). https://doi.org/10.1016/j.watbs.2023.100232

Anne E Magurran. ([s.d.]). Measuring_Biological_Diversity (3).

Arsenieff, L., Simon, N., Rigaut-Jalabert, F., Le Gall, F., Chaffron, S., Corre, E., Com, E., Bigeard, E., & Baudoux, A. C. (2019). First viruses infecting the marine diatom *Guinardia delicatula*. *Frontiers in Microbiology*, 10 (JAN). https://doi.org/10.3389/fmicb.2018.03235

Azaman, F., Juahir, H., Yunus, K., Azid, A., Khalit, S. I., Mustafa, A. D., & Yusri, M. A. M. (2017). Metal concentration at surface water using multivariate analysis and human health risk assessment. *Journal of Fundamental and Applied Sciences*, 9(2S), 217-237.

Begum, M., Sahu, B. K., Das, A. K., Vinithkumar, N. V., & Kirubagaran, R. (2015). Extensive *Chaetoceros curvisetus* bloom in relation to water quality in Port Blair Bay, Andaman Islands. *Environmental Monitoring and Assessment* 187(5), 1–14. https://doi.org/10.1007/s10661-015-4461-2

Bellinger, E. G., & Sigee, D. C. (2010). Algae as Bioindicators. Em Freshwater Algae (p. 99–136). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470689554.ch3

Bosak, S., Godrijan, J., & Šilović, T. (2016). Dynamics of the marine planktonic diatom family Chaetocerotaceae in a Mediterranean coastal zone. *Estuarine, Coastal and Shelf Science* 180: 69–81. https://doi.org/10.1016/j.ecss.2016.06.026

Canini, N. D., Metillo, E. B., & Azanza, R. V. (2013). Monsoon-influenced phytoplankton community structure in a Philippine mangrove estuary. *Tropical Ecology,* 54(3): 331–343.

Chowdhury, A.J., Mohamed, A.A., John, A., & Yunus, K.B. (2011). Spatial Variation and Community Composition of Phytoplankton along the Pahang Estuary, Malaysia. *Asian Journal of Biological Sciences, 4*, 468-476.

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

C.K., H., Hardikar, R., Thomas, J., Pradhan, U. K., T.A., A., P.S., R., M.A., V., Sahu, B., & Rafeeq, M. (2022). Bloom report of *Noctiluca scintillans, Gymnodinium wulfii* and *Guinardia delicatula* from Shastri Estuary, West Coast of India. *Regional Studies in Marine Science* 56: 102689. https://doi.org/10.1016/j.rsma.2022.102689

Essien, T. P., S. P. Antai & N. U. Benson. 2007. Microalgae biodiversity and biomass status in Qua Iboe Estuary Mangrove Swamp, Nigeria. *Aquatic Ecology* 42: 71-81.

Ettoubi, E., Akif, F., Blaghen, M., & Genten, F. (2020). Marine Biotoxins: Origins, Effects, Distribution, Prevention and Treatment. In *International Journal of Innovative Science and Research Technology* (Vol. 5, Issue 11). www.ijisrt.com928

Evans JH. 1972. A modified sedimentation system for counting algae with an inverted microscope. *Hydrobiologia* 40: 247-250. DOI: 10.1007/BF00016796

Fang, X., & Sommer, U. (2017). Overwintering effects on the spring bloom dynamics of phytoplankton. *Journal of Plankton Research* 39(5), 772–780. https://doi.org/10.1093/plankt/fbx046

Flynn, R. F., Haraguchi, L., Mcquaid, J., Burger, J. M., Lunga, P. M., Stirnimann, L., Samanta, S., Roychoudhury, A. N., & Fawcett, S. E. (2023). Oceanography Nanoplankton: The dominant vector for carbon export across the Atlantic Southern Ocean in spring. https://oceancolor.gsfc.nasa.gov/l3/

Graham, L. E. & L. W. Wilcox. 2000. Algae. Prentice Hall. New Jersey.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9pp.

Haridevi, C.K., Hardikar, R., Thomas, J., Pradhan, U. K., T.A., A., P.S., R., M.A., V., Sahu, B., & Rafeeq, M. (2022). Bloom report of *Noctiluca scintillans*, *Gymnodinium w*ulfii and *Guinardia delicatula* from Shastri Estuary, West Coast of India. *Regional Studies in Marine Science* 56: 102689. https://doi.org/10.1016/j.rsma.2022.102689

Hilaluddin, F., Yusoff, F. M., Natrah, F. M. I., & Lim, P. T. (2020). Disturbance of mangrove forests causes alterations in estuarine phytoplankton community structure in Malaysian Matang mangrove forests. *Marine Environmental Research* 158. https://doi.org/10.1016/j.marenvres.2020.104935

Hilaluddin, F., Yusoff, F. M., & Toda, T. (2020). Shifts in diatom dominance associated with seasonal changes in an estuarine-mangrove phytoplankton community. *Journal of Marine Science and Engineering* 8(7). https://doi.org/10.3390/JMSE8070528

Jeslin, I. J., Benny, N., Thomas, L. C., & Padmakumar, K. B. (2021). Short term spatio-temporal variabilities of microphytobenthic assemblages in the mangrove ecosystems along the southwest coast of India. *Wetlands Ecology and Management* 29(5): 689–702. https://doi.org/10.1007/s11273-021-09787-8

Kamal, A. H. M., Idris, M. H., Al-Asif, A., Bhuiyan, M. K. A., Rasdi, N. W., Piah, R. M., Lah, R. A., Musa, N., Wahid, M. E. A., & Abualreesh, M. H. (2022). Phytoplankton Community Structure and Seasonality in a Tropical Mangrove Estuarine and Coastal Waters, South China Sea. *Thalassas*, *38*(1), 511–526. https://doi.org/10.1007/s41208-021-00386-1

Lau, W. L. S, Lawa, I. K., Liow, G. R., Hiia, K. S., Usup, G., Lim, P. T. and Leaw, C. P. (2017). Life-history stages of natural bloom populations and the bloom dynamics of a tropical Asian ribotype of *Alexandrium minutum*. *Harmful Algae,* 70, 52-63

Law, I.K., Hii, K.S., Lau, W.L.S., Leaw, C.P., & Lim, P.T. (2023). Coastal micro-phytoplankton community changes during the toxigenic *Alexandrium minutum* blooms in a semi-enclosed tropical coastal lagoon (Malaysia, South China Sea). *Reg. Stud. Mar. Sci*. 57: 102733

Lim, P. T., Usup, G., & Leaw, C. P. (2012). Harmful algal blooms in Malaysian waters. *Sains Malaysiana* 41: 1509-1515.

Lim, H. C., Leaw, C. P., Tan, T. H., Kon, N. F., Yek, L. H., Hii, K. S., Teng, S. T., Razali, R. M., Usup, G., Iwataki, M., & Lim, P. T. (2014). A bloom of *Karlodinium australe* (Gymnodiniales, Dinophyceae) associated with mass mortality of cage-cultured fishes in West Johor Strait, Malaysia. *Harmful Algae* 40: 51-62.

Liow GR, Lau WLS, Law IK, Hii KS, Mohammad Noor N, Leaw CP, Lim PT (2019) Phytoplankton community changes in Kuantan Port (Malaysia), with emphasis on the paralytic-shellfish toxinproducing dinoflagellate *Alexandrium tamiyavanichii*. Reg Stud Mar Sci 26:100504. https://doi.org/10.1016/j.rsma.2019.100504

Magurran, A. E. (2004). Measuring_Biological_Diversity, pp. 215, Blackwell Publishing Company.

Manna, S., K. Chaudhuri, S. Bhattacharyya & M. Bhattacharyya, 2010. Dynamics of Sundarban estuarine ecosystem: eutrophication induced threat to mangroves. Saline System **6**: 1-16.

Murty, K. N., Sarma, N. S., Pandi, S. R., Chiranjeevulu, G., Kiran, R., & Muralikrishna, R. (2017). Hydrodynamic control of microphytoplankton bloom in a coastal sea. Journal of Earth System Science, 126(6), 82. https://doi.org/10.1007/s12040-017-0867-2

Mohd-Din, M., Hii, K.S., Abdul-Wahab, M.F., Mohamad, S.E., Gu, H., Leaw, C.P., & Lim, P.T. (2022) Spatial-temporal variability of microphytoplankton assemblages including harmful microalgae in a tropical semi-enclosed strait (Johor Strait, Malaysia). *Mar. Environ. Res*. 175: 105589. https://doi.10.1016/j.marenvres.2022.105589

Mohammad-Noor, N., Adam, A., Lim, P. T., Leaw, C. P., Lau, W. L. S., Liow, G. R., Mohammad-Bunnori, N., Hamdan, N. A., Md-Noor, A., Kemat, N., & Muniandi, D. (2018). First report of paralytic shellfish poisoning (PSP) caused by *Alexandrium tamiyavanichii* in Kuantan Port, Pahang, East Coastof Malaysia. *Phycological Research* 66: 37-44

Mohd-Razali R. M., Mustapa N. I., Noordin W. N., Rahim M. A., Hii K. S., Lim P. T., Leaw, C. P., Muhd-Farouk, H., & Ku-Yaacob, K. (2022). Report of a fish kill due to a dinoflagellate bloom in Perak and Penang, Malaysia. *Asian Fish. Sci.* 35: 257–268.

Norhana Noordin, W., Nor Azman Ayub, M., Nor, A., & Mui Hua, L. ([s.d.]). Marine Biotoxins in Malaysia: Occurrence, Toxicity Cases, Analytical Capabilities and Regulatory Limits.

Onitsuka, G., Yoshikawa, Y., Shikata, T., Yufu, K., Abe, K., Tokunaga, T., Kimoto, K., & Matsuno, T. (2018). Development of a thin diatom layer observed in a stratified embayment in Japan. Journal of Oceanography, 74(4), 351–365. https://doi.org/10.1007/s10872-018-0466-0

Odido, M.; Appeltans, W.; BelHassen, M.; Mussai, P.; Nsiangango, S.E.; Vandepitte, L.; Wambiji, N.; Zamouri, N. Jiddou, A.M. (Eds) (2024). African Register of Marine Species. *Frustulia cuspidata* Kutzing,1833.Accessedat:https://www.marinespecies.org/afremas/aphia.php?p=taxdetails&id=163908 on 2024-09-10

Owen, R. B., R. W. Renaut, V. C. Hover, G. M. Ashley & A. M. Muasya. (2004). Swamps, springs and diatoms: wetlands o the semi-arid Bogoria-baringo Rift, Kenya. Hydrobiologia 518: 59-78.

Perumal, N. V., M. Rajkumar, P. Perumal & K. T Rajasekar. (2009). Seasonal variations of plankton diversity in Kaduviyar estuary, Nagapattinam, southeast coast of India. Journal of Environmental Biology **30**: 1035-1046.ES

Rajkumar, M., P. Perumal, V. Ashok Prabu, N. Vengadesh Perumal & K. Thillai Rajasekar. (2009). Phytoplankton diversity in Pichavaram mangrove waters from south-east coast of India. Journal of Environmental Biology **30**: 489-498.

Razali, R. M., Leaw, C. P., Lim, H. C., Nyanti, L., Ishak, I., & Lim, P. T. (2015). Harmful microalgae assemblage in the aquaculture area of Aman Island, Northen Strait of Malacca. Malaysian Journal of Science, 34(1), 20–32.

Razali, R.M., Mustapa, N.I., Yaacob, K.K.K., Yusof, F., Teng, S.T., Hanafiah, A.H., Hii, K.S., Mohd-Din, M., Gu, H., Leaw, C.P., & Lim, P. T. (2022). Diversity of *Heterocapsa* (Dinophyceae) and the algal bloom event in the mariculture areas of Johor Strait, Malaysia. *Plankton Benthos Res*. 17: 290–300.

Redzuan, N. S. (2012). Spatial and temporal distribution of phytoplankton at Carey Island mangrove. University of Malaya.

Redzuan, N. S., & Milow, P. (2021). Monthly chaetocerotales diversity and abundance, and its relationship with water physicochemical parameters and phytoplankton diversity in Carey Island mangrove ecosystem, Malaysia. Biodiversitas, 22(7), 2919–2927. https://doi.org/10.13057/biodiv/d220744

Saifullah, A. S. M., Hena, M. K. A., Idris, M. H., Halimah, A. R., & Johan, I. (2014). Diversity of Phytoplankton from Mangrove Estuaries of Sarawak, Malaysia. 31(5), 915–924. https://doi.org/10.5829/idosi.wasj.2014.31.05.2010

Saifullah, A.S., Kamal, A.H., Idris, M.H., Rajaee, A.H., & Bhuiyan, M.K. (2016). Phytoplankton in tropical mangrove estuaries: role and interdependency. *Forest Science and Technology, 12*, 104 - 113.

Salleh, A., Tajuddin, Z. M. (2006). Phytoplankton of Carey Island. Golden Hope Plantations Berhad and Institute of Biological Sciences (UM). 207 pp.

Saravanakumar, A., Rajkumar, M., Thivakaran, G. A., & Sesh Serebiah, J. (2008). Abundance and seasonal variations of phytoplankton in the creek waters of western mangrove of Kachchh-Gujarat. Journal of Environmental Biology, 29(2), 271–274.

Shao, Q., Zhu, Z., & Zhou, C. (2023). Alteration in Community Dynamics of Chaetoceros curvisetus and Bacterioplankton Communities in Response to Surfactin Exposure. Microorganisms, 11(10). https://doi.org/10.3390/microorganisms11102596

Somsap, N., Gajaseni, N., & Piumsomboon, A. (2015). Physico-Chemical Factors Influencing Blooms of Chaetoceros spp. and Ceratium furca in the Inner Gulf of Thailand. Em Nat. Sci.) (Vol. 49).

Spaulding, S. A., Potapova, M. G., Bishop, I. W., Lee, S. S., Gasperak, T. S., Jovanoska, E., Furey, P. C., & Edlund, M. B. (2021). Diatoms.org: supporting taxonomists, connecting communities. Diatom Research, 36(4), 291–304. https://doi.org/10.1080/0269249X.2021.2006790

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

Stanković, I., Gligora Udovič, M., Žutinić, P., Hanžek, N., & Plenković-Moraj, A. (2024). Is salinity a driving factor for the phytoplankton community structure of a brackish shallow Mediterranean lake? Hydrobiologia, 851(4), 999–1013. https://doi.org/10.1007/s10750-023-05300-9

Supriatna, J. (2018). Biodiversity Indexes: Value and Evaluation Purposes. E3S Web of Conferences, 48. https://doi.org/10.1051/e3sconf/20184801001

Tanaka, K. & P. Choo. 2000. Influences of nutrient outwelling from the mangrove swamp on the distribution of phytoplankton in the Matang Mangrove Estuary, Malaysia. Journal of Oceanography **56**: 69-78

Taucher, J., Arístegui, J., Bach, L. T., Guan, W., Montero, M. F., Nauendorf, A., Achterberg, E. P., & Riebesell, U. (2018). Response of subtropical phytoplankton communities to ocean acidification under oligotrophic conditions and during nutrient fertilization. Frontiers in Marine Science, 5(SEP). https://doi.org/10.3389/fmars.2018.00330

Underwood, G. J. C. (1995). Seasonal and spatial variation in epipelic diatom assemblages in the Severn Estuary. Oceanographic Literature Review, 42(7), 559–560.

United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Prospects 2019, Volume I: Comprehensive Tables (ST/ESA/SER.A/426).

Usup, G., Leaw, C. P., Lim, P. T., & Ahmad, A. (2002). Probable toxin producer responsible for the first occurrence of paralytic shellfish poisoning on the east coast of peninsula Malaysia. *Malaysian Applied Biology* 31(2): 29-35

Utami, F., Hasan, Z., Rizal, A., & Herawati, H. (2019). Spatial distribution of plankton in Cikeruh River, Jatinangor District, West Java Province, Indonesia. Asian Journal of Fisheries and Aquatic Research, 1-9.

Xu, S. J.-L., Chan, S. C.-Y., Wong, B. Y.-K., Zhou, H.-C., Li, F.-L., Tam, N. F.-Y., & Lee, F. W.-F. (2022). Relationship between phytoplankton community and water parameters in planted fringing mangrove area in South China. Science of The Total Environment, 817, 152838. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.152838

Yaakob, F. K. K., & Zakaria, M. P. (2017). Determination of heavy metals in water samples from Sungai Kerteh and Sungai Paka, Terengganu, Malaysia. Journal of Tropical Resource and Sustainable Science, 5 ,74-78.

Yñiguez, A. T., Lim, P. T., Leaw, C. P., Jipanin, S. J., Iwataki, M., Benico, G. & Azanza, R. V. (2021). Over 30 years of HABs in the Philippines and Malaysia: What have we learned? *Harmful Algae* 102: 101776. https://doi.org/10.1016/j.hal.2020.101776

DOI:https//doi.org/10.22452/mjs.vol45no1.3
Malaysian Journal of Science 45(1): 17-26 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 27-32 (March 2026) https://mjs.um.edu.my

Comparative Analysis of Antioxidant Properties of Cold and Hot Water Leaf Extract of *Rauvolfia vomitoria* Afzel

Onyeukwu, O. B.[1ae]*, Dennis-Eboh, U.[2b], Chijindu, P. C.[3c], Njideaka, O. T.[4a], Ohwokevwo, O. A.[5d], Achuba, F. I.[6e] and Okoro, I. O.[7e]

Abstract: *Rauvolfia vomitoria* Afzel has attracted significant research interest due to its multiple health benefits. Its antioxidant properties are important in preventing oxidative stress, cancer, heart problems, and neurological disorders. This study compares the antioxidant capacity of *R. vomitoria* leaf extract in both hot and cold water. Standard methods were used to prepare leaf extracts in both hot and cold water, which were subjected to different antioxidant assays. The hot water leaf extract had a better capacity to snare free radicals produced by stable DPPH's free radical at 50% inhibition concentration (3.07 ± 0.54 mg/ml) than the cold water leaf extract (9.82 ± 0.01mg/ml). The nitric oxide inhibitory capacity of *R. vomitoria* at 50% inhibition concentration in cold and hot water leaf extract (3.64 ± 0.38 and 4.24 ± 0.11) showed no significant difference. The hot water leaf extract had higher ferric-reducing antioxidant power (IC_{50} of 7.21 ± 0.07 mg/ml) than the cold water extract (10.85 ± 0.50 mg/ml). The hot water leaf extract showed a higher total antioxidant capacity (IC50 of 6.01 ± 0.21 mg/ml) than the cold water extract (9.01 ± 0.07 mg/ml). These antioxidant evaluations show that *R. vomitoria* hot water leaf extracts were superior to cold water leaf extracts as antioxidants and free radical scavengers. The findings show that *R. vomitoria's* hot-water extract has greater antioxidant activity than its cold-water extract.

Keywords: *Rauvolfia vomitoria, antioxidant, free radicals, inhibition concentration, extract.*

1. Introduction

Rauvolfia vomitoria Afzel, a rainforest tree in the *Apocynaceae* family, grows to approximately 12 meters in height and features a whorled trunk with visible, red, globose fruit. The tree also produces clusters of flowers and oval leaves with straight venation (Ekarika et al., 2020). It is commonly known as serpent wood, swizzle stick, or poison devil's pepper and is widely distributed and cultured in Africa, India, China, and Bangladesh (Yu et al., 2012; Owoade et al., 2021). Yoruba people in Nigeria refer to it as asofeyeje, which implies producing food for birds. The Igbo people in Nigeria refer to it as akanta, while the Ashantes people in Ghana call it pempe (Ajayi, 2021). Over the years, *R. vomitoria* has been generally regarded as a weed, but in recent years, numerous investigations have confirmed its various uses in different countries (Okereke et al., 2015). According to traditional healers in Nigeria and Africa, decoctions of *R. vomitoria* leaves possess strong emetic and anti-inflammatory properties and are used to treat a wide range of ailments, including fever, general weakness, liver issues, digestive illnesses, mental disorders, impotence, piles, rheumatism, gastrointestinal diseases, cancer, hypertension, insanity, snakebite, cholera, diarrhea, jaundice, and venereal diseases (Kumar et al., 2022; Ajayi, 2021; Chinonye et al., 2021; Kumar et al., 2020, 2016, 2015,). Numerous extracts and fractions have been shown to possess anti-inflammatory, anticancer, antibacterial, anti-diabetic, antioxidant, and antipsychotic properties due to their bioactive components (Surendran et al., 2021; Yu & Chen, 2014; Fannang et al., 2011; Bemis et al., 2006).

Oxidative stress is caused by an imbalance in the relative quantities of essential elements involved in oxidative metabolism and is the root cause of many diseases (Fatima et al., 2021). It occurs when a system's capacity to eliminate reactive oxygen species and functional metabolites is exceeded (Sies, 1985; Goodarzi et al., 2018). The delicate balance between pro- and antioxidants in the cellular environment governs an organism's overall health (Fatima et al., 2021). Even at very low concentrations, antioxidants play several physiological roles in the body and contribute to the immune system's defence against illnesses caused by unregulated radical invasion (Pisoschi & Negulescu, 2011; Sunil, 2014). To inhibit oxidative processes, antioxidants act as reducing agents, typically by eliminating reactive oxygen species before they can harm cells (Wolf, 2005).

Authors information:
[a]Department of Chemical Sciences, University of Delta, PMB 2090, Agbor, NIGERIA.
[b]Department of Medical Biochemistry, Delta State University, PMB 1, Abraka, NIGERIA.
[c]Department of Biological Sciences, University of Delta, PMB 2090, Agbor, NIGERIA.
[d]Department of Biochemistry, Southern Delta University, PMB 05, Ozoro, NIGERIA.
[e]Department of Biochemistry, Delta State University, PMB 1, Abraka, NIGERIA.
*Corresponding Author: benjamin.onyeukwu@unidel.edu.ng

Received: June, 2024
Accepted: October, 2024
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.4
Malaysian Journal of Science 45(1): 27-32 (March 2026)

They perform a key function in the body's protective system and are essential for preventing diseases and disorders in plants and animals, as well as protecting plants from pollution (Ahmed & Beigh, 2009). Some chronic and degenerative ailments significantly impacted by oxidative damage are heart disease, the nervous system, cancer, ageing, autoimmune disorders, and rheumatoid arthritis (Lien et al., 2008; Goodarzi et al., 2018). Heat treatment can enhance or degrade the nutritional value of food (Chukwu et al., 2010; Oghbaei & Prakash, 2016).

Some people use the cold-water extract of *R. vomitoria Afzel* for medicinal purposes, while others prefer the hot-water extract. Therefore, the goal of this investigation is to compare the antioxidant capabilities of cold and hot water extracts of *R. vomitoria Afze*l to determine which extract has greater medicinal or therapeutic value.

2. Materials and Methods

Collection and Preparation of the Sample

We collected fresh *R. vomitoria* Afzel leaves from Delta State University in Abraka, Delta State, Nigeria. Prof. Aigbokhan Emmanuel Izaka identified the leaves, and Dr Akinnibosun Henry Adewale issued a voucher number (UBH-R421) at the Herbarium Unit of the Plant Biology and Biotechnology Department, University of Benin, Edo State. The fresh leaves were rinsed with distilled water to remove any remaining debris.

Cold Water Extract

A cold-water leaf extract of *R. vomitoria* Afzel was obtained by homogenization (Onyeukwu et al., 2024). 125g of fresh leaves were blended with a mortar and pestle and extracted with 500 mL of distilled water (25% w/v). The extract was subsequently filtered through a double-folded clean sieve cloth, and the cold-water filtrate was employed in the investigation.

Hot Water Extract

Hot water leaf extract of *R. vomitoria* Afzel was obtained by the decoction procedure outlined by Onyeukwu et al. (2024). 125g of fresh leaf was placed in 500 mL of boiling water (25% w/v) and allowed to stand for 15 minutes. The extract was subsequently filtered through double-folded muslin cloth, and the hot-water filtrate was cooled and used in the investigation.

Antioxidant Activity Evaluation

1, 1-diphenyl -2-picryl hydrazyl (DPPH) Assay

The determination of antioxidant capacity followed the methodology of Owoade et al. (2021). To 2 ml of DPPH solution (0.3 mM), 0.2 ml of various *R. vomitoria* aqueous leaf extract concentrations (0.2 - 1.0% w/v) was added, followed by 30 min of incubation in the dark. The absorbance was measured at 517 nm. As a reference, ascorbic acid (0.02-0.10 mg/ml) was employed. Using the following equation, the % inhibition of DPPH radical scavenging was determined: $([A_o - A_1]/A_o) \times 100$ equals the percentage of DPPH radical inhibition. A_1 is the absorbance in the presence of the extract, and A_o is the absorbance of the control (blank, without extract).

Nitric Oxide (NO) Free Radical Scavenging Activity

Using the approach developed by Samuel et al. (2021), the ability of nitric oxide to scavenge free radicals was estimated. Different quantities of the aqueous extract of *R. vomitoria* (0.2 - 1.0% w/v) were combined with 0.5 ml of the 10 mM phosphate buffer saline (pH 7.4), 2 ml of 10 mM sodium nitroprusside, and 0.2 ml of 10 mM sodium nitroprusside. After that, the mixture was incubated at 25^0 °C, and 0.5 ml of the incubated solution was taken out after 150 minutes and combined with 0.5 ml of the Griess reagent [1.0 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride (0.1% w/v)]. After 30 minutes of incubation at room temperature, the mixture's absorbance at 546 nm was measured against a blank. With the use of the following equation, the percentage inhibition of nitric oxide radical scavenging was determined: % inhibition of NO radical = $([A_0 – A_1]/ A_0) \times 100$. A_1 is the absorbance in the presence of the extract, and A_0 is the absorbance of the control (blank, without extract).

Ferric Reducing Antioxidant Power (FRAP) Assay

The technique of Oborirhovo et al. (2023) was employed to assess the antioxidant activity via ferric reduction. 1.0 ml of the aqueous leaf extract of *R. vomitoria* (0.2 - 1.0% w/v) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v). After 20 minutes of incubation at 50^0 °C, 2.5 ml of 10% w/v trichloroacetic acid is added to the resultant mixture. 0.5 mL of $FeCl_3$ (0.1%, w/v) and 2.5 mL of distilled water were added to the mixture after it was centrifuged at 3000 rpm for 10 minutes, and the upper layer (2.5 mL) was collected. Following that, the absorbance was measured at 700 nm relative to a blank sample containing distilled water and sodium phosphate buffer. 0.02-0.10 mg/ml of ascorbic acid was employed as a reference.

Total Antioxidant Capacity

The approach employed by Oborirhovo et al. (2023) was applied. In screw-capped tubes, 1.0 mL (0.2–1.0% w/v) of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was taken, to which 0.1 mL of the aqueous extract of the leaf of *R. vomitoria* was added and dissolved. The tubes were sealed and incubated at 95^0 °C for 90 minutes in a thermal block. After cooling to room temperature, the absorbance of the aqueous solution in each tube was measured at 695 nm against a blank. The total antioxidant capacity was reported as equivalent to gallic acid (GAE) and was determined using gallic acid standards (0.02-0.10 mg/mL).

Statistical Analyses

Mean ± SD of triplicate values was used to report data. The LSD test was used in analyses of variance (ANOVA) to compare the outcomes. Statistical significance was established at $P < 0.05$. The graph was plotted in Microsoft Excel, and the linear regression of the extract's concentration against the percentage of inhibition was used to determine the IC_{50}.

DOI:https//doi.org/10.22452/mjs.vol45no1.4
Malaysian Journal of Science 45(1): 27-32 (March 2026)

3. Results

The IC_{50} values for the leaf extracts in hot and cold water of *R. vomitoria* Afzel for DPPH radical are presented in Figure 1. *R. vomitoria* Afzel hot water leaf extract had a better capacity to snare free radicals produced by stable DPPH's free radical at 50% inhibition concentration (3.07 ± 0.54 mg/ml) than the cold water leaf extract (9.82 ± 0.01mg/ml). $P < 0.05$ indicated a significant difference.

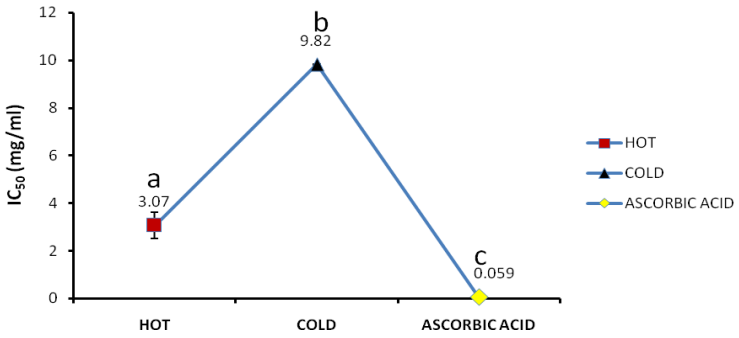


Figure 1. IC_{50} of leaf extract for cold and hot water of *R. vomitoria* Afzel for DPPH radical. Findings presented as Mean ± SD. Data with distinct letters (a-c) exhibit a significant difference ($P < 0.05$).

The IC_{50} values for the leaf extracts in hot and cold water of *R. vomitoria* Afzel showed the ability to scavenge free radicals produced by nitric oxide at IC_{50} of 3.64 ± 0.38 mg/ml and 4.24 ± 0.11 mg/ml, respectively (Figure 2). There was no significant difference between the IC_{50} of leaf extract for cold and hot water ($P < 0.05$). Both extracts had a significantly lower ability to scavenge free radicals produced by nitric oxide than the catechin standard, which had an IC_{50} of 0.076 ± 0.001 mg/ml.

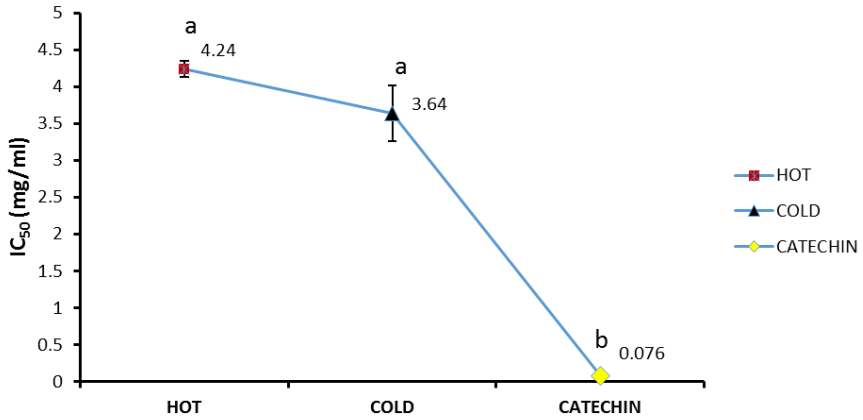


Figure 2. IC_{50} of leaf extract for cold and hot water of *R. vomitoria* Afzel for Nitric oxide radical. Findings presented as Mean ± SD. Data with distinct letters (a-b) exhibit a significant difference ($P < 0.05$).

The IC_{50} of the hot water leaf extracts of *R. vomitoria* Afzel (7.21± 0.07 mg/ml) exceeded the value of the cold extract. (10.85 ± 0.50 mg/ml) However, it was lower than the ascorbic acid standard with an IC_{50} of 0.05 mg/ml for ferric-reducing power (Figure 3). *R. vomitoria* Afzel leaf extracts in standard, hot, and cold water differed significantly ($P < 0.05$).

DOI:https//doi.org/10.22452/mjs.vol45no1.4
Malaysian Journal of Science 45(1): 27-32 (March 2026)

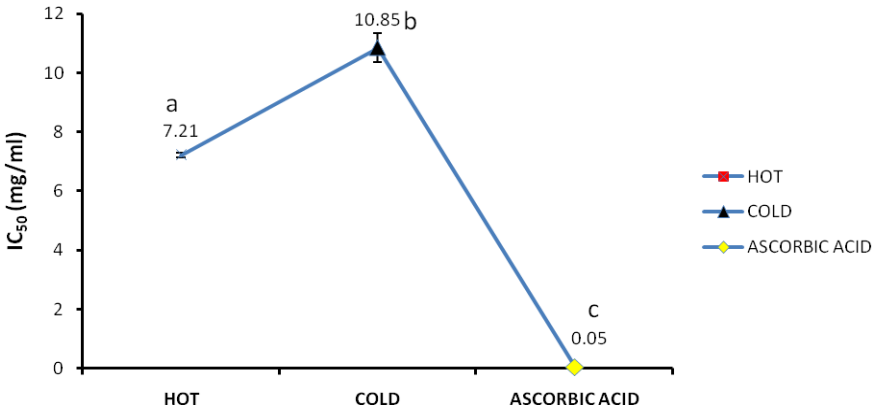


Figure 3. Inhibition concentration at 50% of leaf extract for cold and hot water of *R. vomitoria* Afzel for Ferric reducing power findings presented as Mean ± SD. Data with distinct letters (a-b) exhibit a significant difference ($P < 0.05$).

The hot-water extract's total antioxidant capacity (IC_{50}: 6.01 ± 0.21 mg/ml) was higher than that of the cold-water extract (9.01 ± 0.07 mg/ml). However, it was lower than that of gallic acid, which had an IC_{50} of 0.051 ± 0.001 mg/mL, exhibiting significant variation ($P < 0.05$) (Figure 4). The test samples' total antioxidant capability range in this order: Gallic acid > Hot water extract > Cold water extract.

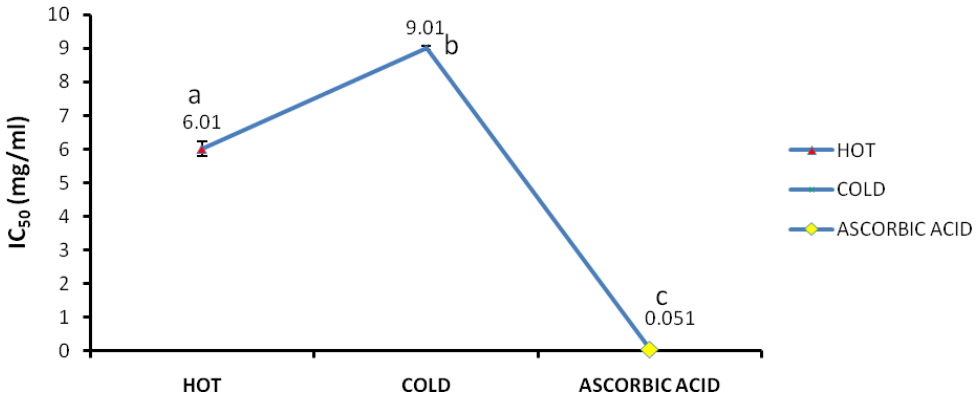


Figure 4. Total antioxidant capacity IC_{50} of leaf extract for cold and hot water of R. vomitoria Afzel. Findings presented as Mean ± SD. Data with distinct letters (a-b) exhibit a signficant difference ($P < 0.05$).

4. Discussion

It has been established that single assays are typically insufficient for determining the antioxidant ability of natural substances (Rahman et al., 2015). Hence, we evaluated the antioxidant potency of leaf extracts of *R. vomitoria* Afzel in cold and hot water in this study. The results show that *R. vomitoria* Afzel leaf extract, in both cold and hot water, can reduce the effects of free radical chain reactions. *R. vomitoria* Afzel leaf extract from hot water outperformed leaf extract from cold water in its capacity to snare free radicals, producing stable DPPH• free radicals, although lower than standards employed as a benchmark antioxidant. *R. vomitoria* Afzel's hot water leaf extract demonstrated greater nitric oxide scavenging efficacy than cold water leaf extract. Earlier research by Owoade et al. (2021) and Samuel et al. (2021) opined that *R. vomitoria* Afzel leaf extract shows excellent antioxidant capacity. A molecule's ability to give an atom of hydrogen to a radical is one of the processes behind antioxidant action, and the likelihood of hydrogen donation is a key element in neutralizing free radicals (Hu et al., 2000). The capacity of plant extracts to donate hydrogen is likely what makes them effective at scavenging DPPH and nitric oxide. Total flavonoids, total phenols, tannins, and DPPH radical scavenging ability of *R. vomitoria* Afzel showed a positive correlation, according to Agbodjogbé et al. (2022) and Sofidiya et al. (2012), indicating that these compounds were heavily involved in the scavenging activity of *R. vomitoria* Afzel. Yam et al. (2008) revealed that the antioxidant capacity of *R. vomitoria* Afzel may potentially be attributed to non-phenolic compounds.

In a study by Mier and coworkers (1995), they observed that an effective indicator of a compound's possible antioxidant capacity could be its capacity to reduce. Phenolic components in the *R. vomitoria* Afzel leaf extracts may determine the ferric ion-reducing antioxidant ability. This position was supported by Erasto et al. (2011), who observed a strong correlation between

DOI:https//doi.org/10.22452/mjs.vol45no1.4
Malaysian Journal of Science 45(1): 27-32 (March 2026)

reducing the potential of the phenolic content of *R. vomitoria* Afzel. The formation of the green phosphate/Mo(V) complex at an acidic pH after the reduction of Mo(VI) to Mo(V) provided the basis for the total antioxidant capability of the cold and hot water leaf extract of *R. vomitoria* Afzel. Total antioxidant capacity analyses both fat- and water-soluble antioxidants (Aliyu et al., 2013). Our investigation shows that the hot-water leaf extract of *R. vomitoria* Afzel has a greater overall antioxidant capacity, explained by variations in phytochemical components in extracts from plant leaves obtained with both hot and cold water. This corroborates the work of Akpojotor and Ebomoyi (2021) and Chinonye et al. (2021), who documented the phytochemical constituents of *R. vomitoria* Afzel.

Boiling can enhance the extraction of phytochemicals and antioxidants, boosting their bioavailability, or it can reduce substances that are thought to be antagonists of nutrients, like phytic acid and oxalic acid (Southon & Faulks, 2002; Van Boekel et al., 2010). This may be the cause of the hot-water leaf extracts of *R. vomitoria* Afzel having stronger antioxidant activity than the cold-water leaf extract.

5. Conclusion

The results of the antioxidant assays done in this work indicate that *R. vomitoria* Afzel leaf extract from hot water is superior to leaf extract from cold water as a scavenger of free radicals. The results of this investigation demonstrate the antioxidative capacity of the hot-water leaf extract of *R. vomitoria* Afzel, as well as that of the cold-water extract. It is therefore advised to use *R. vomitoria* Afzel hot-water leaf extracts rather than cold-water leaf extracts for therapeutic applications.

6. Acknowledgement

I appreciate the University of Delta, Agbor, and TETFUND for the Academic Staff Training and Development Intervention Fund for my PhD programme. Thanks to Dr Adu M. E. for proofreading this manuscript.

7. References

Agbodjogbé, K. W. D., Houngbeme, G. A., Oussouami, S. I., Tchikezo, M. and Messan, F. (2022). Phytochemistry, total polyphenol content and antiradical activity of Rauvolfia vomitoria (Apocynaceae), a plant used against asthma in Southern Benin. *International Journal of Advanced Research in Biological Science*, 9(9): 127-136.

Ahmed, S. & Beigh, S. H. (2009). Ascorbic acid, carotenoids, total phenolic content and antioxidant activity of various genotypes of Brassica Oleracea encephala. *Journal of Medical and Biological Sciences*, 3(1): 1–8.

Ajayi, O. A. (2021). Phytochemical and gc-ms analysis of bioactive components in ethanolic extract of Rauvolfia vomitoria leaves. Journal of the Chemical Society of Nigeria, 46(4): 0656 – 0660.

Akpojotor, P. & Ebomoyi, M. I. (2021). Investigating the anti-diabetic phytoconstituents of Rauvolfia vomitoria leaves by Gas Chromatography-Mass Spectrometry (GC-MS). *International Journal of Innovative Research and Advanced Studies*, 8(5): 1–8.

Aliyu, A. B., Ibrahim, M. A., Musa, A. M., Musa, A. O., Kiplimo, J. J., & Oyewale, A. O. (2013). Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis Engl. (Araceae). *Acta Poloniae Pharmaceutica- Drug Research*, 70(1):115–121.

Bemis, D. L., Capodice, J. L, Gorroochurn, P., Katz, A. E., & Buttyan, R. (2006). Anti-prostate cancer activity of a β-carboline alkaloid-enriched extract from Rauwolfia vomitoria. *International Journal of Oncology*, 29: 1065–1073.

Chinonye, I. I., Chijioke, C., Iwuji, C. S., Ifeoma, O., Lynda, U. O., Augusta, U. A., Maureen, C., & Oluchukwu, E. M. (2021). Chemical and medicinal properties of Rauwolfia vomitoria (Afzel) harvested from southeastern Nigeria. *Asian Journal of Chemical Sciences*, 10(4): 56–71.

Chukwu, O., Orhevba, B. & Mahmood, B. (2010). Influence of Hydrothermal Treatments on Proximate Compositions of Fermented Locust Bean (Dawadawa). *Journal of Food Technology*, 8(3), 99–101.

Ekarika, C. J., Emmanuel, I. E., Anwanabasi, E. U., & Ubong, S. B. (2020). Analysis of the Constituents of Rauwolfia vomitoria Ethanol Root Extract using GC-MS. *World Journal of Innovative Research*, 9(2): 32–34.

Erasto, P., Mbwambo, Z. H., Nondo, R. S. O., Lall, N. & Lubschagne, A. (2011). Antimycobacterial, antioxidant activity and toxicity of extracts from the roots of Rauvolfia vomitoria and R. caffra. Spatula DD, 1(2):73-80.

Fannang, S. V., Kuete, V., Mbazoa, C. D., Momo, J. I., Van-Dufat, H. T., Tillequin, F., Seguin, E., *et al.*, (2011). A new acylated triterpene with antimicrobial activity from the leaves of Rauvolfia vomitoria. *Chemical and Natural Compound*, 47: 404.

Fatima, N., Baqri, S.S.R., Alsulimani, A., Fagoonee, S., Slama, P., Kesari, K.K., Roychoudhury, S. & Haque, S. (2021). Phytochemicals from Indian Ethnomedicines: Promising Prospects for the Management of Oxidative Stress and Cancer. *Antioxidants*, 10:1606-1609

Goodarzi, S., Rafiei, S., Javadi, M., Haghighian, H. k., Noroozi, S. A. (2018). Review on Antioxidants and Their Health Effects. *Journal of Nutrition and Food Security*, 3 (2): 106–112.

Hu, C., Zhang, Y. and Kitts, D. D. (2000). Evaluation of antioxidant and prooxidant activities of bamboo Phyllostachys niger Var. Henonis leaf extract in vitro. *Journal of Agriculture, Food, and Chemistry*, 48: 3170–3176.

DOI:https//doi.org/10.22452/mjs.vol45no1.4
Malaysian Journal of Science 45(1): 27-32 (March 2026)

Kumar, B., Kumar, S., Bajpai, V., & Madhusudanan, K. P. (2020). Phytochemistry of Plants of the Genus Rauvolfia. CRC Press, Boca Raton, Florida, United States.

Kumar, S., Bajpai, V., Singh, A., Bindu, S., Srivastava, M., Rameshkumar, K. B., & Kumar, B. (2015). Rapid fingerprinting of Rauwolfia species using direct analysis in real time mass spectrometry combined with principal component analysis for their discrimination. *Analytical Methods*, 7: 6021–6026.

Kumar, S., Kumari, D. & Singh, B. (2022). Genus Rauvolfia: A review of its ethnopharmacology, phytochemistry, quality control/quality assurance, pharmacological activities and clinical evidence. *Journal of ethnopharmacology*, 295: 115327.

Kumar, S., Singh, A., Bajpai, V., & Kumar, B. (2016). Identification, characterization, and distribution of monoterpene indole alkaloids in Rauwolfia species by Orbitrap Velos Pro-Mass Spectrometer. *Journal of Pharmacy and Biomedicals*, 118, 183–194.

Lien, A. P., Hua, H. and Chuong, P. (2008). Free radicals and antioxidants in disease and health: a review. *International Journal of Biomedical Science*, 4(2):89-96.

Mier, S., Kanner, J., Akira, B. & Hdas, S. P. (1995). Antioxidant action of indole compounds during the autoxidation of linoleic acid. *Eiyo Shokuryo*, 19: 210–214.

Oborirhovo O, Anigboro AA, Avwioroko OJ, Akeghware O, Okafor BJ, Ovowa FO. Tonukari NJ. GC-MS characterized bioactive constituents and antioxidant capacities of aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria*: A comparative study. *Nigerian Journal of Science and Environment*, 2023; 21(2): 479 – 499.

Oghbaei, M. & Prakash, J. (2016). Effect of primary processing of cereals and legumes on their nutritional quality: A comprehensive review. *Cogent Food and Agriculture*, 2: 1136015.

Okereke, N. C., Iroka, F. C., Chukwuma, O. M., Kenneth, U. E., & Clement, U. O. (2015). The effect of boiling on the phytochemical and nutritional content of Rauvolfia vomitoria. *Journal of Global Biosciences*, 4(6): 2561–2568.

Onyeukwu, O. B., Ugbebor, G. C. & Iyeh, U. P (2024). Evaluation of the amino acid composition of aqueous and ethanol extracts of *Phyllanthus niruri* stem from Agbor, Nigeria. *FUDMA Journal of Sciences*, 8(4): 62 – 69.

Owoade, A. O., Adetutu, A., Ogundipe, O. O. and Owoade, A. W. (2021). Hypolipidemic potentials of the methanolic extract of Rauvolfia vomitoria leaves in rats fed with high cholesterol. *Asian Plant Research Journal*, 8(4): 15-25.

Pisoschi, A.M., & Negulescu, G.P. (2011). Methods for Total Antioxidant Activity Determination: A Review. *Biochemistry and Analytical Biochemistry*, 1(1):106–118.

Rahman, M., Badrul, I., Mohitosh, B. & Khurshid, A.H.M. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Research Notes*, 8: 621.

Samuel T. A., James, A.B., Adebesin, O., Okafor, I. & Iwalokun, B. A. (2021). Phytochemical Investigation and Anti-Proliferative Effects of Rauvolfia vomitoria, Calliandra portoriscensis and Anthocleista djalonensis on Human Breast Cancer (MCF-7) Cell Line. *African Journal of Biomedical Research*, 24: 403–411.

Sies, H. (1985). Oxidative stress. Academic Press, San Diego, Pg. 1–8.

Sofidiya, M.O., Jimoh, F.O., Aliero, A.A. Afolayan, A.J. Odukoya, O.A. and Familoni, O.B. (2012). Evaluation of antioxidant and antibacterial properties of six Sapindaceae members. *Journal of Medicinal Plants Research*, 6(1):154-160.

Southon, S. & Faulks, R. (2002). Health benefits of increased fruit and vegetable consumption. In Fruit and Vegetable Processing: Improving Quality, 9th ed.; Jongen, W., Ed.; Woodhead Publishing Ltd.: Cambridge, UK, 1, pp. 2–20.

Sunil Kumar (2014). The Importance of Antioxidants and Their Role in Pharmaceutical Science - A Review. *Asian Journal of Research in Chemical and Pharmaceutical Sciences*, 1: 27–44.

Surendran, S., Raju, R., Prasannan, P. & Surendran, A. (2021). A Comprehensive Review on Ethnobotany, Phytochemistry and Pharmacology of Rauvolfia L. (Apocynaceae). *The Botanical Review*, 87(3): 311–376.

Van Boekel, M., Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., Lalljie, S., Somoza, V., Knorr, D. & Jasti, P. R. (2010). A review on the beneficial aspects of food processing. *Molecular Nutrition and Food Research*, 54(9) 1215–1247.

Wolf G, (2005). The discovery of the antioxidant function of vitamin E: the contribution of Henry A. Mattill. *Journal of Nutrition*, 135 (3): 363–366.

Yam, M. F., Basir, R., Asmawi, M. Z., RosidahAhmad, M., & Akowuah, G. A. (2008). Antioxidant and hepatoprotective activities of Elephantopus tomentosus ethanol extract. *Pharmaceutical Biology*, 46: 199–206.

DOI:https//doi.org/10.22452/mjs.vol45no1.4
Malaysian Journal of Science 45(1): 27-32 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 33-44 (March 2026) https://mjs.um.edu.my

Response Surface Methodology–Based Optimization of HPLC Conditions for Quantification of Paracetamol in Indonesian Traditional Medicines (*Jamu*)

Ni Komang Tri Widya Putri[1a], Anggita Rosiana Putri[2ab]* and Bachtiar Rifai Pratita Ihsan[3ab]

Abstract: Indonesian traditional herbal medicines (Jamu) are prohibited from containing active pharmaceutical ingredients (APIs). Nevertheless, reports indicate that some hyperuricemia jamu products still contain such substances, including paracetamol This study aimed to optimize High-Performance Liquid Chromatography (HPLC) conditions and to validate an analytical method for the determination of paracetamol in jamu for hyperuricemia obtained from Pasar Besar, Malang City. Response Surface Methodology (RSM) combined with a Box–Behnken Design (BBD) was employed to optimize the HPLC conditions. The investigated parameters were the methanol proportion in the mobile phase (X_1, 30–90% v/v), flow rate (X_2, 0.5–1.0 mL/min), and column temperature (X_3, 20–30 °C). The evaluated response variables included peak area (Y_1), resolution (Y_2), tailing factor (Y_3), and theoretical plate number (Y_4). The optimal HPLC conditions consisted of 30% v/v methanol in aquadest, a flow rate of 1.0 mL/min, and a column temperature of 25.18 °C. Method validation demonstrated satisfactory selectivity (λmax 245 nm; paracetamol retention time ± 3.33 min), linearity (r^2 = 0.99), limit of detection (LOD) of 1.51 ppm, and limit of quantification (LOQ) of 5.03 ppm. The method also showed acceptable accuracy (101.75–104.61%) and precision (0.53–1.59%), fulfilling the

Keywords: *Jamu for hyperuricemia, paracetamol, HPLC, response surface methodology, Box-Behnken design.*

1. Introduction

Jamu is a form of Indonesian traditional herbal medicine that has been widely consumed, as its efficacy is traditionally believed to be based on hereditary knowledge and long-standing empirical use (Sumarni et al., 2019). According to the Regulation of the Minister of Health of the Republic of Indonesia No. 7 of 2012 on the registration of traditional medicines, such products are strictly prohibited from containing active pharmaceutical ingredients (APIs), defined as isolated or synthetic substances with pharmacological activity. Nevertheless, the intentional addition of APIs to traditional herbal medicines is often carried out to enhance or provide rapid therapeutic effects. A public report released by the Indonesian Food and Drug Authority (BPOM) in 2022 revealed that 95 traditional medicines and health supplements were found to contain undeclared APIs. The long-term consumption of such adulterated products may lead to serious health risks, including organ dysfunction and even death (Putri et al., 2023).

One of the APIs frequently detected in traditional herbal medicines is paracetamol. Paracetamol is a widely used analgesic and antipyretic agent. Due to its pharmacological effects, paracetamol is often illicitly added to herbal products intended to relieve pain and inflammation, including *Jamu* for hyperuricemia (Ashraful Islam et al., 2011). *Jamu* for hyperuricemia is formulated to alleviate symptoms associated with elevated uric acid levels and to reduce serum uric acid concentration. Hyperuricemia can cause various clinical manifestations, such as musculoskeletal pain, joint stiffness, and inflammatory responses in the joints (Savitri, 2017). Consequently, the presence of paracetamol in such products may provide symptomatic relief, thereby misleading consumers regarding the true efficacy of the herbal formulation. However, excessive or prolonged intake of paracetamol is associated with adverse effects, including gastrointestinal disturbances, renal impairment, cardiovascular disorders, and hepatotoxicity (Chidiac et al., 2023; McCrae et al., 2018).

Several analytical techniques have been reported for the determination of paracetamol in herbal medicines, including High-Performance Liquid Chromatography (HPLC) (Pratama et al., 2022; Wisnuwardhani et al., 2018), thin-layer chromatography (Fitrianasari et al., 2023), liquid chromatography–mass spectrometry (LC–MS) (Taupik et al., 2022), and UV–Vis spectrophotometry (Husain et al., 2023). In the present study, reverse-phase High-Performance Liquid Chromatography (RP-HPLC) was selected for paracetamol analysis. Compared with other analytical techniques, HPLC offers superior sensitivity and selectivity, particularly for quantitative analysis, enabling the detection and accurate quantification of analytes at low concentration levels. Moreover, HPLC provides efficient

Authors information:
[a]Department of Pharmacy, Faculty of Medicine, Universitas Brawijaya, Malang, INDONESIA. E-mail: komangputri112@student.ub.ac.id[1]; anggita.rosiana@ub.ac.id[2]; bachtiar_pharm@ub.ac.id[3]
[b]Drug Development and Analytical Methods Research group, Faculty of Medicine, Universitas Brawijaya, INDONESIA. E-mail: anggita.rosiana@ub.ac.id[2]; bachtiar_pharm@ub.ac.id[3]
*Correspondence: anggita.rosiana@ub.ac.id

Received: August, 2024
Accepted: November, 2024
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

separation of complex matrices and shorter analysis times compared to thin-layer chromatography, making it more suitable for routine analysis.

RP-HPLC was chosen due to its versatility and applicability to a wide range of analytes, particularly polar and moderately ionic compounds (Hameedat et al., 2022; Gupta et al., 2022). This characteristic makes RP-HPLC especially suitable for analyzing paracetamol in *Jamu*, which contains a complex mixture of bioactive constituents. To achieve optimal chromatographic performance and comply with analytical validation requirements, RP-HPLC conditions must be carefully optimized. Therefore, this study applied Response Surface Methodology (RSM) using a Box–Behnken Design (BBD) to optimize key RP-HPLC parameters, including methanol composition, flow rate, and column temperature. The evaluated response variables were peak area, resolution, tailing factor, and the number of theoretical plates.

2. Experimental Section

Apparatus

An HPLC i-Series LC-2030C 3D Plus system (Shimadzu, Japan) was employed for method development and validation. The system was equipped with a quaternary pump, autosampler, column oven, and a photodiode array (PDA) UV detector. Chromatographic separation was performed using a C18 ODS column (150 mm × 4.6 mm i.d., 5 µm particle size) as the stationary phase.

The mobile phase consisted of methanol and water for injection (WFI) and was delivered under gradient elution conditions. The aqueous phase was filtered twice prior to use. All solutions were filtered through a 0.45 µm nylon syringe filter (13 mm diameter) before analysis. Detection was carried out at a wavelength of 245 nm, and the injection volume was set at 20 µL.

HPLC data acquisition and processing were performed using LabSolutions software (Shimadzu, Japan). Optimization and statistical analysis were conducted using Statgraphics Centurion 18 software (*Statgraphics Centurion* Inc., Virginia, USA).

Reagents and Materials

HPLC-grade methanol was purchased from Fisher Scientific (Fair Lawn, USA). Water for injection (WFI) was obtained from a local pharmacy. The crude drugs, namely *Sonchus arvensis*, *Biancaea sappan*, *Stelechocarpus burahol* L., *Curcuma xanthorrhiza*, *Curcuma longa*, and *Phyllanthus niruri*, were purchased from a local herbal store and obtained in powdered form.

Jamu samples for hyperuricemia were collected from Pasar Besar, Malang City, and labeled as AU1, AU2, and AU3. The samples originated from different commercial brands with undisclosed ingredient compositions.

Optimization of HPLC Condition

Response Surface Methodology (RSM) combined with a Box–Behnken Design (BBD) was applied to optimize the RP-HPLC conditions. The independent variables investigated were methanol proportion in the mobile phase (X_1, % v/v), flow rate (X_2, mL/min), and column temperature (X_3, °C). The evaluated response variables were peak area (Y_1), resolution (Y_2), tailing factor (Y_3), and the number of theoretical plates (Y_4).

The BBD experimental design, consisting of 15 experimental runs, was generated using Statgraphics Centurion 18 software (Statgraphics Centurion Inc., Virginia, USA). The experimental levels of methanol proportion, flow rate, and column temperature were defined according to the Box–Behnken Design, as presented in Table 1. All experiments were conducted in accordance with the designed matrix, and the response model coefficients were obtained by fitting the experimental data to a second-order polynomial equation. Analysis of variance (ANOVA) was subsequently performed to evaluate the significance and adequacy of the developed model.

Table 1. Independent Variables and Their Levels in the Box–Behnken Design

Variables Independent	Level			Unit
	-1	0	1	
Ratio of methanol (X_1)	30	60	90	% v/v
Flow rate (X_2)	0.5	0.75	1	mL/minutes
Column temperature (X_3)	20	25	30	℃

Preparation of *Jamu* for Hyperuricemia

Preparation of *Jamu* Matrix

The *Jamu* matrix for hyperuricemia was prepared by weighing powdered crude drugs consisting of 10.53% *Sonchus arvensis*, 26.32% *Biancaea sappan*, 15.79% *Stelechocarpus burahol* L., 15.79% *Curcuma xanthorrhiza*, 15.79% *Curcuma longa*, and 15.79% *Phyllanthus niruri* to obtain a total weight of 10 g. All components were thoroughly mixed and homogenized to obtain a uniform *Jamu* powder matrix.

Preparation of *Jamu* Matrix Solution

The *Jamu* matrix solution was prepared by dissolving 100 g of the control herbal powder in 100 mL of HPLC-grade methanol. The mixture was vortexed for 2 min to ensure complete homogenization.

Preparation of *Jamu* Matrix Spiked with Paracetamol (25 ppm)

A spiked *Jamu* matrix solution containing paracetamol at a concentration of 25 ppm was prepared by transferring 2.5 mL of a 100 ppm paracetamol standard solution into a 10 mL volumetric flask and diluting to volume with the *Jamu* matrix solution.

Preparation of Paracetamol Standard Solution

A paracetamol standard solution was prepared by accurately weighing 10 mg of paracetamol and dissolving it in 10 mL of HPLC-grade methanol to obtain a stock solution with a concentration of 1000 ppm. Appropriate dilutions were prepared as required.

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

Sample Preparation

Each *Jamu* sample for hyperuricemia was accurately weighed (100 mg) and dissolved in 100 mL of HPLC-grade methanol. The solution was vortexed for 2 min to ensure homogeneity and subsequently filtered through a 0.45 µm membrane filter into an HPLC vial. Each sample was prepared in triplicate.

System Suitability Test (SST)

The performance of the HPLC system was verified using a system suitability test (SST) in accordance with the Indonesian Pharmacopoeia, 6th edition, which requires reproducible chromatographic performance prior to analysis. The SST was conducted using six replicate injections of paracetamol standard solutions under the optimized chromatographic conditions obtained after BBD optimization.

The evaluated SST parameters included retention time, peak area, resolution, tailing factor, and the number of theoretical plates. System suitability was considered acceptable when the relative standard deviation (RSD) for all parameters was less than 2%.

Analytical Method Validation

The HPLC method was validated by evaluating its performance characteristics according to the guidelines of the International Council for Harmonisation (ICH) and the Association of Official Analytical Chemists (AOAC).

Specificity

Specificity was assessed by injecting the *Jamu* matrix solution, *Jamu* matrix spiked with paracetamol (25 ppm), and three commercial *Jamu* samples (AU1, AU2, and AU3). Specificity was confirmed by comparing retention times, chromatographic profiles, and PDA spectral overlays between the samples and the paracetamol reference standard.

Linearity

Linearity was evaluated by constructing a calibration curve using five paracetamol concentrations (10, 20, 30, 40, and 50 ppm). The peak area was plotted against the corresponding concentration, and linear regression analysis was performed. Linearity was considered acceptable when the correlation coefficient (r) exceeded 0.99, in accordance with AOAC guidelines (AOAC International, 2013).

Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ were calculated using the following equations:.

$$LOD = \frac{Sy}{b} \times 3 \quad (1)$$

$$LOQ = \frac{Sy}{b} \times 10 \quad (2)$$

$$Sy = \sqrt{\frac{\Sigma(y-yi)^2}{n-2}} \quad (3)$$

where b is the slope of the calibration curve, Sy is the standard deviation of regression, y is the observed peak area, y_i is the predicted peak area from the regression equation, and n is the number of calibration points (Ihsan et al., 2022).

Accuracy and Precision

Accuracy and precision were evaluated using *Jamu* matrix solutions spiked with paracetamol at concentrations of 20, 25, and 30 ppm. Accuracy was expressed as percent recovery (% recovery), while precision was expressed as the relative standard deviation (%RSD). According to AOAC Appendix K, acceptable criteria were % recovery in the range of 92–105% and %RSD less than 2%.

Determination of Paracetamol Content in *Jamu* Samples

Quantification of paracetamol in *Jamu* samples was performed for samples that tested positive for paracetamol during the specificity assessment. The paracetamol concentration was calculated by substituting the measured peak area into the linear regression equation obtained from the calibration curve.

3. Result and Discussion

This study applied a quantitative experimental approach to optimize HPLC conditions and validate an analytical method for the determination of paracetamol in *Jamu* for hyperuricemia. The optimized chromatographic conditions fulfilled the predefined acceptance criteria, as summarized in Table 2. These criteria included adequate selectivity, demonstrated by consistent peak shape and retention time of the analyte compared with the reference standard; satisfactory peak resolution; acceptable peak symmetry, expressed as the tailing factor; and high column efficiency, indicated by the number of theoretical plates.

The results indicate that the chromatographic performance was strongly influenced by key HPLC parameters, namely the methanol proportion in the mobile phase, flow rate, and column temperature. Optimization of these parameters was therefore essential to achieve reliable separation and accurate quantification of paracetamol in a complex *Jamu* matrix. Following optimization and method validation, the developed RP-HPLC method was subsequently applied to the analysis of paracetamol in commercial *Jamu* products for hyperuricemia marketed at Pasar Besar, Malang City.

Three *Jamu* samples, coded AU1, AU2, and AU3, were selected based on predefined inclusion and exclusion criteria. The inclusion criteria comprised products with indications related to pain relief, ease of purchase in the local market, and either registered or unregistered status with the Indonesian Food and Drug Authority (BPOM). Products in liquid dosage form, expired products, and samples with damaged packaging were excluded from the analysis.

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

Table 2. Acceptance Criteria for Chromatographic Responses

Responses	Acceptance Criteria
RSD of peak area (Y_1)	< 2.0 %
Resolution (Y_2)	≥ 2.0
Tailing factor (Y_3)	< 2
Theoretical plate (Y_4)	> 2000

Design for HPLC Optimization

Response Surface Methodology combined with a Box–Behnken Design (RSM–BBD) was employed to optimize the RP-HPLC conditions. This design was selected because it requires a relatively small number of experimental runs, thereby reducing analysis time and experimental cost. In addition, the Box–Behnken Design enables the evaluation of linear, quadratic, and interaction effects of independent variables on the selected responses.The independent variables investigated were the methanol proportion in the mobile phase (X_1), flow rate (X_2, mL/min), and column temperature (X_3, °C), each examined at three levels (−1, 0, +1). The evaluated response variables included peak area (Y_1), resolution (Y_2), tailing factor (Y_3), and the number of theoretical plates (Y_4). A summary of the BBD experimental design is presented in Table 3.

The BBD consisted of 15 experimental runs, including three replicates at the center point and twelve runs at factorial (edge) points. The center point replicates were used to estimate the pure experimental error, while the randomized runs minimized the influence of uncontrolled variables and potential systematic bias (Elkady et al., 2022). Based on the preliminary chromatographic evaluation, some experimental runs yielded responses in which resolution and theoretical plate values could not be determined. This result indicates inadequate peak separation and/or unsuitable peak shapes, which prevent reliable calculation of these chromatographic parameters (Barth, 2019; Dolan, 2012; Wahab et al., 2017). The experimental data were subsequently analyzed using multiple regression analysis to model the relationship between the independent variables and the measured responses. The quadratic polynomial equations describing each response are presented below.

Y_1 (Peak area) $= 2.22\times10^6 + 114062A - 9.86\times10^6 \times B + 74262.9C - 232.614A^2 - 77107.2\times AB - 110.35A\,C + 7.01\times10^6 \times B^2 + 40001.8BC - 2086.3C^2$

Y_2 (Resolution) $= -6.32 + 0.07A + 6.34B + 0.21C + 0.00A^2 - 0.11AB - 0.00AC - 0.12B^2 + 0.07BC - 0.00C^2$

Y_3 (Tailing factor) $= -2.56 - 0.04A - 0.8B + 0.29C + 0.00A^2 - 0.0343333AB + 0.0000966667AC + 2.316B^2 + 0.0BC - 0.00579C^2$

Y_4 (Theoretical plates) $= -33810.6 + 597.632A - 15448.0B + 2185.74C - 3.53718A^2 - 145.0\,AB - 1.10833AC + 16892.7B^2 - 104.4BC - 40.2583C^2$

Table 3. Box–Behnken Design Matrix and Experimental Responses

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	0	-1	-1	3885509	0.85	-	7028
2	1	1	0	2246871	-	0.86	3869
3	1	0	1	2993366	-	0.82	3659
4	1	-1	0	4536657	-	0.76	5777
5	0	1	-1	1945259	0.19	-	5603
6	1	0	-1	2977228	-	0.77	3732
7	-1	0	1	1409220	1.76	-	854
8	-1	0	-1	1326872	1.26	-	262
9	0	-1	1	3602825	0.98	-	7390
10	0	0	0	2429075	0.76	-	6425
11	-1	0	0	1930220	-	-	2334
12	0	0	0	2400557	0.75	-	6444
13	0	1	1	1862584	0.67	-	5443
14	-1	1	0	1953650	3.37	1.13	4776
15	0	0	0	2484912	0.81	-	6081

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

Statistical Analysis

The statistical significance of the developed models was evaluated using analysis of variance (ANOVA). The ANOVA results are summarized in Table 4. A model or individual term was considered statistically significant when the corresponding p-value was less than 0.05.

Table 4. Analysis of Variance (ANOVA) Summary for the Developed Models

	P-value for each model of responses			
Source	Y1	Y2	Y3	Y4
A	0.00	0.02	0.04	0.02
B	0.00	0.27	0.16	0.30
C	0.71	0.60	0.94	0.78
AA	0.16	0.84	0.01	0.00
AB	0.01	0.06	0.11	0.05
AC	0.90	0.73	0.92	0.72
BB	0.02	0.98	0.34	0.07
BC	0.70	0.81	1	0.78
CC	0.70	0.80	0.34	0.08

A: methanol proportion in the mobile phase, B: flow rate, C: column temperature, AA: quadratic effect of methanol proportion, AB: interaction effect between methanol proportion and flow rate, AC: interaction effect between methanol proportion and column temperature, BB: quadratic effect of flow rate, BC: interaction effect between flow rate and column temperature, CC: quadratic effect of column temperature.

Optimization and Effects on the Responses

Based on the ANOVA results (Table 4), the methanol proportion exhibited *p*-values < 0.05, indicating that this variable had a statistically significant effect on all evaluated responses. According to the Pareto chart for peak area (Figure 1A), the methanol proportion showed a positive effect on peak area. In chromatographic analysis, a peak represents the presence of a specific compound in a sample, and its area is directly proportional to the concentration of the analyte (Ministry of Health of the Republic of Indonesia, 2020; Chulikhit et al., 2023). In RP-HPLC, nonpolar compounds tend to be retained longer by the stationary phase, whereas polar compounds are eluted earlier, particularly when a polar mobile phase is used (Lundanes et al., 2014). As paracetamol is a polar compound, it interacts more favorably with the polar mobile phase. Increasing the methanol proportion enhances this interaction, resulting in improved elution and higher detector response, which is reflected by an increased peak area. Flow rate also exerted a significant influence on peak area. As shown in the Pareto chart (Figure 1A), flow rate had a negative effect on peak area, indicating that higher flow rates led to a reduction in peak area. This phenomenon occurs because increasing the flow rate shortens the retention time, limiting the establishment of equilibrium between the mobile and stationary phases. Consequently, separation efficiency decreases, leading to peak broadening. Pronounced peak broadening may reduce the overall peak area detected by the system (Koszur, 2023).

Regarding resolution (Figure 1B), the methanol proportion exhibited a negative effect. In RP-HPLC, increasing the methanol content reduces the polarity of the mobile phase, thereby weakening the interaction between polar analytes and the stationary phase. As a result, polar analytes are eluted more rapidly, which reduces the retention factor (*k*) and adversely affects chromatographic resolution. The distribution of analytes between the mobile and stationary phases plays a crucial role in determining both retention behavior and resolution (Ministry of Health of the Republic of Indonesia, 2020; Meyer, 2010).

The Pareto chart for the tailing factor (Figure 1C) indicated that the quadratic effect of methanol proportion positively influenced peak symmetry. Gradual increases in the methanol proportion improved peak symmetry, resulting in lower tailing factors. Methanol, as a polar solvent, enhances analyte solubility in the mobile phase, facilitating mass transfer and reducing analyte adsorption onto the stationary phase. This solvation effect produces narrower and more symmetrical peaks. However, beyond the optimal methanol proportion, further increases resulted in tailing factor values that no longer met the acceptance criteria, indicating deteriorated chromatographic performance (Dolan, 2012; Wahab et al., 2017).

Similarly, the quadratic effect of methanol proportion had a positive influence on the number of theoretical plates (Figure 1D). This relationship followed a quadratic trend rather than a linear one. Initially, increasing the methanol proportion enhanced column efficiency, as indicated by a higher number of theoretical plates, due to improved mass transfer and peak sharpening. However, exceeding the optimal methanol proportion led to band broadening and reduced column efficiency, ultimately decreasing the number of theoretical plates (Kazmouz et al., 2022).

Based on the 15 experimental runs generated by the Box–Behnken Design, the experimental data were processed using Statgraphics Centurion 18 to develop predictive models. Three-dimensional response surface plots (Figure 2) were constructed to visualize the effects of the independent variables on the responses. The optimized RP-HPLC conditions were determined to be a methanol proportion of 30% v/v, a flow rate of 1.0 mL/min, and a column temperature of 25.18 °C. Under these conditions, all evaluated responses met the predefined acceptance criteria. The optimized method was subsequently subjected to validation prior to its application for paracetamol analysis in *Jamu* samples.

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

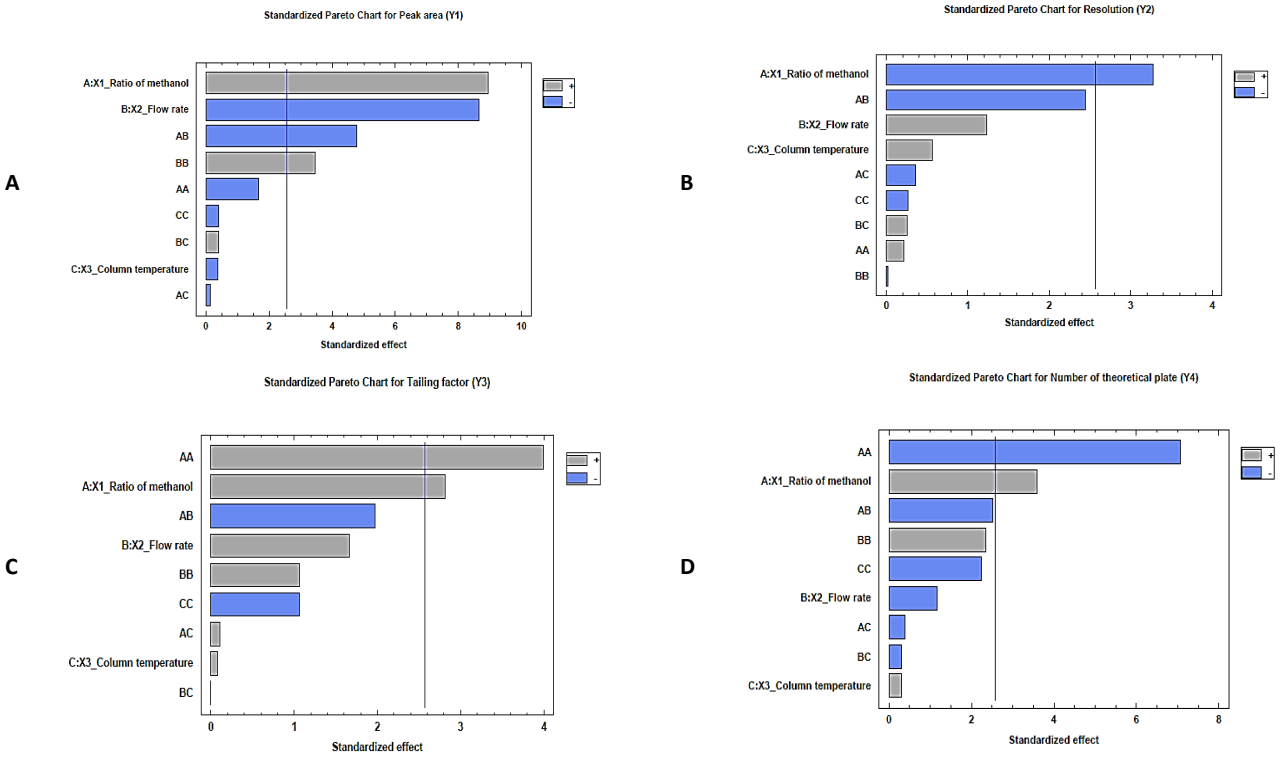
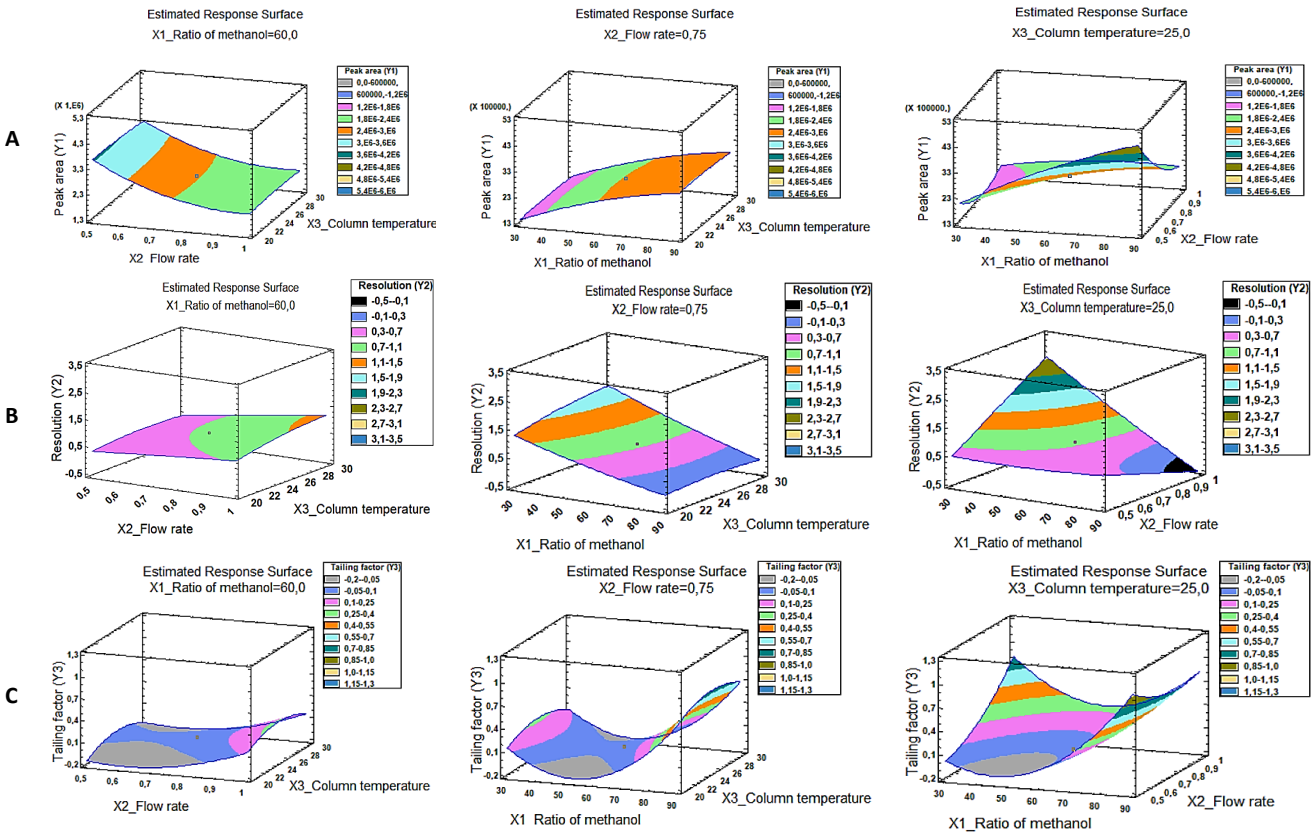


Figure 1. Pareto charts for (A) peak area, (B) resolution, (C) tailing factor, and (D) number of theoretical plates



DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

D

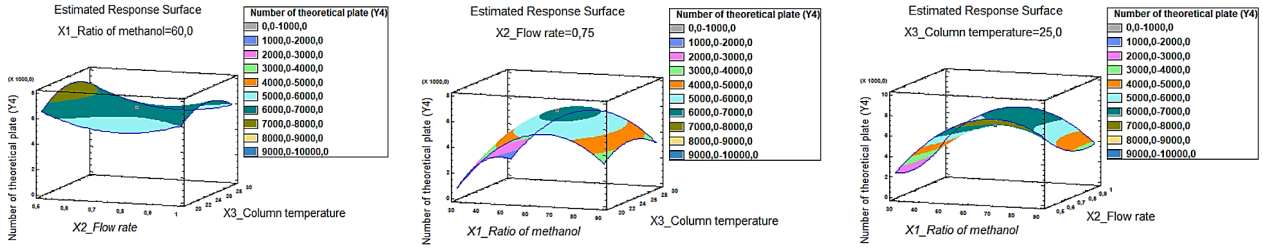


Figure 2. Three-dimensional response surface plots illustrating the effects of HPLC variables on (A) peak area, (B) resolution, (C) tailing factor, and (D) number of theoretical plates

Validation Method

System Suitability Test

The system suitability test (SST) was performed to verify the capability of the chromatographic system to provide reproducible and reliable analytical performance prior to sample analysis. According to the Indonesian Pharmacopoeia (6th edition), acceptable relative standard deviation (RSD) values for retention time, peak area, resolution, tailing factor, and number of theoretical plates should be less than 2% (Ministry of Health of the Republic of Indonesia, 2020).

Retention time consistency ensures reliable analyte identification by preventing peak position shifts. Peak area reflects the method's sensitivity and its ability to detect and quantify low analyte concentrations, which is critical for quantitative analysis. Resolution indicates the degree of peak separation, ensuring accurate quantification of individual components. The tailing factor evaluates peak symmetry, with values close to unity indicating well-shaped peaks suitable for precise integration. The number of theoretical plates represents column efficiency, with higher values indicating improved separation performance (Snow, 2021).

As summarized in Table 5, the RSD values for all evaluated SST parameters were below 2%, confirming that the chromatographic system was suitable for subsequent analysis.

Table 5. Results of the System Suitability Test (SST)

Run	Retention time	Peak Area	Resolution	Tailing Factor	Theoretical Plates
1	3.150	2207219	3.36	1.19	4557
2	3.148	2214299	3.32	1.20	4560
3	3.146	2197133	3.29	1.19	4560
4	3.149	2208261	3.31	1.20	4595
5	3.138	2196590	3.31	1.19	4551
6	3.148	2205841	3.32	1.20	4592
%RSD	0.25	0.00	0.25	0.41	0.01

Selectivity

Selectivity was evaluated using *Jamu* matrix solution for hyperuricemia, *Jamu* matrix spiked with paracetamol (25 ppm), paracetamol reference standard (30 ppm), and commercial *Jamu* samples AU1, AU2, and AU3. Selectivity was assessed by comparing chromatographic profiles, retention times, and PDA spectral overlays between the reference standard and sample solutions.

The selectivity results demonstrated comparable retention times between the paracetamol reference standard (3.33 min), paracetamol in the spiked *Jamu* matrix (3.33 min), and paracetamol detected in the AU1 sample (3.13 min). In addition, the analyte peaks were well resolved from the matrix components, and the PDA spectra of paracetamol in the spiked matrix and AU1 sample closely matched that of the reference standard, confirming the specificity of the method (Figure 3).

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

a

b

c

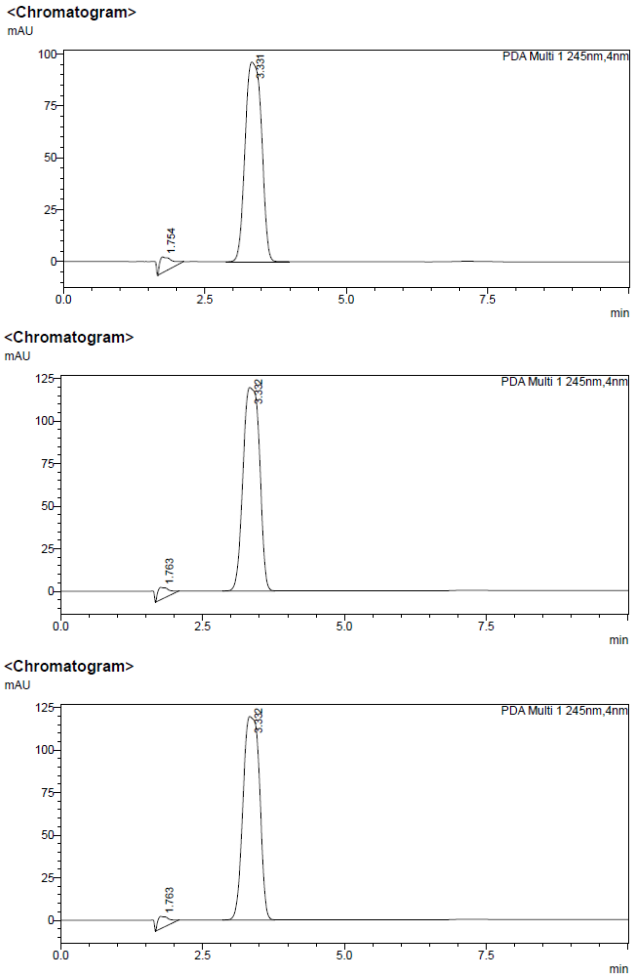


Figure 3. RP-HPLC chromatograms of (a) *Jamu* matrix solution, (b) paracetamol reference standard, and (c) *Jamu* matrix spiked with paracetamol

Linearity

Linearity was evaluated using paracetamol standard solutions at concentrations of 10, 20, 30, 40, and 50 ppm. Then data processing was carried out so that the regression equation y = 88110x - 43113, with a coefficient of determination (r^2) of 0.9992. These results indicate excellent linearity over the investigated concentration range and satisfy the acceptance criteria for quantitative analysis (Figure 4).

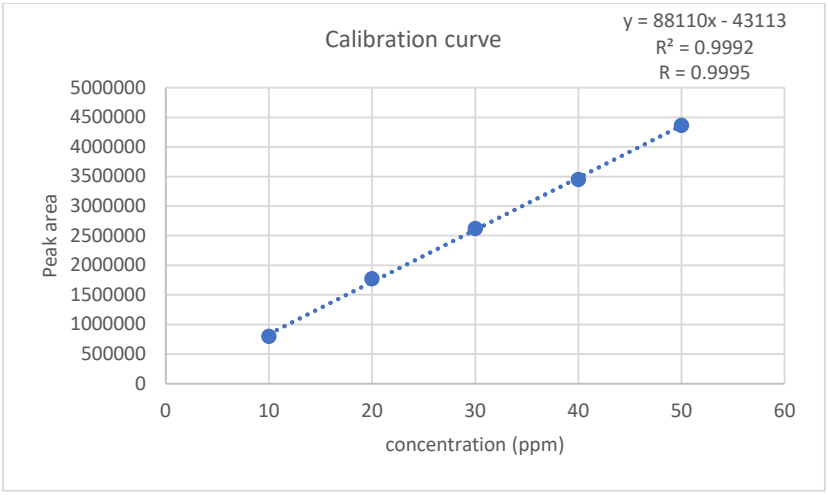


Figure 4. Calibration curve of paracetamol obtained using the optimized RP-HPLC method

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

Limit of Detection and Quantification

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ) based on the standard deviation of the regression and the slope of the calibration curve. The LOD and LOQ values were found to be 1.51 ppm and 5.03 ppm, respectively, demonstrating the suitability of the method for detecting low levels of paracetamol in complex *Jamu* matrices.

Accuracy and Precision

Accuracy represents the closeness of agreement between the measured value and the true value, whereas precision reflects the repeatability of the analytical procedure under identical conditions. According to AOAC guidelines, acceptable accuracy is indicated by percent recovery within the range of 92–105%, while precision is considered acceptable when the %RSD is less than 2% (AOAC International, 2013). Accuracy and precision were evaluated using *Jamu* matrix solutions spiked with paracetamol at concentrations of 20, 25, and 30 ppm. The average percent recoveries ranged from 101.75% to 104.61%, while the %RSD values ranged from 0.53% to 1.59% (Table 6). These results demonstrate that the method exhibits satisfactory accuracy and precision and meets the established validation criteria.

Based on the comprehensive validation results, the developed RP-HPLC method was deemed reliable and suitable for the quantitative determination of paracetamol in *Jamu* for hyperuricemia. Accuracy is the closeness between the results obtained from the validated procedure and the correct value. The acceptance criterion for the accuracy test is % recovery around 92-105%. Precision is the closeness between individual results where the procedure is repeatedly applied to multiple sample or homogeneous samples. The acceptance criterion for the precision test is % RSD < 2% (AOAC International, 2013). Based on the calculation, the average % recovery and %RSD were 101.75 - 104.61% and 0.53 - 1.59%, respectively. Therefore, the result of accuracy and precision tests have met the acceptance requirements (Table 6).

Table 6. The result of the accuracy and precision test

Theoretical Conc. (ppm)	Measured Conc. (ppm)	% Recovery	Average % Recovery	% RSD
	20.95	104.76		
20	21.01	105.07	104.61	0.53
	20.80	103.99		
	25.80	103.20		
25	25.08	100.31	102.18	1.59
	25.76	103.04		
	30.34	101.14		
30	30.53	101.77	101.75	0.59
	30.70	102.35		

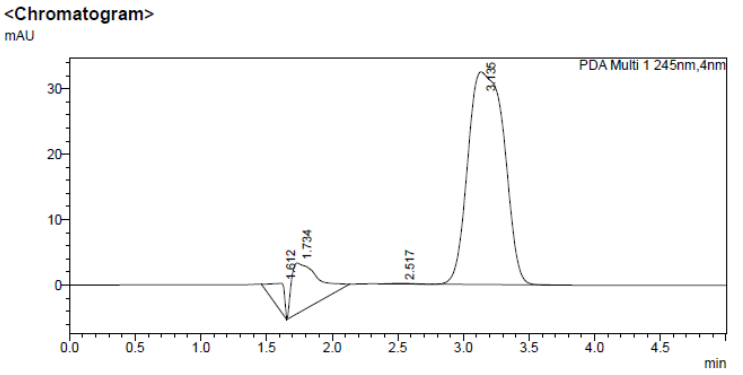
Determination of Paracetamol in Sample

The determination of paracetamol content was performed on sample AU1, as this sample tested positive for paracetamol during the selectivity assessment. Quantification was conducted in triplicate using the validated RP-HPLC method. The average paracetamol content in sample AU1 was found to be 5.08% w/w.

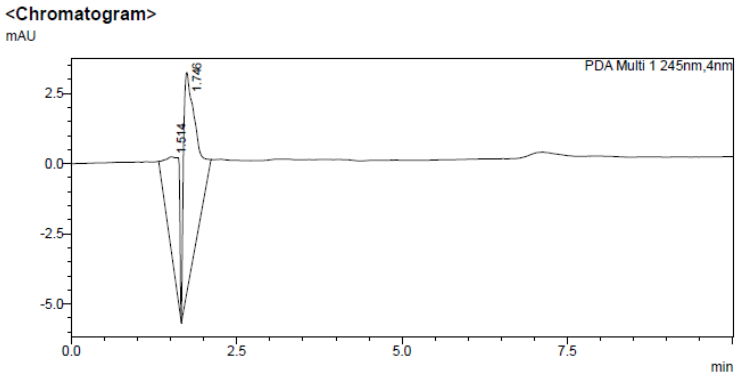
The presence of paracetamol at this concentration indicates that sample AU1 contains an undeclared active pharmaceutical ingredient, thereby violating the applicable regulations governing traditional medicines. Representative chromatograms of AU1, AU2, and AU3 are shown in Figure 5, While the PDA spectral overlay confirming the identity of paracetamol in AU1 is presented in Figure 6.

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

a



c



b

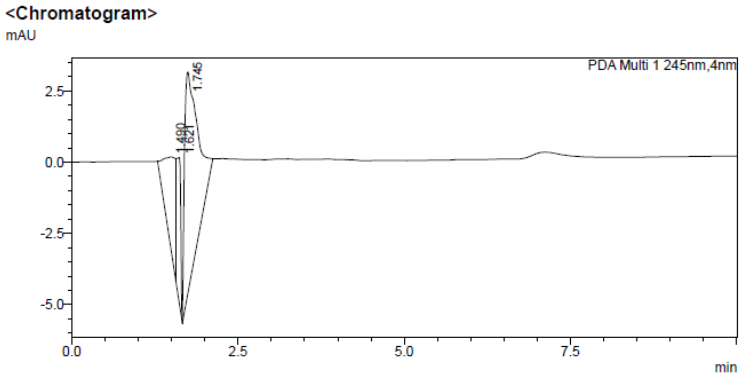


Figure 5. RP-HPLC chromatograms of (a) AU1, (b) AU2, and (c) AU3 samples

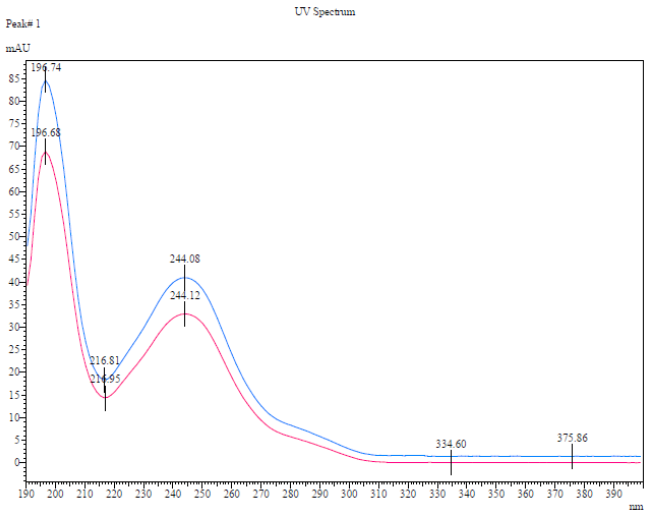


Figure 6. Overlay UV–Vis spectra of paracetamol reference standard (blue) and AU1 sample (pink)

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

4. Conclusion

An RP-HPLC method was successfully optimized using Response Surface Methodology combined with a Box–Behnken Design for the analysis of paracetamol in *Jamu* for hyperuricemia. The optimized chromatographic conditions consisted of a methanol proportion of 30% v/v, a flow rate of 1.0 mL/min, and a column temperature of 25.18 °C. All validation parameters, including system suitability, selectivity, linearity, sensitivity, accuracy, and precision, met the established acceptance criteria.

Application of the validated method to commercial *Jamu* samples obtained from Pasar Besar, Malang City, revealed that sample AU1 contained paracetamol at an average concentration of 5.08% w/w. This finding indicates a violation of the Regulation of the Minister of Health of the Republic of Indonesia No. 7 of 2012 concerning the registration of traditional medicines. The developed method demonstrates strong potential for application in the routine surveillance and analysis of undeclared active pharmaceutical ingredients in traditional medicines containing analytes with physicochemical properties similar to paracetamol.

5. References

AOAC International. (2013). Guideline for Dietary Supplements and Botanical (Appendix K). *AOAC Off. Method Anal*, 8–12.

Ashraful Islam, S., Md Abuzar, S., & Kumar Paul, P. (2011). Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of Paracetamol and Aceclofenac in marketed tablets. *Int. J. of Pharm. & Life Sci. (IJPLS)*, *2*(12), 1267–1275.

Azmi, S. N. H., Al Hoqani, U., Al Mamari, J. O. S., Al Mamari, B. M. S., Al Jassasi, B. S. A. R., Al Rubaiai, A. S. S., Rahman, N., Nasir, M., Haque, S. M., Khan, S. A., Ahmed, Q. U., & Zakaria, Z. A. (2022). Box–Behnken Design Based Development of UV-Reversed Phase High Performance Liquid Chromatographic Method for Determination of Ascorbic Acid in Tablet Formulations. *Separations*, *9*(11), 1–21. https://doi.org/10.3390/separations9110361.

Barth, H., 2019. Chromatography fundamentals, part VIII: The meaning and significance of chromatographic resolution. *LCGC Europe*, *37*(11), 824-828.

Chidiac, A. S., Buckley, N. A., Noghrehchi, F., & Cairns, R. (2023). Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. *Expert Opinion on Drug Metabolism and Toxicology*, *19*(5), 297–317. https://doi.org/10.1080/17425255.2023.2223959.

Dolan, J., 2012. Troubleshooting basics, Part IV: peak shape problems. *LCGC Europe*, *30*(7), 564-569.

Gupta, M.K., Ghuge, A., Parab, M., Al-Refaei, Y., Khandare, A., Dand, N. and Waghmare, N., 2022. A comparative review on high-performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC) & high-performance thin layer chromatography (HPTLC) with current updates. *Current Issues in Pharmacy and Medical Sciences*, *35*(4), pp.224-228.

Fitrianasari, R. D. P., Haresmita, P. P., & Agusta, H. F. (2023). Qualitative Analysis of Drug Substances in Rheumatic Jamu Samples Using Thin Layer Chromatography. *Medical Sains : Jurnal Ilmiah Kefarmasian*, *8*(3), 999–1008. https://doi.org/10.37874/ms.v8i3.862

Hameedat, F., Hawamdeh, S., Alnabulsi, S. and Zayed, A., 2022. High performance liquid chromatography (HPLC) with fluorescence detection for quantification of steroids in clinical, pharmaceutical, and environmental samples: A review. *Molecules*, *27*(6), p.1807.

Husain, F., Ysrafil, Y., Daud, R. P. A., Aisyah, A. N., Fadri, A., & Nur, S. (2023). Authentication of Medicinal Chemicals in Traditional Herbal Products (Jamu) by UV-Vis Spectrophotometry. *Hacettepe University Journal of the Faculty of Pharmacy*, *43*(2), 100–110. https://doi.org/10.52794/hujpharm.1090673

Ihsan, B. R. P., Nurhayati, I. P., & Maysaroh, I. (2022). Performance Chromatography Double Mass Spectrometry (UHPLC-MS / MS) Validasi Metode Ultra High Performance Chromatography Double Mass Spectrometry (UHPLC-MS / MS) untuk Analisis Kurkumin pada Ekstrak Etanol Kunyit (Curcuma longa) dengan Berbagai Per. *Pharmaceutical Journal of Indonesia*, *4*(1), 29–34. https://doi.org/10.21776/ub/pji

Kazmouz, M.Y. and Felinger, A., 2023. The competitive adsorption of water and methanol on a hybrid silica stationary phase in supercritical fluid chromatography. *Separations*, *10*(9), p.492.

Kementerian Kesehatan Republik Indonesia. (2020). Farmakope Indonesia edisi VI. In *Departemen Kesehatan Republik Indonesia*.

Koszur, S. 2021. Real solution to improve your HPLC peak resolution. *Thermo Fischer Scintific.*

McCrae, J. C., Morrison, E. E., MacIntyre, I. M., Dear, J. W., & Webb, D. J. (2018). Long-term adverse effects of paracetamol – a review. *British Journal of Clinical Pharmacology*, *84*(10), 2218–2230. https://doi.org/10.1111/bcp.13656

Pratama, M., Harisman, D., Labasy, L., Seniwati, & Fawwaz, M. (2022). Determination of Acetaminophen in Jamu Pegal Linu by High Performance Liquid Chromatography. *Research Artikel*, *Iii*, 12–15.

Putri, N., Sari, D., & Haresmita, P. P. (2023). PEGAL LINU DI WILAYAH MAGELANG. *Jurnal Ilmu Farmasi Dan Farmasi Klinik (JIFFK)*, *20*(1), 53–59.

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

Saragiotto, B. T., Abdel Shaheed, C., & Maher, C. G. (2019). Paracetamol for pain in adults. *The BMJ*, *367*(December), 1–5. https://doi.org/10.1136/bmj.l6693

Savitri, D. (2017). *Diam-diam Mematikan, Cegah Asam Urat dan Hipertensi*. Penerbit Anak Hebat Indonesia.

Snow, N., 2021. What Chromatograms Can Tell Us About Our Analytes. *LCGC Europe*, *30*(12), 670-678. *LCGC Europe*, *34*(1), 25-28.

Sumarni, W., Sudarmin, S., & Sumarti, S. S. (2019). The scientification of jamu: A study of Indonesian's traditional medicine. *Journal of Physics: Conference Series*, *1321*(3). https://doi.org/10.1088/1742-6596/1321/3/032057

Taupik, M., Djuwarno, E. N., Mustapa, M. A., Kunusa, W. R., La Kilo, J., & Sahumena, M. H. (2022). The Type Fragmentation Patterns Confirmed Acetaminophen By Using Liquid Chromatography-Mass Spectroscopy (LCMS) From Herbal Medicine (Jamu). *Elkawnie: Journal of Islamic Science and Technology*, *7*(2), 341. https://doi.org/10.22373/ekw.v7i2.7492.

Wahab, M.F., Armstrong, D. and Patel, D.C., 2017. Peak shapes and their measurements: the need and the concept behind total peak shape analysis. *LCGC Europe*, *30*(12), 670-678.

Wisnuwardhani, H. A., Rusdi, B., & Yuliawati, K. M. (2018). Method validation for simultaneous quantitative analysis of acetaminophen and dexamethasone in jamu pegal linu using spe-hplc method. *Journal of Pharmaceutical Sciences and Research*, *10*(11), 2693–2696.

DOI:https//doi.org/10.22452/mjs.vol45no1.5
Malaysian Journal of Science 45(1): 33-44 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 45-53 (March 2026) https://mjs.um.edu.my

Ground Penetrating Radar Method for Estimating Peat Thickness and Volume: Case Study in Kubu Raya, Indonesia

Riska Rais[1a], Muhardi[2a]* and Hans Elmaury Andreas Siregar[3b]

Abstract: Tropical peatlands in Indonesia are very large, covering an area of about 13.4-14.9 million hectares. Kubu Raya Regency, West Kalimantan Province, is known to have significant natural resources and large regions of peatland. Therefore, this study aimed to estimate the thickness and volume of peat soil in the Rasau Jaya and Sungai Raya Subdistricts of Kubu Raya Regency. The method adopted was the Ground Penetrating Radar (GPR), where the Plug-In Cobra GPR SE70 tool with a frequency of 80 MHz was used. In the process, a total of 23 tracks was applied with validation by three drill points. This consisted of nine tracks in Sungai Raya Subdistrict and 14 in Rasau Jaya Subdistrict. GPR data was processed through several processes, including static correction, dewow, Butterworth bandpass, background removal, gain, subtracting the average, and horizon picking. The results showed that the subsurface electromagnetic wave propagation velocity at the study location was 0.026 m/ns, with a peat thickness of 0.8-4.2m. The deepest peat layer was in the east, with a thickness of 3.8-4.2m, while the shallowest was in the west, at a thickness of 0.8-1.1m. Based on observation, the volume of peat in the study location was 4.8×107 m^3.

Keywords: *Electromagnetic wave, ground penetrating radar, peat thickness, volume.*

1. Introduction

Peat soils are formed from organic plant fragments subjected to chemical processes. These soils are compressible, with a high water content and a distinctive color ranging from dark brown to black (Wahab et al., 2022). They are generally discovered around tropical forests in lowland areas with moist conditions, abundant water, and relatively low temperatures. Tropical peatlands in Indonesia are very large, covering an area of approximately 13.4-14.9 million hectares. The lands are spread over several islands, including Sumatra, Kalimantan, Papua, and Sulawesi, with areas of 5.85 million hectares (43.5%), 4.54 million hectares (33.8%), 3.01 million hectares (22.4%), and 0.03 million hectares (0.3%), respectively (Yuwati et al., 2021). Based on records, West Kalimantan Province has an area of approximately 1.73 million hectares (Putri, 2017). Rasau Jaya Subdistrict, located in Kubu Raya Regency, West Kalimantan, is known to possess significant natural resources, specifically extensive peatlands. In the Rasau Jaya Subdistrict, the land reaches approximately 14,371.392 hectares, making it an essential area for peat ecosystem management in Indonesia (Tampubolon et al., 2020).

Peatland restoration has become a global concern, specifically in tropical regions such as Indonesia and Malaysia. This is because more than 20 million hectares are thought to have been disturbed by human activities. Restoration efforts through maintenance of soil moisture levels at optimal levels not only help prevent fires but also allow peat ecosystems to recover and resume functioning as effective carbon sinks. This process includes restoring natural hydrology, increasing water content, and reducing greenhouse gas emissions such as carbon dioxide and methane released when peat becomes dry (Damanik et al., 2024). The restoration is also essential for maintaining biodiversity, as these ecosystems support a variety of endemic species that depend on a moisture-rich environment. Furthermore, other benefits include reducing the risk of peatland fires. Restoring soil moisture levels can significantly reduce fires while preserving carbon stocks in deep peat layers and preventing the loss of large amounts of carbon to the atmosphere (Vernimmen et al., 2020). These efforts are crucial to achieving global carbon emission reduction targets and climate change mitigation. Peatlands have a deficient soil-bearing capacity in infrastructure development, making building foundations vulnerable to subsidence. The thicker the peat layer, the greater the potential for subsidence in building foundations (Tanjung et al., 2017).

A method that is often used to estimate peat thickness and volume is the drilling method. It is considered inefficient because the equipment is challenging to move and requires a lot of personnel. Therefore, peatland analysis needs to be conducted using geophysical surveys. Ground Penetrating Radar (GPR) is a geophysical method suitable for peatland studies (Sinyutkina, 2021). The method has the advantages of effectiveness, practicality, and non-destructiveness (Rais et al., 2024). It works by using electromagnetic waves propagating to the subsurface and detecting signals reflected by sediment interfaces with

Authors information:
[a]Geophysics Study Program, Department of Physics, Universitas Tanjungpura, Jalan Prof. Dr. H. Hadari Nawawi, Pontianak, West Kalimantan, 78124, INDONESIA. E-mail: riskaraiss13@gmail.com[1]; muhardi@physics.untan.ac.id[2]
[b]Center for Mineral, Coal, and Geothermal Resources (PSDMBP), Jalan Soekarno-Hatta No. 444, Bandung, West Java, 40254, INDONESIA. E-mail: hans.elmaury@esdm.go.id[3]
*Correspondence: muhardi@physics.untan.ac.id

Received: October, 2024
Accepted: January, 2025
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

different electromagnetic properties (Ryazantsev & Ignashov, 2022; Knödel et al., 2007). The high water content of the peat layer causes electromagnetic waves to propagate at a low velocity. Significant differences in water content at the peat-sediment interface lead to high-amplitude reflections, which contribute to the signal response (Pezdir et al., 2021). Good signal reflections are generally produced when there is a significant difference in the dielectric constant between the peat layer and other layers below. However, when the layer is clay, the electromagnetic wave may have significant attenuation, reducing the reflectance clarity at the interface.

This study aims to apply the GPR method to estimate the thickness and volume of peat soil in Rasau Jaya and Sungai Raya Subdistricts, Kubu Raya Regency. The result is expected to produce more accurate information on peat thickness and volume to support more optimal restoration, by estimating carbon reserves, reducing the risk of peatland fires, and building infrastructure foundations. However, limited data on the thickness and volume in the region remains a significant obstacle to restoration planning and environmental impact mitigation. The inefficient use of conventional methods often takes a long time. Therefore, the application of the GPR enables the mapping of peat thickness over a larger area in a relatively short time, increasing the effectiveness of data collection in the field. The results of this study are also expected to help local governments, analysts, activists, and the general public in planning more targeted and sustainable restoration strategies.

2. Material and Method

The Study Location

This study was conducted in Kubu Raya Regency, West Kalimantan Province. Data were collected through 23 tracks, consisting of nine in the Sungai Raya Subdistrict and 14 in the Rasau Raya. A track in the Rasau Raya Subdistrict (L22) with a length from 192 m to 887 m was used as a reference to measure the velocity wave propagation in the peat layer. Furthermore, this study applied four drill points, with three being adopted for interpolation in areas not covered by the GPR survey track. The remaining drill point was located on L22 to validate the thickness of the peat layer on that track. The measurement track and drill point locations are shown in Figure 1. Equipment used in this study was a Plug-In Cobra GPR SE70 with a frequency of 80 MHz, as presented in Figure 2.

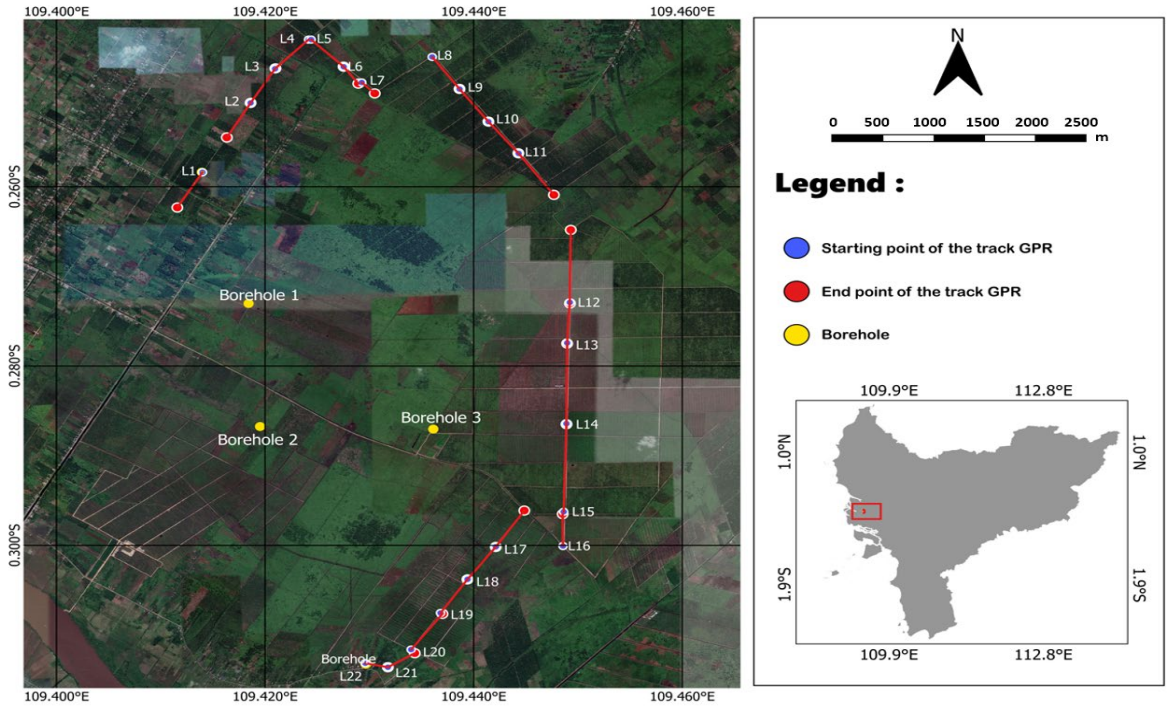


Figure 1. Study design; measurement track and drill point locations

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

Figure 2. The GPR survey equipment, i.e., Plug-In Cobra GPR SE70

Data Processing

The propagation velocity of electromagnetic waves in peat layers was determined based on drill depth data by using the concept of Two-Way Time (TWT). The time measured is the wave's travel duration from the surface to the boundary of the subsurface layer and back to the surface. Therefore, the total time measured was twice the layer distance. By identifying the peat layer thickness d (m) and wave travel time t (ns), the electromagnetic wave velocity v was determined using Equation (1).

$$v = 2\frac{d}{t} \quad (1)$$

Peat thickness was identified by showing the peat layer boundary on the radargram. The thickness boundary was visible from the amplitude contrast marked by the color difference in the radargram profile. Processing of radargram data includes a series of processes aimed at improving the quality of the details obtained from the measurements. It began with raw data processing through static correction to account for positional and topographical variations, including time-zero correction based on the initial wave arrival. A dewow procedure was subsequently applied to eliminate low-frequency noise (Koyan et al., 2023). A Butterworth bandpass filter was applied to filter frequencies outside the desired range, while the background removal process eliminated signals unrelated to the subsurface structure (Wu et al., 2022). The energy decay effect of the radar signal generated during penetration into the subsurface was removed. This was followed by subtracting the average method to remove the horizontal coherent energy appearing with low frequencies (L. Zhang et al., 2021). The final step was manual gain, which ensured reflections at various depths could be seen.

Peat thickness profiles were generally classified into four classes based on the depth/thickness of the layer. This includes shallow (0.5-1 m), medium (1-2 m), deep (2-3 m), and very deep (3-5 m) (Suryani et al., 2022). The kriging interpolation method in 3D modeling facilitates more accurate mapping of the peat layer's volume in the study location. The method has been used effectively in several studies to accurately determine soil layer thickness. It can be adopted to map peat thickness and carbon stock in degraded peatlands (Fiantis et al., 2024; Beucher et al., 2020). The kriging method was also applied to estimate soil layer thickness by incorporating topographic and vegetation variables with significant results (S. Zhang et al., 2021). Therefore, the 3D peat layer profiles were obtained using this method. This provides more accurate results in mapping peat thickness and estimating the total volume, specifically in areas with topography.

3. Results and Discussion

Electromagnetic Wave Propagation Velocity

A clear reflector was identified at a depth of about 2.1 m, signified by the dashed white line, as shown in Figure 3. This suggests a significant change in the characteristics of the subsurface material, which could be due to differences in density or moisture content. Therefore, the difference in physical properties was interpreted as a boundary between peat and clay layers. High-amplitude subsurface layers signified the stronger reflections of these layers (blue and purple), while the underlying layers had weaker reflections (yellow). This showed a decrease in material contrast with increasing depth. Electromagnetic waves propagate at velocities that depend on the dielectric properties of the material. In general, the propagation velocity of electromagnetic waves in peat layers was 0.026 m/ns. The thickness of the peat layer was accurately determined through a boring test conducted on track L22 at a trace distance of 102 out of 196 traces. Meanwhile, the travel time t of 161.8 ns was obtained from the radargram recording. The wave velocity was relatively low, hence, the material in the area was suspected to possess a high water content or be organic (Sumargana et al., 2019; Zhou et al., 2019).

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

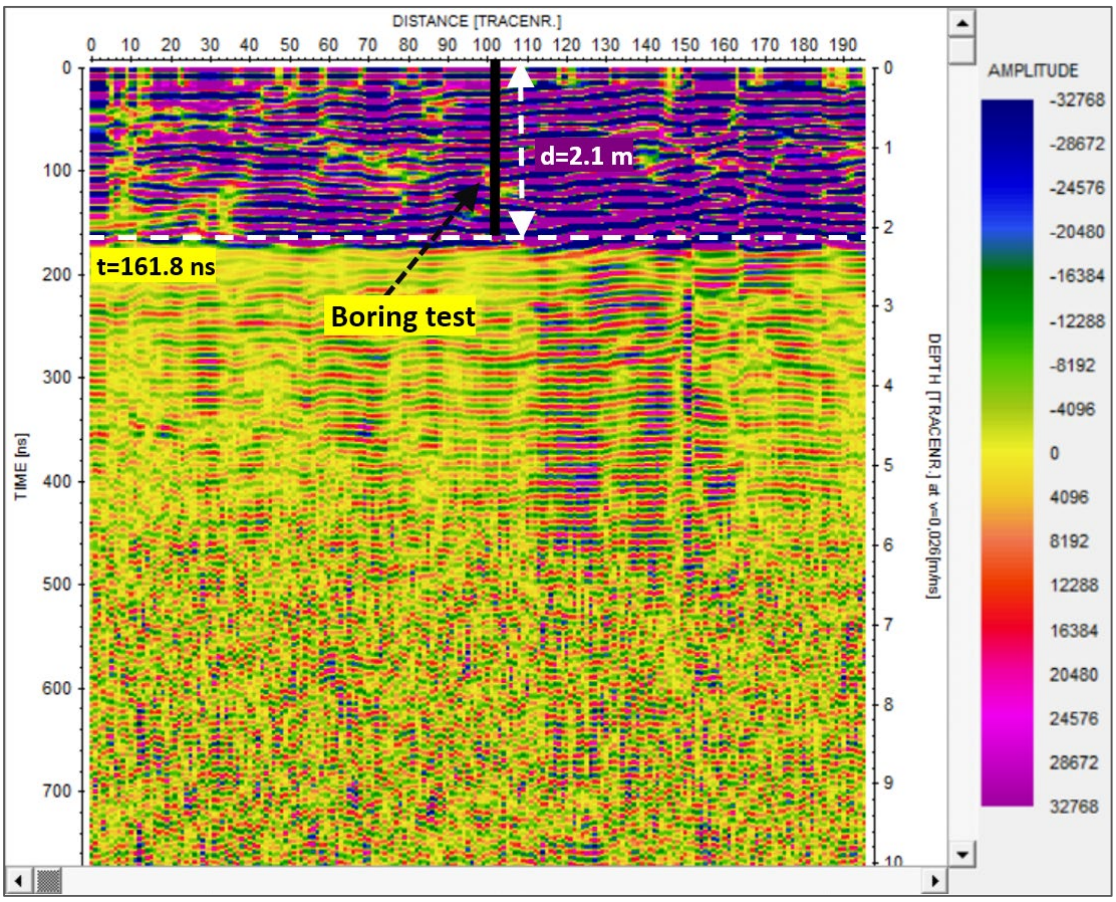


Figure 3. Parameters on the radargram L22 to obtain the wave propagation velocity

Data Processing to Identify Peat Layer Boundaries

The radargram data (raw data) processing consists of several steps, namely static correction, dewow, Butterworth bandpass, background removal, energy decay, subtracting average, and manual gain, as shown in Figure 4. The whole process was essential in estimating peat thickness, as it can improve the accuracy of detecting different subsurface layers, including peat layers. Removing noise and clarifying the reflection signal helped identify the boundary between the peat layer and the layers below.

Peat Thickness

The peat thickness was identified by marking the horizontal layer boundaries (horizons) on the radargram data from the manual gain process through horizon picking. An example of the results of the horizon-picking process used to identify the thickness in each layer is shown in Figure 5. The boundary between the peat and clay layers was obtained from the dominant contrast between the high and low amplitudes. The peat layer at the study location was classified as very deep because most of it had a thickness of more than 1 m. The deepest peat had a thickness of 4.2m on track L12, and the shallowest was 0.8m on track L1.

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

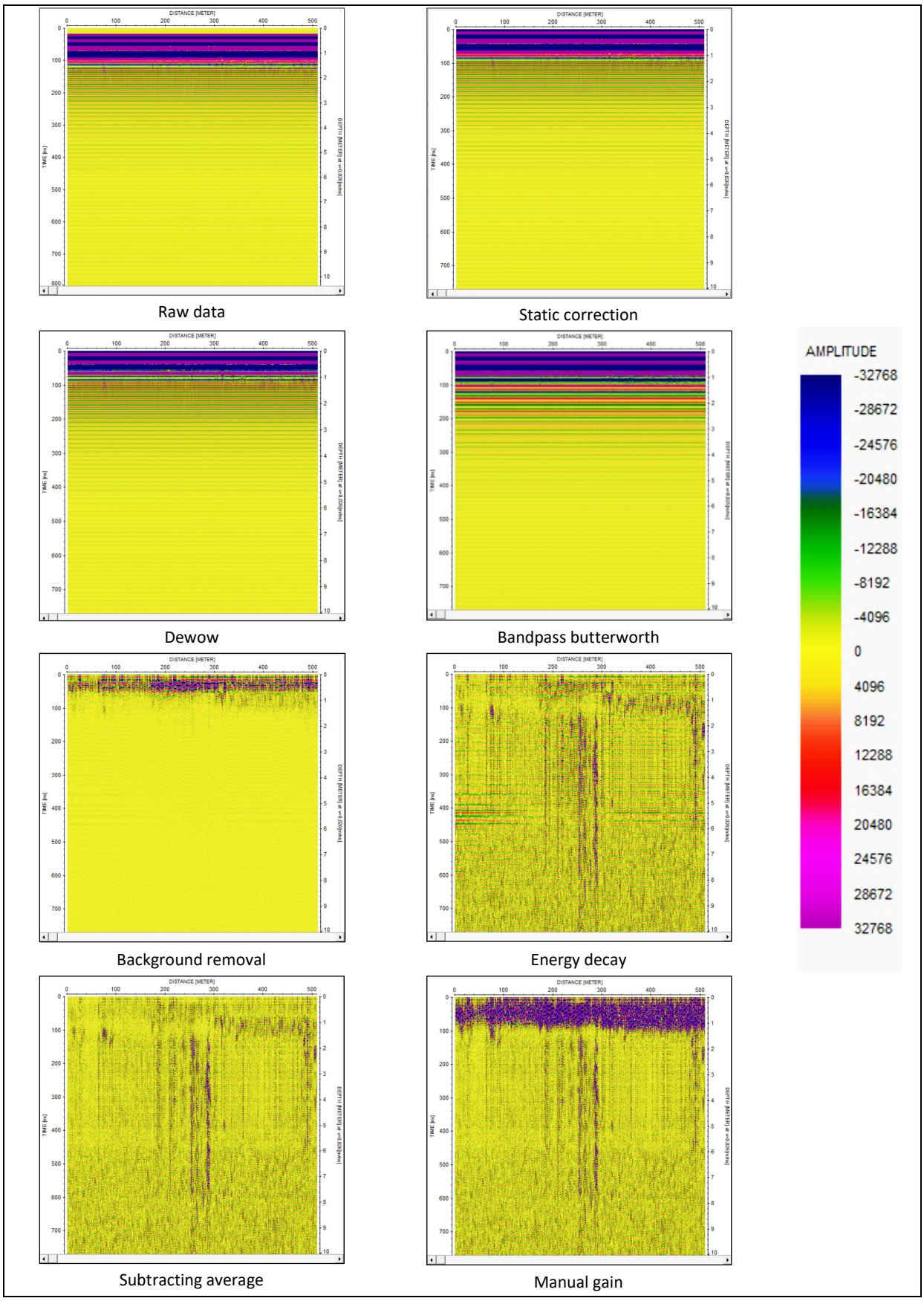


Figure 4. Examples of radargram processing results at each stage

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

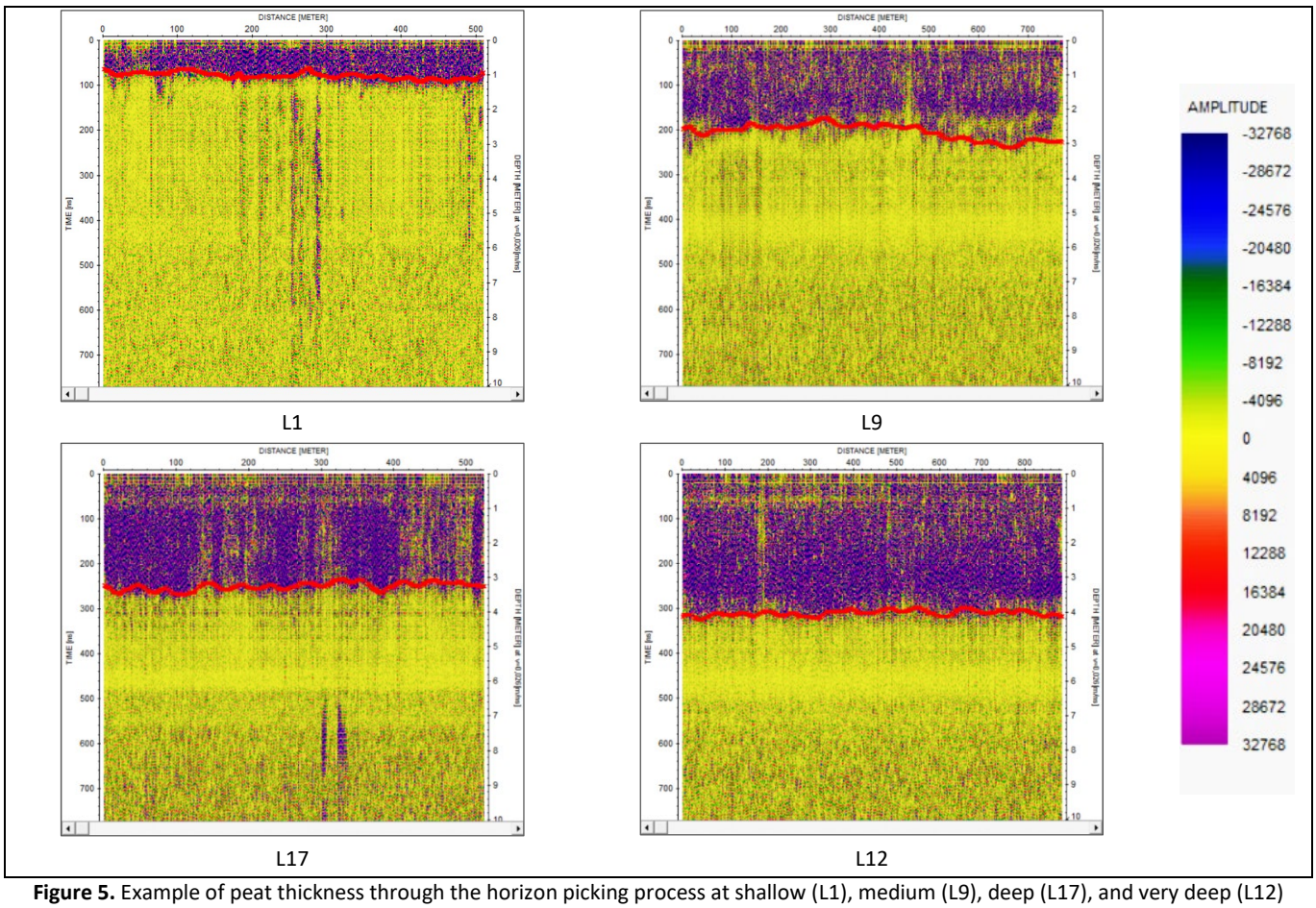


Figure 5. Example of peat thickness through the horizon picking process at shallow (L1), medium (L9), deep (L17), and very deep (L12)

This study obtained the wave propagation velocity by calculating the drill depth and trace data at the drill point. The radargram was adjusted to the depth of the trace point to obtain the travel time, leading to a wave propagation velocity of 0.026 m/ns. Wave propagation velocity possessed different values for each medium it passed through. The velocity in a medium was influenced by the permittivity of the materials composing the medium. The higher the ability of a material to store electrical energy in an electrical field (permittivity of the material), the smaller the wave propagation velocity (Mbango et al., 2022). Peat thickness in each track at the study location is shown in Table 1. At the location, the pear had different thicknesses and was classified as very deep because it is dominated by thicknesses of >3-5m.

The distribution of peat thickness in 2D contours is shown in Figure 6 and distinguished by color variations. Based on the thickness, light purple is signified as shallow peat with a 0.8-1m thickness. Dark blue reflected a medium peat with a thickness of 1-2 m. The yellow color suggested a deep peat with a thickness of 2-3 m. Orange to red signified a very deep peat with a 3-4 m thickness. In general, the thickness of peat in the eastern and northern parts was thicker than in the west and south. This was observed from the dominance of the yellow to red in the east and north, while the light purple to dark blue dominated in the west and south.

Table 1. Estimation of peat thickness for each track

Track	Track length (m)	Number of Traces	Thickness (m)
L1	511	2.871	0,8 – 1,1
L2	495	3.015	3,1 – 3,4
L3	500	3.263	2,7 – 3,1
L4	527	3.539	2,5 – 3,0
L5	557	4.310	2,9 – 3,6
L6	261	2.249	2,8 – 3,3
L7	192	1.681	2,8 – 3,2
L8	501	4.114	2,8 – 3,2
L9	510	4.511	2,4 – 2,9
L10	495	3.258	2,2 – 2,9
L11	632	3.079	1,9 – 2,9
L12	887	3.361	3,8 – 4,2
L13	484	2.064	2,7 – 3,2
L14	759	3.585	2,8 – 3,1
L15	837	4.319	2,6 – 3,0
L16	402	2.072	2,7 – 3,7
L17	525	5.143	3,1 – 3,5
L18	496	5.805	2,6 – 3,4
L19	494	5.088	3,0 – 3,3
L20	550	5.165	1,9 – 2,5
L21	328	3.989	1,8 – 2,6
L22	246	3.032	1,8 – 2,1

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

Figure 6 shows the distribution map of peat thickness in the study area, categorized from shallow (<1 m) to very deep (>3 m). These characteristics had important implications for carbon storage potential. Areas with thickness of more than 3 m had the potential to possess high carbon stocks compared to the shallow areas (Vernimmen et al., 2020). Peat is also often associated with a greater risk of forest fires, specifically when it dries out due to human activities or climate change. Areas with shallow thickness tended to have a higher fire risk than deep peat. This is because shallow peat dries out more quickly (Nizam et al., 2023). Dried peat is highly fire-prone due to its high organic matter content. The blue and purple areas in the study location are potentially fire-prone.

The uneven distribution of thickness in the study location was caused by several factors, namely topography, drainage, and maturity (Word et al., 2022; Suryani et al., 2022). Low areas or basins were prone to having deeper thicknesses because of their ability to retain more water and support the accumulation of organic material. Poor drainage allowed the accumulation of more organic matter, leading to deeper peat. Maturity also affected its thickness and distribution characteristics. More mature peat generally had a denser and more compact structure because the organic material was subjected to further decomposition. In contrast, young or immature peat tended to be softer and porous and may have a deeper thickness because it has not reached significant compaction (Novrianti & Harisuseno, 2024).

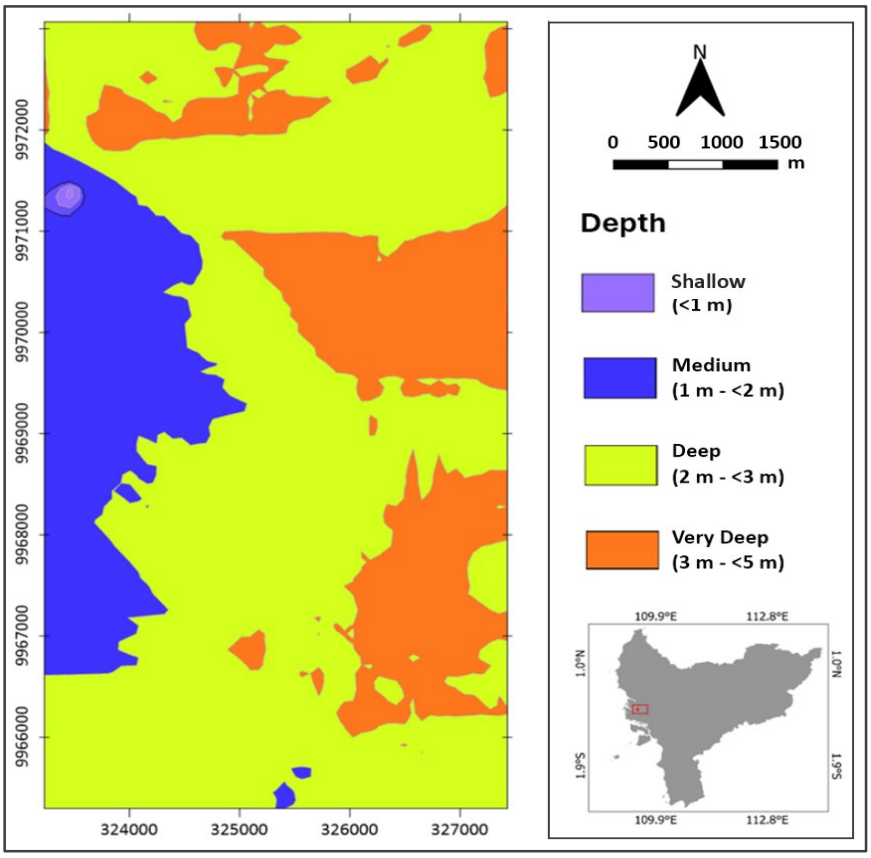


Figure 6. Distribution of peat thickness and its classification

Peat Volume

Volume of peat was obtained based on the distribution of thickness and the area of the study location. The interpolation process through the kriging method was conducted using the Surfer Software to produce a three-dimensional (3D) profile of the peat layer for estimating its volume. The method was used because it can estimate values between data points with a high level of accuracy (Fiantis et al., 2024). Based on the calculation of thickness distribution and the study area, the total volume was estimated to be approximately 4.8 x 10^7 m^3, as shown in Figure 7. This estimated value was influenced by the thickness distribution, which depended on factors such as topography, drainage, and maturity level. With such a significant volume, peatlands have a large capacity to store carbon, playing an essential role in climate change mitigation. Peat possesses the ability to absorb atmospheric carbon for thousands of years. When the lands are disturbed by fire, the stored carbon can be released back into the atmosphere as greenhouse gases, worsening global warming. Therefore, maintaining a moist area is essential to prevent fires, often a problem in tropical peat ecosystems.

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

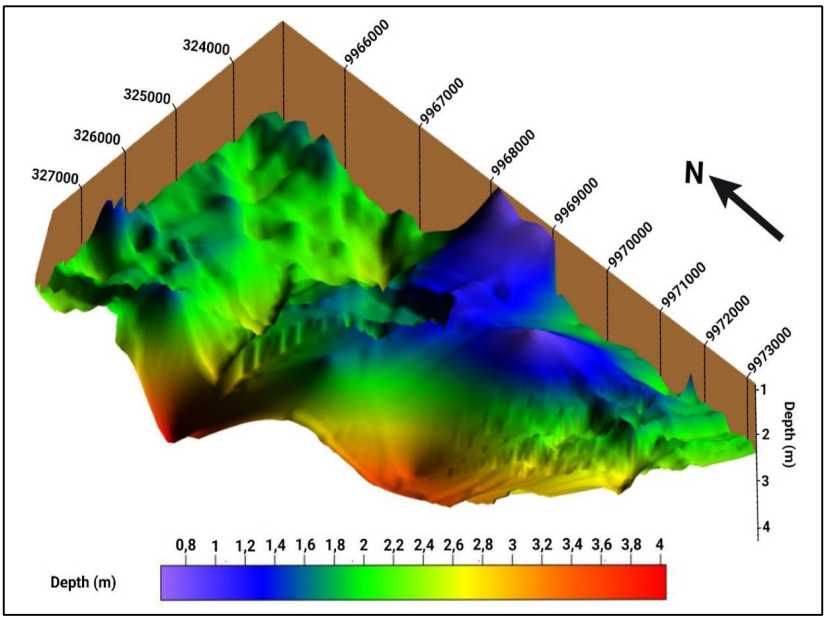


Figure 7. The 3D profile of the peat layer for estimating its volume

4. Conclusion

In conclusion, the GPR method was applicable for estimating peat thickness and volume. The radargram data (raw data) processing consisted of several steps, namely static correction, dewow, Butterworth bandpass, background removal, energy decay, subtracting the average, and manual gain. The thickness of peat was identified by marking the horizontal layer boundaries (horizons) on the radargram data from the manual gain process through horizon picking. The results showed that the subsurface electromagnetic wave propagation velocity at the study location was 0.026 m/ns, with a peat thickness of 0.8-4.2m. The deepest layer was in the east, with a thickness of 3.8-4.2m, while the shallowest was in the west, recording 0.8-1.1m. The results also show that the volume of peat in the study location was 4.8×107 m^3.

5. Acknowledgement

The authors are grateful to the head of the Center for Mineral, Coal, and Geothermal Resources (PSDMBP) Bandung and the head of the Geophysics and Geographic Information System Laboratory of Universitas Tanjungpura for facilitating this study.

6. References

Beucher, A., Koganti, T., Iversen, B. V., & Greve, M. H. (2020). Mapping of Peat Thickness Using a Multi-Receiver Electromagnetic Induction Instrument. *Remote Sensing*, *12*(15), 1–21. https://doi.org/10.3390/RS12152458

Damanik, Z., Jaya, A., Radjagukguk, B., & Adam, C. (2024). Influence of Water Table and Peat Thickness on Dissolved Organic Carbon of Tropical Peat Soil with Sulfidic Substratum from Central Kalimantan, Indonesia. *Journal of Multidisciplinary Applied Natural Science*, *4*(1), 76–89. https://doi.org/10.47352/jmans.2774-3047.189

Fiantis, D., Rudiyanto, Ginting, F. I., Agtalarik, A., Arianto, D. T., Wichaksono, P., Irfan, R., Nelson, M., Gusnidar, G., Jeon, S., & Minasny, B. (2024). Mapping Peat Thickness and Carbon Stock of a Degraded Peatland in West Sumatra, Indonesia. *Soil Use and Management*, *40*(1), 1–13. https://doi.org/10.1111/sum.12954

Knödel, K., Lange, G., & Voigt, H.-J. (2007). *Ground Penetrating Radar* (1st ed.). Springer Berlin, Heidelberg. https://doi.org/https://doi.org/10.1007/978-3-540-74671-3

Koyan, P., Tronicke, J., Klose, T., & Guillemoteau, J. (2023). 3D GPR to Explore Peat Deposits: Strategies for Data Acquisition, Processing, and Interpretation. *12th International Workshop on Advanced Ground Penetrating Radar (IWAGPR)*, 1–4. https://doi.org/10.1109/IWAGPR57138.2023.10329077

Mbango, F. M., Bouesse, G. F., & Ndagijimana, F. (2022). Extraction of the Complex Relative Permittivity from the Characteristic Impedance of Transmission Line by Resolving Discontinuities. *Electronics (Switzerland)*, *11*(23), 1–15. https://doi.org/10.3390/electronics11234035

Nizam, A. M. A. H., Taib, M. S., Yunus, M. N. Z., & Saman, N. (2023). Assessment of Peat Fire Susceptibility for Carbon Emission Reduction. *IOP Conference Series: Earth and Environmental Science*, *1144*(012014 IOP), 1–8. https://doi.org/10.1088/1755-1315/1144/1/012014

Novrianti, & Harisuseno, D. (2024). Correlation Between Volume Weight, Porosity and Moisture Content in Central Kalimantan's Peatlands. *IOP Conference Series: Earth and Environmental Science*, *1311*(012047), 1–15. https://doi.org/10.1088/1755-1315/1311/1/012047

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

Pezdir, V., Čeru, T., Horn, B., & Gosar, M. (2021). Investigating Peatland Stratigraphy and Development of the Šijec Bog (Slovenia) Using Near-Surface Geophysical Methods. *Catena*, *206*(105484), 1–15. https://doi.org/10.1016/j.catena.2021.105484

Putri, T. T. A. (2017). Pengelolaan Sumberdaya Lahan Gambut di Kubu Raya Kalimantan Barat Menuju Lahan Tanpa Bakar. *Jurnal Penelitian Agrosamudra*, *4*(2), 92–109.

Rais, R., Muhardi, & Siregar, H. E. A. (2024). Analisis Tingkat Kematangan Gambut Berdasarkan Refleksi Radargram di Kecamatan Rasau Jaya Kabupaten Kubu Raya. *Prisma Fisika*, *12*(1), 25–29. https://doi.org/10.26418/pf.v12i1.73789

Ryazantsev, P. A., & Ignashov, P. A. (2022). Integrating TDR and GPR Methods to Study The Structure of Peatland. *Limnology and Freshwater Biology*, *5*(4), 1556–1558. https://doi.org/10.31951/2658-3518-2022-A-4-1556

Sinyutkina, A. (2021). Investigating Peat Deposits with Ground Penetrating Radar-A Case Study of Drained Bogs in Western Siberia, Russia. *E3S Web of Conferences*, *258*, 1–7. https://doi.org/10.1051/e3sconf/202125812007

Sumargana, L., Frederik, M., Sumirah, & Nugroho, D. (2019). Ground Penetrating Radar Mapping of Peat Depth at Burnai-Sibumbung Rivers Peatland Hydrological Unit Ogan Komering Ilir Regency. *2nd IEEE Asia-Pacific Conference on Geoscience, Electronics and Remote Sensing Technology (AGERS)*, 15–18. https://doi.org/10.1109/AGERS48446.2019.9034262

Suryani, E., Pratamaningsih, M. M., Muslim, R. Q., Erwinda, Hati, D. P., Kricella, P., Nugroho, E. S., Subandiono, R. E., & Anda, M. (2022). Variations in Peat Soil Properties at the West Coast of Sumatra Island. *IOP Conference Series: Earth and Environmental Science*, *1025*(012025), 1–11. https://doi.org/10.1088/1755-1315/1025/1/012025

Tampubolon, B., Harjanti, D. T., Adlika, N. M., & Hari, M. L. C. (2020). Pemanfaatan Lahan Gambut Menjadi Lahan Potensial untuk Menjaga Ketahanan Pangan di Kalimantan Barat. *Geodika: Jurnal Kajian Ilmu dan Pendidikan Geografi*, *4*(2), 182–191. https://doi.org/10.29408/geodika.v4i2.2765

Tanjung, M. I., Sadikin, N., & Indrawan, D. (2017). Foundation Technology Development of Hydraulic Structure on Peat Soil. *Jurnal Teknik Hidraulik*, *7*(2), 115–130.

Vernimmen, R., Hooijer, A., Akmalia, R., Fitranatanegara, N., Mulyadi, D., Yuherdha, A., Andreas, H., & Page, S. (2020). Mapping Deep Peat Carbon Stock from a LiDAR based DTM and Field Measurements, with Application to Eastern Sumatra. *Carbon Balance and Management*, *15*(1), 1–18. https://doi.org/10.1186/s13021-020-00139-2

Wahab, A., Hasan, M., Kusin, F. M., Embong, Z., Zaman, Q. U., Babar, Z. U., & Imran, M. S. (2022). Physical Properties of Undisturbed Tropical Peat Soil at Pekan District, Pahang, West Malaysia. *International Journal of Integrated Engineering*, *14*(4), 403–414. https://doi.org/10.30880/ijie.2022.14.04.031

Word, C. S., McLaughlin, D. L., Strahm, B. D., Stewart, R. D., Varner, J. M., Wurster, F. C., Amestoy, T. J., & Link, N. T. (2022). Peatland Drainage Alters Soil Structure and Water Retention Properties: Implications for Ecosystem Function and Management. *Hydrological Processes*, *36*(3), 1–12. https://doi.org/10.1002/hyp.14533

Wu, S., Wang, L., Zeng, X., Wang, F., Liang, Z., & Ye, H. (2022). UAV-Mounted GPR for Object Detection Based on Cross-Correlation Background Subtraction Method. *Remote Sensing*, *14*(20), 1–20. https://doi.org/10.3390/rs14205132

Yuwati, T. W., Rachmanadi, D., Pratiwi, Turjaman, M., Indrajaya, Y., Nugroho, H. Y. S. H., Qirom, M. A., Narendra, B. H., Winarno, B., Lestari, S., Santosa, P. B., Adi, R. N., Savitri, E., Putra, P. B., Wahyuningtyas, R. S., Prayudyaningsih, R., Halwany, W., Nasrul, B., Bastoni, & Mendham, D. (2021). Restoration of Degraded Tropical Peatland in Indonesia: A Review. *Land*, *10*(11), 1–31. https://doi.org/10.3390/land10111170

Zhang, L., Ling, T., Yu, B., Huang, F., & Zhang, S. (2021). Intensive Interferences Processing for GPR Signal Based on the Wavelet Transform and F-K Filtering. *Journal of Applied Geophysics*, *186*(104273), 1–9. https://doi.org/10.1016/j.jappgeo.2021.104273

Zhang, S., Liu, G., Chen, S., Rasmussen, C., & Liu, B. (2021). Assessing Soil Thickness in a Black Soil Watershed in Northeast China Using Random Forest and Field Observations. *International Soil and Water Conservation Research*, *9*(1), 49–57. https://doi.org/10.1016/j.iswcr.2020.09.004

Zhou, L., Yu, D., Wang, Z., & Wang, X. (2019). Soil Water Content Estimation Using High-Frequency Ground Penetrating Radar Ligang. *Water (Switzerland)*, *11*(5), 1–16. https://doi.org/10.3390/w11051036

DOI:https//doi.org/10.22452/mjs.vol45no1.6
Malaysian Journal of Science 45(1): 45-53 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45 (1): 54-63 (March 2026) https://mjs.um.edu.my

An Evaluation of The Impact of An Engineering Service-Learning Implementation on Learning

Tarek Riaji[1a]*, Sanae El Hassani[2a], Youngbong Seo[3b] and Fatima Ezzahrae M'hamdi Alaoui[4c]

Abstract: This study aims to examine the impact of the Service-Learning approach on the engineering education system and student learning. The study is conducted through the example of a specific implementation of this approach, namely the "Smile project," a collaborative initiative between universities in Morocco and South Korea. This study also provides valuable insights and recommendations to further develop this educational implementation. To assess the effectiveness of the program in enhancing student learning, the study uses pre- and post-questionnaires administered to the participating students. The results of this analysis reveal a strong positive effect of engineering service-learning as a learning approach, improving both technical and interpersonal skills of engineering students. The impact on these skills, however, varies among individuals.

Keywords: *Engineering Service-Learning, engineering education system, student learning, learning approach, educational approach.*

1. Introduction

Service-Learning is a learning approach that associates community service with educational objectives to create a practical, progressive learning experience by addressing social needs. In fact, service learning resembles problem-based learning: combining an educational experience content with an organized service activity that addresses identified community needs, and reflecting on the service activity to develop a deeper understanding of the course content, a greater appreciation of the discipline, and an increased sense of civic responsibility.

The Smile Project is a component of the Beyond Engineering Education (BEE) program, whose objective is to provide service through engineering. The collaborative educational platform of Project BEE Community integrates an Engineering Service-Learning (ESL) model, which is based on the design process, to address tangible real-world challenges. The BEE program consists of many projects, and aims to have an international committee acting as event organizers from the Community Service Hub Center. Smile project is a collaborative initiative between the University of Chouaib Doukkali in Morocco and the Pusan National University in South Korea. Since its inception in 2016, this project has brought together engineering students from both universities, representing various fields.

This study uses a qualitative approach based on questionnaires, interviews, observations, and documents. As a study example, the Smile Project last edition in face-to-face mode, which was organized in Morocco in 2019, is considered. The objective of this study is to evaluate the impact of this approach in the 2019 edition on participating students, through the comparison of different skills and competencies of these students before and after this project.

The structure of the remaining sections in this paper is outlined as follows. The second section describes the design project of our study example, the Smile project. The third section presents the methods that were used in this study, and the fourth section details the study results, discussion, recommendations, and limitations. The last section provides a brief conclusion on the study.

Authors information:
[a]Laboratory of Information Technology (LTI), Department of Telecommunications, Computer Sciences and Networks, ENSA El Jadida, Chouaib Doukkali University, MOROCCO. E-mail: riaji.t@ucd.ac.ma[1]; elhassani.s@ucd.ac.ma[2]
[b]Office for Education Accreditation, Pusan National University, Busan, KOREA. E-mail: ybseo@pusan.ac.kr[3]
[c]Energy Laboratory, Department of Physics, Faculty of Sciences, Abdelmalek Essaadi University, Tetouan, MOROCCO. E-mail: f.mhamdialaoui@uae.ac.ma[4]
*Correspondence: riaji.t@ucd.ac.ma

2. Presentation of Smile Project

The Smile project follows a predefined process, which is executed in a face-to-face format over 10 to 11 days, with students and managers gathering in or near a rural area where the service is intended to be offered. This section describes this process.

Design Process

This project is organized as a five-task process. 'Task 1: Building the Best Team' focuses on creating the best teams in terms of composition, cohesion, and harmony. Several factors are considered to achieve this, including specialties, skills, and personality types. 'Task 2: Looking at Problems from Different Perspectives' trains students to examine problems from various angles through creative invention, design, and technical writing methods. In 'Task 3: Solving Community Problems', the project helps solve problems faced by local people. This includes developing a new concept, designing a product, purchasing materials, building the product, writing instructions, and installing it. 'Task 4: Becoming One with the Local Community' focuses on

Received: April, 2024
Accepted: June, 2024
Published: December, 2025

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

effective communication to ensure that locals understand who the project participants are and why they are in their community. Finally, 'Task 5: Organizing and Sharing Ideas' assists students in evaluating their personal creations, organizing them, and disseminating their ideas so that their work is valued and similar projects can be completed in future years. Throughout the project's duration, engineering students follow a structured procedure to complete these tasks. Figure 1 illustrates the steps of this procedure.

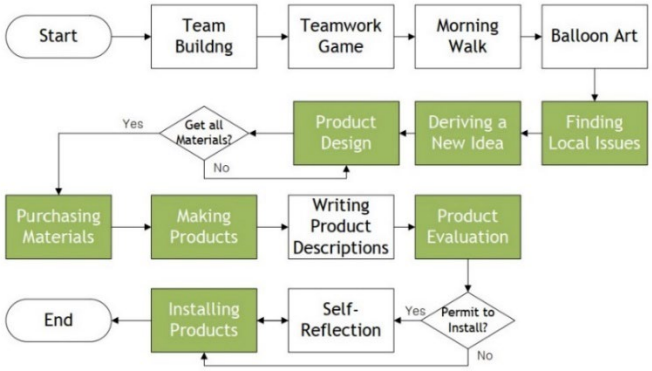


Figure 1. Design process of Smile project.

Students are selected based on their stated interest in the project, followed by interviews to assess their communication skills, particularly their English proficiency, which is the primary language of communication throughout the project duration. The participants in the 2019 version were divided into two teams of five students each, considering their personality, gender, and nationality to create balanced and homogeneous teams. Table 1 provides a summary of students' demographics in the Smile project 2019 edition. According to Table 1, it can be observed that the teams' distribution is balanced in terms of gender, nationalities, and age. Moreover, the students' majors are varied, which enables them to complement each other and foster an environment of competition and enthusiasm.

Table 1. Demographics of Students in the Smile Project 2019.

Demographics	Team 1 (EMJOY)	% (n=5)	Team 2 (MISO)	% (n=5)
Sex				
M	2	60%	3	60%
F	3	40%	2	40%
Age				
≤19	2	40%	1	20%
20	1	20%	2	40%
21	1	20%	2	40%
≥22	1	20%		
Grade				
Sophomore			2	40%
Junior	3	60%	2	40%
Senior	2	40%	1	20%
Specialty				
Industrial Engineering	1	20%		
Energetic and Electrical Engineering	1	20%	1	20%
Network and telecommunication Engineering			1	20%
Electronic Engineering	1	20%		
Materials Science and Engineering	1	20%		
Polymer Science and Engineering	1	20%	1	20%
Software Engineering			1	20%
Mechanical Engineering			1	20%
University				
PUSAN - South Korea	2	40%	3	60%
CHOUAIB DOUKKALI - Morocco	3	60%	2	40%

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

To optimize role specification within a team, team members have previously completed the Myers-Briggs Type Indicator (MBTI) test. The purpose of this test is to assess an individual's psychological preferences regarding how they perceive the world and make decisions, and it serves as a self-reflective tool (Cohen et al., 2013; Edwards et al., 2002; Kusuma et al., 2018). The underlying hypothesis of MBTI is that we all possess specific preferences in how we live our experiences, and these inclinations influence our needs, interests, motivation, and values (Kaplan & Saccuzzo, 2001). Each team member is assigned one of six specified roles according to their talents, majors, and psychological preferences, based on the test results (Table 2). Note that one individual is assigned two roles since the total number for each team is five engineering students.

Table 2. The Six Roles Assigned by The MBTI Test.

Job	Description
Mapper	Mapping all activity online
Journalist	Recording all progress at sheet
Designer	Drawing the product design
Scheduler	Check the Schedule and activity
Communicator	Reporting and communicating others
Accountant	Managing materials and budget

Project design based on engineering service-learning.

Using their engineering background and higher education knowledge, students create their own perceptions of the proposed solutions. Then, they specify the materials, tools, design process, and also estimate the costs of the product design (Figure 2). The proposed solution must satisfy several criteria. In particular, it must:

- provide a radical solution to the chosen problem;
- represent an engineering solution;
- use available equipment, resources, and funds;
- be installable;
- be accessible to the local community and easy to use (Figure 3); and
- represent a sustainable solution.

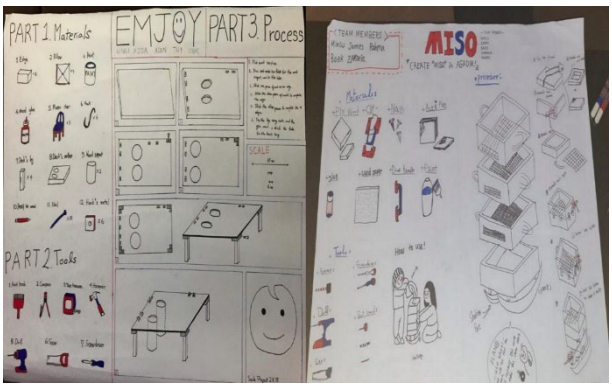


Figure 2. Product design plan.

Figure 3. Final product.

3. Study Methods

Ethics

This study was approved by students through the signing of consent forms. These forms were attached to the surveys and accompanied by a statement explaining that data collected would be used for research and program improvement, and that anonymized data may be shared through publication. Responsibility for ethical integrity was assumed by professors and managers throughout the project.

Data Collection and Recruitment

This research utilizes a mixed-method case study design to gain a comprehensive understanding of the impact of the engineering service-learning approach on the competencies and skills of engineering students. This impact was examined through data collected from all participants in the Smile project. A paper survey was administered to the participating engineering students, achieving a 100% response rate. The survey was given to students twice: first before the project began (pre-survey) to determine the initial state of the competencies, attitudes, and skills to be assessed, and second, after the project was completed (post-survey) to evaluate the level of improvement and degree of achievement.

Survey questions assessed participation, cultural intelligence, experimental analysis, design analysis, engineering knowledge, use of engineering tools, problem solving, multidisciplinary function, communication skills, lifelong learning, professional responsibility, and engineering impact. Additional questions gathered demographic information about the participants. Responses were fully anonymized, and the survey required 8-10 minutes to complete.

Survey Instrument Design and Measures

The survey includes eight main measures, which were then divided into twelve categories, each containing four Likert scale questions, shown in Table 3. This psychometric response scale requires respondents to express their degree of agreement with a statement using a five-point scale: (1) Strongly disagree; (2) Disagree; (3) Neither agree nor disagree; (4) Agree; (5) Strongly agree. The eight measures, also designated by Program Outcomes (POs), are:

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

Ability to utilize engineering major: The basis on which the importance of any engineering major is built is the need for it, and understanding when and how to apply it. This is how an engineering student realizes the importance of their specialization and how they can adapt their knowledge acquisitions to various situations. This measure was divided into three categories and assigned the largest number of questions (Q1-12).

Ability to solve problems: Engineers must use problem-solving skills to determine the best actions for any given situation. The type of problems that engineers face can differ greatly depending on their engineering specialty. Therefore, there is not a one-size-fits-all approach to solving all problems (Q13-16). Wright suggests a list of commonly included steps that most engineers use in their design process, though not all engineers follow the same steps.

1. Problem identifying.
2. Gather the necessary information.
3. Creative solutions searching.
4. Overcome barriers to creative thinking.
5. Progress from ideas to initial designs (including modelling).
6. Assess and choose a preferred solution.
7. Create reports, plans, and specifications for project planning.
8. Execute the design for project implementation.

Engineering tools usage capability: The ability to use engineering tools properly and to understand when and how to apply them is an essential criterion for assessing an engineer's technical competence and capability (Q17-20).

Interpersonal skills: It refers to behaviours and strategies used by a person to have an effective interaction with others. These skills are essential for an employee in a business context to be able to work well with colleagues. They can include communication, listening, attitude, and deportment (Q21-24).

Communication skills: Having strong communication skills is essential for engineers who wish to practice their profession in a global setting. These skills include fluency in English and a basic understanding of visual communication. Educators and industry professionals have increasingly emphasized the importance of both professional and technical skills for engineering students to improve community engagement and career success (Q25-28).

Self-management capability: Self-appraisal and the ability to plan, evaluate, and make necessary adjustments and revisions to work are all part of the scope of self-management that contribute to successful cognitive and learning processes (Q29-32) and (Q41-44).

Ability to think synthetically: It requires knowledge of contemporary issues and the contribution of engineering solutions to the economy, the environment, and society (Q33-36) and (Q37-40).

Global capability: It aims to normalize global relations between different nationalities of students, creating an atmosphere of harmony and avoiding cross-cultural misunderstandings (Q45-48).

Statistical Analysis

Data were first compiled in papers and then exported to Statistical Package for the Social Sciences (SPSS) version 28 (IBM, Chicago, IL) for analysis. The reliability of the performance indicators scale was assessed using Cronbach's alpha coefficient, a measure of internal consistency that evaluates how closely related a set of items is as a group. A value close to 1 indicates high reliability, while values below 0.7 are generally considered inadequate (Cronbach, L. J., 1951). The results of the reliability statistics showed high levels of internal consistency, as demonstrated in Table 3, which presents Cronbach's alpha scores for both surveys and their respective subscales.

A normality test was adopted to check whether the data were normally distributed (Table 4). The Kolmogorov-Smirnov test, a non-parametric test, compares the empirical distribution function of the data to the expected theoretical distribution, typically the normal distribution (Massey Jr, F. J., 1951). The Shapiro-Wilk test is used to assess the normality of data, particularly effective for small sample sizes, by evaluating the relationship between observed and expected values under normality assumptions (Shapiro, S. S., & Wilk, M.B., 1965). For the comparison of the means between the two measurements taken from the pre- and post-surveys, the Paired Samples t-Test was applied (Table 5).

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

Table 3. Cronbach's Alpha for Scales in Both Pre-and Post-Survey.

	Cronbach 's alpha	
	Pre-survey	Post-survey
Ability to apply knowledge of mathematics, basic science, engineering, and information technology. I can comprehend related knowledge such as mathematics, basic science, and engineering which is used for solving the engineering issues. I can comprehend and solve the engineering issues with my specialty. I can apply engineering knowledge and theory to make a prototype. I can put knowledge and theory into practice in order to demonstrate the effect of made prototype.	0.841	0.863
Ability to design and conduct experiments, as well as to analyze and interpret data. I can collect the data needed for the making the product and analyse it. I can organize elements and detailed plan with analysed data. I can construct and conduct the experiment systematically. I can make a report on issue and result of the engineering experiment.	0.617	0.823
Ability to devise a system, component, or process to meet desired needs within realistic constraints. I can design the products with safety for people in mind. I can define the necessary elements and carry out the phased plan. I can calculate the total time based on the time taken to make each part. I can make a prototype with common materials.	0.630	0.570
Ability to identify, formulate, and solve engineering problems. I am interested in public issue, water, energy, pollution, and food. I can see public issue with my specialty. I can suggest idea and organize detail plan for solving the problems. I can demonstrate and validate the result of solved problem with criteria.	0.224	0.854
Ability to use techniques, skills, and engineering tools necessary for engineering practice. I can make a detail drawing of product by engineering software. I know how to deal with tools needed to make product. I can fix the drawing of product when it needs. I can organize process of making product efficiently with PowerPoint.	0.563	0.754
Ability to function in multi-disciplinary teams. I can organize conducting plan for project on discussion with teammates. I can show leadership to raise quality of our project to a higher level. I can carry out my duty, being assigned within my team. I can do my best to strengthen the unity of the team that consists of different university and nationality.	0.723	0.312
Ability to communicate effectively. I can explain efficiently how to operate and maintain the products. I can organize my idea on systematical document. I can write Product Descriptions so the users can understand. I can make my intention clear to my teammates in project.	0.676	0.628
A recognition of the need for, and ability to engage in life-long learning. I can make continuous study plan based on my future. I can collect learning data and study on my own. I can explain requirement of life-lasting education for self-improvement. I can contribute to the regional society by continuous participating in engineering service.	0.896	0.757
A broad understanding of the impact of engineering solutions in economic, environmental, and societal context I understand how engineering method influence on regional society. I can explain how my specialty will be used in real life. I know how engineering awareness of problem and attempts to solve the problem will be recognized to people. I can understand and explain that recent engineering technology will affect society, economy, and environment.	0.875	0.681
A knowledge of contemporary issues I can explain logically global issues on water, energy, environment pollution, and food. I can collect information on various approaches and use it to solve engineering issues. I understand about the common issues in regional society or underdeveloped country. I can explain about 'engineering service' that solves common issues based on engineering technology.	0.756	0.658
Understanding of professional and ethical responsibilities I can understand if engineers lost their moral sense, it would be severe harm to man. I can explain how sense of responsibility affect society. I can understand importance of moral sense when I perform engineering technology. I can perform my duty with responsibility and work ethic as engineers.	0.728	0.870
Understanding of other cultures and an ability to engage in international cooperation. I can work as team with other countries teammates and accomplish the work together. I can explain about the produced work in English. I can share my thoughts and experience in English. I understand the diversity in culture as citizen of world.	0.955	0.583
Total survey scale	**0.931**	**0.966**

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

Table 4. Normality Tests.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Pre	0.111	10	0.200*	0.959	10	0.775
Post	0.187	10	0.200*	0.943	10	0.587

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Table 5. Paired Samples T-test.

		Paired Differences							Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	One-Sided p	Two-Sided p
Pair1	Pre-Post	0.80720	0.42239	0.13357	0.50504	1.10936	6.043	9	<0.001	<0.001

Quantitative Analysis: The study included descriptive statistics and exploratory data analysis for demographic variables, as well as the capacity to solve problems, using engineering major, self-management, applying engineering tools, and thinking synthetically, and skills of interpersonal, communication, and global capability in socially engaged design were conducted. Regarding normality, the Shapiro–Wilk test results indicate that the data are normally distributed, as the p-value is above 0.05. Paired Sample t-Test indicates a statistically significant difference between pre- and post-survey results, with an advantage to the post-survey. The considered variable is at 1% level of significance as $p < 0.01$. On the other hand, considering Hedges' correction estimate, the pre-and post-scores have a mean difference situated around 1.7 with a Confidence Interval of 95% (0.76-2.10) (Riaji et al., 2022, 2024).

Validation and Reliability of Survey Instrument: All the participants completed the survey. Participation measures included eight domains. We note "n", the number of questions related to each domain. These domains are: Ability to utilize engineering major (n=12), Ability to solve problems (n=4), Engineering Tools Usage capability (n=4), Interpersonal skill (n=4), Communication skill (n=4), Self-management capability (n=8), Ability to think synthetically (n=8), and Global capability (n=4). In the pre-survey, both the ability to solve problems and the ability to use engineering tools have unacceptable internal consistency (standard Cronbach's α) of 0.224 and 0.563, respectively, while only interpersonal skills have unacceptable internal consistency (α= 0.312) in the post-survey, whereas the rest of the areas received acceptable to high rates. Both the pre- and post-surveys obtained 0.931 and 0.966, respectively, of the Cronbach's alpha value, which indicates an excellent level of survey validity and reliability (Taber, 2018; Tavakol & Dennick, 2011). Due to the limited number of responses, confirmatory factor analysis was not conducted (Hurley et al., 1997).

4. Results and Discussion

The average results of the answers for each question have been calculated independently for the pre- and post-surveys. The degree of achievement for each competency and skill was calculated, all of which, except for the skill of controlling engineering software (Q17), had an achievement rate equal to or greater than 0.5. Table 6 shows the results of the average responses from pre- and post-surveys categorized into program outcomes.

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

Table 6. Descriptive Statistics for Measures of Various Competences in Smile Project 2019.

Competence measure	POs*	Point			Percentage		
		Group 1 Pre (a)	Group 2 Post (b)	achievement degree (b-a)	Group 1 Pre (a)	Group 2 Pre (b)	achievement degree (b-a)
Ability to utilize engineering major	1,2,3	3.14	4.39	1.25	63%	88%	25%
Ability to solve problem	4	3.47	4.19	0.72	69%	84%	14%
Engineering Tools usage capability	5	3.13	3.78	0.66	63%	76%	13%
Interpersonal skill	6	3.47	4.41	0.94	69%	88%	19%
Communication skill	7	3.38	4.38	1.00	68%	88%	20%
Self-management capability	8,11	3.53	4.34	0.81	71%	87%	16%
Ability to think synthetically	9,10	3.30	4.22	0.92	66%	84%	18%
Global capability	12	3.78	4.63	0.84	76%	93%	17%
Total average					**68%**	**86%**	**18%**

*POs- Program Outcomes

The results presented in Table 6 demonstrate significant improvements across all measured competencies, with achievement levels ranging from 13% to 25%, confirming the positive impact of the Smile project on students' engineering skills. The overall paired samples t-test result ($p < 0.001$) supports the statistical relevance of these outcomes. Notably, the ability to utilize engineering majors showed the greatest improvement at 25%, reflecting students' enhanced proficiency in applying theoretical knowledge to practical challenges. Communication skills improved by 20%, highlighting the project's role in fostering teamwork and effective information exchange. Similarly, the ability to solve problems and the capability to use engineering tools increased by 14% and 13%, respectively, illustrating strengthened technical and analytical skills. Gains in interpersonal skills (19%) and self-management capability (16%) indicate better collaboration and personal responsibility. Additionally, improvements in synthetic thinking (18%) and global capability (17%) demonstrate enhanced critical thinking and cross-cultural collaboration. These results collectively underscore the effectiveness of the Smile project in providing a comprehensive learning experience that advances both technical and soft skills within the framework of Engineering Service-Learning.

The results of Group 1 demonstrate that the participants possess the least competencies and skills, with all target competencies exceeding 63%. This fact suggests that the participating students are carefully selected with a particular set of abilities and skills. Group 2's results are consistently higher than those of Group 1, with all target skills above 76%, indicating that the project had a positive and significant influence on the engineering students. The achievement degree for all target competencies and skills is at least 13%, with an average of 18% (Figure 4) (Riaji et al., 2022).

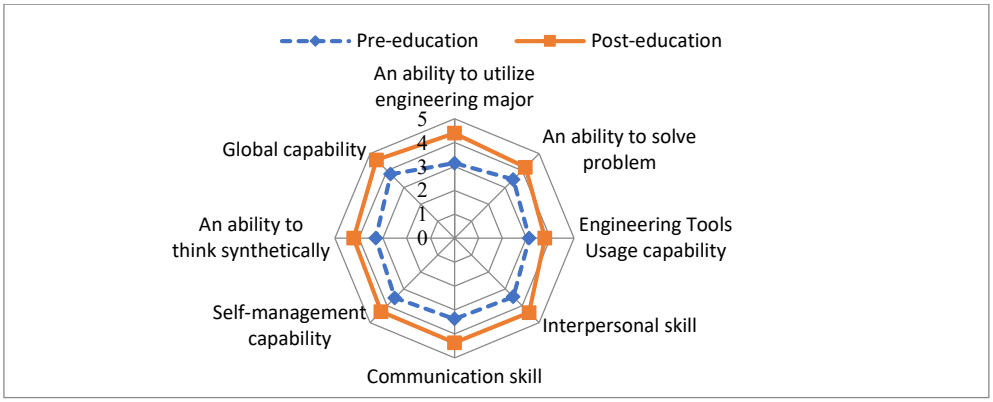


Figure 4. The overall development level of all the skills and competences targeted after the benefits of Smile project 2019.

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

Satisfaction Level

The post-survey included additional questions (Q49-59) to assess the level of satisfaction of engineering students with respect to the various processes associated with the project and the project as a whole, as shown in Table 7.

Table 7. Satisfaction Post-Survey Result from Smile Project 2019.

Contents of Survey Questionnaire	Result	Cronbach's alpha
The number of reconstructed team members was proper.	4.8	
I enjoyed staying in the accommodations (dormitory).	4.6	
I am satisfied with the team formation.	4.5	
I communicated well.	4.9	
It helped me improve my specialty.	4.4	
After team reconstruction with students in other countries, we could have better teamwork for the mission.	4.6	0.926
The dispatched period was proper.	4.4	
I performed all my duties in the schedule properly.	4.4	
I would like to recommend this program to friends.	4.6	
If I have another chance, I would like to participate again.	4.8	
How satisfied are you with the Smile Project overall?	4.9	

The Cronbach's alpha value (≈ 93 %) confirms the excellent reliability and validity of this section of the survey. It also indicates the extent of students' satisfaction (≥ 4.4) with the various processes accompanying the project and the project as a whole (4.9). The students were highly satisfied with the distribution of the teams, the activities and tasks they performed, the positive atmosphere and organization, and their willingness to participate again and to recommend participation to their engineering friends in future editions of the project (Taber, 2018; Tavakol & Dennick, 2011). Note that the satisfaction of professors and managers, along with the satisfaction of engineering students and the local community (based on an interview), is a key factor in evaluating the success of the Smile project in achieving the targeted objectives.

Results Discussion

The conducted study aimed to answer the following question: Do projects involving the engineering service-learning approach have a significant and lasting impact on engineering students' skills and competencies? This research targets the Smile project, which is an implementation of the pedagogical approach of engineering service-learning, in the 2019 edition of this project. A survey was conducted involving participating engineering students of various levels, majors, genders, universities, and countries. Eight skills and competencies, identified as the most important for an engineering student (Samavedham & Ragupathi, 2012), were targeted. A pre-survey was conducted to assess the initial skills status of participating students, and the obtained results were compared with their counterparts in the post-survey. The statistical study found a significant difference between the pre- and post-survey results, favoring the post-survey. This indicates a significant development of the targeted skills and competencies, and therefore a positive impact of the engineering service-learning approach on engineering education (Hedberg & Ayers, 2015; Mowery, 2011). Note that further research with a larger sample size can yield more statistically significant results.

This analysis provided additional insight into the impact of the Smile project in helping students develop their competencies and skills as civic-minded and socially responsible members of society. The quantitative and qualitative results suggest that for the participants, the objectives of engineering service-learning extend well beyond competencies and skills to include citizenship and social responsibility.

To deepen the comparative analysis of our findings, we reference the comprehensive bibliometric study by Hallinger and Kongpiwatana, which examines the evolution of service-learning research indexed in the Scopus database between 1950 and 2022 (Hallinger, P., & Narong, D.K., 2024). This study systematically analyzes 5,815 scholarly documents, highlighting the considerable growth and maturation of the field while noting its primary geographic concentration in economically developed Western countries. The research mainly investigates the design and outcomes of service-learning initiatives, focusing on their influence on students' personal development, social competencies, and academic performance (Astin & Sax, 1998; Billig, 2017). Key reported benefits include improved teamwork, leadership, cultural sensitivity, and a reduction in social biases. Furthermore, service-learning has been demonstrated to foster stronger student-faculty relationships, higher student satisfaction, and deeper community engagement (Bandy, 2016; Gray et al., 1998). These findings collectively emphasize the pedagogical value and societal impact of service-learning as a transformative educational method.

Suppose academics and practitioners are motivated to fully understand and document the value of service-learning as a pedagogical model in engineering education and as a framework for developing skills, competencies, and social responsibility in all disciplines. In that case, it will serve as a strong impetus for engineering education development, and consequently, the graduation of highly qualified students from engineering schools (Dukhan et al., 2008; Maloney et al., 2013; Oz-Medina et al., 2021; Queiruga-Dios et al., 2021; Ropers-Huilman et al., 2005).

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

Recommendations

Following the obtained results, and in order to improve the Smile project and develop the engineering service-learning approach, we recommend:

- Select participating students through stratified random sampling, a method where items in the target population are divided into distinct groups or strata. Within each stratum, items share similarities in specific characteristics considered important for the survey.
- Conduct a personality test to determine each student's role within the team, in order to place the right person in the correct position.
- Provide students with a broad choice of local communities or issues in order to give them a wide panel of opportunities to apply their specialties.
- Adopt more reliable and accurate methods for assessing the competencies of engineering students, as well as include assessment of the services provided and their impact on the local community.
- Allow students to participate multiple times by multiplying project editions, which will allow an objective observation of outcome improvement for the same students over editions.
- Expand the number of participants to be able to generalize the obtained results.

Limitations

This design study carries limitations regarding the value of this research due to the international nature of the Smile project work, which tends to attract students willing to participate in the project, and participants are selected through an oral interview to ensure they possess a minimum level of skills and abilities required. The latter influences the random method by which participants must be selected and may represent significant viability for the learning development of the selected sample. Furthermore, there is potential for self-selection bias among people who are more favorable to the Smile Project. Due to the small size of the teams compared to the number of data points desired, confirmatory factor analysis was not conducted in our survey.

The method adopted to evaluate different competencies is self-appraisals (by survey), which do not provide precise results. The students' self-appraisals may not correspond closely to their actual performances. Note that this is sometimes referred to as Calibration Error, which is the difference between values indicated by an instrument—the survey in our case—and the actual values. This phenomenon has been repeatedly observed in multiple contexts, including the Kruger-Dunning effect. In psychology, this is a cognitive bias where individuals overestimate their knowledge or competence in a given intellectual or social domain when they initially have limited knowledge or competence in that domain, relative to objective criteria, their peers' performance, or the general population. Similarly, when maintaining a consistent competency assessment model, the principle of bias mitigation strengthens and improves the validity of results.

Finally, it is important to remember that this is a small-scale, practitioner-driven study limited by time, funds, and staffing resources. Therefore, it does not aim to provide generalizable knowledge but intends to present a perspective yet to be explored. It is hoped that the Engineering Service-Learning methodology will be extended by researchers, professors, and evaluators in various settings to build on participants' experiences in engineering education. While the study is limited in scope and does not aim to produce generalizable knowledge, discussing scalability offers valuable insights. Expanding the participant base would broaden the range of local communities and geographical areas involved, introducing greater diversity in the issues addressed. However, this expansion would complicate project management, making monitoring and follow-up more difficult. Strengthening partnerships between academic institutions and local communities would be essential to ensure effective coordination and sustainable collaboration. Additionally, larger-scale implementations would require enhanced cross-cultural training, improved resource management strategies, and scalable team dynamics, possibly using peer-led leadership or technology-assisted structures. Finally, comprehensive and automated assessment frameworks would be necessary to handle larger data sets while maintaining consistency and accuracy. Addressing these considerations would support the successful adaptation of the Smile project to larger and more diverse educational contexts, enhancing its impact while adhering to the principles of Engineering Service-Learning.

5. Conclusion

This study examines the educational approach known as "Engineering Service-Learning," which can enhance the diverse competencies and skills of engineering students along with traditional educational methods. In their universities, engineering students benefit from theoretical and practical lessons constrained by time and place. The Engineering Service-Learning approach improves the hard and soft skills of engineering students by providing them with the opportunity and suitable conditions to complete and apply what they have learned during their engineering education. The benefit of this approach is not limited to engineering students; it also impacts the local community, which receives sustainable and free solutions to address its issues and develop its activities or projects. This approach also assists professors in giving real meaning to their courses while serving a supervisory and mentoring role. Finally, engineering service-learning remains an effective educational approach that can be adapted to various engineering disciplines. It is also scalable by adding, removing, or modifying its stages and methods to achieve better results.

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

5. References

Cohen, Y., Ornoy, H., & Keren, B. (2013). MBTI Personality Types of Project Managers and Their Success: A Field Survey. *Project Management Journal*, *44*(3), 78–87. https://doi.org/10.1002/pmj.21338

Dukhan, N., Schumack, M. R., & Daniels, J. J. (2008). Implementation of service-learning in engineering and its impact on students' attitudes and identity. *European Journal of Engineering Education*, *33*(1), 21–31. https://doi.org/10.1080/03043790701746132

Edwards, J. A., Lanning, K., & Hooker, K. (2002). The MBTI and Social Information Processing: An Incremental Validity Study. *Journal of Personality Assessment*, *78*(3). https://www.researchgate.net/profile/Kevin-Lanning/publication/11235513_The_MBTI_and_Social_Information_Processing_An_Incremental_Validity_Study/links/58b0b212aca2725b5413e131/The-MBTI-and-Social-Information-Processing-An-Incremental-Validity-Study.pdf

Hedberg, E. C., & Ayers, S. (2015). The power of a paired t-test with a covariate. *Social Science Research*, *50*, 277–291.

Hurley, A. E., Scandura, T. A., Schriesheim, C. A., Brannick, M. T., Seers, A., Vandenberg, R. J., & Williams, L. J. (1997). Exploratory and confirmatory factor analysis: Guidelines, issues, and alternatives. *Journal of Organizational Behavior*, 667–683.

Kaplan, R. M., & Saccuzzo, D. P. (2001). *Psychological testing: Principles, applications, and issues*. Wadsworth/Thomson Learning. https://psycnet.apa.org/record/2001-06953-000

Kusuma, D. W. Y., Raharjo, H. P., & Mugiyo, H. (2018). Personality Profiles Using MBTI Test for Sport Talent Identification for Students. *International Seminar on Public Health and Education 2018 (ISPHE 2018)*, 168–171. https://www.atlantis-press.com/proceedings/isphe-18/25899771

Maloney, P., Dent, L., & Karp, T. (2013). A new method of assessing the effects of a service-learning class on engineering undergraduate students. *International Journal for Service Learning in Engineering, Humanitarian Engineering and Social Entrepreneurship*, 29–47.

Mowery, B. D. (2011). The paired t-test. *Pediatric Nursing*, *37*(6), 320–322.

Oz-Medina, B. N. M., Blanco, S., & Alberti, M. G. (2021). Impact of service-learning on the motivation of engineering and high school students. *International Journal of Engineering Education*, *37*(4), 1060–1070.

Queiruga-Dios, M., Santos Sánchez, M. J., Queiruga-Dios, M. Á., Acosta Castellanos, P. M., & Queiruga-Dios, A. (2021). Assessment methods for service-learning projects in engineering in higher education: A systematic review. *Frontiers in Psychology*, *12*, 629231.

Riaji, T., El Hassani, S., & Alaoui, F. E. M. (2022). Application of paired samples t-test in engineering service-learning project. *2022 11th International Symposium on Signal, Image, Video and Communications (ISIVC)*, 1–4. https://doi.org/10.1109/ISIVC54825.2022.9800209

Riaji, T., Hassani, S. E., Seo, Y. B., & Alaoui, F. E. M. (2024). A comparative study of virtual and insite engineering service-learning implementations. *Journal of Education and Learning (EduLearn)*, *18*(2), Article 2. https://doi.org/10.11591/edulearn.v18i2.21164

Ropers-huilman, B., Carwile, L., & Lima, M. (2005). Service-learning in engineering: A valuable pedagogy for meeting learning objectives. *European Journal of Engineering Education*, *30*(2), 155–165. https://doi.org/10.1080/03043790410001664363

Samavedham, L., & Ragupathi, K. (2012). Facilitating 21st century skills in engineering students. *The Journal of Engineering Education*, *26*(1), 38–49.

Taber, K. S. (2018). The Use of Cronbach's Alpha When Developing and Reporting Research Instruments in Science Education. *Research in Science Education*, *48*(6), 1273–1296. https://doi.org/10.1007/s11165-016-9602-2

Tavakol, M., & Dennick, R. (2011). Making sense of Cronbach's alpha. *International Journal of Medical Education*, *2*, 53.

DOI:https//doi.org/10.22452/mjs.vol45no1.7
Malaysian Journal of Science 44(4): 54-63 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45 (1): 64-102 (March 2026) https://mjs.um.edu.my

The Belum-Temengor Forest Complex: A Comprehensive Review of The Floral Diversity, Ecology, Indigenous Community and Ecotourism

Hemaharshni Nagarajan[1a], Iman Batrisyia Manarus Maddin[2a], Ainnur Fateha A Hamid[3a], Jayaraj Vijaya Kumaran[4a], Wan Juliana Wan Ahmad[5b], Ahmad Fitri Zohari[6c], Sreeramanan Subramaniam[7de], Visweswara Rao Pasupuleti[8f], Mohammed Abdus Salam[9g], Jaya Seelan Sathiya Seelan[10h], Muneeb M. Musthafa[11i], Komala Thirumalai[12j], Liew Ju Neng[13k], Zubir Ismail[14l], Ching Fong Lau[15l], Siti-Munirah Mat Yunoh[16m], Nivaarani Arumugam[17a], Nurul Wahida Binti Abdullah[18a], and Suganthi Appalasamy[19ao*]

Abstract: The Belum-Temengor Forest Complex (BTFC) is a recognised biodiversity nexus, home to numerous endemic and threatened species. This forest complex includes diverse ecosystems, ranging from lowland dipterocarp forests to highland areas, which are pivotal in ecological balance and local climate processes. The forest has been designated as an Environmentally Sensitive Area (ESA) Rank 1 due to its unique environmental value, highlighting the need for rigorous protection and conservation measures. The current study provided a comprehensive analysis of the existing literature on the BTFC, covering vital aspects such as floral composition, focusing on several important species, ecological role, ecotourism, and the interrelationships among the Orang Asli community within this forest complex. The findings revealed that the diverse floral species composition within the forest complex significantly enhances the ecotourism potential and supports the socio-economic development of the Orang Asli communities. Additionally, the current study highlighted the crucial ecological role of the forest and addressed several threats, including deforestation and habitat loss.

Keywords: *Belum-Temengor Forest Complex, floral composition, ecological role, Orang Asli, ecotourism.*

Authors information:

[a]Faculty of Earth Science, Universiti Malaysia Kelantan (UMK), Jeli Campus, 17600 Jeli, Kelantan, MALAYSIA. Email: hemanaga31@gmail.com[1], imanbatrisyia.mm@gmail.com[2], ainnurfatehahamid@gmail.com[3], jayaraj@umk.edu.my[4], niva035@gmail.com[17]; nrl.wahida00@gmail.com[18]; suganthi.a@umk.edu.my[19]

[b]Institute of Climate Change, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, MALAYSIA. Email: wanjulianawanahmad@gmail.com[5]

[c]Department of Biological Science and Biotechnology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, MALAYSIA. Email: ahmadfitri@ukm.edu.my[6]

[d]School of Biological Sciences, Universiti Sains Malaysia (USM), 11800, Gelugor, Pulau Pinang, MALAYSIA. Email: sreeramanan@gmail.com[7]

[e]Chemical Centre Biology (CCB), Universiti Sains Malaysia (USM), 11900 Bayan Lepas, Pulau Pinang, MALAYSIA. Email: sreeramanan@gmail.com[7]

[f]Centre for International Relations and Research Collaborations, REVA University, Rukmini Knowledge Park, Kattigenahalli, Yelahanka, Bangalore 560064, Karnataka, INDIA. Email: visweswararao@reva.edu.in[8]

[g] Department of Environmental Science and Disaster Management, Noakhali Science and Technology University, Noakhali, BANGLADESH. Email: masalam.esdm@nstu.edu.bd[9]

[h] Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, MALAYSIA. Email: seelan80@ums.edu.my[10]

[i] Department of Biosystems Technology, Faculty of Technology, South Eastern University of Sri Lanka, University Park, Oluvil, SRI LANKA. Email: muneeb@seu.ac.lk[11]

[j] Department of Biochemistry, Faculty of Medicine, Quest International University, 30250 Ipoh, Perak, MALAYSIA. Email: komala.thirumalai@qiu.edu.my[12]

[k]Department of Earth Sciences and Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, MALAYSIA. Email: juneng@ukm.edu.my[13]

[l]Perak State Park Corporation (PSPC), 30010 Ipoh, Perak, MALAYSIA. Email: zubirgerik@gmail.com[14], lauchingfong@perak.gov.my[15]

[m]Forest Research Institute Malaysia (FRIM), 68100 Kuala Lumpur, MALAYSIA. Email: sitimunirah@frim.gov.my[16]

[o]Institute of Food Security and Sustainable Agriculture (IFFSA), Universiti Malaysia Kelantan (UMK), Jeli Campus, 17600 Jeli, Kelantan, MALAYSIA. Email: suganthi.a@umk.edu.my[19]

*Correspondence: suganthi.a@umk.edu.my

Received: January, 2024
Accepted: January, 2025
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

1. Introduction to Belum-Temengor Forest Complex

Tropical rainforests that once covered 12% of the Earth's surface now occupy less than 5%. Despite this drastic reduction, these forests continue to provide essential ecosystem functions, supporting more species and biomass than any other forest type (Brandon, 2015). Tropical forest formation is distinguished by high temperatures, continuous sunlight year-round, abundant rainfall, and a diverse range of species. Brandon (2015) described tropical rainforests as ecosystems characterised by elevated temperatures and substantial annual rainfall of 1,500-3,000 mm, with a short dry season lasting three months or less, or potentially none. For instance, Southeast Asian rainforests form the world's third-largest tropical rainforest block, encompassing tropical, evergreen, and deciduous biomes in areas affecred by seasonal droughts (Dong et al., 2012).

Southeast Asian rainforests also have dense canopies, with trees often exceeding 35 metres in height, while their extraordinary species diversity has made them a research focus, especially in Malaysia (Nakamura et al., 2017; Walsh, 1996). Malaysia is prominent in the region, being one of the world's 17 megadiverse countries due to its exceptional biodiversity, scenic landscapes, and unique ecosystems (Abdullah et al., 2013). The remarkable level of endemism in the country further enhances its global ecological importance. The BTFC is one of the largest and most intact tropical rainforest regions in Peninsular Malaysia, covering over 300,000 hectares of rainforest (Schwabe, 2015).

The Royal Belum State Park (RBSP) was first gazetted in 2007, encompassing 117,500 hectares. The rainforest covers approximately all of the former Belum Forest Reserve (132,133 ha), excluding a narrow southern strip named the Banding Forest Reserve. Collectively, the Gerik, Amanjaya, and Temenggor Forest Reserves form the continuous rainforest landscape of the BTFC (see Figure 1.1). The BTFC contains various forest types, distributed by elevation, ranging from lowland dipterocarp forests (0–300m) to hill dipterocarp forests (300–750m), upper dipterocarp forests (750–1200m), and montane forests (above 1200m). Meanwhile, altitudes within the BTFC range from 130 to 2161 metres above sea level (ASL), with Gunung Ulu Sepat being the highest peak (Latiff & Mat Salleh, 2001).

The BTFC is critical within the Central Forest Spine (CFS) of Malaysia, serving as the backbone for ecological connectivity by linking ESAs and other protected regions. As an ESA, the park and the BTFC are vital for protecting endangered species, such as the Asian elephant (*Elephas maximus indicus*), Malayan tiger (*Panthera tigris jacksoni*), Malayan tapir (*Tapirus indicus*), and helmeted hornbill (*Rhinoplax vigil*). The forest also contains significant plant diversity, with approximately 3,000 species of flowering plants, including four species of the large *Rafflesia*, 304 endemic species, and 104 threatened species (PLANMalaysia, 2023). The forest complex underwent significant structural changes during the 1970s. First, the construction of the 124-kilometre East-West Highway in 1975, which bisected the BTFC into two sections: The RBSP in the north and the Temengor Forest Reserve (TFR) in the south. This highway was built for strategic defense during the communist insurgency, fragmenting the forest and creating distinct ecological zones. The second major development was the completion of the Temengor Dam in 1977, which submerged vast forest areas, forming the Temengor Lake and leaving numerous islands that had once been hilltops (Yeap & Lim, 2020). This lake covers approximately 15,200 hectares and is the second-largest man-made lake in Peninsular Malaysia, serving as a vital water source for numerous states within the country.

The BTFC remains one of the largest intact forest complexes in Peninsular Malaysia despite these developments. The forest complex serves as a crucial ecological corridor, supporting biodiversity conservation and hydrological functions. This key component of the nation's ecological network underscores the importance of sustained conservation efforts amid ongoing environmental pressures.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

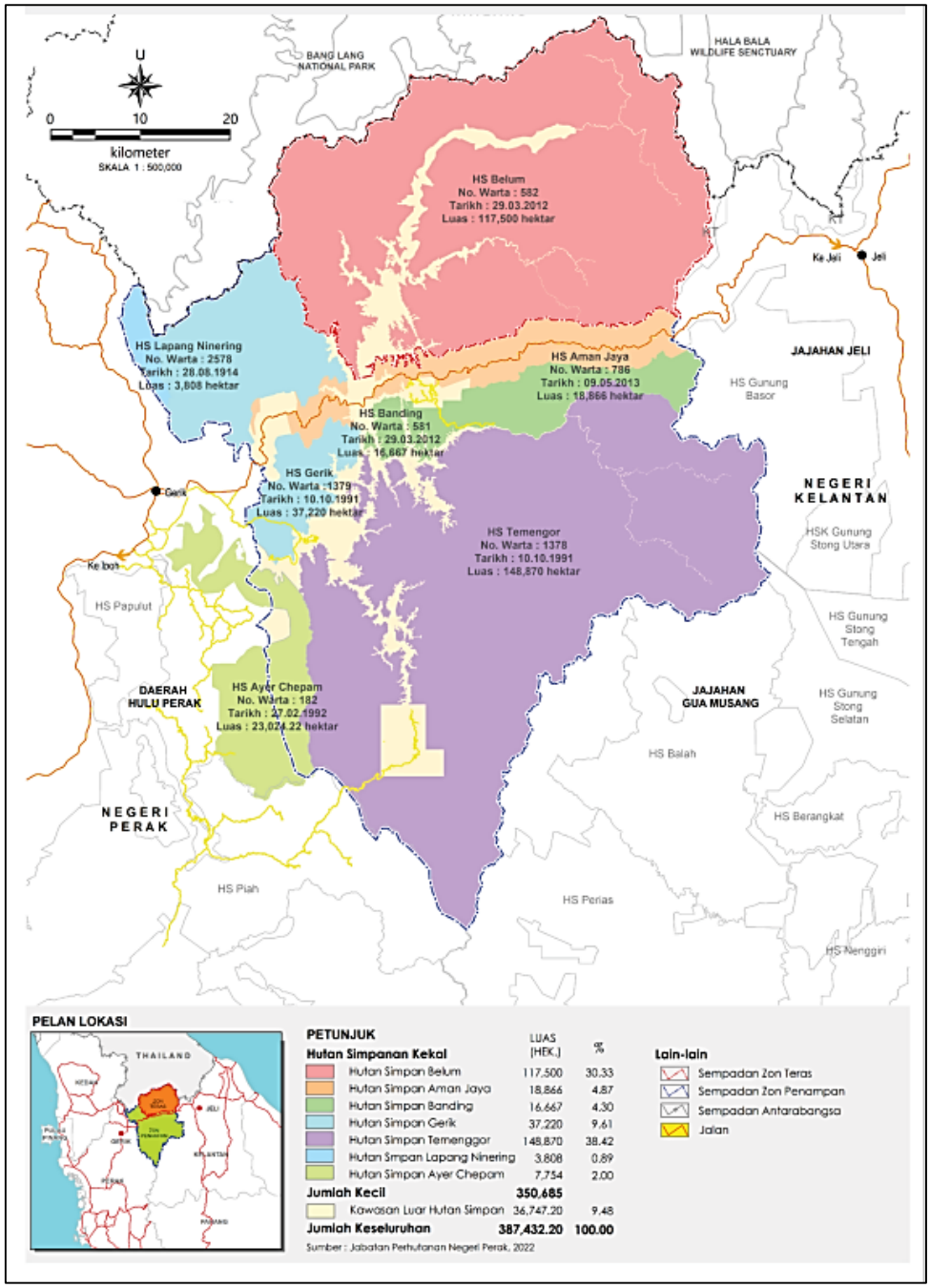


Figure 1.1. Map of the BTFC highlighting the various forest blocks in the area, including RBSP, Gerik, Temengor, Amanjaya, and Banding Forest Reserve, connected by the Temengor Lake (PLANMalaysia, 2023)

Table 1.1. Key components of BTFC

Area	Forest Classification	Size
Royal Belum State Park	State park	128,272 ha
Gerik Forest Reserve	Production forest and partially protected forest	37 220 ha
Temengor Forest Reserve (TFR)	Production forest	148 870 ha
Banding Forest Reserve	Production forest	16 667 ha
Amanjaya Forest Reserve	Production forest	18 866 ha
Temengor Lake		15 200 ha
East -West Highway		124 km

(Ching & Leong, 2011; PLANMalaysia, 2023)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

The BTFC is critical within the Central Forest Spine (CFS) of Malaysia, serving as the backbone for ecological connectivity by linking ESAs and other protected regions. As an ESA, the park and the BTFC are vital for protecting endangered species, such as the Asian elephant (*Elephas maximus indicus*), Malayan tiger (*Panthera tigris jacksoni*), Malayan tapir (*Tapirus indicus*), and helmeted hornbill (*Rhinoplax vigil*). The forest also contains significant plant diversity, with approximately 3,000 species of flowering plants, including four species of the large *Rafflesia*, 304 endemic species, and 104 threatened species (PLANMalaysia, 2023). The forest complex underwent significant structural changes during the 1970s. First, the construction of the 124-kilometre East-West Highway in 1975, which bisected the BTFC into two sections: The RBSP in the north and the Temengor Forest Reserve (TFR) in the south. This highway was built for strategic defense during the communist insurgency, fragmenting the forest and creating distinct ecological zones. The second major development was the completion of the Temengor Dam in 1977, which submerged vast forest areas, forming the Temengor Lake and leaving numerous islands that had once been hilltops (Yeap & Lim, 2020). This lake covers approximately 15,200 hectares and is the second-largest man-made lake in Peninsular Malaysia, serving as a vital water source for numerous states within the country.

The BTFC remains one of the largest intact forest complexes in Peninsular Malaysia despite these developments. The forest complex serves as a crucial ecological corridor, supporting biodiversity conservation and hydrological functions. This key component of the nation's ecological network underscores the importance of sustained conservation efforts amid ongoing environmental pressures.

2. Floral and Tree Species Composition and Diversity in Belum-Temengor Forest Complex

Asian tropical rainforests possess a rich and diverse tree species, representing families such as Burseraceae, Arecaceae, Annonaceae, Clusiaceae (mangosteen family), Fabaceae, Euphorbiaceae (spurge family), Ebenaceae (ebony family), Myristicaceae, Moraceae, Lauraceae, Meliaceae, Myrtaceae, Olacaceae, Rutaceae, Phyllanthaceae (phyllanthus family), Rubiaceae, Sapotaceae, and Sapindaceae (litchi family). In areas recovering from natural or human disturbances, young forests are usually dominated by pioneer species, such as *Macaranga* speciesand bamboo in Southeast Asia. Family Dipterocarpaceae (see Figure 2.1) trees dominate the forests in the Malay Peninsula and contribute to regional ecology and economy (Corlett & Primack, 2011).

The BTFC is a sanctuary for multiple ecologically significant and unique plant species. Towering dipterocarps cover the forest canopy, while vibrant orchids and other flora enrich its understorey. The forest complex is a biodiversity hotspot in Southeast Asia, harbouring rare and endemic species that showcase its global conservation value. Approximately 3,000 plant species, representing 171 families and 850 genera, have been recorded in BTFC, including 304 species endemic to Peninsular Malaysia and 104 species under the International Union for Conservation of Nature (IUCN) Red List. Zaki et al. (2014), Ahmad Fitri et al. (2017a), and Awang et al. (2023) identified dominant plant families, such as Dipterocarpaceae, Rubiaceae, and Euphorbiaceae, which significantly shape the ecological dynamics of the forest. Intriguing species include *Rafflesia*, *Thismia belumensis*, and *Johannesteijmannia perakensis,* thus accentuating the unique floral assemblage in this forest complex. Detailed plant lists in BTFC are provided in Appendices A, B, and C.The BTFC features various forest types, including lowland dipterocarp forests (below 300m ASL), hill dipterocarp forests (300–750m ASL), upper dipterocarp forests (750–1200m ASL), montane forests (over 1,200m ASL), riparian zones, and limestone forests. These forest types host diverse tree species and exhibit distinct structural characteristics that vary with elevation (Siti Eryani et al., 2023; Syahida-Emiza et al., 2023).

Figure 2.1. Tree species composition at the Royal Belum State Park, primarily dominated by the Dipterocarpaceae family

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

The riverine areas near boat drop-off points are rich in species, such as *Hibiscus floccosus* and *Scaphium linearicarpum* (Malvaceae), *Mallotus muticus* (Euphorbiaceae), and the dominant *Monocarpia marginalis* (Annonaceae). Riparian zones feature abundant *Saraca indica* (Fabaceae) and *Pometia pinnata* (Sapindaceae), while rocks and boulders along streams produce microhabitats for lithophytic ferns, such as *Bolbitis appendiculata* and *Selaginella* species. Additionally, palms, rattans, and bamboos are prevalent in these areas (Siti Eryani et al., 2023; Syahida-Emiza et al., 2023).

At an elevation of approximately 300 metres ASL, the forest comprises mainly Annonaceae, Malvaceae, Leguminosae, and Euphorbiaceae, with emergent trees, namely *Rubroshorea* and *Dipterocarpus* species. This zone supports numerous plants, including *Neesia altissima*, *Intsia palembanica*, and *Memecylon amplexicaule*. Forest gaps caused by fallen trees foster the growth of pioneer species, such as *Balakata baccata*, *Endospermum diadenum*, and *Macaranga hypoleuca*. Climbers (genus *Gnetum* and *Aristolochia*), shrubs ((*Cyrtandra cupulata* (Gesneriaceae) and *Clerodendron deflexum* (Labiatae)), and the herbaceous *Orchidantha longiflora* (Lowiaceae) cover the forest floor (Siti Eryani et al., 2023; Syahida-Emiza et al., 2023).

The floristic composition changes at an elevation of 900 metres ASL, particularly in the upper and main canopy, where trees have smaller girths and a thinner canopy. Species characteristic of the hill dipterocarp forest, such as *Rubroshorea curtisii* (Dipterocarpaceae), are commonly found on ridges, distinguished by their greyish-blue crowns. Several lowland species persist but are present in much lower abundance. The understorey in this area is enriched with edible non-dipterocarp species, including *Archidendron bubalinum* (kerdas), *Diplospora malaccense*, *Elaeocarpus nitidus*, and *Garcinia urophyll*. Gesneriads and ginger species are common along the hill ridges, adding to the botanical diversity. Palms such as *Iguanura polymorpha* and *I. wallichiana* extend from the lowlands into these upper hills, showcasing the adaptive range of these species within the dipterocarp forest (Siti Eryani et al., 2023; Syahida-Emiza et al., 2023).

The forest transitions into a lower stature 1,200 metres ASL, with trees typically not exceeding 30 metres in height. The canopy becomes more open, with reduced girth and density, and tall emergent trees are rare. Oaks and laurels, including *Syzygium* species (Myrtaceae) and *Lithocarpus* species (Fagaceae), dominate the landscape. In forest gaps formed by landslides or fallen trees, *Oxyspora curtisii*, *O. exigua* (Melastomataceae) and *Alpinia glabra* (Zingiberaceae) commonly establish themselves. On steep, damp, and shady slopes, tree ferns such as *Cyathea borneensis* (Cyatheaceae) are occasionally observed. The forest floor is covered with dense litter and layers of humus, which provide an ideal substrate for lithophytic ferns, lycophytes, orchids, and aroids. Moreover, mosses and liverworts thrive on numerous surfaces, including rocks, leaves, and tree trunks (Syahida-Emiza et al., 2023).

Towards the summits of Gunung Hulu Temin and Gunung Hulu Tan Hain, trees become smaller in diametre, reaching approximately six metres in height. The shaded understorey supports diverse herbaceous plants, such as gesneriads, gingers, and ferns. Notably, *Codonoboea oreophila*, which grows in rock crevices, and *Phyllagathis hispida* (Melastomataceae), carpets the forest floor along slopes near the summit. Near the peak, a thick humus layer interspersed with patches of *Sphagnum* moss emerges, along with striking montane plants such as the pitcher plant *Nepenthes sanguinea* (Nepenthaceae). The summit landscape is adorned with blooming flowers of *Spathoglottis aurea* (Orchidaceae) and *Rhododendron klossii* (Ericaceae), thriving amid conifers, such as *Podocarpus neriifolius* and *Dacrydium elatum* (Podocarpaceae) (Syahida-Emiza et al., 2023).

The limestone hills near the Temengor Dam in BTFC are ecologically significant due to their unique flora and fauna. Most of these hills are partially submerged by the lake and host various species that thrive in the karst environments. For instance, *Cnesmone subpeltata* (Euphorbiaceae) is a vine species restricted to limestone habitats and has been recorded in the area, marking the species as the third locality in Peninsular Malaysia. *Homalium undulatum* (Salicaceae) is abundant on limestone slopes and features spiny leaves and is listed as vulnerable on the IUCN Red List. Another species is *Grewia sclerophylla*, a small shrub or tree found in these limestone regions, with tough, leathery leaves typical of its adaptation to harsh environments. Other notable species include *Cratoxylum arborescens* (Clusiaceae), *Diospyros lotus* (Ebenaceae), and *Phyllanthus tesselatus* (Phyllanthaceae), all adapted to the rocky terrain. Additionally, the hills are home to rare species, such as *Aporosa villosa* (Euphorbiaceae) and *Streblus elongatus* (Moraceae) (Turner et al., 1995). The limestone hills are critical as a significant tourist attraction and an essential habitat for endemic and specialised species. These karst formations provide a unique ecological niche, with each hill supporting diverse plant and animal species. The diversity of flora and fauna in these areas outlines the high conservation value of the limestone hills in BTFC, highlighting the need to protect these fragile ecosystems.

Tropical rainforests display distinct stratification, namely the understorey, canopy, emergent, and forest floor, each with unique structural and ecological characteristics. The emergent layer contains mature, light-demanding, and long-lived trees growing over the main canopy. This layer often exceeds 30 metres in height and features wide-spreading crowns. The main canopy layer below consists of mature trees reaching 20–30 metres. This layer is light-demanding and long-lived, forming a dense, interlocking cover that dominates the forest structure. The understorey layer lies beneath, where shade-tolerant species thrive in the limited light available, growing below 20 metres and forming the lower stratum of the forest (Ahmad Fitri et al., 2017a).

A study examined the lowland dipterocarp forest (260–440 m ASL) of the RBSP and recorded notable species across these layers. The emergent and primary canopy layers were dominated by members of the Dipterocarpaceae family, including *Shorea leprosula*, *S. guiso*, and *Anisoptera laevis*, alongside species from Leguminosae (*Intsia palembanica*, *Sindora coriacea*), Burseraceae (*Canarium littorale*, *Santiria laevigata*), and Fagaceae

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

(*Lithocarpus wallichianus*). In contrast, the understorey layer was predominantly composed of species from families, such as Annonaceae (*Alphonsea elliptica*, *Goniothalamus macrophyllus*), Ebenaceae (*Diospyros wallichii*), Euphorbiaceae (*Antidesma montanum*, *Aporosa* spp.), Lauraceae (*Beilschmiedia palembanica*, *Cryptocarya infectoria*), and Sapindaceae (*Nephelium cuspidatum*, *Paranephelium xestophyllum*). An endangered dipterocarp species, *Shorea farinosa*, was also recorded for the first time in Perak along Sungai Beruak. Furthermore, palms such as *Calamus castaneus* (Arecaceae) were abundant (Chua et al., 2000).

Stratification patterns were observed in the hill dipterocarp forest of the TFR (560–810 m ASL). The emergent layer was dominated by Dipterocarpaceae species, including *Anisoptera*, *Dipterocarpus*, *Parashorea*, and *Shorea*, as well as Leguminosae species, such as *Dialium platysepalum*, *Intsia palembanica*, and *Koompassia malaccensis*. Meanwhile, the main canopy layer featured species, such as *Hydnocarpus woodii* and *Elateriospermum tapos*, reflecting a different composition at higher elevations. These stratification patterns highlight the structural and species diversity of tropical forests, influenced by elevation and ecological conditions (Ahmad Fitri et al., 2017a).

The Rafflesiaceae family is renowned for its extraordinary flowers, namely *Rafflesia arnoldii*, which is the largest individual flower in the world (Kedri et al., 2018). The tropical rainforests in Southeast Asia host 42 *Rafflesia* species (Malabrigo et al., 2023), with three species: *R. azlanii*, *R. cantleyi*, and *R. kerrii* endemic to Peninsular Malaysia and the BTFC (Siti-Munirah, 2020). *R. cantleyi* and *R. azlanii* were discovered in Kampung Sungai Raba and Kampung Bongor in BTFC, with notable findings such as an individual *R. azlanii* flower displaying 10 petals in Sungai Gadong, Perak (Siti-Munirah, 2020).

The distribution of *R. azlanii* extends across Perak (Sungkai, Kinta, Sungai Halong, and Temengor) and Pahang (Taman Negara, Ulu Sungai Forest Reserve, and Sungai Peleting), while *R. cantleyi* spans Perak (Gerik, BTFC, and Ulu Geroh), Pahang (Raub, Jerantut, Taman Negara, and Pulau Tioman), Kelantan (Kuala Koh, Kuala Betis, and Ulu Sat), and Terengganu (Tasik Kenyir, Pasir Akar, and Besut) (Norhazlini et al., 2021). These species occupy distinct ecological niches, with *R. azlanii* favouring primary lowland dipterocarp forests and lower montane forests at altitudes of 150-400 m, while *R. cantleyi* thrives in primary and logged lowland dipterocarp forests at altitudes of 200-610 m. The survival of *Rafflesia* in these regions is connected to the availability and health of *Tetrastigma* vines, reinforcing its role as a vital species in these ecosystems.

Figure 2.2: *Rafflesia azlanii* of Royal Belum State Park

Note: A = flower in situ; B = a side view of the flower showing the eyeball-like tube perigone and diaphragm dome; C = flower from side view; D and F = perigone lobe (petal); E = warts (blotches) on perigone lobes; G = diaphragm (upper surface) and aperture (opening of diaphragm); H = wart dots on the diaphragm surface; I = wart dots (Siti-Munirah, 2020)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

The BTFC is a critical sanctuary for endemic and threatened species, outlining its unparalleled ecological importance. For instance, endemic flora, such as *Actinodaphne cuspidata*, *Ardisia perakensis*, *Thismia belumensis*, and *Johannesteijsmannia perakensis* underscore the unique biodiversity of BTFC. The *T. belumensis is* a recently discovered achlorophyllous herb that thrives in the lowland forests of the RBSP (Siti-Munirah et al., 2021). Meanwhile, the towering erect stem and striking diamond-shaped leaves of *J. perakensis a*re found only in Perak and parts of Kedah. Critically endangered species, such as *Pseuduvaria taipingensis*, *Aquilaria malaccensis* (agarwood), and *Gonystylus bancanus* are safeguarded within BTFC. *A. malaccensis* are threatened by overharvesting, habitat loss, and low regeneration rates. Meanwhile, *P. taipingensis,* endemic to Peninsular Malaysia, has a limited distribution and is subject to habitat degradation. The BTFC provides a refuge for these rare and valuable species, critical in sustaining their populations and preventing extinction, emphasising the need for its continued conservation.

The BTFC is a vast, biodiverse region that remains largely unexplored, with new species continually being discovered, such as *Castanopsis corallocarpus*, highlighting the rich potential of the area for scientific discovery. Tan et al. (2023) discovered this new species from the Fagaceae family in the RBSP. *Castanopsis* (D.Don) Spach ranks third among the genus in Fagaceae, with over 134 species. Click or tap here to enter text.The Fagaceae family is a significant component of the tropical rainforest in Peninsular Malaysia, comprising 72 species distributed across four genera, namely *Castanopsis*, *Trigonobalanus, Quercus,* and *Lithocarpus*. The genus is locally referred to as *berangan* in Malay or the Malayan chestnut. In Peninsular Malaysia, a total of 20 identified species of *Castanopsis* existClick or tap here to enter text..

In Tan et al. (2023)**,** the team encountered a previously unknown species during the 2018 RBSP expidition, which was subsequently classified under the genus *Castanopsis*. The species is geographically limited to low to mid-elevation forests within the BTFC, specifically in Sungai Papan and Sungai Tiang. Thriving in lowland and hillside dipterocarp forests 300-450 m ASL, the species is characterised by low-nutrient, clay-rich soils typical of most dipterocarp ecosystems. *Castanopsis corallocarpus* (see Figure 3.5) is a moderate-sized tree distinguished from similar ones by its fruits, with distinct rows of blunt, coral-like spines on the outside surface of the cupule and unusual, rounded, rectangular nuts that are asymmetric. The IUCN Red List issued an initial assessment of the species conservation status as Critically Endangered (CR) based on two records from RBSP (Tan et al., 2023).

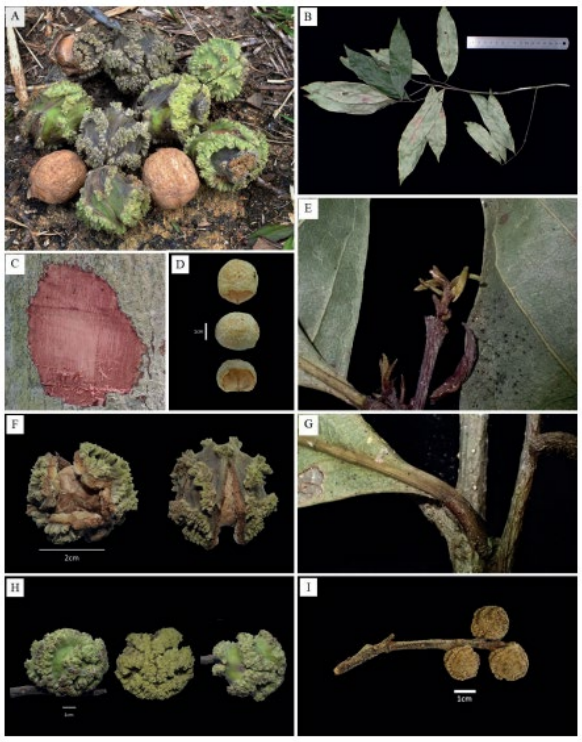


Figure 2.3: *Castanopsis corallocarpus* W.H.Tan & Strijk, sp. nov. *W.H.Tan TWH002* (KEP)

Note: A and B = Fruits and leaves harvested during sample collection; C = Sapwood and bark; D = Nut from different view (top, side, front); E = New young leaf; F = Cupule of matured fruit with split-valves; G = Petiole; H = Mature fruit from different view (top, side, front); I = Infructescence spike (Tan et al., 2023)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

The trees of BTFC are vital in carbon sequestration, contributing significantly to global climate regulation. As a tropical rainforest, BTFC is a vital carbon sink, absorbing and storing substantial amounts of carbon dioxide from the atmosphere. The forest exhibits exceptional carbon storage capacity due to the high wood density and substantial biomass of its tree species, dominated by towering trees, particularly from families such as Dipterocarpaceae. For instance, *Shorea* and *Dipterocarpus* species grow to impressive heights and store vast amounts of carbon in their trunks, branches, and roots. Other large tree species in BTFC, such as *Koompassia excelsa* (tualang) and *Neobalanocarpus heimii* (chengal), further enhance this capacity. These trees have long lifespans and dense wood and accumulate carbon over decades, making them critical to mitigating climate change. The preservation of the extensive forests in BTFC is essential for biodiversity conservation and maintaining its role as a major carbon reservoir in Southeast Asia.

3. Ecology, Environmental Status, and Management of Resources Within The Belum-Temengor Forest Complex

Tropical rainforests are characterised by tall, dense, and evergreen vegetation, serving as the primary natural cover in wet tropical regions. These ecosystems thrive in consistently warm climates with minimal or no dry season, which is a unique adaptation to high levels of humidity and year-round precipitation (Corlett & Primack, 2011). Chapin et al. (2012) stated that tropical forests provide a vast array of ecosystem services due to climate, terrain, soil composition, water and nutrient availability, natural disturbances, including fires, various species, and human activities. The global comparison on the main regions of tropical rainforest is presented in Table 3.1.

Table 3.1: Characteristics of the main global rainforest regions

	Neotropics	Africa	Madagascar	Southeast Asia	New Guinea
Main Geographical features	Amazon River basin and Andes Mountains	Congo River basin	Forests along eastern edge of island	Peninsula and islands on Sunda Shelf	Large, mountainous island
Largest country	Brazil	Democratic Republic of Congo	Malagasy Republic	Indonesia	Papua New Guinea
Annual rainfall (mm)*	2,000-3,000	1,500-2,500	2,000-3,000	2,000-3,000, often > 3,000	2,000-3,000, often > 3,000
Annual temperature	Ranges increase with distance from equator, ranges decrease with increasing altitude from ASL				
Wind	Affected by tropical cyclones				
Light availability	Vary between sites, seasons and phases of El Nino-Southern Oscillation (ENSO) cycles				

* Rainfall is highly variable within each region. These are the ranges over most of the core rainforest area (1,000 mm equals 40 inches). (Corlett & Primack, 2011).

The BTFC situated in Perak, Malaysia, is the largest intact forest complex in Peninsular Malaysia and one of the tropical rainforests in Southeast Asia. The climate in BTFC is mainly tropical, with elevation ranging from 140-2,161 m and characterised by dipterocarp trees. The region maintains a consistently warm and humid climate year-round, with an average temperature ranging from 24 to 29.9℃ throughout the year. Meanwhile, the humidity levels in the area fluctuate between 70% and 98%, with significant rainfall occurring in April and October, and minimal rain in February and July (Aiman Hanis et al., 2014, Kanniah et al., 2018). This forest complex contains multiple ecosystems, including lowland dipterocarp woods and highland areas, which contribute to the distinctive environment that sustains a high degree of biodiversity.

The BTFC is one of the most biodiverse regions in Malaysia, hosting over 3,000 species of flowering plants. The rich biodiversity in the reserve extends to its animal population, with over 140 mammal species, including the critically endangered Malayan tiger, Asian elephant, and Sunda pangolin (Lazarus et al., 2021; PLANMalaysia, 2023). The BTFC is home to nine species of primates, the highest diversity of primates in the Malay Peninsula. Birdlife is equally diverse, with over 336 species recorded (Yeap & Lim, 2020), including 10 hornbill species, making this landscape part of the larger landscape with the highest hornbill diversity globally. Additionally, the area supports numerous amphibians, reptiles, and freshwater fish, contributing to its status as a vital ecological hotspot.

The comparable biodiversity hotspot in Peninsular Malaysia is Taman Negara National Park (TNNP), the first and largest national park in the country, gazetted in 1938/1939. The TNNP is renowned for its rich biodiversity, hosting spectacular arrays of flora and fauna within one of the oldest rainforests in the world (UNESCO, 2014). This park exemplifies the national commitment to conservation and symbolises ecological preservation. Conversely, BTFC holds a unique position in regional conservation efforts due to its vital transboundary conservation role. As part of the Transboundary ASEAN Heritage Park (AHP), BTFC links Malaysian forests with protected areas in Thailand (Hala Bala

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Wildlife Sanctuary and Bang Lang National Park) to facilitate wildlife migration and encourage genetic diversity across borders (PLANMalaysia, 2023). This connectivity underscores the significance of BTFC in the broader ecological network of Southeast Asia, making it an indispensable asset for regional biodiversity conservation.

In terms of the significance of forest reserves, a comparison between the BTFC and the Ulu Muda Forest Reserve (UMFR) emphasises their distinct ecological roles. Mei et al. (2017) mentioned that UMFR is a crucial water catchment area for the northern Peninsular Malaysian states of Kedah, Penang, and Perlis, while BTFC mainly supports localised ecosystems. Consequently, the biodiversity of UMFR is more centered on freshwater-dependent species. The rivers within UMFR also contribute to maintaining vital ecosystems, including the mudflats along the Kedah coast, which are important habitats for migratory birds and economically valuable shellfish (Rajoo et al., 2021).

Apart from being a biodiversity hotspot, the forest complex provides various ecosystem services, including regulating the hydrological cycle, providing clean water, and preventing erosion and sedimentation. The National Physical Plan has classified the forest as an ESA-Rank 1, meaning that development, farming, and logging are strictly forbidden, except for research, educational activities, and minimal impact tourism (Kanniah et al., 2018). The ESA is a land-use strategy originally developed by the United Kingdom and later adopted by Malaysia**.** The specific definition of ESA varies depending on the type of governance framework in place, which refers to a specific region vulnerable to any alterations in its ecology caused by internal or external natural processes, directly or indirectly (Munian et al., 2023)**.**

The BTFC has significant economic value, direct and indirect. Gwee et al. (2019) expressed that one benefit is the direct value obtained from logging activities that may be instantly utilised. Private corporations could directly benefit from the income generated by logging activities, while Perak and the Malaysian government receive royalties and premiums based on the quantity of timber harvested and the land area used for logging (Schwabe et al., 2015). Furthermore, BTFC adds indirect benefit by acting as a catchment basin and supplying water to most northern states in Malaysia (Abdullah et al., 2013). The forest complex preserves the hydrological cycle and acts as a carbon sink, a flood-control device, and a study site for ecological and forest research. In the current global environment, Hurteau (2021) emphasised that forests are crucial in the uptake and sequestration of carbon dioxide, including overall maintenance of the global stock of carbon storage capacity. Ecotourism increases the utility of the BTFC by offering non-extractive benefits through recreational experiences for visitors (Gwee et al., 2019).

Southeast Asia experiences the highest rates of forest loss and degradation among tropical regions (Corlett & Primack, 2011). Laurance et al. (2010) stated that major threats to deforestation involve the extensive logging industry and the conversion of forests into agricultural plantations, specifically for cash crops such as oil palm. Beyond deforestation, tropical forests are threatened by fragmentation. Rainforests that are cleared for agriculture often leave scattered forest fragments. Laurance et al. (2011) examined the Amazon rainforest and revealed that habitat fragmentation significantly reduces movement between these isolated patches. Numerous species, including birds, mammals, and insects, are unable or unwilling to cross even small open areas, with roads posing a notable barrier (Laurance et al., 2009). Fragmentation also alters the microenvironment at the edges of forest fragments, which can profoundly affect species composition. These outcomes cause the gradual loss of species within fragments. The rate and extent of species loss depend on fragment size, distance from other forested areas, and the surrounding land use.

The Malaysian National Forestry Act of 1984 was enacted in Perak to safeguard forests against the deterioration of BTFC (Kanniah et al., 2018). The National Forest Policy (NFP) 1977, later approved by the National Land Council in 1978, enables Malaysia to maintain its Permanent Reserved Forest (Mundher et al., 2022). The Amanjaya Forest Reserve spans 18,886 hectares and was officially gazetted in 2013 to strengthen ecological linkages between the RBSP and TFR as part of the Central Forest Spine Plan in Malaysia. The gazettement of the reserve aligns with the National Physical Plan, and serves as a crucial corridor to reduce fragmentation between forest blocks. Jewitt et al.'s (2017) research in South Africa applied connectivity corridors to connect protected areas in KwaZulu-Natal (KZN), facilitating species adaptation to environmental changes and preserving floristic variety amid land-cover and climate change.

The forest biodiversity conservation, community involvement in forest development, and genetic resource management were all given legal significance in 1992 due to legislative reforms. Nevertheless, the protection was inadequate and not extensive, resulting in the significant depletion of multiple forest regions (Kanniah, 2017). Globally, community-based conservation successes are demonstrated with the Green Belt Movement, an initiative led by Nobel Peace Prize laureate Wangari Maathai in Kenya, which encourages women to plant trees and restore degraded lands (Clauzel, 2024). Millions of trees were planted throughout this programme, increasing forest cover and boosting local economies. Consumer choices that support responsible producers reduce environmental damage. For example, consumers can choose products from companies with forest certifications, which prove that the forests are managed sustainably. Certifications, such as those from the Forest Stewardship Council (FSC), ensure that wood products meet strict environmental and social standards (Corlett & Primack, 2011). Consumers can help protect forests and encourage sustainable practices by purchasing certified products.

Conservation efforts in areas such as BTFC are vital for protecting wildlife, flora, and the overall ecosystem, preserving biodiversity and mitigating climate change. Nonetheless, these efforts could adversely affect the Indigenous communities that depend on the forest for their livelihoods. Indigenous communities in the Ankeniheny-Zahamena Corridor (CAZ) in Madagascar engage in activities such as swidden agriculture,

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

hunting, and gathering of forest products. These practices are critical for their subsistence and are deeply embedded in their cultural heritage. The implementation of conservation policies, such as those under the REDD+ initiative, aims to reduce deforestation and carbon emissions but impose significant restrictions on these traditional activities (Poudyal et al., 2018).

These conservation policies have caused substantial economic displacement for the local population. The restrictions on access to forest resources have resulted in high opportunity costs, comprising a significant portion of their annual income. Various households fail to secure alternative livelihoods, exacerbating their economic hardships. Compensation provided through initiatives, namely the social safeguard policies of the World Bank has been insufficient, leaving many families without adequate support (Poudyal et al., 2018). This situation underscores the need for a more balanced approach to conservation in areas such as BTFC. Although protecting biodiversity is essential, the socio-economic impacts on Indigenous communities must be considered.

The Agta hunter-gatherers of the Philippines are one of the few remaining Indigenous groups in Southeast Asia that primarily rely on traditional subsistence practices, namely hunting, fishing, and gathering forest resources. Their deep connection to the land and sustainable resource use has been integral to their cultural identity and survival. Nevertheless, the Agta encounter significant challenges due to displacement from their ancestral lands resulting from infrastructure development and extractive industries. This displacement disrupts their access to vital forest resources, threatens their traditional lifestyle, and leaves them vulnerable to poverty and food insecurity (Minter, 2010). Conservation policies should be designed and implemented to support environmental goals and the well-being of the local people, without neglecting their livelihoods and cultural practices.

Conservation efforts should prioritise protecting the most intact and representative examples of remaining rainforest communities. These preserved core areas serve as vital refuges that enable species to migrate and regenerate new forest ecosystems, safeguarding biodiversity for the future.

4. Orang Asli Community in Belum-Temengor Forest Complex

Demographic of Orang Asli In Belum-Temengor Forest Complex

The Orang Asli is the Indigenous population in Peninsular Malaysia who reside in the BTFC. The Orang Asli are divided into three groups: Senoi, Proto Malay, and Negrito or Semang, each divided into six ethnic groups. The Senoi group is divided into Jahut, Che Wong, Semoq Beri, Mahmeri, Temiar, and Semai; the Proto-Malay are Kenaq, Kuala, Semelai, Seletar, Temuan, and Jakun, the Negritos are the least populated Orang Asli group comprising Kensiu, Kintaq, Jahai, Lanoh, Mendriq, and Bateq (Masron et al., 2013). The Orang Asli tribes were classified based on physical attributes, language, cultural customs, and geographic location.

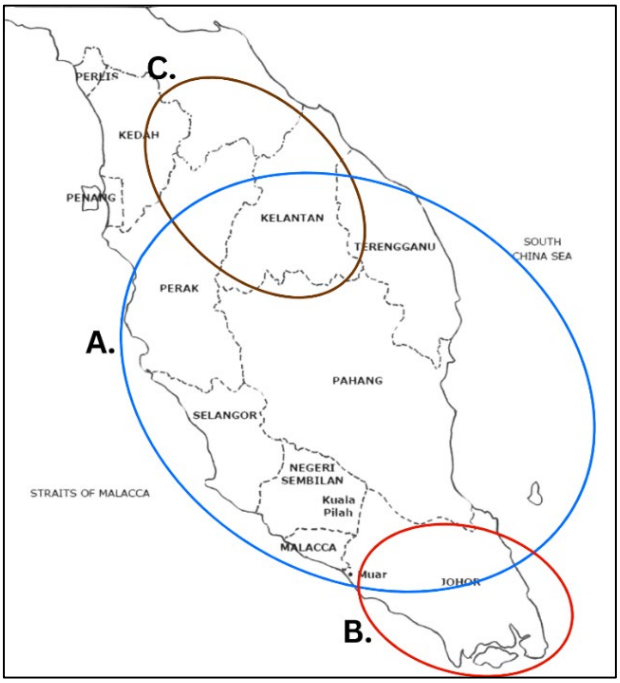


Figure 4.1: Demography of Orang Asli placement in Peninsular Malaysia
Note: A = Senoi; B = Proto-Malay; C = Negrito
(Jabatan Kemajuan Orang Asli, 2022)

Based on the International Work Group for Indigenous Affairs (2021), the number of people from the Orang Asli community accounts for only 0.7% of the overall population in Peninsular Malaysia. Figure 4.1 illustrates that the Senoi tribe holds the highest percentage, occupying the biggest area in Peninsular Malaysia. Masron et al. (2013) discovered that the Negrito tribe is the smallest and most isolated community among all tribes. Nor Awang et al. (2015) stated that Sungai Kejar and Sungai Tiang were the main settlements for the Jahai and Temiar tribes in RBSP, which became the main attraction for Indigenous tourism. The JAKOA has recorded the population of Orang Asli by their ethnicity and subtribes based on the state in Peninsular Malaysia where Jahai and Temiar settlements reside in Perak and Kelantan. Figure 4.2 focused on the population of the sub-tribe Jahai and Temiar from 2020 to 2023, where both communities have lived in the complex for ages.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

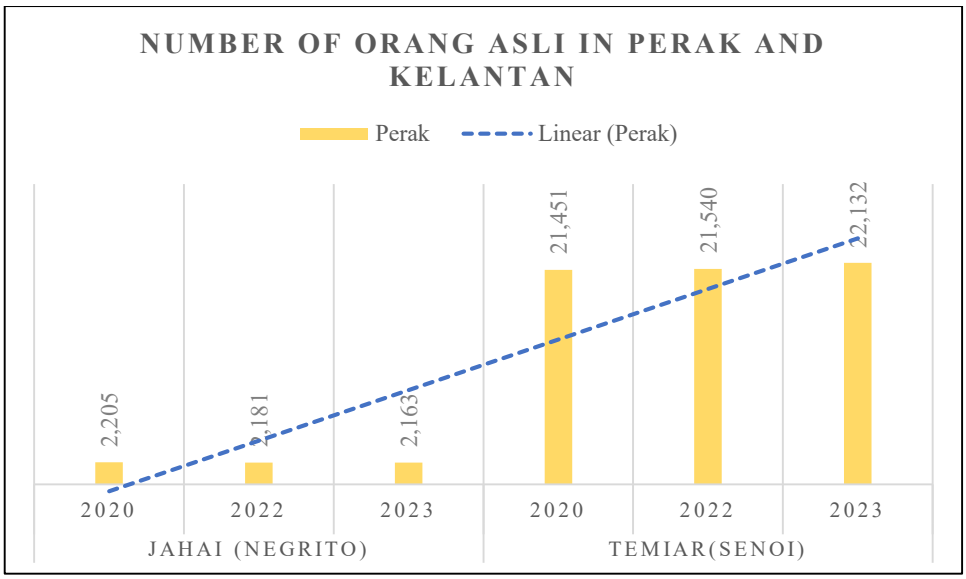


Figure 4.2: Jahai's and Temiar's Population in Perak and Kelantan from 2020 to 2023 (Jabatan Kemajuan Orang Asli, 2024)

The graph depicts the distribution of the Jahai (Negrito) and Temiar (Senoi) tribes in Perak and Kelantan between 2020 and 2023. In Perak, the Temiar population increased steadily from 21,451 in 2020 to 22,132 in 2023, while the Jahai population remained small, with minimal fluctuations around 2,000 individuals. In Kelantan, the Temiar population is smaller but demonstrates gradual growth from 14,628 to 16,406 over the same period, whereas the Jahai population is substantially lower, never exceeding 750 individuals. These trends suggest that the Temiar tribe bear a stronger presence in both states, with Perak as their primary location, while the Jahai tribe is more concentrated in Perak but maintains a smaller, stable population, reflecting their nomadic lifestyle.

Socio-Economy of Orang Asli in Belum-Temengor Forest Complex

The Jahai and Temiar communities have long relied on the forest for their sustenance, engaging in traditional hunter-gatherer activities and collecting a wide range of forest resources. The Jahai are traditionally nomadic hunter-gatherers, but have recently adopted shifting cultivation. Meanwhile, the Temiar have established themselves as a settled agricultural community, with a focus on farming. Jabatan Hal Ehwal Orang Asli (Department of Orang Asli Affairs, JHEOA) and Itam Wali (1993) stated that the primary economic activities of these communities involve gathering rattan, agarwood, resin, honey, and forest fruits, as well as fishing. Additionally, these groups practice subsistence agriculture, cultivating hill rice, cassava, and various food crops, which are integral to their cultural and economic existence in BTFC (Fadzil et al., 2013; Tabi & Zulnaidah, 2019).

Loke et al. (2020) mentioned that the Jahai hunter-gatherers residing in and around the RBSP are critical for conservation efforts as they primarily rely on hunting and fishing for their protein intake. Hunting practices in Malaysia are regulated under the Wildlife Conservation Act 2010, under which hunting protected species without a license constitutes a criminal offence. Special exemption was made for Orang Asli communities that allow them to hunt 10 specific protected mammal and bird species without requiring a license (Hassan, 2015). Nonetheless, this exemption is limited as it does not authorise the commercial trade in these species or hunting within the designated protected areas. Abdullah et al. (2011) highlighted that most of the Orang Asli in BTFC engage in hunting and gathering forest resources for consumption (see Table 4.1). The Orang Asli refrained from participating in these tasks for reasons such as advanced age, deteriorating health, full-time household duties, or a diminished need for forest resources for their sustenance.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Table 4.1: Utilisation of wild animals by Orang Asli in BTFC

Wild animals	Use of Forest Resources			
	Personal consumption only (%)	For sale only (%)	Personal consumption and for sale (%)	Total respondents' involvement (%)
Fish	28.9	7.7	29.2	65.8
Frog	16.5	15.1	28.5	60.2
Soft-shelled terrapin	19.4	9.2	24.6	53.2
Monkey	45.1	0.0	0.4	45.4
Wild boar *Sus scrofa*	39.8	1.8	1.1	42.6
Barking deer *Muntiacus muntjak*	38.7	0.4	2.1	41.2
Bird	36.3	0.4	4.2	40.8
Deer	31.3	0.7	1.8	33.8
Pangolin *Manis javanicus*	9.9	5.7	4.2	19.8
Gaur *Bos gaurus*	11.3	0.0	0.0	11.3
Malayan Sun Bear *Helarctos malayanus*	8.1	0.0	0.7	8.8
Snake	3.9	0.0	0.0	3.9
Sumatran rhinoceros *Dicerorhinus sumatrensis*	2.1	0.4	0.4	2.8
Asian elephant *Elephas maximus*	1.8	0.0	0.0	1.8
Tiger *Panthera tigris jacksoni*	0.4	0.0	0.0	0.4

(Source: Abdullah et al., 2011)

In Abdullah et al. (2011), the Orang Asli noted that the availability of natural resources in the forest began to decline approximately 10 years ago. Although the Jahai people are the sole permanent residents of the area, the armed forces also conduct patrols in the region. Additionally, foreign poachers trespass into the park for hunting and fishing (Clements et al., 2010). According to Ching and Leong (2011), the Orang Asli community, local populations, and individuals from neighbouring countries including Thailand, Cambodia, and Indonesia, are engaged in the illegal hunting and trafficking of animals in the BTFC. The situation is alarming as severe defaunation caused by hunting in tropical rainforests leads to the depletion of wildlife populations and extinction of species, including long-term ecological consequences **(**Dirzo et al., 2014). The disruption of these ecological processes due to hunting-induced defaunation can significantly impact tree regeneration and alter biome-level aboveground biomass, underscoring the urgent need for effective conservation measures (Berzaghi et al., 2019; Harrison et al., 2013).

The Orang Asli Resettlement Plan (*Rancangan Penempatan Semula*, RPS) by JHEOA involves consolidating scattered indigenous communities into a centralised location equipped with essential infrastructure and opportunities for economic activities (Awang et al., 2012). Some resettlement sites, such as Pos Kemar in Temengor, could trace their history to the establishment of jungle forts, whereby army posts were established strategically in the forest to win the hearts and minds of the people through the provision of educational and health services to the nearby communities. The objectives of the resettlement schemes were to alleviate poverty, modernise communities through the provision of basic facilities, reorganise them in suitable centres, and ensure their security against subversive elements, such as communism (Mohamad et al., 2023). Other developments were also implemented through the resettlement scheme, such as the development of rubber plantation projects, the installation of power supply, housing, and schools (see Figure 4.3) (Ramli, 2024).

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Figure 4.3: A Jahai tribe settlement by the lake, allowing for easy access to water and transportation by boat
Note: A = Jahai tribe souvenir booth made out of bamboo and palm leaves; C = Solar panels have been installed at the Jahai tribe villages to power communication facilities for the residents

The Jahai from Kampung Tiang, through the village cooperative called Koperasi Orang Asli Sungai Tiang, were involved in the development and management of the fish sanctuary, *Akekchep*, at Sungai Tiang, RBSP. The Akekchep Fish Sanctuary is a co-management initiative to conserve river ecosystem while generating income through sustainable community-led ecotourism (TNB Research, 2024). The community has stopped commercial fishing in the upper reaches of the river Since the project started in 2017. The community also explores other cultural and experiential tourism activities to offer more diverse options for visitors, including cultural demonstrations, recreational fishing, wildlife watching, rafting, and tubing. This initiative is an adaptation by local communities that shifts traditional extractive activities to new income opportunities by aligning with conservation and management goals (Idris, 2020).

The relationship between Indigenous peoples and ecotourism in BTFC is vital considering that the cultural and economic significance of the forest is increasingly harnessed for tourism, attracting researchers and visitors. The traditional villages of the Jahai tribe within the state park are major draws for tourists, who often purchase forest products collected by the Jahai, namely stingless bee honey, agarwood, and herbs. Moreover, the Jahai actively participate in sustainable tourism by performing traditional Sewang dances, demonstrating the use of blowpipes, cultivating medicinal herbs, and creating handicrafts (Awang et al., 2012; Ramli et al., 2024).

The Orang Asli are pivotal to conservation initiatives within the BTFC, specifically in land use, forest resource management, and ecotourism development. Their traditional knowledge and active involvement are essential for encouraging collaboration among stakeholders to protect the area. The rich biodiversity of the region has made seed-collection training advantageous for the Orang Asli, boosting their entrepreneurial skills, providing employment, and generating income. For example, the Tropical Rainforest Living Collection Banun, Amanjaya project, launched in 2013, which operates a one-hectare nursery within a 500-hectare area managed by the Tropical Rainforest Conservation and Research Centre (TRCTC). The project supports conservation and restoration efforts with the Orang Asli engaging in species selection, germination, nursery cultivation, and outplanting, playing a significant role in preserving endangered tropical rainforest species. Out of the 29,278 seeds collected, 60 were forest tree species, and 26 were listed as endangered on the IUCN Red List (see Figure 4.4). This initiative emphasises the critical role of Orang Asli in maintaining and restoring the forest ecosystem through conservation and sustainable practices (Likin et al., 2018; Idris, 2020).

Figure 4.4 : Seeds collected by the Jahai tribe include A. *Swintonia* sp. (family Anacardiaceae), B. *Hopea* sp., C. *Rubroshorea* sp.

The Orang Asli community is integral to conservation efforts in the BTFC through their extensive knowledge of the forest as patrollers. Several government agencies and non-governmental organisations have engaged the local Orang Asli in anti-poaching activities. For instance, the WWF-Malaysia initiated Project Stampede, hiring the Orang Asli as patrol units to combat poaching, covering over 7,500 kilometres between 2014 and 2017 and revealing numerous traps. These patrols comprised entirely of Orang Asli members from RPS Banun and RPS Kemar, who provide a stable income and benefits, while WWF-Malaysia supports their development with courses in tour guiding, business, and law. This partnership empowers the Orang Asli and strengthens conservation and ecotourism initiatives in the region (WWF-Malaysia, 2023).

The Perak State Parks Corporation, in collaboration with Rimau, established the Menraq Patrol Unit to involve the communities of Kampung Kejar. This patrol unit comprises 30 Jahai men trained to conduct routine patrols to monitor illegal activities and collect vital wildlife data. Furthermore, the Department of Wildlife and National Parks allocated funding to hire Community Rangers for Perak State Parks Corporation, WWF-Malaysia, and RIMAU to perform anti-poaching and conservation activities. As of 2024, approximately 200 Orang Asli in BTFC were recruited for monitoring and protection (Yayasan Sime Darby, 2022). These community patrolling initiatives provide sustainable livelihoods

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

for the Orang Asli communities by recognising and adapting the traditional knowledge for the benefit of protection while achieving conservation targets. In the long run, the patrols create a strong line of defence against illegal activities and instil a sense of ownership among the communities.

Community-based conservation programmes, such as **community forest management (CFM), eco-tourism,** and **wildlife corridors**, could significantly enhance conservation efforts in BTFC. The CFM in Nepal aims to reduce forest degradation, with local communities managing their forests for personal use and benefits based on an operational plan approved by the divisional forest officer (Ghimire & Lamichhane, 2020). Under **CFM**, local communities are trained by officers and experts to monitor biodiversity through citizen science within an assigned forest plot. They could also be trained to use tools such as camera traps and mobile applications to track wildlife and forest health. This training includes tasks such as developing **wildlife corridors** to improve habitat connectivity across BTFC, with communities playing a key role in planting trees, maintaining buffer zones, and monitoring wildlife movement. This participatory approach would empower locals to collect vital data, report illegal activities, and engage in sustainable resource management.

In terms of **eco-tourism**, BTFC could promote indigenous culture by involving local communities as specialised **tour guides** for wildlife and nature tours, providing cultural education and economic opportunities. The Anangu guides at Uluru provide immersive tours that share their cultural heritage, including storytelling about the spiritual significance of the land, bush tucker walks, and art workshops on traditional dot painting. Locals could deepen their connection to the forest and support conservation initiatives by offering additional tourism roles, such as guiding. This holistic approach would foster greater local involvement, ensuring that conservation efforts benefit the environment and the surrounding communities.

Traditional Ethnobotanical Practices of The Orang Asli Community

Natural resources are essential for indigenous peoples who incorporate them into their daily routines and rely on them for sustainability and subsistence. Recent studies have explored how these natural resources are utilised, albeit overlooking the factors influencing their usage and the essential role these resources play in sustaining their lifestyle. Traditional knowledge, referred to as local ecological knowledge, encompasses a profound comprehension of the interactions between living things and their surroundings. Elders typically transmit this knowledge verbally, spanning centuries and including insights into forests, wildlife, and ecosystems. Indigenous peoples possess a detailed comprehension of plant and animal properties, ecosystem functions, and management techniques. Local species in rural communities of developing countries are vital for food, medicine, fuel, and building materials. Meanwhile, their environmental knowledge and cultural practices, such as songs and stories, are integral to their cultural identity and heritage (Bartholomew et al., 2017).

Aweng et al. (2020) explored the traditional vegetable practices of the Jahai tribe, focusing on seven primary species: wild eggplant (*Solanum torvum*), sweet potato (*Ipomoea batatas*), Indian mulberry (*Morinda citrifolia*), cassava (*Manihot esculenta*), pigweed (*Amaranthus viridis*), purple milletia (*Millettia atropurpurea*), and torch ginger (*Etlingera elatior*). The Jahai tribe cultivates *I. batatas*, *M. esculenta*, *E. elatior*, and *S. torvum*, with *M. esculenta* and *I. batatas* as staple foods. *Etlingera elatior* and *S.torvum*, originally wild, are cultivated and served with rice or cassava. In contrast, *M. atropurpurea*, *A. viridis*, and *M. citrifolia* are wild-grown and not widely eaten. The shift of several formerly wild plants to domesticated status emphasises the flexible agricultural methods of the tribe and their integration of these plants into their dietary practices.

Ayuni et al. (2015) provided a detailed ethnobotanical survey of the Jahai tribe in RBSP, identifying 104 plant species across 72 genera and 38 families, highlighting that 91 species are used medicinally. Most listed plants are prepared as decoctions, where leaves, roots, and other plant parts are boiled and consumed for numerous health purposes. For example, *Fissistigma* sp., *Saprosma glomerulata*, *Spondias pinnata*, and *Stachyphrynium spicatum* for fever; *Goniothalamus scortechinii* and *Thottea tricornis* for headaches; *Coptosapelta tomentosa* and *Glycosmis* sp. for worms infections; *Cinnamomum javanicum*, *Morinda elliptica*, and *Psychotria* sp. for body aches; and *Clerodendrum* sp., *Heritiera javanica*, and *Labisia pumila* for female fertility. Socially, plants such as *Artabotrys* sp., *Elettariopsis* sp., *Polyalthia cauliflora*, and *Saprosma* sp. are used in the Sewang ritual and for protection against ill omens. Additionally, eight species, namely *Baccaurea parviflora*, *Baccaurea* sp., *Barringtonia macrostachya, Daemonorops geniculata*, *Drynaria* sp., *Elateriospermum tapos*, *Garcinia malaccensis*, and *G. parvifolia* are consumed raw (Ayuni et al., 2015).

5. Ecotourism Activities Within Belum-Temengor Forest Complex

Ecotourism refers to travelling to peaceful natural areas to appreciate the environment and local culture while protecting nature and improving the livelihoods of nearby communities (Choo & Halim, 2022). The main objective is to minimise environmental damage while ensuring tourism benefits local populations. Jaini et al. (2012) explained that ecotourism promotes wildlife conservation and environmental protection while offering social and economic advantages, such as job creation and increased environmental awareness. Despite these benefits, several economists noted the challenge in assigning a precise economic value to preserving tropical forests (Adhikari & Baral, 2018).

Ecotourism also raises public awareness, where activities such as cultural exchanges, conferences, and interactive tourism experiences educate locals and visitors about environmental and cultural matters (Mudasir et al., 2020). Nevertheless, critics argue that ecotourism generates positive outcomes but not meet its intended goals due to various implementation challenges.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

The diverse natural environment and supportive government policies in Malaysia provides a place conducive for ecotourism development (Mordal, 2014). Tourism has long been vital to the national economy, ranking as the third-largest contributor to national GDP after manufacturing and commodities (Nair & Thomas, 2013; Hirschmann, 2020). Langkawi Geopark was recognised by UNESCO as an ecotourism site and serves as a prime example of sustainable tourism in Southeast Asia, balancing environmental preservation with community benefits and tourist experiences (Mordal, 2014). Similarly, BTFC contributes to eco-friendly tourism by offering activities such as wildlife observation, forest trekking, and visits to indigenous villages. These initiatives create employment opportunities for local communities while highlighting the biodiversity of the area (Schwabe et al., 2015).

The National Ecotourism Plan emphasises the potential of BTFC as a major ecotourism destination, showcasing its indigenous culture, wildlife diversity, and natural attractions such as waterfalls and Rafflesia flowers (Nik Mohamad, 2013). Ecotourism has fostered a sustainable local economy by creating jobs in sectors, namely hospitality, guiding, and food services. Nonetheless, the distribution of financial benefits is not consistently equitable. Although local communities earn income through employment and the sale of local products, most of the revenue is taken by foreign operators, reducing the overall benefit for locals (Mudasir et al., 2020). Kennedy (2012) outlined that approximately one-third of tourists' spending remains within the host country, with the rest going to international airlines and travel agencies.

Ecotourism has several drawbacks despite its positive contributions. A key issue is low environmental awareness among Malaysians, despite efforts to include environmental topics in education (Daniel & Nadeson, 2006). Buckley (2004) cautioned that expanding tourism infrastructure could increase resource consumption and waste generation. Moreover, tourism activities could disturb wildlife and plant life, and tourists moving through forests may inadvertently spread invasive species and diseases.

In BTFC, repeated logging, road construction, illegal hunting, and insufficient boundary monitoring have caused habitat degradation and biodiversity loss, undermining conservation efforts (Schwabe et al., 2015; Gwee et al., 2019). These activities affect Orang Asli communities, who depend on forest resources for their livelihoods. Encroachment on their lands disrupts their cultural practices and diminishes their role in forest conservation (Rozainee Abdullah et al., 2013). Other external threats, including water pollution and forest fires, further endanger these ecosystems. For example, from 1992 to 1998, forest fires driven by natural factors and human activity significantly reduced forest cover in states such as Kedah, Kelantan, and Pahang (Diemont & Hillegers, 2002).

Another concern is the commercialisation of local cultures due to ecotourism. Tourists often expect indigenous communities to perform traditional rituals or pose for photographs, which alters authentic cultural practices and places excessive pressure on local identities (Mudasir et al., 2020). Kennedy (2012) denoted that these expectations disrupt traditional social structures and reduce cultural heritage to mere commercial goods. Nonetheless, initiatives such as the establishment of RBSP in 2007 demonstrate that a balance between environmental preservation and socio-economic development is achievable. Adopting sustainable tourism strategies enables destinations such as BTFC and Langkawi Geopark to maximise their natural and cultural resources while safeguarding their ecosystems for future generations. A well-managed ecotourism could enhance economic growth, strengthen cultural identity, and foster environmental stewardship by involving local communities in decision-making processes.

6. Conclusion

The BTFC boasts a wealth of flora species, encompassing a broad spectrum of species across numerous genera and families. The varying conservation status of these species, as noted by the IUCN Red List, highlight the urgent need for ongoing data collection and research. Expeditions in this region are critical for acquiring the data necessary to inform conservation strategies and protect the unique biodiversity of the forest, which is vital to ecological balance.The rich diversity of flora in the BTFC significantly enhances its ecotourism appeal, attracting visitors globally who come to experience its botanical diversity, including the rare Rafflesia. The presence of lakes also enhances their appeal, providing opportunities for water-based activities that complement the ecotourism experience. This tourist influx raises awareness about the importance of preserving such habitats and contributes economically to the region, supporting local conservation efforts.

The Orang Asli community plays an integral role in the ecosystem of BTFC. Traditionally reliant on the forest for hunting and foraging, the Jahai have adapted to modern roles within the forest, serving as rangers and participating in aquaculture and reforestation projects. Their deep connection to the forest underscores the importance of their involvement in conservation and sustainable management.

Given the ecological significance of BTFC and its value to tourism and local communities, maintaining the natural habitats is critical. Efforts should focus on balancing the growth of ecotourism with rigorous conservation measures to protect the diverse flora and ensure the continued provision of vital ecosystem services. All plant species, including those overlooked, play essential roles in maintaining ecosystem health, such as preventing soil erosion and supporting future plant growth. Protecting the BTFC is vital to preserving its natural beauty and sustaining the livelihoods of the Orang Asli, including supporting the broader goals of conservation and sustainable development.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

7. Acknowledgement

This work was supported by funding from the European Commission via the call for action, Securing the Resiliency and Sustainability of Royal Belum Forest against the Impact of Climate Change (NDICI CSO/2022/438-429) awarded to Project Coordinator Dr Jayaraj Vijaya Kumaran. Pictures of Royal Belum State Park are courtesy of Dr. Muhammad Firdaus Bin Abdul Karim.

8. References

Abdullah, A. R., & Chan, N. W. (2011). Ecotourism activities: Threats to sustainability of Belum Temengor Forest Complex (BTFC) natural heritage. *Malaysian Journal of Environmental Management*, 12(1), pp 97-108.

Abdullah, A., Or, O. C., & Fadzil, K. S. (2011). *Collectors and Traders: A study of Orang Asli involvement in wildlife trade in the Belum-Temengor Complex, Perak*. Centre for Malaysian Indigenous Studies, University of Malaya.

Abdullah, A., Weng, C. N., & Mohamed, B. (2013). Potentials and challenges of involving indigenous communities in ecotourism in Belum-Temengor forest complex, Perak, Malaysia. In Mohamed, B. & Bahauddin, A. (eds.): Proceedings of International Conference on Tourism Development, pp. 332-344, Suistainable Tourism Research Cluster, Universiti Sains Malaysia.

Abdullah, M. F., Ahmad, B. E., Iqbal, M., Noor, M., Jamru, L. R., Yusoh, M. P., Mara, S., & Alam, M. (2023). The Socioeconomics Support on Indigenous Community in Malaysia. *JATI-Journal of Southeast Asian Studies*, *28*(1), pp. 28–49. https://doi.org/10.22452/jati.vol28no1.2

Adhikari, S., & Baral, H. (2018). Governing forest ecosystem services for sustainable environmental governance: A review. *Environments*, *5*(5), 53. pp. 1–13. https://doi.org/10.3390/environments5050053

Ahmad Fitri, Z., Nizam, M. S., Latiff, A., Abd, R. K., & Shamsudin, I. (2017a). Floristic composition and biomass of trees in Temengor Forest Reserve, Perak, Peninsular Malaysia. *The Malaysian Forester*, *80*(2), pp. 178-197.

Ahmad Fitri, Z., Nizam, M. S., Latiff, A., Abd, R. K., & Shamsudin, I. (2017b). Wild Fruit Trees in An Upper Hill Dipterocarp Forest At Temengor Forest Reserve, Perak, Malaysia. *The Malaysian Forester*, *80*(2), pp. 129-135.

Aiman Hanis, J., Abu Hassan, A., Nurita, A. T., & Che Salmah, M. R. (2014). Community structure of termites in a hill dipterocarp forest of Belum- Temengor Forest Complex, Malaysia: Emergence of pest species. *Raffles Bulletin of Zoology*, *62*.

Ang, K. C., Leow, J. W. H., Yeap, W. K., Hood, S., Mahani, M. C., & Md-Zain, B. M. (2011). Phylogenetic Relationships of The Orang Asli And Iban Of Malaysia Based on Maternal Markers. *Genetics and Molecular Research*, *10*(2), pp. 640–649. https://doi.org/10.4238/vol10-2gmr1011

Awang, A., Zulkeflee, N. H., Adek, N. B. N., Hamzah, Z., Rak, A. E., Karim, M. F. A., & Amaludin, N. A. (2023). Composition and Diversity of Tree Species with DBH of 5 cm and above at Pulau Banding, Gerik, Perak, Malaysia. In *BIO Web of Conferences*, 73, pp. 01001, EDP Sciences

Awang, S. N., Fadzil, K. S., Edo, J., Zainol, R., & Firuza, M. (2012). 50 Tahun Berlalu Ke mana Jahai dan Temiar di Hutan Hujan Belum Temengor. *Perancangan dan Pembangunan Komuniti*, pp. 61-87, UUM Press, 10.32890/9789670876108

Awang, S. N., Fadzil, K. S., Ibrahim, W. S. W., Zainol, R., & Edo, J. (2015). Indigineous tourism-Satu tinjauan awal penglibatan masyarakat Orang Asli dalam industri pelancongan (Orang Asli (aboriginal) community participation in the tourism industry-A preliminary survey of Belum-Temengor). *Geografia*, *11*(9).

Aweng, E. R., Suganthi, A., Nivaarani, A., Aisyah, S. S., Choo, A. M. R., Salam, M. A., & Zulhazman, H. (2020). Short Notes on Traditional Vegetables Consumed by Jahai Tribe in Klewang Village, Royal Belum State Park, Malaysia. In *IOP Conference Series: Earth and Environmental Science*, 596, 012066. IOP Publishing. 10.1088/1755-1315/596/1/012066.

Ayuni, N., Faridah, Q. Z., Anisa, S. A., Rosimah, N., Norhidayah, M. H., & Shamsul, K. (2015). Ethnobotanical documentation of plants used by the Jahai tribe in Royal Belum State Park, Perak. *Unravelling Nature's Treasures & Secrets: Current Species of Interest*, 2.

Bartholomew, C. V., Abdullah, M. T., Abdullah, R., & Clements, G. R. (2017). Ethno-Tourism: A Review On Natural Resources Use Pattern By Indigenous Community In Peninsular Malaysia. *Ecotourism Potentials In Malaysia*, pp. 84-95 Faculty of Forestry, Universiti Putra Malaysia.

Belum Rainforest Resort Malaysia. (2016). Exploring Belum. From Belum Rainforest Management SDN BHD.: https://www.belumrainforestresort.com/explore (accessed August 6, 2024).

Berzaghi, F., Longo, M., Ciais, P., Blake, S., Bretagnolle, F., Vieira, S., Scaranello, M., Scarascia-Mugnozza, G., & Doughty, C. E. (2019). Carbon stocks in central African forests enhanced by elephant disturbance. *Nature Geoscience*, 12(9), pp. 725-729, https://doi.org/10.1038/s41561-019-0395-6

Bhore, S. J., Husin, N. A., Nurul, A. H., & Shah, F. H. (2009). Genetic Variability Based on Randomly Amplified Polymorphic DNA in Kacip Fatimah (Labisia pumila Benth & Hook f) collected from Melaka and Negeri Sembilan States of Malaysia. *Journal of Forest Science*, 25(2), pp. 93-100, www.tropicos.org/Name/22002192

Brandon, K. (2015). Ecosystem Services from Tropical Forests: Review of Current Science. *Center for Global Development Working Paper*, (380), https://doi.org/10.2139/ssrn.2622749

Burenhult, N. (2005). *A grammar of Jahai*. http://openresearch-repository.anu.edu.au/bitstream/1885/146729/1/PL-566.pdf

Chapin, F. S., Matson, P. A., Mooney, H. A., & Vitousek, P. M. (2002). Principles of terrestrial ecosystem ecology, Springer. https://doi.org/10.1007/978-1-4419-9504-9

Ching, O. O., & Leong, T. F. (2011). Orang Asli and wildlife conservation in the Belum-Temengor forest complex, Malaysia. *Traffic Bulletin*, 23(3), pp. 94-104.

Choo, S. L., & Halim, T. B. (2022). Advancing Sustainable Tourism Development and Its Contribution to Poverty Reduction and Development: Perspective from Malaysia. *Journal of Hospitality & Tourism Management*, *5*(1), pp. 1–13. https://doi.org/10.53819/81018102t4032

Chua, L. S. L., Kamarudin, S., & Damahuri, S. (2000). A preliminary checklist of seed plants of the Belum Forest Reserve, Perak, Peninsular Malaysia. *Malayan Nature Journal*, *54*(3), pp. 171-186.

Chua, L. S. L., Suhaida, M., Hamidah, M. & Saw, L.G. (2010). Malaysia Plant Red List: Peninsular Malaysian Dipterocarpaceae. Forest Research Institute Malaysia (FRIM) & Ministry of Natural Resources and Environment Malaysia, Malaysia.

Chua, L. S., Abdullah, F. I., & Salleh, F. M. (2023). Comparing Three Varieties of Labisia pumila (Primulaceae): Phytochemicals and Antioxidation. *Trends in Sciences*, 20(2), https://doi.org/10.48048/tis.2023.4042

Clauzel, R. (2024). Community-led Approaches to Address Forest Fragmentation and Promote Reforestation. *Journal of Biodiversity & Endangered Species*, *12*(02).

Clements, R., Rayan, D. M., Ahmad Zafir, A. W., Venkataraman, A., Alfred, R., Payne, J., Ambu, L., & Sharma, D. S. K. (2010). Trio under threat: Can we secure the future of rhinos, elephants and tigers in Malaysia?. *Biodiversity and Conservation*, 19(4), pp. 1115-1136, https://doi.org/10.1007/s10531-009-9775-3

Corlett, R. T. (2019). The Ecology of Tropical East Asia. In *The Ecology of Tropical East Asia*. https://doi.org/10.1093/oso/9780198817017.001.0001

Corlett, R. T., & Primack, R. B. (2011). Tropical Rain Forests: An Ecological and Biogeographical Comparison: Second Edition. In *Tropical Rain Forests: An Ecological and Biogeographical Comparison: Second Edition*. https://doi.org/10.1002/9781444392296

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science* 345(6195). https://doi.org/10.1126/science.1251817

Dong, J., Xiao, X., Sheldon, S., Biradar, C., Duong, N. D., & Hazarika, M. (2012). A comparison of forest cover maps in Mainland Southeast Asia from multiple sources: PALSAR, MERIS, MODIS and FRA. *Remote Sensing of Environment*, *127*, pp. 60–73, https://doi.org/10.1016/j.rse.2012.08.022

Ghazalli, M. N., Hasrani, M., Rusli, N. M., Talip, N., & Latiff, A. (2016). Rare Fruit and Wild Ginger Diversity at Sungai Tiang, Royal Belum State Park, Perak, Malaysia: Proceedings of the Fourth Royal Belum Scientific Expedition 2015, pp. 66-70, Pulau Banding Foundation

Ghimire, P., & Lamichhane, U. (2020). Community based forest management in Nepal: Current status, successes and challenges. *Grassroots Journal of Natural Resources*, *3*(2), 16-29.

Gwee, S. L., Tan, A. K. G., & Narayanan, S. (2019). Sustainable tourism and forest conservation: The case of the Belum-Temengor Rainforest Complex in Perak, Malaysia. *Journal of Sustainable Forestry*, *38*(4). https://doi.org/10.1080/10549811.2018.1549498

Hairi, H. A., Sofi, N. S. M., Khodari, S. N. K., Jamal, J. A., Mohamed, I. N., & Shuid, A. N. (2016). Therapeutic Effects of Labisia pumila on Estrogen-deficiency related disorders: An evidence based review. *International Journal of Pharmacology*, *12*(5), pp 451-460.

Harrison, R. D., Tan, S., Plotkin, J. B., Slik, F., Detto, M., Brenes, T., ... & Davies, S. J. (2013). Consequences of defaunation for a tropical tree community. *Ecology letters*, *16*(5), pp 687-694.

Hashim, M., Pour, A. B., & Wei, C. K. (2014). Comparison of ETM+ and MODIS Data for Tropical Forest Degradation Monitoring in the Peninsular Malaysia. *Journal of the Indian Society of Remote Sensing*, *42*(2), pp 383–396. https://doi.org/10.1007/s12524-013-0314-z

Hassan, K. H. (2015). Application of the wildlife conservation 2010 (act 716): The Malaysian experience. *American Journal of Animal and Veterinary Sciences*, 10(1), pp 43-46, https://doi.org/10.3844/ajavsp.2015.43.46

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Hazandy, A. (2014). Identification Of Potential Species To Be Planted In Poor Forests Of The Central Forest Spine (CFS) Wildlife Corridor Gerik, Perak. *The Malaysian Forester*, *77*(2), pp 147-160.

Hirschmann, R. (2020). Travel and tourism in Malaysia - Statistics & Facts. From Statista.: https://www.statista.com/topics/5741/travel-and-tourism-in-malaysia/ (accessed August 2, 2024)

Hurteau, M. D. (2021). The role of forests in the carbon cycle and in climate change. *Climate Change: Observed Impacts on Planet Earth, Third Edition*, 561–579. Elsevier. https://doi.org/10.1016/B978-0-12-821575-3.00027-X

Idris, S. N. (2022). *Socio-Economic Status Of Orang Asli Communities Pre-Cbo Project In Sungai Yu Corridor, Merapoh, Pahang And Gerik Corridor, Belum-Temengor, Perak*. Department Of Wildlife And National Parks (DWNP) Peninsular Malaysia.

International Work Group for Indigenous Affairs (IWGIA). (2021). *The Indigenous World 2021: Malaysia*. From International Work Group for Indegenous Affairs (IWGIA): https://www.iwgia.org/en/malaysia/4231-iw-2021-malaysia.html (accessed August 10, 2024)

Ismail, S. N., Hamid, M., Zainol, M. Z., Kamaruszaman, S. A. & Mansor, M. (2016). The Distribution of Weed Species at Sungai Kejar, Royal Belum State Park: Proceedings of the Fourth Royal Belum Scientific Expedition 2015, pp. 87-92, Pulau Banding Foundation

Jabatan Kemajuan Orang Asli. (2022). *Suku Kaum* - Laman web rasmi Jabatan Kemajuan Orang Asli. Laman Web Rasmi Jabatan Kemajuan Orang Asli. https://www.jakoa.gov.my/orang-asli/suku-kaum/ (accessed August 11, 2024)

Jabatan Kemajuan Orang Asli. (2024). *Taburan Etnik Orang Asli Mengikut Etnik/Sub Etnik Mengikut Negeri* - Laman web rasmi Jabatan Kemajuan Orang Asli. Laman Web Rasmi Jabatan Kemajuan Orang Asli. https://www.jakoa.gov.my/orang-asli/taburan-etnik-orang-asli-mengikut-etnik-sub-etnik-mengikut-negeri/ (accessed August 11, 2024)

Jaini, N., Anuar, A. N. A., & Daim, M. S. (2012). The practice of sustainable tourism in ecotourism sites among ecotourism providers. *Asian Social Science*, *8*(4), pp. 175–179. https://doi.org/10.5539/ass.v8n4p175

Jewitt, D., Goodman, P. S., Erasmus, B. F. N., O'Connor, T. G., & Witkowski, E. T. F. (2017). Planning for the Maintenance of Floristic Diversity in the Face of Land Cover and Climate Change. *Environmental Management*, *59*(5). https://doi.org/10.1007/s00267-017-0829-0

Kadir, A. H. A. (2006). The distribution of the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and threats to their population in Peninsular Malaysia. *Management and status of resources in protected areas of Peninsular Malaysia*, pp. 111-123, Department of Wildlife, Kuala Lumpur.

Kamal, S. H. S., Suratman, M. N., Khamis, S., Hassan, A. N. N., & Mohammad, M. S. (2021). Host-Parasitic Relationships between *Tetrastigma rafflesiae* and *Rafflesia azlanii* and *Rafflesia cantleyi* in Belum-Temenggor Forest Complex, Perak, Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *44*(4).

Kanchanapoom, T., Kamel, M. S., Picheansoonthon, C., Luecha, P., Kasai, R., & Yamasaki, K. (2007). Hydrolyzable tannins and phenylpropanoid from *Rafflesia kerrii* Meijer (Rafflesiaceae). *Journal of natural medicines*, 61(4), pp 478-479. https://doi.org/10.1007/s11418-007-0181-4

Kanniah, K. D. (2017). Quantifying green cover change for sustainable urban planning: A case of Kuala Lumpur, Malaysia, *Urban Forestry and Urban Greening*, 27 pp. 287-304, https://doi.org/10.1016/j.ufug.2017.08.016

Kanniah, K. D., Tan, K. P., Cracknell, A. P., Huete, A. R., Idris, N. H., Lau, A. M. S., ... & Ahmad, A. (2018). Assessment of biophysical properties of Royal Belum tropical forest, Malaysia. *Singapore Journal of Tropical Geography*, *39*(1), pp. 90-106, https://doi.org/10.1111/sjtg.12215

Kedri, F. K., Hamzah, Z., Sukri, N. S., Yaacob, S. H., Abd Majid, N. K. S., Mokhtar, N., & Amir, S. F. (2018). Distribution and ecology of Rafflesia in Royal Belum State Park, Perak, Malaysia. *International Journal of Engineering and Technology*, 7(2), pp. 292-296, https://doi.org/10.14419/ijet.v7i2.29.13335

Latiff A, L. A. (2018). Viability of having the gigantic Rafflesia flowers in our park. *Open Access Journal of Science*, 2(2), pp. 106-107, https://doi.org/10.15406/oajs.2018.02.00053

Latiff, A., & Mat Salleh, K. (2001). Notes on the discovery of Rafflesia hasseltii Suringar (Rafflesiaceae) in taman negara (National park), Malaysia. *Flora Malesiana Bulletin*, *12*(7/8), 393-395.

Laurance, W. F., Camargo, J. L. C., Fearnside, P. M., Lovejoy, T. E., Williamson, G. B., Mesquita, R. C. G., Meyer, C. F. J., Bobrowiec, P. E. D., & Laurance, S. G. W. (2018). An Amazonian rainforest and its fragments as a laboratory of global change. *Biological Reviews*, *93*(1). https://doi.org/10.1111/brv.12343

Laurance, W. F., Camargo, J. L. C., Luizão, R. C. C., Laurance, S. G., Pimm, S. L., Bruna, E. M., Stouffer, P. C., Bruce Williamson, G., Benítez-Malvido, J., Vasconcelos, H. L., Van Houtan, K. S., Zartman, C. E., Boyle, S. A., Didham, R. K., Andrade, A., &

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Lovejoy, T. E. (2011). The fate of Amazonian forest fragments: A 32-year investigation. In *Biological Conservation* (Vol. 144, Issue 1). https://doi.org/10.1016/j.biocon.2010.09.021

Laurance, W. F., Goosem, M., & Laurance, S. G. W. (2009). Impacts of roads and linear clearings on tropical forests. In *Trends in Ecology and Evolution* (Vol. 24, Issue 12). https://doi.org/10.1016/j.tree.2009.06.009

Laurance, W. F., Koh, L. P., Butler, R., Sodhi, N. S., Bradshaw, C. J. A., Neidel, J. D., Consunji, H., & Vega, J. M. (2010). Improving the performance of the roundtable on sustainable palm oil for nature conservation. *Conservation Biology*, *24*(2). https://doi.org/10.1111/j.1523-1739.2010.01448.x

Lazarus, B. A., Che-Amat, A., Abdul Halim Shah, M. M., Hamdan, A., Abu Hassim, H., Mustaffa Kamal, F., ... & Ahmad, H. (2021). Impact of natural salt lick on the home range of Panthera tigris at the Royal Belum Rainforest, Malaysia. *Scientific reports*, *11*(1), 10596, https://doi.org/10.1038/s41598-021-89980-0

Leong, M. C., James, P. T., & Muhammad, M. (2023). Preliminary assessment on climatological conditions in Royal Belum-Temengor Rainforest Complex, Malaysia using MERRA-2 reanalysis datasets. *BIO Web of Conferences*, 73(05026), https://doi.org/10.1051/bioconf/20237305026

Li, R., Sun, B., Wang, Q., Ma, F., Xu, X., Wang, Y., & Jia, H. (2015). Two new Castanopsis (Fagaceae) species based on cupule and foliage from the upper Miocene of eastern Zhejiang, China. *Plant Systematics and Evolution*, 301(1), pp 25-39, https://doi.org/10.1007/s00606-014-1051-7

Likin, S., Zainun, N., & Jaafar, F. B. (2020). Traditional Knowledge of Jahai Tribe at Kampung Semelor, Gerik, Perak. *International Conference on Multidisciplinary Research*, pp. 676-680. 10.5220/0008892306760680.

Lim, L. S., Ang, K. C., Mahani, M. C., Shahrom, A. W., & Md-Zain, B. M. (2010). Mitochondrial DNA polymorphism and phylogenetic relationships of Proto Malays in Peninsular Malaysia. *Journal of Biological Sciences*, 10(2), pp. 71–83. https://doi.org/10.3923/jbs.2010.71.83

Lim, T., Loke, V. P. W., Solana-Mena, A., Pura, P., Angah, R., Tan, A. S. L., & Campos-arceiz, A. (2017). Mapping the Distribution of People, Elephants, and Human-Elephant Conflict in Temengor Forest Complex, Peninsular Malaysia. *Malayan Nature Journal*, *Special Edition 2017*, pp 25-43.

Loke, V. P. W., Lim, T., & Campos-Arceiz, A. (2020). Hunting practices of the Jahai indigenous community in northern peninsular Malaysia. *Global Ecology and Conservation*, 21(e00815), https://doi.org/10.1016/j.gecco.2019.e00815

Maideen, H., Nur Aliah, M. K., Rohani, A., & Damanhuri, A. (2016). Diversity of Ferns and Lycophytes of Sungai Kejar and It's Vicinity in Royal Belum State Park: Proceedings of the Fourth Royal Belum Scientific Expedition 2015, pp. 61-65, Pulau Banding Foundation

Masron, T., Masami, F., & Ismail, N. (2013). Orang Asli in Peninsular Malaysia: population, spatial distribution and socio-economic condition. *J Ritsumeikan Soc Sci Humanit*, *6*, pp. 75-115.

Mei, S. L., Carr, G., & Weng, C. N. (2017). Resolving water disputes via interstate co-operation and stakeholders' engagement: A case study from Muda river basin. *Advanced Science Letters*, *23*(9). https://doi.org/10.1166/asl.2017.10037

Miyamoto, M. (2020). Poverty reduction saves forests sustainably: Lessons for deforestation policies, *World Development*, 127, https://doi.org/10.1016/j.worlddev.2019.104746

Mohamad, S., Lau, C. F. and Rayan, D. M. (2023). *Wild Perak: Belum-Temengor*. WWF-Malaysia, Selangor, Malaysia.

Mohd Rusdi, N. D., Mansor, A., Mohd Sah, S. A., Zakaria, R., & Nik Rosely, N. F. (2022). Environmental Impact on Duration of Flowering and Fruiting of Rattan (*Calamus castaneus*) in Peninsular Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *45*(1), pp. 133–151. https://doi.org/10.47836/pjtas.45.1.08

Mundher, R., Abu Bakar, S., Maulan, S., Mohd Yusof, M. J., Osman, S., Al-Sharaa, A., & Gao, H. (2022). Exploring Awareness and Public Perception towards the Importance of Visual Aesthetics for Preservation of Permanent Forest Reserve (PFR) in Malaysia. *Land*, 11(8). https://doi.org/10.3390/land11081280

Munian, K., Mahyudin, N. A. A., & Azman, S. M. (2023). Understorey bird assemblages in selected environmentally sensitive areas (ESA) of Selangor, Peninsular Malaysia. *Biodiversity Data Journal*, 11. https://doi.org/10.3897/BDJ.11.E95670

Nair, P. K., & Thomas, T. K. (2013). Sustainable tourism in Malaysia: Policies and practices, *Mondes du Tourisme*, 8(8), pp. 60-69, 10.4000/tourisme.88

Nakamura, A., Kitching, R. L., Cao, M., Creedy, T. J., Fayle, T. M., Freiberg, M., ... & Ashton, L. A. (2017). Forests and their canopies: achievements and horizons in canopy science. *Trends in ecology & evolution*, *32*(6), pp. 438–451, Elsevier Ltd, https://doi.org/10.1016/j.tree.2017.02.020

Nik Hussain, N. H., & Kadir, A. A. (2013). Potential Role of Labisia pumila in the Prevention and Treatment of Chronic Diseases.

Journal of Food Research, *2*(4), pp. 55, https://doi.org/10.5539/jfr.v2n4p55

Nik Mohamad, M. M. (2013). Some Preliminary Thoughts on Tourism in Belum Temengor Complex (BTC): Proceedings of the Second Temengor Scientific Expedition 2012, pp. 61-71, Pulau Banding Foundation.

Minter, T. (2010). *The Agta of the Northern Sierra Madre: livelihood strategies and resilience among Philippine hunter-gatherers*. Faculty of Social Sciences, Leiden University.

Norhayati, M. N., George, A., Nik Hazlina, N. H., Azidah, A. K., Idiana, H. I., Law, K. S., Bahari, I. S., Wan Zahiruddin, W. M., Liske, E., & Azreena, A. (2014). Efficacy and safety of Labisia pumila var alata water extract among pre- and postmenopausal women. *Journal of Medicinal Food*, 17(8), pp. 929–938. https://doi.org/10.1089/jmf.2013.2953

Norhazlini, M. Z., Latiff, A., Farah, K. K., Nasihah, M., Siti-Munirah, M. Y., & Zulhazman, H. (2021). A review of species diversity of Rafflesia in Malaysia. *Malayan Nature Journal*, 73(4), pp. 531–552.

Pelser, P. B., Nickrent, D. L., & Barcelona, J. F. (2016). Untangling a vine and its parasite: Host specificity of Philippine Rafflesia (Rafflesiaceae). *Taxon*, *65*(4), 739-758.

Peters, R. F., & Ting, Y. Y. (2016). Protection of Rafflesia through the Appreciation of the Dusunâ€TMs Indigenous Knowledge; A Preliminary Case Study at Poring-Sabah. *Journal of Tropical Biology & Conservation (JTBC)*, 13. https://doi.org/10.51200/jtbc.v13i0.395

PLANMalaysia. (2023). Draft Rancangan Kawasan Khas: Taman Negeri Royal Belum, Perak Darul Ridzuan. Jabatan Perancangan Bandar dan Desa Negeri Perak.

Poudyal, M., Jones, J. P., Rakotonarivo, O. S., Hockley, N., Gibbons, J. M., Mandimbiniaina, R., ... & Ramamonjisoa, B. S. (2018). Who bears the cost of forest conservation?. *PeerJ*, *6*, e5106.

Rahmad, Z., Johari, S., & Akomolafe, G. F. (2018). Tree Stands and Liana Community in Royal Belum State Park, Malaysia. *Borneo Journal of Resource Science and Technology*, *8*(2), pp. 75-83. 10.33736/bjrst.1198.2018

Rahman, M. Z. A., Bakar, M. A. A., Razak, K. A., Rasib, A. W., Kanniah, K. D., Kadir, W. H. W., Omar, H., Faidi, A., Kassim, A. R., & Latif, Z. A. (2017). Non-destructive, laser-based individual tree aboveground biomass estimation in a tropical rainforest. *Forests*, 8(3). https://doi.org/10.3390/f8030086

Raihan, A., Begum, R. A., Mohd Said, M. N., & Pereira, J. J. (2021). Assessment of carbon stock in forest biomass and emission reduction potential in Malaysia. *Forests*, 12(10). https://doi.org/10.3390/f12101294

Rajoo, K. S., Ismail, A., Abdu, A., & Karam, D. S. (2021). Ulu Muda Forest Reserve: A systematic literature review of research pertaining to the 160,000-hectare tropical rain forest. In *Malayan Nature Journal* (Vol. 73, Issue 3).

Ramli, R. R., Kamal, N. A., & Zafik, N. N. K. (2024). The assessment of Royal Belum State Park as a World Heritage Site. *Geografia*, *20*(2), 82-94.

Razak, K. A., RozaimiChe Hasan, Khairul Hisyam Kamarudin, Habibah @ Norehan Haron, Rudzidatul Akmam Dziyauddin, Shamsul Sarip, & Sharihan Fathi. (2015). Transroyal: Multi-inter-trans-disciplinary Geo-biosphere Research Initiatives in The Royal Belum and Temengor Forest Complex (RBTFC) Gerik Perak. International Conference on Sustainability Initiatives (ICSI) 2015 in Conjunction with 8th ASEAN Environmental.

Rozali, F. Z., Masum, K. M., Mansor, M. S., & Mansor, A. (2021). Rattan composition and diversity assessment in tropical rainforests of Peninsular Malaysia for conservation. *Biodiversity and Conservation*, *30*(11), pp. 2907–2928. https://doi.org/10.1007/s10531-021-02226-3

Rusdi, N. D, M., Mansor, A., Azmi, N., Johari, M. S., Zakaria, R., & Harun, A. H. (2019). Diversity and distribution of rattans (Arecaceae subfam. Calamoideae) in selected habitats of Pulau Pangkor, Perak, Peninsular Malaysia, *The Malaysian Forester*, 82(1), pp. 204–211.

Rusdi, N. D. M., Mansor, A., Sah, S. A. M., Zakaria, R., Rosely, N. F. N., & Ismail, W. R. (2020). Associations of Growth and Phenology Cycles with Environmental Variables on the Population Dynamics of Non-climbing Rattan Calamus castaneus Griff. *Pertanika Journal of Tropical Agricultural Science*, *43*(3), http://www.pertanika.upm.edu.my/

Rusea, G., Wong, W. N., Mohd Raffi, M. A., Shaipudin, N. Kamaludin, F., Mustafa, M., & Abdullah, J. O. (2013). An Inventory of Wild Orchids Found in Sg. Enam Basin, Temengor, Perak: Proceedings of the Second Temengor Scientific Expedition 2012, pp. 137-147, Pulau Banding Foundation

Schwabe, K. A., Carson, R. T., DeShazo, J. R., Potts, M. D., Reese, A. N., & Vincent, J. R. (2015). Creation of Malaysia's Royal Belum State Park: A Case Study of Conservation in a Developing Country. *Journal of Environment and Development*, *24*(1), pp. 54–81. https://doi.org/10.1177/1070496514551173

Shahril Azhalin Sam, A. A., Muhamad Nor, A. N., Rafaai, N. H., Muhammad Jamil, R., Nawawi, S. A., Hassin, N. H., Abas, M. A., Hambali, K. A., Subki, N. S., Mohamad Amir Hamzah, A. S., Ibrahim, N., & Abdullah, S. A. (2023). Habitat quality assessment

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

in the Royal Belum rainforest, Malaysia using spatial analysis. *BIO Web of Conferences*, *73*, 05020. https://doi.org/10.1051/bioconf/20237305020

Shahriza, S., Ibrahim, J., Anuar, S., & Muin, A. (2012). Herpetofauna of Peta Area of Endau-Rompin National Park, Johor, Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *35*(3), pp. 553–567, http://www.pertanika.upm.edu.my/

Shamsul, K., Sarun, A., Mansor, A. K. & Samuri, B. H. (2016). Some Flowering Plants Collected in Sungai Tiang and Sungai Kejar Royal Belum State Park, Perak: Proceedings of the Fourth Royal Belum Scientific Expedition 2015, pp. 81-86, Pulau Banding Foundation

Sheikh Kamal, S. H., Suratman, M. N., Khamis, S., Nik Hassan, A. N., & Mohammad, M. S. (2022). Growth Rate, Mortality Rate and Life Cycle of Rafflesia azlanii and R. cantleyi in Belum-Temengor Forest Complex, Perak, Malaysia. *Sains Malaysiana*, 51(4). https://doi.org/10.17576/jsm-2022-5104-01

Siti Eryani, S., Muhammad-Alif Azyraf, A., Norzamli, A., & Norazmi, A. (2023). Tree Species along the trail to Gunung Hulu Temin. In Chua, L. S. L., Ong, S. P., & Lau, K. H. (eds.), *Conservation Malaysia*, 36, pp 14-16, Forest Research Institute Malaysia (FRIM), Malaysia.

Siti Munirah, M.Y. (2012). *Rafflesia* blooms in Royal Belum. *Conservation Malaysia* 16 : 6-10.

Siti-Munirah M. Y. (2020). Notes on a ten-perigoned *Rafflesia azlanii* from the Royal Belum State Park, Perak, Peninsular Malaysia. *Malayan Nature Journal*, 72(1), pp. 11-17.

Siti-Munirah, M. Y., Suhaimi-Miloko, Z., & Ahmad, M. I. Z. (2021). Thismia belumensis (Thismiaceae), a remarkable new species from The Royal Belum State Park, Gerik, Perak, Peninsular Malaysia. *PhytoKeys*, *172*, 121.

Siti-Munirah, M. Y., Ummul-Nazrah, A. R., Imin, K. & Mohd-Nazri, A. (2013). The Diversity of Flowering Plants in Sungai Enam Basin, Temengor Forest Reserve, Perak: Proceedings of the Second Temengor Scientific Expedition 2012, pp. 123-136, Pulau Banding Foundation

Stone, B. C. (1988). Notes on the genus Labisia Lindyl. (Myrsinaceae). *Malayan Nature Journal*, 42(1), 43-51.

Stuart Chapin, F., Matson, P. A., & Vitoausek, P. M. (2012). Principles of terrestrial ecosystem ecology. In *Principles of Terrestrial Ecosystem Ecology*. https://doi.org/10.1007/978-1-4419-9504-9

Subramaniam, Y. (2015). Ethnicity, Indigeneity and Indigenous Rights: The 'Orang Asli' Experience. QUT Law Review, 15(1), pp. 71-91. https://doi.org/10.5204/qutlr.v15i1.562

Sunarno, B. (2005). Revision of the genus Labisia (Myrsinaceae). *Blumea: Journal of Plant Taxonomy and Plant Geography*, 50(3), pp. 579–597. https://doi.org/10.3767/000651905X622879

Syahida-Emiza, S., Siti Munirah, M. Y., Cheah, Y. H., Suhaida, M., Imin, K., & Saw, L. G. (2023). Into the eainforest of Temin in the Royal Belum State Park. In Chua, L. S. L., Ong, S. P., & Lau, K. H. (eds.), *Conservation Malaysia*, 36, pp 1-3, Forest Research Institute Malaysia (FRIM), Malaysia.

Taib, M. F., & Zulnaidah, M. (2019). Availability of the local communities towards the implementation of the Central Forest Spine (CFS) project in Gerik, Perak. *The Malaysian Forester*, 82(2), pp. 335–339.

Tan, W. H., Hii, A., Solana-Mena, A., Wong, E. P., Loke, V. P., Tan, A. S., ... & Campos-Arceiz, A. (2021). Long-term monitoring of seed dispersal by Asian elephants in a Sundaland rainforest. *Biotropica*, *53*(2), 453-465.

Tan, W. H., Ong, L., & Strijk, J. S. (2023). Castanopsis corallocarpus (Fagaceae), a new species from Royal Belum (Perak) in Peninsular Malaysia. *PhytoKeys*, 219, pp 1-10. https://doi.org/10.3897/phytokeys.219.95991

The Star. (2023). Biodiversity: Our Life, Our Heritage, Our Future. The Star. https://www.thestar.com.my/news/nation/2023/05/30/biodiversity-our-life-our-heritage-our-future (accessed August 7, 2024).

The Importance of Uluru to Australian Indigenous Culture. (2024). From Voyages Indigenous Tourism Australia.: https://www.ayersrockresort.com.au/stories/the-importance-of-uluru-to-australian-indigenous-culture (accessed January 1, 2025)

TNB Research. (2024) Regulatory & Environmental Science. TNB Research. https://www.tnbr.com.my/sustainable-environment-1/regulatory-%26-environmental-science (accessed October 15, 2024)

Tropical Rainforest Conservation & Research Centre. (2020). *Tropical Rainforest Living Collection*. https://www.trcrc.org/trlc (accessed August 12, 2024)

Turner, I. M., Tan, H., & Yong, J. (1995). The Limestone Flora of Temengor, Hulu Perak, Malaysia. *Malayan Nature Journal*. 48. 189-196.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Ummul-Nazrah, A. R., Imin, K., Norzielawati, S., & Mohd-Nazri, A. (2016). Flora of Sungai Tiang, Royal Belum State Park, Perak, Malaysia): Proceedings of the Fourth Royal Belum Scientific Expedition 2015, pp. 97-105, Pulau Banding Foundation

UNESCO. (2014). *National Park (Taman Negara) of Peninsular Malaysia*.

Vincent, J. R., Carson, R. T., DeShazo, J. R., Schwabe, K. A., Ahmad, I., Chong, S. K., ... & Potts, M. D. (2014). Tropical countries may be willing to pay more to protect their forests. Proceedings of the National Academy of Sciences of the United States of America, 111(28), pp. 10113-10118. https://doi.org/10.1073/pnas.1312246111

Walsh, R.P.D. (1996). Climate. In Richards, P.W. (ed.), The tropical rain forest: An ecological study, Journal of Tropical Ecology, 13(5), pp 159–205, Cambridge: Cambridge University Press, https://doi.org/10.1017/S0266467400010944

Wan Rozali, W. N. F. Z., Mohamad Syafiq, M. J., Norahizah, A. R., Rahmad, Z., Asyraf, M., Nur Zhafarina, A. (2016). The Diversity and Abundance of Climbers and Medicinal Value of Selected Liana in Sungai Tiang, Royal Belum State Park, Perak: Proceedings of the Fourth Royal Belum Scientific Expedition 2015, pp. 57-60, Pulau Banding Foundation

Wan Zakaria, W. N. F., Ahmad Puad, A. S., Geri, C., Zainudin, R., & Latiff, A. (2016). Tetrastigma diepenhorstii (Miq.) Latiff (Vitaceae), a New Host of Rafflesia tuan-mudae Becc. (Rafflesiaceae) in Borneo. *Journal of Botany*, 2016(1), 3952323, https://doi.org/10.1155/2016/3952323

Wong, S. L. (2003). The Royal Belum: crowning glory of the peninsula, Ipoh, Perak: Perbadanan Taman Negeri Perak; Petaling Jaya, Selangor: Sasyaz Holdings Sdn. Bhd.

WWF-Malaysia. (2024). WWF-Malaysia Annual Review 2023. WWF-Malaysia. https://www.wwf.org.my/media_and_information/publications/wwf_malaysia_annual_review/ (accessed August 6, 2024).

Yamamoto-Ebina, S., Saaban, S., Campos-Arceiz, A., & Takatsuki, S. (2016). Food habits of Asian elephants Elephas maximus in a rainforest of Northern Peninsular Malaysia. *Mammal Study*, 41(3), pp. 155-161. https://doi.org/10.3106/041.041.0306

Yayasan Sime Darby. (2022). *Malayan Tiger Protection receives RM1.2 million in funding for enhancing Orang Asli Forest Patrol Teams - Media*. https://www.yayasansimedarby.com/media/malayan-tiger-protection-receives-rm12-million-in-funding-for-enhancing-orang-asli-forest-patrol-teams (accessed 15 October, 2024)

Yeap, C. A., & Lim, K. C. (2020). Birds of Belum-Temengor Forest Complex, Perak State, Peninsular Malaysia: An Overview of Ornithological Research, Checklist & Selected Bibliography, MNS Conservation Publications,21, Malaysia Natura Society.

Yeap, C. A., Lim, K., Noramly, G., Carang, R. O. S. L. A. N., Carang, A. Z. A. M., & Pandak, M. A. R. I. S. A. N. (2016). The Malaysian nature society hornbill conservation project. *Malayan Nature Journal*, *68*(4), 149-159.

Yunoh, S. M. M. (2020). Notes on a ten-perigoned Rafflesia azlanii from the Royal Belum State Park, Perak, Peninsular Malaysia. *Malayan Nature Journal*, 72(1), pp. 11-17.

Zakaria, A. A., Noor, M. H. M., Ahmad, H., Hassim, H. A., Mazlan, M., & Latip, M. Q. A. (2021). A Review on Therapeutic Effects of Labisia pumila on Female Reproductive Diseases, *BioMed Research International*, 2021(1), 9928199, Hindawi Limited. https://doi.org/10.1155/2021/9928199

Zaki, N. A. M., Abd Latif, Z., & Suratman, N. (2014). Allometric Equations for Estimating the Above-Ground Biomass and Carbon Stock in Tropical Lowland Dipterocarp: Case study of Royal Belum Forest Reserve, Proceedings of the National Conference on Royal Belum 2014.

Zen, I. S., Ahmad, Z. A., Hassan, Z., & Afifi, A. (2014). Contextualize the Community Based Conservation Practices of Royal Belum Forest Reserve, in Perak State, Malaysia, Proceedings of the National Conference on Royal Belum 2014.

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64- (March 2026)

Appendix A: List of Floral Diversity (Phylum: Ptrediophyta) Recorded in Belum-Temengor Forest Complex

Family name	Species name	Common Name	Global IUCN Red List Status	Habit	Location	References
Adiantaceae	*Adiantum latifolium*	Paku Sisik	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Haplopteris ensiformis		NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Apleniaceae	*Asplenium longissimum*		NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Asplenium scortechinii		NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Aspleniaceae	*Asplenium macrophyllum*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Asplenium nidus	Paku Langsuyar	NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Asplenium pellucidum		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Asplenium sp.		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Athyriaceae	*Diplazium accedens*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Diplazium crenatoserratum	Paku Naga	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Diplazium tomentosum	Paku Binet	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Diplazium sp.		NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Blechnaceae	*Stenochlaena palustris*	Paku Miding	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Davalliaceae	*Davallia angustata*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Davallia repens		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Dennstaedtiaceae	*Microlepia speluncae*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Dryopteridaceae	*Bolbitis appendiculata*		LC	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Bolbitis heteroclita		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Bolbitis simplicifolia		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Bolbitis sinuata		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Hymenophyllaceae	*Hymenophyllum penangianum*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Lomariopsidaceae	*Cyclopeltis crenata*		NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
Lycopodiaceae	*Huperzia phlegmaria (L.)*	Sri Gading	NE	Lycophytes	Royal Belum State Park	Maideen et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Palhinhaea cernua	Paku Serani	LC	Lycophytes	Royal Belum State Park	Maideen et al. (2015)
	Phlegmariurus phlegmaria		NE	Lycophytes	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Lygodiaceae	*Lygodium flexuosum*	Ribu-Ribu Gajah	NE	Lycophytes	Royal Belum State Park	Maideen et al. (2015)
	Lygodium microphyllum	Akar Sidin Kecil	NE	Lycophytes	Royal Belum State Park	Maideen et al. (2015)
	Lygodium salicifolium	Ribu-Ribu Gajah	NE	Lycophytes	Royal Belum State Park	Maideen et al. (2015)
Marattiaceae	*Angiopteris elliptica*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Angiopteris evecta	Paku Gajah	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Nephrolepidaceae	*Nephrolepis biserrata*	Paku Uban	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Nephrolepis falciformis	Paku Uban	NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Oleandraceae	*Oleandra cumingii*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Ophioglossaceae	*Helminthostachys zeylanica*	Akar Tunjuk Langit	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Ophioglossum costatum		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Ophioglossum pedunculosum		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Polypodiaceae	*Colypsis* sp.		NE		Royal Belum State Park	Maideen et al. (2015)
	Drynaria rigidula		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Drynaria sparsisora		NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Lecanopteris crustacea		NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Leptochilus macrophyllus var. pedunculatus		NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Leptochilus sp.		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Loxogramme subcostata		NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Microsorum membranifolium	Paku Chai	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Microsorum pteropus		LC	Fern	Royal Belum State Park	Maideen et al. (2015)
	Platycerium ridleyi	Tanduk Rusa	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Pyrrosia lanceolata	Bulu Ayam	NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

Pteridaceae	*Antrophyum callifolium*	Akar Selempar	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Antrophyum latifolium		NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Ceratopteris thalictroides	Paku Roman	LC	Fern	Royal Belum State Park	Maideen et al. (2015)
	Pteris ensiformis	Paku Mega	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Taenitis blechnoides	Paku Pijai	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
Selaginellaceae	*Selaginella delicatula*		NE	Spike moss	Royal Belum State Park	Maideen et al. (2015)
	Selaginella frondosa		NE	Spike moss	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Selaginella mayeri		NE	Spike moss	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Selaginella padangensis		NE	Spike moss	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Selaginella plana		NE	Spike moss	Royal Belum State Park	Maideen et al. (2015)
	Selaginella roxburghii		NE	Spike moss	Royal Belum State Park	Maideen et al. (2015)
	Selaginella stipulata		NE	Spike moss	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Selaginella wallichii	Paku Merak	NE	Spike moss	Royal Belum State Park	Ummul-Nazrah et al., 2015
	Selaginella willdenowii	Paku Merak, Paku Lipan	NE	Spike moss	Royal Belum State Park	Ummul-Nazrah et al., 2015
Tectariaceae	*Pleocnemia conjugata*	Paku Gading	NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Tectaria brachiata		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Tectaria crenata		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Tectaria impressa		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Tectaria semipinnata		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Tectaria sp.		NE	Fern	Royal Belum State Park	Ummul-Nazrah et al., 2015
Thelypteridaceae	*Pneumatopteris* sp.		NE	Fern	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Pneumatopteris truncate		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Pronephrium menisciicarpon		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Reholttumia truncata		NE	Fern	Royal Belum State Park	Maideen et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

Vittariaceae	*Haplopteris angustifolia*		NE	Fern	Royal Belum State Park	Maideen et al. (2015)
	Haplopteris angustissima		NE	Fern	Royal Belum State Park	Maideen et al. (2015)

*NE = Not Evaluated, LC = Least Concern

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

Appendix B: List of Floral Diversity (Phylum: Gymnosperm) Recorded in Belum-Temengor Forest Complex

Family name	Species name	Common Name	Global IUCN Red List Status	Habit	Location	References
Cycadaceae	*Cycas macrocarpa*	Bogak	VU	Palm-like	Royal Belum State Park	Ghazalli et al. (2015)
	Cyperus pilosus	Rumput Para-Para	LC	Herb	Royal Belum State Park	Ismail et al. (2015)
	Cyperus sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Cyperus sp. 1			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Cyperus sp. 2			Herb	Royal Belum State Park	Ghazalli et al. (2015)
Gnetaceae	*Gnetum gnemonoides*		LC	Liana	Royal Belum State Park	Rahmad et al. (2018)
	Gnetum latifolium	Akar Melinjau	LC	Liana	Royal Belum State Park	Rahmad et al. (2018)
	Gnetum macrostachyum		LC	Liana	Royal Belum State Park	Ghazalli et al. (2015)
	Gnetum microcarpum		LC	Liana	Royal Belum State Park	Rahmad et al. (2018)
	Gnetum sp.			Liana	Royal Belum State Park	Ghazalli et al. (2015)
Podocarpaceae	*Podocarpus* sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)

*LC = Least Concern, VU = Vulnerable

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

Appendix C: List of Floral Diversity (Phylum: Anthophyta) Recorded in Belum-Temengor Forest Complex

Family name	Species name	Common Name	Global IUCN Red List Status	Habit	Location	References
Achariaceae	*Hydnocarpus nana*	Setumpol	NT	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Hydnocarpus woodii	Senumpul	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Pangium edule	Kepayang	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017b)
Amarylidaceae	*Crinum asiaticum*	Bakung Putih	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
Anacardiaceae	*Bouea macrophylla*	Kundang Hutan	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Dracontomelon dao	Sengkuang	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Mangifera microphylla	Rawa	NE	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Mangifera quadrifida var. quadrifida	Macang Hutan	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Melanochyla sp.	Rengas		Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Spondias pinnata	Kedondong	NE	Tree	Royal Belum State Park	Ghazalli et al. (2015)
Anisophylleaceae	*Anisophyllea corneri*	Delek	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Annonaceae	*Alphonsea elliptica*	Mempisang	LC	Tree	Royal Belum State Park	Chua et al. (2000)
	Alphonsea lucida	..	VU	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Alphonsea maingayi	Pisang-Pisang Batu	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Anaxagorea javanica	Akar Rarak	LC	Shrub/Tree	Royal Belum State Park	Ismail et al. (2015)
	Desmos chinensis	Akar Mempisang	NE	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Enicosanthum fuscum		NT	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Fissistigma manubriatum	Akar Larak	NE	Climber	Royal Belum State Park	Wan Rozali et al. (2015)
	Friesodielsia sp.			Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Goniothalamus curtisii	Cagau	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Goniothalamus macrophyllus	Lukai Kampong	NE	Tree	Royal Belum State Park	Chua et al. (2000)
	Goniothalamus scortechinii	Selayar Hitam	NE	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Goniothalamus sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Goniothalamus uvarioides	Belindung	NE	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Mezzettia parviflora	Mempisang	LC	Tree	Royal Belum State Park	Chua et al. (2000)
	Mezzettia sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Miliusa longipes	Kayu Hamat Bawa	NE	Tree	Royal Belum State Park	Ismail et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Monocarpia maingayi	Mempisang	NE	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Orophea cf. *hirsuta*	Pialu	NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Orophea cuneiformis		NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Orophea enterocarpa		NE	Shrub	Royal Belum State Park	Ismail et al. (2015)
	Polyalthia bullata	Tongkat Ali Hitam	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Polyalthia cauliflora var. cauliflora	Karai Larak Merah	LC	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Polyalthia clavigera	Mempisang	NE	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Polyalthia sp.			Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Polyalthia stenopetala	Jambul Cicit	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Popowia fusca		NE	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
Apocynaceae	*Rauvolfia verticillata*	Beras-Beras	LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Tabernaemontana corymbosa	Jelutung Badak	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Aglaonema nitidum	Keladi Hutan	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Alocasia denudata	Keladi Birah	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Amorphophallus campanulatus (Amorphophallus paeoniifolius)	Lekir	LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Amorphophallus prainii	..	NE	Herb	Royal Belum State Park	Ismail et al. (2015)
	Arisaema laminatum		NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Colocasia esculenta	Keladi	LC	Herb	Royal Belum State Park	Ismail et al. (2015)
Araceae	*Epipremnum giganteum*	Akar Resdung	NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Homalomena humilis	Keladi Hutan	NE	Aroid	Royal Belum State Park	Ismail et al. (2015)
	Homalomena pontederiifolia		NE	Aroid	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Homalomena sp.			Aroid	Royal Belum State Park	Ghazalli et al. (2015)
	Licuala triphylla		NE	Aroid	Royal Belum State Park	Ghazalli et al. (2015)
	Pinanga paradoxa		NE	Aroid	Royal Belum State Park	Ghazalli et al. (2015)
	Piptospatha perakensis		NE	Aroid	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Schismatoglottis calyptrata		NE	Aroid	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Araliaceae	*Trevesia burckii*	Jari hantu	LC	Tree	Banding Forest Reserve	Hazandy (2014)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

Arecaceae	*Arenga hookeriana*	Miniature Sugar Palm	NE	Palm	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Calamus castaneus	Rotan Cucur	NE	Rattan	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Licuala sp.			Palm	Royal Belum State Park	Ghazalli et al. (2015)
	Pinanga disticha	Pinang	NE	Palm	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Pinanga malaiana	Lagong	NE	Palm	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Pinanga perakensis		NE	Palm	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Aristolochiaceae	*Thottea parviflora*	Kemed Kawit	NE	Shrub	Royal Belum State Park	Ismail et al. (2015)
	Thottea tomentosa	Tapak Gajah	LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
Asparagaceae	*Peliosanthes* sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
Asteraceae	*Ageratum conyzoides*	Berenyol	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Erechtites hieraciifolia	Fireweed	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Mikania micrantha	Akar Lupang	NE	Herb	Royal Belum State Park	Ismail et al. (2015)
	Vernonia arborea	Medang Gambong	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
Begoniceae	*Begonia* sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Begonia wrayi	Riang Batu	NT	Herb	Sg Kuak, Temengor Forest Reserve	Ummul-Nazrah et al., 2015
Burseraceae	*Canarium littorale*	Kedondong Bulan	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Canarium patentinervium		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Dacryodes costata	Kedondong Bulu Costata	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Dacryodes laxa	Kedondong Bulu Laxa	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Dacryodes puberula	Kedondong	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Dacryodes rostrata	Kedondong Kerut	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Dacryodes rugosa	Kedondong Matahari	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Santiria apiculata	Kedondong Kerantai	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Santiria laevigata	Kedondong Kerantai Licin	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Santiria tomentosa	Kedondong Kerantai Bulu	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Calophyllaceae	*Calophyllum inophyllum*	Bintangor Laut	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Mesua ferrea	Lenggapus	NE	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Cannabaceae	*Celtis rigescens*		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Gironniera parvifolia	Hampas Tebu	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Chloranthaceae	*Chloranthus erectus*	Sambau Paya	NE	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
Cleomaceae	*Cleome rutidosperma*		NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
Clusiaceae	*Garcinia atroviridis*	Asam Gelugor	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017b)
	Garcinia bancana	Tengkawan	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Garcinia cowa	Kandis	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017b)
	Garcinia nervosa	Kandis	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Garcinia prainiana	Mencupu	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Garcinia sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Garcinia urophyll	Kandis	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Commelinaceae	*Amischotolype glabrata*	Segambut Merah	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Pollia secundiflora		NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Rhopalephora scaberrima		NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Costaceae	*Costus globosus*		LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
Cucurbitaceae	*Gymnopetalum chinense*	Sipam	NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
Dilleniaceae	*Dillenia ovata*	Simpoh Beludu	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Dillenia reticulata	Simpoh Gajah	LC	Tree	Royal Belum State Park	Ismail et al. (2015)
	Tetracera indica	Mempelas	NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
Dioscoreaceae	*Dioscorea bulbifera*		NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
Dipterocarpaceae	*Anisoptera costata*	Mersawa kesat	EN	Tree	Royal Belum State Park	Zaki et al. (2014)
	Anisoptera laevis	Mersawa durian	VU	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Dipterocarpus acutangulus	Keruing Merkah	EN	Tree	Royal Belum State Park	Zaki et al. (2014)
	Dipterocarpus baudii	Keruing Bulu	VU	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Dipterocarpus chartaceus	Keruing Kertas	EN	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Dipterocarpus costulatus	Keruing Kipas	NT	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Dipterocarpus fagineus	Keruing Pipit	VU	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Dipterocarpus gracilis	Keruing Kesat	VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Dipterocarpus grandiflorus	Keruing Belimbing	EN	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Dipterocarpus kunstleri	Keruing Gombang Merah	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Hopea coriacea	Giam Hantu	VU	Tree	Royal Belum State Park	Zaki et al. (2014)
	Hopea latifolia	Merawan Daun Bulat	DD	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Hopea pubescens	Merawan Bunga	VU	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Hopea sangal	Merawan Siput	VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Hopea sublanceolata	Merawan Jeruai	VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Parashorea densiflora	Meranti Pasir	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Parashorea stellata	Gerutu Gerutu	VU	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Rubroshorea curtisii ssp. *curtisii*	Meranti Seraya	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Rubroshorea macroptera	Meranti Melantai	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Shorea bentongensis (Anthoshorea bentongensis)	Meranti Mengkai	VU	Tree	Royal Belum State Park	Zaki et al. (2014)
	Shorea dasyphylla (Rubroshorea dasyphylla)	Meranti Batu	NT	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Shorea farinosa	Temak Merah	EN	Tree	Royal Belum State Park	Chua et al. (2000)
	Shorea guiso	Balau Membatu	VU	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Shorea laevis	Balau Kumus	VU	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Shorea lepidota (Rubroshorea lepidota)	Meranti Langgang	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Shorea leprosula (Rubroshorea leprosula)	Meranti Tembaga	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Shorea maxima (Richetia maxima)	Damar Sengkawang Puteh	NT	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Shorea multiflora (Richetia multiflora)	Damar Hitam Pipit	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Shorea ovata (Rubroshorea ovata)	Meranti Sarang Punai	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Shorea parvifolia	Meranti Sarang Punai	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Shorea pauciflora (Rubroshorea pauciflora)	Meranti Nemesu	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Shorea platyclados (Rubroshorea platyclados)	Meranti Bukit	NT	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Vatica bella	Resak Keluangas	DD	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Vatica pauciflora	Resak Laru	VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
Ebenaceae	*Diospyros dictyoneura*		NE	Tree	Royal Belum State Park	Ghazalli et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Diospyros ridleyi	Meribut	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Diospyros scortechinii	Tembakar	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Diospyros singaporensis	Meribut	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Diospyros sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Diospyros wallichii	Tuba buah	LC	Tree	Royal Belum State Park	Chua et al. (2000)
Elaeocarpaceae	*Elaeocarpus nitidus*	Mendong	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Sloanea sigun		LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Antidesma cuspidatum	Bruni	LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Antidesma montanum	Berunai	LC	Shrub	Royal Belum State Park	Chua et al. (2000)
	Balakata baccata	Ludai	NE	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Blumeodendron sp.	Gaham Badak		Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Croton argyratus	Hamba Raja	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Elateriospermum tapos	Perah	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Endospermum diadenum	Sesendok	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Macaranga conifera	Mahang	LC	Tree	Banding Forest Reserve	Hazandy (2014)
Euphorbiaceae	*Macaranga denticulata*	Mahang	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Macaranga hullettii	Mahang	LC	Tree	Banding Forest Reserve	Hazandy (2014)
	Macaranga hypoleuca	Mahang Putih	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Macaranga triloba	Mahang Merah	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Mallotus griffithianus	..	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Mallotus muticus	Mallotus Paya	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Paracroton pendulus		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Sapium baccatum	Ludai	LC	Tree	Banding Forest Reserve	Hazandy (2014)
	Archidendron bubalinum	Kerdas	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017b)
	Bauhinia acuminata	Bunga Perak	LC	Climber	Royal Belum State Park	Rahmad et al. (2018)
Fabaceae	*Bauhinia bidentata*	Katup Katup	NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Desmodium heterocarpon	Kacang Kayu Betina	NE	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Dialium indum	Keranji	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Dialium platysepalum	Keranji Kuning Besar	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Intsia palembanica	Merbau	NT	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Koompassia malaccensis	Kempas	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Mucuna pruriens		NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Ormosia macrodisca		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Parkia speciosa	Petai	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017b)
	Saraca indica	Ashoka tree	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Sindora coriacea	Sepetir Licin	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Sindora sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Spatholobus gyrocarpus		LC	Liana	Royal Belum State Park	Rahmad et al. (2018)
	Uraria crinita		NE	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Castanopsis argentea		EN	Tree	Royal Belum State Park	Zaki et al. (2014)
Fagaceae	*Castanopsis curtisii*	Berangan Babi	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Lithocarpus wallichianus	Mempening	NE	Tree	Royal Belum State Park	Chua et al. (2000)
	Cyrtandra gimlettii		NE	Herb	Royal Belum State Park	Ismail et al. (2015)
	Epithema parvibracteatum		NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
Gesneriaceae	*Epithema saxatile*		NE	Herb	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Henckelia sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Stauranthera grandiflora		NE	Herb	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Hanguanaceae	*Hanguana malayana*	Susum	LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
Hypericaceae	*Cratoxylum arborescens var. arborescens*	Geronggang Geronggang	LC	Tree	Royal Belum State Park	Ismail et al. (2015)
Irvingiaceae	*Irvingia malayana*	Pauh Kijang	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Lamiaceae	*Vitex vestita*	Leban Hutan	LC	Shrub	Royal Belum State Park	Siti Eryani et al. (2023)
	Actinodaphne sp.	Medang		Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Beilschmiedia dictyoneura		VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Beilschmiedia insignis		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Lauraceae	*Beilschmiedia palembanica*	Medang	LC	Tree	Royal Belum State Park	Chua et al. (2000)
	Beilschmiedia sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Cryptocarya infectoria		NE	Tree	Royal Belum State Park	Chua et al. (2000)
	Endiandra maingayi		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Litsea umbellata	Medang	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
Lecythidaceae	*Barringtonia macrostachya*	Putat Gajah	LC	Shrub/Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Barringtonia scortechinii	Putat Gajah	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
Loganiaceae	*Strychnos ignatii*	Akar Ipuh	NE	Climber	Royal Belum State Park	Wan Rozali et al. (2015)
Lowiaceae	*Orchidantha longiflora*		NE	Herb	Royal Belum State Park	Syahida-Emiza et al. (2023)
	Durio griffithii	Durian Tupai	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Hibiscus floccosus	Kangsar	NE	Shrub	Royal Belum State Park	Siti Eryani et al. (2023)
	Hibiscus macrophyllus	Tutur	NE	Shrub	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Microcos tomentosa	Cenderai	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Neesia altissima		NE	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Malvaceae	*Pentace perakensis*		VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Scaphium linearicarpum	Kembang Semangkok	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Scaphium macropodum	Kembang Semangkok Jantong	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Schoutenia kunstleri		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Urena lobata	Pepulut	LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Memecylon amplexicaule		LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Melastomataceae	*Memecylon* sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Pternandra echinata	Sial Menahun	NE	Tree	Banding Forest Reserve	Hazandy (2014)
	Aglaia eximia	Bekak	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Aglaia foveolata	Bekak	NT	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Aglaia korthalsii	Bekak	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Aglaia leucophylla	Bekak Kedondong	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Aglaia oligophylla	Bekak	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Meliaceae	*Aglaia palembanica*	Memberas	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Aglaia rubiginosa	Bekak	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Aglaia squamulosa	Bekak	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Azadirachta excelsa	Sentang	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Chisocheton tomentosus		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Lansium domesticum	Langsat	LC	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017b)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Sandoricum koetjape	Sentul	VU	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Xylocarpus moluccensis	Nyireh Batu	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Menispermaceae	*Coscinium fenestratum*	Akar Mengkunyit	DD	Climber	Royal Belum State Park	Wan Rozali et al. (2015)
Moraceae	*Artocarpus elasticus*	Terap Nasi	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Artocarpus gomezianus	Keledang Tampang Hitam	NE	Tree	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
	Artocarpus rigidus	Temponek	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Ficus ischnopoda		LC	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Myristicaceae	*Endocomia canarioides*	Pianggu Talang	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Horsfieldia polyspherula	Penarahan	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Horsfieldia sucosa	Penarahan	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Knema conferta		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Myristica iners	Penarahan Arang	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Myristica sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
Myrsinaceae	*Ardisia* sp.	Mata Pelanduk		Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Labisia pumila	Kacip Fatimah	NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
Myrtaceae	*Rhodamnia cinerea*	Mempoyan	NE	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Syzygium sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
Olacaceae	*Erythropalum scandens*	Akar Kulim	LC	Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Ochanostachys amentacea	Petaling	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Scorodocarpus borneensis	Kulim	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
Onagraceae	*Ludwigia hyssopifolia*	Inai Paya	LC	Weed	Royal Belum State Park	Ismail et al. (2015)
Orchidaceae	*Corymborkis veratrifolia*	Hencing Ali	NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Didymoplexiella ornata		NE	Shrub	Royal Belum State Park	Ismail et al. (2015)
Pandaceae	*Galearia* sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
Passifloraceae	*Adenia* sp.	Akar Lempedu Gajah		Herb/Vine	Royal Belum State Park	Ghazalli et al. (2015)
Phyllanthaceae	*Antidesma pendulum*	Berunai	LC	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Antidesma sp.	Berunai		Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Aporosa sp.	Sebasah	..	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Phyllanthus sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Piper sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
Poaceae	*Gigantochloa scortechinii*	Buluh Galah	NE	Bamboo	Royal Belum State Park	Ghazalli et al. (2015)
Primulaceae	*Ardisia colorata*	Mata Pelanduk	LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Ardisia korthalsiana	Mata Pelanduk	NE	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
Rafflesiaceae	*Rafflesia azlanii*		NE	Parasite	Royal Belum State Park	Siti-Munirah et al. (2012)
	Rafflesia cantleyi		NE	Parasite	Royal Belum State Park	Wan Zakaria et al. (2016)
	Rafflesia kerrii	Pakma	NE	Parasite	Royal Belum State Park	Wan Zakaria et al. (2016)
Rhamnaceae	*Zizyphus* sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
Rosaceae	*Prunus arborea*	Pepijat	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Prunus grisea	Pepijat	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Rubiaceae	*Argostemma* sp.		NE	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Canthium aciculatum		NE	Tree	Royal Belum State Park	Ismail et al. (2015)
	Chassalia chartacea	Pengugur	NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Chassalia sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Diplospora malaccense	..	NE	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
	Fagerlindia fasciculata		NE	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Gardeniopsis longifolia	..	LC	Shrub	Royal Belum State Park	Siti Eryani et al. (2023)
	Ixora brunonis		LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Ixora javanica var. javanica	Jenjarum	LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Ixora nigricans		LC	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Ixora sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Lasianthus montanus		NE	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Lasianthus sp.			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Lasianthus sp. 1			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Lasianthus sp. 2			Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Lasianthus sp. 3			Tree	Royal Belum State Park	Ismail et al. (2015)
	Lasianthus stercorarius		NE	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Neolamarckia cadamba	Kelempayan	NE	Tree	Royal Belum State Park	Ismail et al. (2015)
	Ophiorrhiza communis		NE	Shrub	Royal Belum State Park	Ismail et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

	Ophiorrhiza discolor		NE	Shrub	Royal Belum State Park	Ismail et al. (2015)
	Pavetta salicina		NE	Shrub	Royal Belum State Park	Ismail et al. (2015)
	Pavetta wallichiana		NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Porterandia anisophylla	Tinjau Belukar	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Prismatomeris sp.			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Psychotria calocarpa		NE	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Psychotria rhinocerotis		LC	Shrub	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Psychotria sp. 1			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Psychotria sp. 2			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Psychotria sp. 3			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Psychotria sp. 4			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Saprosma distans		NE	Shrub	Royal Belum State Park	Ismail et al. (2015)
	Saprosma sp. 1			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Saprosma sp. 2			Shrub	Royal Belum State Park	Ghazalli et al. (2015)
	Timonius wallichianus	Kaum Kopi	NE	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Urophyllum glabrum	Melukut	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Rutaceae	*Citrus halimii*	Limau Hutan	LC	Tree	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Murraya paniculata	Kamuning	NE	Shrub	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
Salicaceae	*Flacourtia rukam*	Rukam	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
Sapindaceae	*Nephelium cuspidatum var. ophiodes*	Rambutan Hutan	LC	Tree	Royal Belum State Park	Chua et al. (2000)
	Paranephelium macrophyllum	Gesiar	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Paranephelium xestophyllum		LC	Tree	Royal Belum State Park	Chua et al. (2000)
	Pometia pinnata	Kasai	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)
	Xerospermum noronhianum	Rambutan Pacat	NE	Tree	Royal Belum State Park	Ghazalli et al. (2015)
Sapotaceae	*Palaquium hexandrum*	Nyatoh Jambak	NT	Tree	Royal Belum State Park	Rahmad et al. (2018)
	Payena maingayi	Nyatoh Durian	LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Stemonuraceae	*Gomphandra quadrifida*		LC	Shrub	Royal Belum State Park	Ghazalli et al. (2015)
Thymelaeaceae	*Gonystylus confusus*	Ramin	LC	Tree	Royal Belum State Park	Siti Eryani et al. (2023)
Torricelliaceae	*Aralidium pinnatifidum*	Sebalai	LC	Tree	Royal Belum State Park	Ghazalli et al. (2015)

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

Urticaceae	*Dendrocnide stimulans*	Jelantang Gajah	LC	Shrub	Temengor Forest Reserve	Ahmad Fitri et al. (2017a)
Violaceae	*Rinorea horneri*		LC	Tree	Royal Belum State Park	Rahmad et al. (2018)
Vitaceae	*Cissus repens*		NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Cissus sp.			Climber	Royal Belum State Park	Ghazalli et al. (2015)
	Tetrastigma leucostaphylum		NE	Climber	Royal Belum State Park	Ghazalli et al. (2015)
Zingiberaceae	*Alpinia* sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Amomum sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Boesenbergia jahaiana		NE	Herb	Royal Belum State Park	Ismail et al. (2015)
	Boesenbergia plicata		LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Boesenbergia prainian		LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Elettaria sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Elettariopsis sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Globba curtisii		NE	Herb	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Globba pendula	Meroyan Tinggal	LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Globba sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Scaphochlamys sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Zingiber cf. *kunstleri*		LC	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Zingiber griffithii		NT	Herb	Royal Belum State Park	Ismail et al. (2015)
	Zingiber puberulum		NT	Herb	Royal Belum State Park	Ghazalli et al. (2015)
	Zingiber raja		EN	Herb	Temengor Forest Reserve	Siti-Munirah et al. (2013)
	Zingiber sp.			Herb	Royal Belum State Park	Ghazalli et al. (2015)

*NE = Not Evaluated, DD = Data Deficient, LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered

DOI:https//doi.org/10.22452/mjs.vol45no1.8
Malaysian Journal of Science 44(4): 64-102 (March 2026)

MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 45(1): 103-127 (March 2026) https://mjs.um.edu.my

A Review: Role of Silkworm (*Tubifex Tubifex Müller*, 1774) as Bioremediator in Freshwater Ecosystem

Andri Kurniawan[1a]*, Sudirman Adibrata[2b], Rahmad Lingga[3a], Jhodi Setiadi[4b], Ufi A. Wulandari[5b], Reysya S.N. Hidayah[6a]

Abstract: *Tubifex tubifex Müller*, 1774 is an oligochaete that is extensively dispersed, in particular in freshwater ecosystems, and plays an important role in the ecosystem. It is one of natural or live feeds for aquatic organisms in nature or cultivation media. Furthermore, *T. tubifex* could be a reasonable living being for the inquiry about the organic impact of different contaminants due to its capacity to decompose matter as well as accumulate metals or chemical pollutants. This review aimed to elaborate on the information about the biology of *T. tubifex* and its role, focusing on its ability as a decomposer in freshwater ecosystems. In the arrangement of this paper, many relevant scientific articles were cited. This paper examined the biology of *T. tubifex* (i.e., morphology, chaetae, segmentation, respiratory, reproduction, and habitat of Tubifex) and also the role of Tubifex as a bioremediator. This review informed that Tubifex has a reddish color due to the presence of erythrocruorin. As a group of clitellates, Tubifex has a clitellum at segments X, XI, and XII. Furthermore, as a group of oligochaetes, it has chaetae that play an important role in burrowing, hooking to the substrate, swimming, crawling, and bioturbating. Tubificidae have the capability of autotomization (autotomy) and regeneration of their missing body parts. *T. tubifex* spends its whole life cycle within the substrate, with its head down within the substrate, whereas part of its posterior may extend over the water column-substrate interface. T. tubifex develops a mixed reproductive strategy, self-fertilization or parthenogenesis (pseudogamy), and is hermaphrodite. Tubifex undergoes fertilization and produces eggs that are stored in cocoon sacs. The eggs will hatch and then develop into juveniles, adult worms, and individuals that are mature enough to reproduce. This reproductive stage takes about 57-60 days. Tubifex is a meiobenthic species of aquatic worm and an important key for organic matter decomposition, heavy metal, or chemical substances reduction and detoxification. It can perform vermicomposting on organic matter and also accumulate chemical substances through its metabolic compounds such as superoxide dismutase enzyme, catalase enzyme, carboxylesterase enzyme, glutathione-S-transferase enzyme, and metallothionein enzyme.

Keywords: *Biology, bioremediator, metabolic compound, role, Tubifex.*

1. Introduction

Tubifex worm (*Tubifex tubifex* Müller, 1774) is included as a group of water worms, generally known as silkworm or hair because it has a soft and very soft body like silk or hair (Yanar et al., 2003; Mahendra et al., 2019). *T. tubifex* is one of the natural feeds commonly used and the most important live feed used in aquaculture or fish cultivation (Mandal et al., 2018; Simangunsong et al., 2023; Kurniawan et al., 2024). This species can play an important role as a supplement because of its high nutritional content (Kautsar et al., 2022). Yanar et al., (2003) explained that this worm contains a percentage of crude protein 11.02 ± 0.58, lipid 2.14 ± 0.06, ash 1.83 ± 0.16, and moisture 18.78 ± 0.83, and also some fatty acid and amino acid. Oplinger et al., (2011) and Rech et al., (2013) revealed *T. Tubifex* contains protein (50-55%), fat (8-10 %), crude fibre (2-5%), ash (4-7%), and water (8-10%). This nutrient content is higher in quantity and of the same quality compared to *Artemia* sp. Mashudi et al., (2023) explained that *T. tubifex* is small in size according to larval mouths, easier to be digested for larvae of aquatic biota, and according to Conceição et al. (2010) *Tubifex* sp. can supply fundamental nutrients for the great growth performance of larvae.

T. tubifex is grouped in the Oligochaeta class and Naididae (Tubificidae) family, so it is also known as tubificid worms (Erséus & Gustavsson, 2002; Alam et al., 2021). It also has an ability to live in in variety of waters from clean to very contaminated water, especially a habitat with a lot of organic materials (Singh et al., 2007; Yang et al., 2020a) and in the mud or sludge containing rich organic compound particularly in waste water, canals, and drainage basin so that make them known as sewage or sludge worms (Mandal et al., 2016; Haque et al., 2020).

T. tubifex plays a vital role in the ecosystem, in addition to being a natural feed (e.g., for fish, crustacea, etc.), the capability of *T. tubifex* to decompose organic matter has explored through various researches like the pollutant removal of wastewater treatment (Yang et al., 2020a; Yang et al., 2023), include ecotoxicity assessment for metal reduction (Thit et al., 2020). Its bioturbation activity can impact water movement (Mao et al., 2020), which generally influences the materials exchange in the water column-substrate interface (Lagauzère et al., 2009a). *Tubifex*, which lives with its head-down and partially submerged

Authors information:
[a]Department of Biology, Bangka Belitung University, Bangka, Indonesia. E-mail: andri_pangkal@yahoo.co.id[1]; linkgarahmad@gmail.com[3]; reysya_snh@gmail.com[6]
[b]Department of Aquaculture, Bangka Belitung University, Bangka, Indonesia. E-mail: sadibrata@gmail.com[2]; jhodisetiadi3232@gmail.com[4]; ufiayuwulndri@gmail.com[5]
*Corresponding Author: andri_pangkal@yahoo.co.id

Received: September, 2024
Accepted: December, 2024
Published: March, 2026

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

in the substrate (e.g., sediment, clay, silt), can function as a conveyor-belt deposit-feeder. Its posterior of the body is exposed to the water above, enabling cutaneous breathing. It consumes substrate particles when foraging in the substrate and excretes them to the surface in mucus-bound fecal pellets, which causes mixing of the substrate particles and influences the distribution of dissolved particles (Lagauzère et al., 2009b). Further, it also improves the bacterial diversity (Yang et al., 2020b), and this association is an effective bioremediation strategy by accelerating organic matter and nitrogen loss in the ecosystem without substantially increasing the nitrogen level in water (Yang et al., 2021). To maintain the quality of the biophysical features of the ecosystem, the remediation can degrade, break down, convert, and/or essentially eliminate toxins from the contaminated environment (Masciandaro et al., 2013).

T. tubifex is a widely distributed invertebrate and can tolerate to the stress factors, so it that, resulting as being a significant bioindicator species (Mermillod-Blondin et al., 2005). Its metabolisms, both of physiological and biological activities, are significantly mediated by many environmental parameters, like hydrodynamics (Mermillod-Blondin et al., 2010), temperature (Przeslawski et al., 2009), low oxygen concentration (Fowler & Goodnight, 1965; Simangunsong et al., 2024), and the contamination of pollutants (Lagauzère et al., 2009a; Mermillod-Blondin et al., 2013; Nie et al., 2017).

T. tubifex is a cosmopolitan worm of freshwater (Beauchamp et al., 2001), it could be a common inhabitant of organic-rich dregs around the world (Hallett et al., 2005), and it has been studied as a bioassay in the aquatic environment (Scopetani et al., 2020). Since as early as 1745, *T. tubifex* has become well-known for its capacity to survive in deoxygenated and often poisonous conditions. Furthermore, Tubifex may survive in extremely polluted waterways with large quantities of complex organic chemicals or metals, and it could be the sole metazoan animal there (Palmer, 1968).

Based on the arguments, the presence and role of *T. tubifex* in the environment, in particular in freshwater ecosystems, were important to be studied. This review was written to elaborate on the information about the biology of *T. tubifex* and its role, focusing on its ability as a decomposer in freshwater ecosystems.

2. Literature Search

The cited articles are the relevant scientific articles. There were 224 cited articles that were published from 1921 to 2024 that were retrieved from Google Scholar based on several keywords, including *Tubifex* sp., *Tubifex tubifex*, role of *Tubifex* sp., Oligochaeta, silkworm, tubificid worms, bioturbation, natural feed for aquatic biota and aquaculture, Tubifex as bioassay and bioaccumulator, and other articles related to it.

The collected information of those articles was systematically analyzed to ensure a comprehensive and detailed review. We have cited articles ranging from Bouché et al., (1999) to Simangunsong et al., (2024), with 20 relevant scientific articles in discussion about the morphology of *Tubifex* sp. We cited Foxon (1936) to Tilic & Bartolomaeus (2016) with total a of 18 relevant scientific articles in discussion about chaetae of *Tubifex* sp. We also cited Goto et al., (1999) and Bouché et al., (1999) to Walczyńska et al., (2023), with a total of 28 relevant scientific articles in discussion about the segmentation of *Tubifex* sp. We found Zattara & Bely (2015) and Simangunsong et al., 2024) that focus on elaborating about the nervous system of *Tubifex* sp. We cited Palmer (1968) until Méndez-Fernández et al., (2014) with a total of 13 relevant scientific articles in discussion about the respiratory system of *Tubifex* sp. There were 29 relevant scientific articles in discussion about the reproductive system of *Tubifex* sp, ranging from Welch (1921) to Shimizu (2020). Aston (1973) until Simangunsong et al., (2024), with 23 relevant scientific articles, have revealed the habitat of *Tubifex* sp. We cited Aston (1973) until Hertika et al., (2023) and Ratnasari et al., (2023), with a total of 75 the relevant scientific articles in discussion about the role of *Tubifex* sp. as a bioremediator.

The references had partially explained the existence of Tubifex biologically and ecologically. Therefore, through this article review, a fairly comprehensive synopsis was made about the biological aspects of Tubifex and the ecological aspects related to its role in waters, especially its bioremediation role.

3. The Biology of *Tubifex tubifex*

Tubifex is the benthic oligochaete worm, one of the important natural foodsfor water organisms, in particular fish. Some of the Tubificidae were found in marine euryhaline environments, e.g. *Tubifex costatus* (Brinkhurst, 1964), and species of *Ainudrilus* sp. and *Heterodrilus* sp. are found in intertidal and shallow-water subtidal (Wang & Erséus. 2003). However, more of Tubificidae were found in freshwater, e.g. *Tubifex laxus* n. sp., *T. gracilentus* n. sp., *T. conicus* He, Wang & Cui, 2012, *T. tubifex* (Müller, 1774) (Hallett et al., 2005; Peng et al., 2017), *T. montanus* Kowalewski, 1919 (Peng et al., 2014), *T. blanchardi* Vejdovský, 1891 (Marotta et al., 2009), *T. ignotus* (Beauchamp et al., 2001; Negredo et al., 2003), *T. newaensis* (Yıldız et al., 2007), and *T. nerthus* (Şahin & Yıldız, 2011). Many studies were conducted on *T. tubifex* compared to other species. It has been studied for several reasons, including freshwater indicator, live and natural food for fish, culturable species, host for fish parasites, genetic diversity, and water-polluted resistance.

Tubifex tubifex (Müller, 1774), species of Genus Tubifex, Subfamily Tubificinae, Family Naididae (Tubificidae), Suborder Tubificina, Order Tubificida, Subclass Oligochaeta, Class Clitellata, Phylum Annelida, and Kingdom Animalia. In this taxonomy, many studies have explained that *T. tubifex* is grouped in Family Tubificidae; however, Erséus & Gustavsson (2002) declared Naididae is older than Tubificidae, and Christensen and Theisen (1998) postulated that Naididae arose relatively early in Tubificidae evolution based on relative genetic distances. Benbow (2009) explained different statements that Naididae and Tubificidae are two different families of 10 families in the Subclass Oligochaeta found in freshwater. Referring to various publications, it generally uses the terminology of the Family Turbificidae (e.g., Beaucham et al., 2001; Erséus & Källersjö, 2004) and up to the latest publication to reveal the family of Tubifex

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

species. Further, Erséus et al., (2022) have analyzed the Tubificidae'18S rDNA. The result suggests that the Naididae is formed as an equivalent word of the Family Tubificidae, and even Kaster (1980) has written *Tubifex tubifex* Müller, 1774 is a species of Family Tubificidae.

Morphology

At first glance, the Tubifex colony looks like a red wave. This is due to the reddish color of its body (Mandal et al., 2016, Haque et al., 2020), particularly the presence of erythrocruorin (Singh et al., 2010) or dissolved haemoglobin (Vytlačilová et al., 2004) in its blood. Erythrocruorin (Ec) is a metalloprotein that provides more oxygen-carrying capacity due to the high amount of oxygen binding and high concentration in the blood (Weber & Vinogradov, 2001; Royer et al., 2006; Kruczkowska et al., 2023).

Tubifex sp. has the body length ranged 1-2 cm (Mahendra et al., 2019; Simangunsong et al., 2024), 2-3 cm (Ragi & Jaya, 2014), 4-5 cm (Zhang et al., 2017; Nuraisyah et al., 2023), 2-8.5 cm (Vytlačilová et al., 2004), 5-10 cm (Mewekani & Tampobulon, 2019), and even can reach grow up to 20 cm long (Singh et al., 2010), whereas 0.5 mm in diameter (Kang et al., 2017). *Tubifex* sp. as well as all oligochaetes, has a segmented body ranging from 30-60 segments (Mahendra et al., 2019), 32 to 120 segments (Mariom et al., 2016), or about 130 segments (Vytlačilová et al., 2004).

There are appendages, a prostomium, and a mouth on the segments of its body. There is a peristomium on the second segment of its body (Mariom et al., 2016); however, Bouché et al., (1999) drew that the peristomium is located on the first segment of the body, together with the mouth and prostomium. There is an anus on the end segment of its body. Each segment of the body has four bundles of chaetae (setae), namely two chaetae in dorsal and two chaetae in ventral position (Mariom et al., 2016). Based on the position of the peristomium at the second segment according to Mariom et al., (2016), they explained that this segment was free of chaetae. Meanwhile, according to other researchers, there are no or free chaetae at the first segment, and the chaetae appear at segment II (Bouché et al., 1999; Vytlačilová et al., 2004; Ragi & Jaya, 2014). Although there is a difference in determining the position of the peristomium, there is a similarity in that there are no chaetae in the segment where the peristomium is located. In addition, there is a clitellum at segments X, XI, and XII, while the ovisac for mature eggs is stored at segments XII to XIV (Bouché et al., 1999; Marotta et al., 2009; Shimizu, 2020).

Clitellates have a clitellum (hence called Clitellata), a glandular structure modification of the epidermis (Schmelz & Collado, 2010) that produces cocoons, namely a sac for encapsulating the eggs. Other characters of Clitellates are the absence of the parapodium and all clitellate species of Clitellates do not have the sensory nuchal organ, asprostomial appendages, and epidermal ciliation (Kuo, 2017). The morphology of *Tubifex* sp. was illustrated in Figure 1 with abbreviations AR: anterior region, consist of prostomium (pr), peristomium (pe), mouth (m), and brain (b); RSR: reproductive system regions (look Figure 7 for detail); CL: clitellum; PR: posterior region; dc: dorsal chaetae; vc: ventral chaetae; and g/i: gut/intestine.

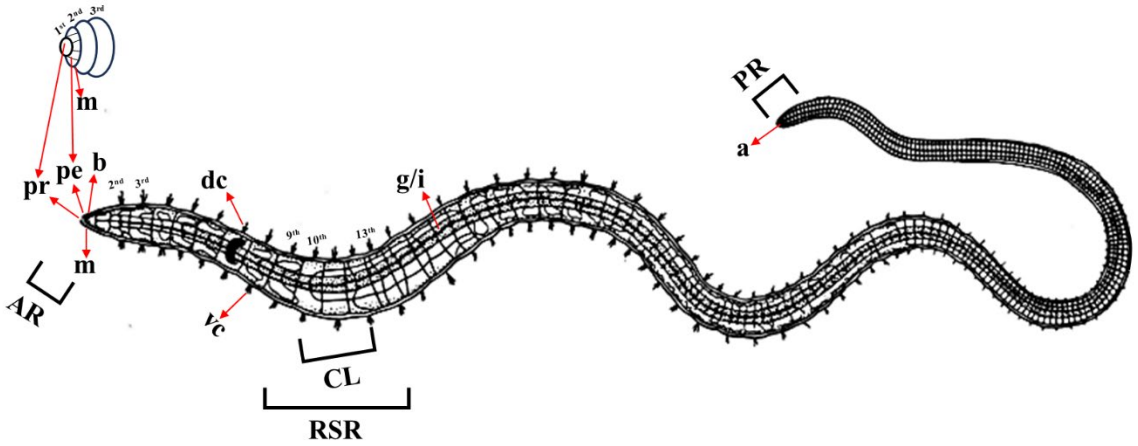


Figure 1. Morphology of *Tubifex* sp.

Chaetae

Oligochaetes have body segments with a small number of chitinous bristles (Bouché et al., 1999) called chaetae or setae. Chaetae are chitinous extracellular structures, and the presence of chitinous chaetae as well as a clitellum is one of the specific features of oligochaete (e.g., Tubifex), and the chaetae are an important diagnostic character in Annelida, including oligochaetes. Merz & Edwards (1998) explained that most annelid species contain dorsal and ventral structures of chaetae connected with the parapodia of the majority of body segments.

The chaetae have important functions for annelids, including Tubifex. The chaetae serve a function in creating rubbing between the worm and the substrate when crawling (Gustus & Cloney, 1973; Merz & Edwards, 1998; Hesselberg & Vincent, 2006). The movement of chaetae in crawling is supported by parapodium (parapodia) (Foxon, 1936) Furthermore, Merz & Edwards (1998) revealed that setal sacs hold and bundle the chaetae of

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

polychaete parapodia. A parpodium may have some setal sacs, each with a connection to muscles, in particular the intrinsic parapodial protractor. These muscles are located in the setal sac to the parapodial wall. An aciculum facilitates conectivity between the setal sacs and tissue, a strong internal chaetae that is connected to protractor muscles and the intrinsic parapodial retractor. The chaetal retractor muscles' contraction reflex can pull the aciculum. The aciculum comes into the parapodium, causing the setal sacs and their associated chaetae to retract. Meanwhile, the protractor muscle's contraction reflex might draw the setal sacs toward the parapodial surface. This condition causes the chaetae to lengthen and the aciculum to shift further away. Thus, the movement of chaetae is indirectly performed by the attachment of muscle to the setal sacs and aciculum, with the worm controlling the movement.

Tubifex uses the chaetae for burrowing (Trevor, 1977) and hooking to the burrow substrate (Woodin & Merz, 1987). The chaetae activity allows the Tubifex to rapidly withdraw the body (Clark, 1960), thus it can tribulate a predator from pulling Tubifex out of its substrate (Knight-Jones & Fordy, 1979; Woodin & Merz, 1987). The chaeta is also used for bioturbating (Mao et al., 2020), meanwhile the parapodia generate thrust for the worm's movement during swimming (Gray, 1939; Clark & Tritton, 1970). Hesselberg & Vincent (2006) have investigated a correlation of the morphology of chaetae to habitat and locomotory behavior.

The chaetae are epidermal extracellular structures that are in general clearly visible from the exterior of annelids (Annelida). The Polychaeta have highly diverse of chaetae' structure. Each species has a specific pattern of chaetae that can be used as an instrument for species determination (Hausen, 2005). Dumnicka & Poznańska (2006) explained the absence, the changes in shape, number, and dimensions of chaetae depending on environmental conditions.

Furthermore, Hausen (2005) discovered that the microvilli pattern of a single cell—the chaetoblast, the most basal cell of the chaetal follicle—is what dictates the morphology of chaetae. It expands when new chitinous material is deposited at the base. The microvilli produced by the chaetoblast are a highly distinctive category. On these microvilli's exterior, new chitinous material assembles. In spite of the absence of vesicular transport within the chaetoblast' microvilli, the material for apposition to the base of the chaetae may not be directly supplied by the microvilli. On the other hand, the chaetoblast and follicle cells secrete the chitinous material, which then enters into the lumen of the chaetal follicle. After that, it reaches the surface of microvilli by diffusion mechanism, and it releases the substance between the chaetoblast part, i.e., the bases of the microvilli.

Tilic & Bartolomaeus (2016) completed the explanations that chaetae are generated within an ectodermal invagination, namely the chaetal follicle. It is composed of a terminal chaetoblast and follicle cells. Each chaetoblast contains an array of apical microvilli that are changed across time and space as chitin polymerizes along the microvilli. Therefore, the ultimate shape of the chaetae is determined by a controlled alteration of the microvilli pattern. Many distinct chaetal forms, ranging from extremely complex compound hooked chaetae to simple capillaries, can emerge as a result of changes in spatiotemporal patterns. Chitin release freezes changes in the microvilli pattern, so that the definitive chaetae's structure reflects the changes in the microvilli pattern throughout time. The structure of the chaetae is also influenced by cellular interactions that occur during chaetogenesis.

The chaetae are segmentally arranged bristles. There are many morphologically distinct types of chaetae; however, within a species the chaetae of one type are very similar. The chaetae are classified as either simple (unjointed) chaetae or compound (jointed) chaetae (Gustus & Cloney, 1973). Tubifex has pectinate chaetae, hair chaetae, and bifid chaetae on its anterior segments, while bifid chaetae are on the posterior segments (Bouché et al., 1999). The chaetae are illustrated by Timm (2012) and Ragi & Jaya (2014) (Figure 2).

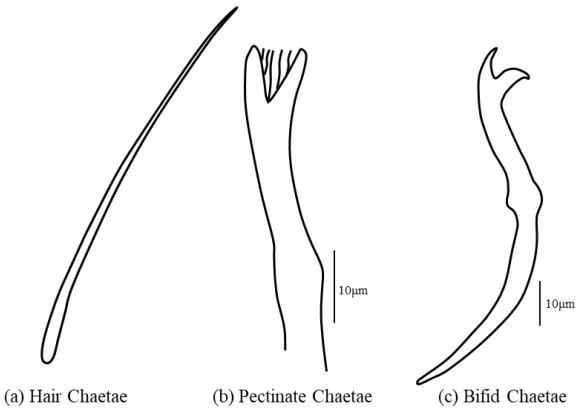


Figure 2. Morphology of *T. tubifex*' chaetae. Dorsal chaetae: (a) hair chaetae and (b) pectinate chaetae (modification of short locomotory chaetae); Ventral chaetae: (c) bifid chaetae (Redrawn from Timm, 2012; Ragi & Jaya, 2014).

Segmentation

Oligochaete worms of Phylum Annelida are segmented coelomates that are bilaterally symmetrical, and there are bundles of chaetae on all body segments, however absent on the prostomium at the first segment (Vytlačilová et al., 2004), and an invertebrate whose body plan has a high degree of metamerism (Shimizu & Nakamoto, 2001; Balavoine, 2014). Metamerism or segmentation refers to translational symmetry (Klingenberg, 2015). Zattara & Bely (2015) drew the fundamental annelid body plan that consists of non-segmental regions and segmental regions. There is one non-segmental region at the anterior end composed of the prostomium (pr), peristomium (pe), and mouth (mo), and there is one non-segmental region at the posterior end, called the pygidium (py), composed of the anus. Regions are located between the anterior non-segmental and the posterior non-segmental and consist of segmental units. It also has a growth zone in front of the pygidium at the posterior segment of its body, called the posterior growth zone (pgz) (Figure 3).

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

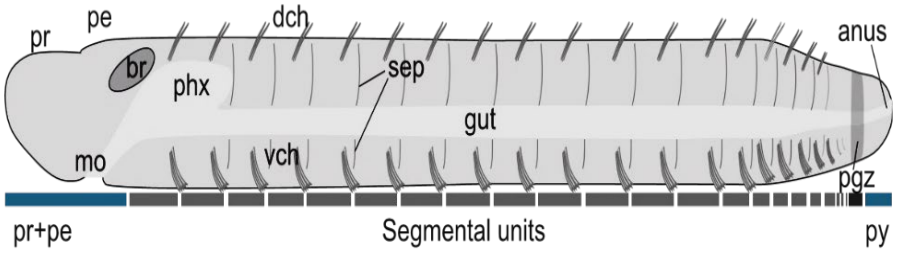


Figure 3. Overview of basic annelid body plan. pr: prostomium; pe: peristomium; mo: mouth; br: brain; phx: pharynx; dch: dorsal chaetae; vch: ventral chaetae; sep: intersegmental septum; gut: cillated gut; pgz: posterior growth zone; py: pygidium (Zattara & Bely, 2015, cited with permission).

Segmentation involves the repetition of units that are arranged along the longitudinal (anterior-posterior) (A-P) axis. Each unit consists of elements from some organ systems and is serially homologous to each other (Fedonkin, 2003; Minelli & Fusco, 2004; Hannibal & Patel, 2013; Isaeva & Kasyanov, 2021). The body part is segmented and limited to the trunk, the head, and the terminal. Its head is represented by the presence of a prostomium, including a brain which is located at no-segment, nor is the pygidium. While the terminal of the body segment is a part of the anus. The prostomium in the anterior part and the pygidium in the posterior part are non-segmental (Starunov et al., 2015). This segment repeats many units of tissues, cells, or organs along the A-P axis, grouping units of various sorts into distinct segments. These units can be excretory organs, sensory organs, skeletal units, nerve cells or ganglia, muscles, and locomotory organs (Chipman, 2010). Externally, the trunk segmentation looks like rings or annuli, and internally, it is separated by intersegmental septa on a serial arrangement of coelomic compartments and also organs and system components in metameric arrangement (Goto et al., 1999; Shimizu & Nakamoto, 2001).

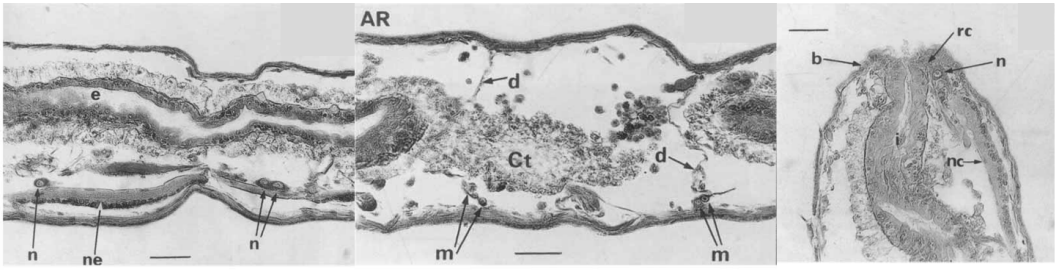
The Oligochaeta, Tubificidae (e.g., *Tubifex tubifex*) have the capability of autotomization (autotomy) and regeneration of their missing body parts. Regeneration is a physiological process that can occur daily or as a direct response to traumatic injury following segmentation or autotomy, regrowth, and restoration of missing body parts. This process occurs in all the different phases of development, from the embryo and larva to the adult phase of an organism undergoing autotomy. Although almost all animals have physiological regeneration (periodic regeneration of cells or tissues, such as epidermal tissue), the ability to regenerate body parts varies greatly, even between closely related species (Kostyuchenko et al., 2016; Kostyuchenko & Kozin, 2021; Walczyńska et al., 2023).

Regeneration, as a result of damaged structures after injury or amputation has been studied in phyla of invertebrates, one of them is annelid that are capable of regeneration at cell until tissue levels, including cell repairing, germ cell rebuilding, structures regrowing, or the regeneration of the all parts of body from the small fragments (Bely, 2006; Bely, 2014; Nikanorova et al., 2020). The regeneration is a complex process, including wound closure and healing, immune system reaction, aggregation of cells for regeneration, growth of body part generations, patterning, and differentiation. However, generally, there are three processes or steps of this regeneration, namely wound healing phases, dedifferentiation phases, and redevelopment phases. While the duration for each regeneration phase can vary greatly, which can be influenced by the anatomy of the organism, nature of the wound, the location and cause of the amputation, the developmental phase, or the individual age (Acosta et al., 2021; Kostyuchenko & Kozin, 2021; (Walczyńska et al., 2023).

The posterior segment of the *T. tubifex* is the main part for autotomy, such as post-predation, toxification, etc. This worm can regenerate its functional posterior end as well as a prepygidial segment, which becomes a new regenerated zone. The missing part of body regeneration in *T. tubifex* involves activation of mesoblastocytes and an increasing amount of neoblasts, and there is an increased number of migration of these cells (Bouché et al., 2003). Teloblasts are embryonic stem cells which produce five bilateral pairs of coherent longitudinal columns (bandlets) in the same number of bilateral pairs and then initiate the segmental structures of ectodermal and mesodermal. At the posterior end of the embryo, the teloblasts undergo asymmetrical cell divisions to generate daughter cells. The daughter cells move to the anterior end, so the oldest daughter cells are found at this side of each coherent longitudinal column (Goto et al., 1999; Nakamoto et al., 2000; Takahashi et al., 2008).

Following autotomy, the septum-level circular muscles contracted, and the epidermis then covered them. Large cell migration into the regenerate was observed following the first day of regeneration. Huge nuclei with huge, extremely thick nucleoli and highly basophilic cytoplasm were the characteristics of these cells. The neoblast cell production forms the blastema at the place of section, and these cells have migrated along the nerve cord and are found in the fifth metamere anterior to the site of section (Figure 4A). Neoblasts that were migrating had a pear or spindle shape, whereas those that were immobilized and stuck to the septa (Figure 4B) had a circular shape. The migrating neoblasts started dividing into smaller cells at 24 hours when neoblasts were organized in rows of several cells past the nerve cord's tip (Figure 4C) (Bouché et al., 2003).

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)



(A) (B) (C)

Figure 4. Regeneration in worm (Bouché et al., 2003, cited with permission).

Figure 4 showed (A) worms' regeneration: day 1. Migration of neoblasts (n) to the wound along the nerve cord (ne). Scale bar is 50 µm, and (e) is the intestinal lumen. (B). worms' regeneration: day 1. inesoblastocytes position (m) at the postero-ventral face of the dissepiments (d). Scale bar is 50 µm, (ct) is chloragogen tissue, and (AR) is the anterior region. (C). worms' regeneration: day 1. neoblasts accumulation (n) beyond the broken nerve cord (nc). These neoblasts are regulated in rows of some cells and generate into smaller regeneration cells (rc). Scale bar is 50 µm, (i) is intestine and (b) is blastema.

Tubifex sp., like all oligochaetes, is a body-segmented worm with 30-130 segments (Vytlačilová et al., 2004; Mariom et al., 2016; Mahendra et al., 2019). The annelids, in particular, have a body that is segmented into pre-clitellar (pre-clitellum), clitellar (clitellum), and post-clitellar (post-clitellum) segments (Paul et al., 2022).

Tubifex has a mount at the first segment, which has appendages. The prostomium's shape is round or triangular, meanwhile. the terminal of its segment contains the anus. Each segment of the body has four bundles of chaetae (also called setae), namely two chaetae in the dorso-lateral and two chaetae in the ventro-lateral position; however, there are no chaetae in Tubifex, which appeared at segment II (Mariom et al., 2016; Bouché et al., 1999; Gline et al., 2011). Marotta et al., (2009) revealed that *T. tubifex* has 2-4 (mostly 3) capillary chaetae for each bundle that is found at the dorsal bundle of the pre-clitellum and also 2-3 (mostly 3) pectinate chaetae, which contain 3 intermediate denticles for each chaeta. Bouché et al., (1999) scanned the distribution of the putative chemoreceptor chaetae (arrowheads) over the anterior region of *T. tubifex*. The many small chaetae are grouped in tufts on the prostomium (P) and peristomium (Pe), and are less dense in other areas. II: segment II (bar = 50 µm) (Figure 5.3).

The brain of Tubifex is shifted anteriorly, passing the prostomium or peristomium. There are three pairs of serotonin immunoreactive (SIR), in which the asymmetric trunk pattern was found from the fourth segment, have non-septate ganglia, and the full complement of nerves from the third segment. In the brain of Tubifex' juveniles, there are two serotonin-positive cells, meanwhile, the adults have six cells. This fact shows that the number of serotonin-positive cells can change post-embryonically. In addition, the anterior segments of Tubifex show a symmetrical pattern of ectodermal segmentation including the autonomous morphogenetic at an early stage, followed by the mesoderm-dependent alignment (Zattara & Bely, 2015).

Furthermore, Mariom et al., (2016) explained there two kinds of chaetae, i.e., pectinate and hair chaetae at the dorsal-lateral. The pectinate chaetae have hooks at the end terminal, formed like combs, and their interior teeth are smaller than the exterior teeth. The hair chaetae have fine elements, called serrations. Meanwhile, all of the ventro-lateral chaetae have the same type, and the chaetae have bifid tips.

Bouché et al., (1999) revealed that there are differences in the dorsal and ventral chaetae of Tubifex. The dorsal chaetae consist of hair and pectinate chaetae. There are fine elements, serrations that adorn the hair chaetae (Figure 5.4), meanwhile pectinate chaetae have an S shape, a distal swelling on the shaft hooks-like combs at the ends with the two exterior larger than the interior teeth (3-4 intermediate teeth). The hair chaetae were interspersed with pectinate chaetae, so that pectinate chaetae were the most external chaetae in the bundles (Figure 5.5 and 5.6). The ventral chaetae have only one type, namely bifid tips (Figure 5.7), which have a longer upper branch than the lower one and also have an S-shaped curve and a swelling, as also owned by the dorsal pectinate chaetae (Figure 5.8).

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

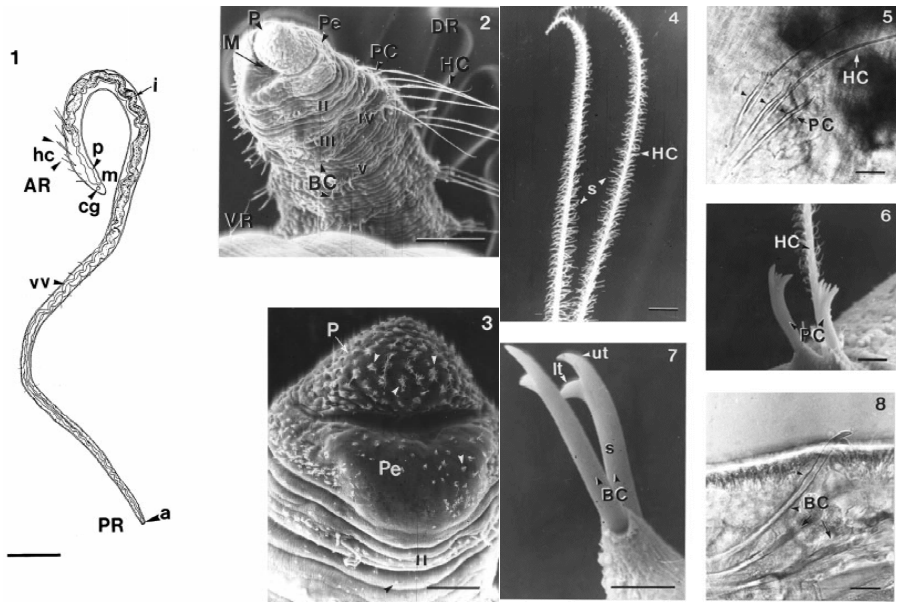


Figure 5. Microscopic electron scanning of the anterior region of *T. tubifex*. Each metamere bears two bundles of ventro-lateral chaetae (bifid chaetae: BC) and two dorso-lateral bundles composed of pectinate chaetae (PC) and hair chaetae (HC) (only one of the two dorso-lateral bundles is visible in the picture). Mouth (M), prostomium (P), peristomium (Pe), dorsal region (DR), ventral region (VR), and metameres II-V (II, III, IV, and V) (scale bar=200 µm)
(Bouché et al., 1999).

Bouché et al., (1999) have revealed that there is a variation in the number of chaetae in the dorsal and ventral bundles in animals and the parts of individuals. There are many chaetae in the front part or anterior of the body and decrease to the rear or posterior of the body. Tubifex has bundles that contain 2-4 pectinate chaetae, 1-3 hair chaetae, and 2-4 bifid chaetae on its anterior segments. However, there is only a single chaeta on the bundles of dorsal and ventral at its third posterior. It's all of the posterior chaetae are bifid type. The size of the chaetae changes along the body, following its diameter function. The chaetae size at the segments II to V becomes larger, then it is the same size up to the clitellum at the segments X-XII, and finally gradually becomes smaller in size until the posterior end. Ventral bifid chaetae have diameter of 300 µm in the anterior zone and 100 µm in the posterior zone. The size of hair chaetae was 2-4 times larger than that of the pectinate chaetae.

Nervous System

Tubifex has a segmented body with distinctive anatomical structures. Each segment contains a simple and effective nervous system, while the nervous system consists of ganglia in each segment connected through longitudinal nerve cords, allowing the worms to respond to environmental conditions and stimuli (Simangunsong et al., 2024). Zattara & Bely (2015) revealed that *T. tubifex* has three main parts of the nervous system, namely the anterior brain, and it is joined with the peripheral nervous system, the ventral nerve cord with ganglia, and the segmented peripheral nerves.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

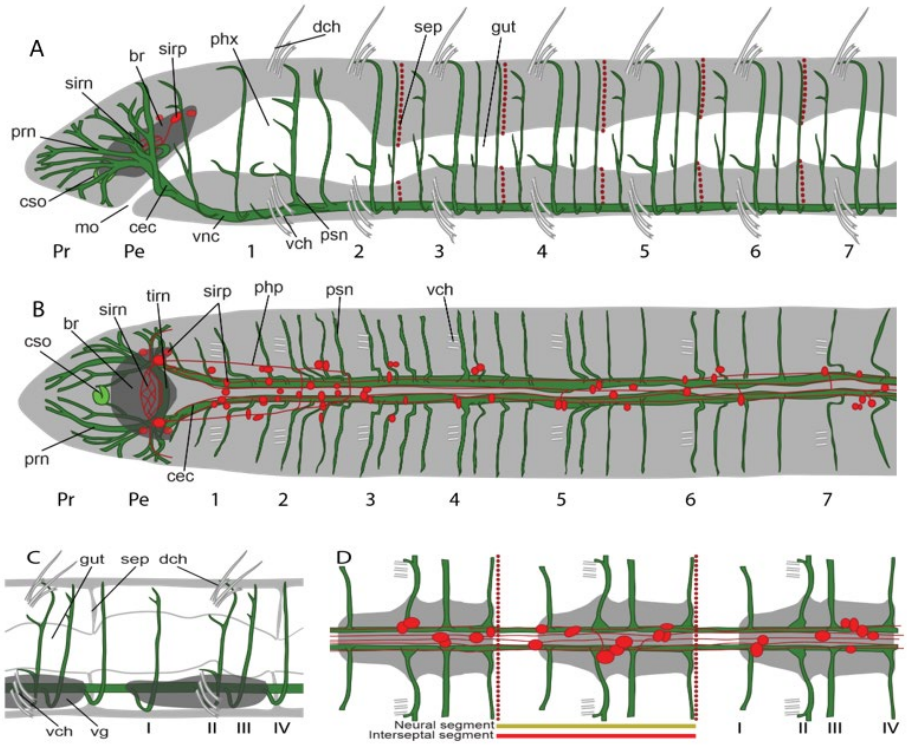


Figure 6. The *T. Tubifex*'s nervous system. A) the anterior terminal' lateral view, B) the anterior terminal' dorsal view, C) lateral view of a typical trunk body segment, and D) schematic of the ventral nerve cord structure (Zattara & Bely, 2015, cited with permission).

Figures 6A and 6B clarify that the green color indicates Acetylated-tubulin immunoreactive (acTIR) neuropil, the red color indicates perikarya and serotonin immunoreactive (SIR) neurites, and the dark gray color indicates the brain. At the anterior end, there is a nervous system that consists of the ventral nerve cord, the segmental peripheral nerve, the prostomial nerve, the circumesophageal connective, the pharyngeal plexus, and the brain. Meanwhile, Figures 6C and 6D reveal the dark gray color showing ventral nerve cord ganglia and the dashed dark red line showing intersegmental septa. These pictures show the position of ganglia, septa, and chaeta relative to peripheral nerve roots (Zattara & Bely, 2015).

Zattara & Bely (2015) discovered that the brain of *T. tubifex* consists of two bilobed structures inside the anterior nervous system. The location of the brain is dorsal to the mouth, with the peristomium encircling the rear edge and the prostomium housing the anterior side. The ventral nerve cord and paired prostomial peripheral nerve cords are connected to the inner neuropil of the brain's external cell cortex via a pair of circumesophageal connectives. The trough-shaped cell cortex is located within the ventral nerve cord, which extends lengthwise along the worm's body. Each piece of its body has a single ganglion and a subesophageal ganglion at the anterior terminal of the cord. The cortical cell is traversed by the neuropil that contains SIR and acTIR neurites. Four peripheral acTIR nerves and recurrent segmental in each fragment's peripheral segmental nerves are present in Tubifex. After emerging from the ganglia and passing ventrally through the body wall's muscular layer, the recurrent segmental nerves run subepidermally to the dorsal segment of the ventral nerve cord. Each nerve innervates many epidermal sensory hairs. On the front side of the worm, the segmental nerve pattern differs, with segments 1-2 having three nerves rather than four.

Respiratory System

T. tubifex usually lives and feeds with the head inserted into the substrate, while some part of its posterior may appear above the water column-substrate interface (Pelegri & Blackburn, 1995). The posterior part undulates freely in the overlying waters, while partially its part of the body is submerged in the substrate (Bouché et al., 2000; Hare et al., 2001; Lagauzère et al., 2009a). The feed preferences indicate that its feeding behavior is that of an infaunal species. It consumes on the fine-substrate fraction (< 63 µm) (Rodriguez et al., 2001), ingests particles, and digests the attached microflora (e.g., bacteria) mainly on the top 2 cm until 8 cm of substrate e.g., sediment (Pelegri & Blackburn, 1995), and includes organic matter-associated particles in the fractions of the substrate such as sediment, silt, or clay (Méndez-Fernández et al., 2014). Based on this feeding behavior, *Tubifex* is classified as a deposit feeder (Kaster & Wolff, 1982).

The head-down deposit feeder is a species that consumes the organic debris and substrate-associated microorganisms as food by burrowing and swallowing huge volumes of substrate (Lopez & Levinton, 1987). It utilizes the organic matter directly at the substrate surface and interacts with the microbial community inhabiting this area (Amon & Herndl, 1991). The deposit-feeding worm can ingest the substrate, even reaching a depth of 15 cm under the water column-substrate interface (Clough & Lopez,

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

1993), by burrowing into the substrate and processing it through its digestive tract for substrate alteration. Thus, the deposit feeder can be described as an organism that moves along the surface and burrows into the substrate (e.g., sediment) and digests or consumes organic matter, including living or non-living (dead) organic materials from the substrate.

Therefore, Tubifex spends its entire life cycle in the substrate, e.g., sediment, so it has to develop a respiratory system. Its respiratory activity is also carried out in the substrate through the posterior body portion that overlays above the water column-substrate interface to allow cutaneous respiration (Lagauzère et al., 2009b), where its gases exchange with water occurs by diffusion mechanism through the epidermis (Vytlačilová et al., 2004).

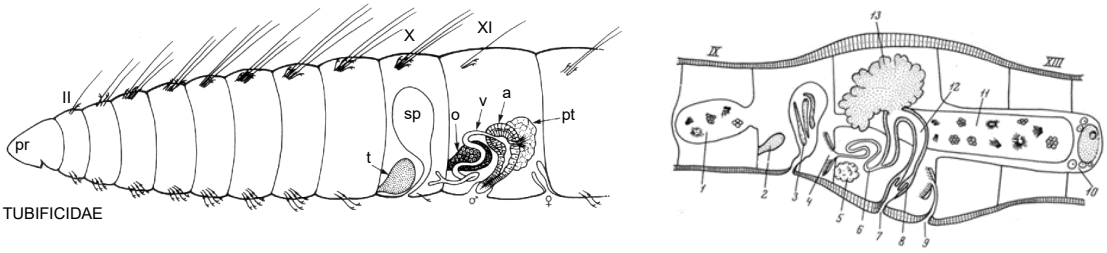
T. tubifex uses the permeable integument as the gas exchange organ in its respiratory system. As a deposit feeder, *T. tubifex* places the terminal of the anterior part in the bottom substrate, while its terminal of the posterior part is extended several millimeters into the contiguous water where gas exchange occurs. Its posterior portion's ventilatory movements are used to enhance convective transport of respiratory media, from and to the exchange media surfaces. Ventilatory function can be effective only if an adequate surface area for gas exchange exists and the posterior end has a larger surface area to diffuse the gas. Integumentary convolutions of Tubifex's segments of the posterior play a role to enhance the availability of surface area for gas exchange in the respiratory process. The increase in surface area in this part is more than three times than in the anterior segments. A complicated network of furrows and ridges dominates this convoluted area. The combination of movement of ventilatory and the structure of this area may increase the gas exchange in the respiratory process (Kaster & Wolff, 1982). *T. tubifex* is independent in consuming oxygen in the external environment at an level oxygen of about 1.5 %. This consumption can change to the dependent type if the oxygen is at 2.5% (Palmer, 1968). In the hypoxia condition, Tubifex can survive about 48 hours (Vytlačilová et al., 2004).

Reproduction System

T. tubifex can develop a selfing and outcrossing strategy as a mixed reproductive strategy and a self-fertilization or parthenogenesis (pseudogamy) (Lüscher & Milinski, 2003; Baldo & Ferraguti, 2005; Marotta et al., 2014). Meanwhile, Mahendra et al., (2019) explained *Tubifex* sp. is a hermaphrodite species which has two genital organs in one individual, and it regenerates itself by egg production from mature eggs. Singh et al., 2010 also revealed that *T. tubifex* reproduces through hermaphroditism and sexually matures at about 42 days, and Kaster (1980) explained maturity of Tubifex's sexual was reached in 67 days at low temperature (15º C). Lazim & Learner (1986) revealed Tubifex is indicated as a univoltine species (i.e., apparently one sexual generation, one brood of offspring in a year). Although its breeding activity can last for several months, its period is different in each location.

Furthermore, Lazim & Learner (1986) explained Tubifex has a reproduction activity over a long period throughout the winter and spring seasons. Tubifex is most abundant during April and May, while it is least abundant in October and early November. However, Learner et al., (1978) revealed that Tubifex undergoes individual maturity usually during summer and autumn. The adults of Tubifex die soon after laying the cocoons.

In our observation, we found Tubifex to be most abundant during the dry season and least abundant during the rainy season. It was confirmed by Herawati et al., (2020) that the population growth of *T. tubifex* is influenced by the seasons, so it is easier to find during the dry season than the rainy season. Govedich et al., (2010) drew the reproductive system of Oligochaeta, in particular Tubificidae and Meshcheryakov (1990) for Tubifex (Figure 7).



(A) (B)

Figure 7. Reproduction organ systems and external characteristics of (A) Tubificidae. Prostomium (pr); spermatheca (sp); testis (t); ovary (o); vas deferens (v); prostate (pt) (Govedich et al., 2010) and (B) 1) anterior seminal sac; 2) testis; 3) spermatheca containing spermatozeugmata; 4) seminal funnel; 5) ovary; 6) seminal duct; 7) male genital pore; 8) penis in the penial sac; 9) female genital pore; 10) egg sac; 11) posterior seminal sac; 12) atrium; 13) prostate (Meshcheryakov, 1990).

In the terminology, parthenogenesis from the Greek, namely parthenos, means virgin, while genesis means origin or creation, interpreted as virgin birth (Kono, 2008; Vijverberg et al., 2019). The parthenogenesis, parthenogenone, parthenogen, or parthenote refers to embryogenesis in unfertilized eggs. It indicates an unfertilized embryo (Parker & McDaniel, 2009) or the spontaneous embryonic development and naturally occurs in a variety of organisms (Vijverberg et al., 2019), and is a common mechanism of reproduction in most lower species (e.g. rotifers, nematodes, invertebrates) or can also in mammals through inducing process (in-vitro mechanism) (Kharche & Birade, 2013; Ramachandran & McDaniel, 2018).

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

The terminology of parthenogenesis is used to explain pseudogamy. Pseudogamy is a reproductive mechanism where sperm of males activate females' oocytes, but there is no contribution of the genome from the sperm to the offspring's genome (Launay et al., 2020). Pseudogamy was also known as gynogenesis, namely, the natural parthenogenetic development. In this mechanism, sperm only stimulate or trigger the embryogenesis phases without the contribution of the sperm's genome in this process (Schlupp, 2005). This condition is also called pseudogamous parthenogenesis (sperm-independent reproduction). Sperms penetrate ovum and initiate embryogenesis development. However, sperms do not contribute their genetic material to the zygote, including the syngamy process i.e., the fusion process between the egg and sperm pronuclei which results in no parental genetic expression in the fertilization (Beukeboom & Vrijenhoek, 1998).

Hermaphrodite is the expression of reproductive function in a single individual, both male and female (Nakadera & Koene, 2013). It is capable of self-fertilization (parthenogenesis); however, it is also capable of cross-fertilization by copulation (Hill et al., 2018). Its entire life cycle takes place within the substrate (Méndez-Fernández et al., 2014). The *T. tubifex,* oligochaetes, Clitellata, a monophyletic group of Annelida, reproduce through hermaphroditism (i.e., an individual, male or female, contains a single reproductive system). Clitellata produce gonopores that protrude from the clitellum of the worm, both male and female. Then, there is a slime secretion and a protective cocoon to protect embryo development. The sexual way matures at about 42 days (Singh et al., 2010; Hill et al., 2018; Iyer et al., 2019).

This worm has two genital organs in one individual and regenerates itself by egg production. Tubifex can produce cocoons, and this production can occur for 40-45 days since first copulation. The ovoid-shaped cocoons with a diameter of 0.7 mm and 1 mm in length deposit the eggs. Furthermore, the eggs in the cocoons undergo division and develop into segments. There are 4-5 grains in each cocoon, which are bred until they hatch into embryos for 10-12 days. Tubifex needs 50-57 days through all phases of its life cycle from the copulation, laying eggs and cocooning phase, hatching to adulthood phase, until producing and removing its cocoons (Mahendra et al., 2019). Kaster (1980) revealed that cocoons of Tubifex commonly contain 4-9 embryos, and they can contain 14-17 embryos in each cocoon. Referring to those arguments, we proposed an illustration of the life cycle of Tubifex as oviparous in cross-fertilization by copulation (Figure 8).

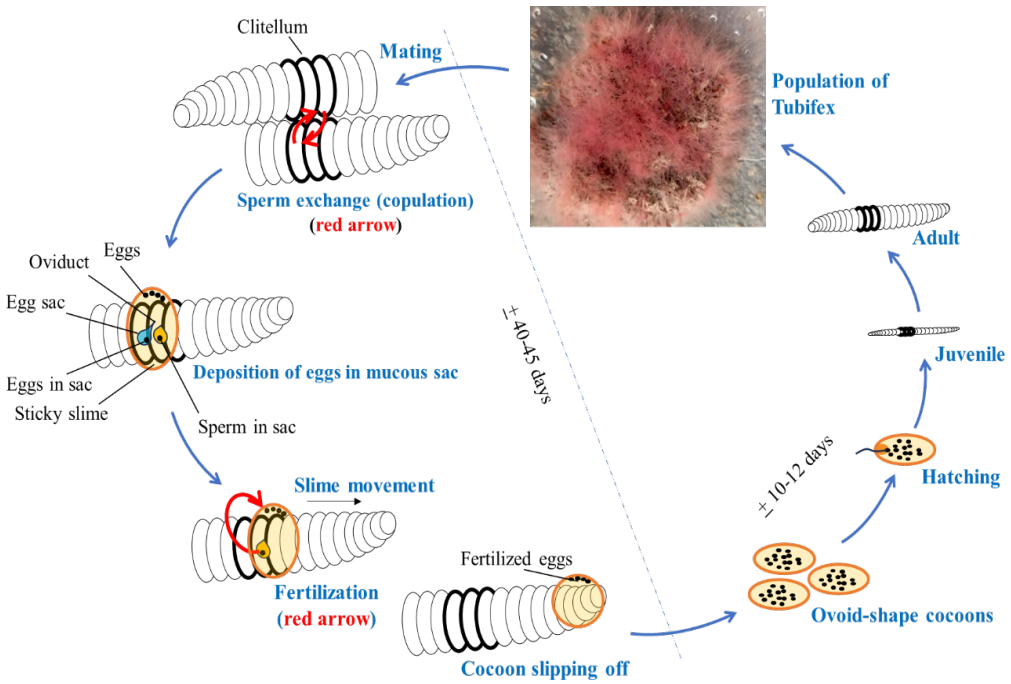


Figure 8. Schematic of life cycle of Tubifex as oviparous in cross fertilization by copulation.

Shimizu (2020) explained that Tubifex has oviposition behaviour. This behavior consists of four phases, i.e., cocoons move around the area of the clitellum, ovisac eggs budge to the ovarian coelom at around segment 11, coelomic eggs are deposited to outside of the cocoons, and then the cocoons are released to the environment. Meanwhile, Hirao (1965) explained Tubifex has two main successive phases of the oviposition behaviour, i.e., the forming of cocoons around the clitellum and the releasing or depositing of the cocoons.

The embryonic development of Tubifex starts from stages 1-cell, 2-cell, 4-cell, quadrant micromeres, bilateral cell division, mesoteloblasts, endodermal precursors, and ectoteloblast precursors. Tubifex produces eggs in metaphase I, and then the zygote forms two polar bodies. After that, the process gets into the first mitosis phase. The cytoplasm of the egg cell lacking yolk is concentrated at both poles of the egg cell's polar plasma before the first cell division. In the early development, there is a stereotyped sequence of cell divisions. In the second division, cells undergo division and separation. In the third division, there

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

is an unequal repetition of the quadrants to produce D quadrant micromeres on the animal side in three and four production and macromeres on the vegetal side. During cell division, the polar plasma is segregated, and these cells undergo bilateral cell divisions in the next divisions. Meanwhile, there is quadrantal cells dividing evenly in the sixth division (Nakamoto et al., 2011; Nakamura et al., 2017). Urbisz et al., (2015) found that Tubifex's ovary consists of only one large ovary with more than 2,600 cells.

Clitellate have a special reproductive organ, which is distinguished from other worms, namely the clitellum. The egg capsule (i.e., cocoon) is produced in this organ. The clitellum looks like a thickened sleeve or saddle (Hill et al., 2018) at segments X, XI, and XII of the anterior regions (Bouché et al., 1999; Marotta et al., 2009; Shimizu, 2020). The clitellum produces proteinaceous material for the protection of eggs, building the cocoons in the mature sexual periods within thousands of glands, or provides a microenvironment for embryonic development (Rossi et al., 2013; Mc Loughlin et al., 2016). The reproduction mechanism, hermaphrodite involve gonopores of male and female that are discharged from the clitellum (Parish, 1981).

The fertilization process of clitellate can also involve physical contact. The copulation occurs between two individuals, and both of them mutually transfer their sperm from a spermatophore (i.e., sperm-filled sac) that involves an intromittent organ (Hill et al., 2018). Kelly & Moore (2016) explained that males have intromittent organs. These organs enter the genital tract of the female, and in this tract, the female deposits sperm in internal fertilization. Rodriguez & Fend (2018) explained that the spermatophores are attached to the prostate bulb. The sperms are transferred to the clitellar area of its partner at the ventral body surface.

Hill et al., (2018) explained that sperm come out of the spermatophore and move to the ovaries via the coelomic sinuses for egg fertilization internally. The mechanism of spermatogenesis occurs in paired testes. It produces a mature spermatozoa, and then the spermatozoa are collected in the epididymis for a longer time. On the other hand, a paired ovisacs produce oogonia and oocytes. They move for congregating and toward the female pore through the oviducts. The sperm is stored in spermathecae, and it will be released during internal fertilization process or into the cocoon. Within egg development, the vitellogenesis process provides yolk for the eggs. Therefore, the yolk can be quite large, so it may be stored in the cocoon fluid. It will become albumenotrophy, a nutritional source during the long embryonic development period. Further, the proliferation of clitellum-specific granular cells occurs as the first process in the secretion of proteinaceous cocoons together with egg laying. It is proliferated to make fibrous protein as material for the cocoon's wall; meanwhile, another cell will produce a sticky matter like glue to seal the terminal regions of the cocoon. The granules of each component, including cocoon walls, are needed to construct a single cocoon. Each cocoon can deposit between 1 and100 eggs, depending on the species. Welch (1921) explained that the cocoons' colour is whitish or greyish. The cocoons appear semi-transparent, although the eggs cause the cocoons to appear opaque.

Dumnicka & Poznańska (2006) revealed *T. tubifex* developed spermathecae containing spermatozeugma, and its spermatozeugma has a different shape in each species (Figure 9). As a group of Annelida, some organisms show diversity of reproductive behaviors, including fertilization or asexual fragmentation. Although almost all of Oligochaeta reproduce asexually as the main form of their life (Timm, 2012), there is no publication or evidence that indicates Tubifex can do asexual fragmentation except the segmentation-autotomization for regeneration of its missing body part.

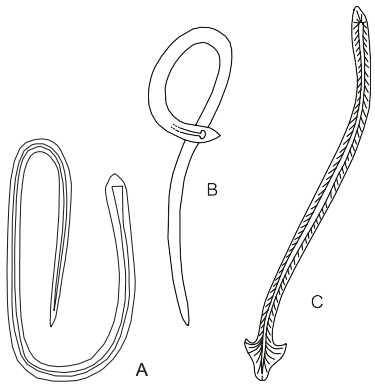


Figure 9. Comparison of the spermatozeugma shapes. A-*Tubifex blanchardi* from Włocławek reservoir; B-*Tubifex tubifex* (after Hrabě 1981); C-*T. tubifex* (after Stephenson 1930) (Dumnicka & Poznańska, 2006).

Habitat of Tubifex

Tubifex is a meiobenthic (meiofauna) species of aquatic worm, dwelling in the substrate or other stationary surfaces and a burrowing organism. *Tubifex tubifex* thrives in silt-clay and fine sand substrate, in mud rich in organic waste, including in waste canals, open drainage, and associated in slow water flow.

It forms reddish colonies in the substrate and consumes particles in the silt-clay, bacteria, and organic debris via its body wall (Rodriguez et al., 2001; Kaeser & Sharpe, 2006; Anlauf & Moffitt, 2008; Mandal et al., 2016, Haque et al., 2020). Tubifex is often found at the lower layer of water bodies, where its optimal habitat characteristics are nutrient-rich muddy substrates that provide organic matter, such as dead leaves, plant debris, and remnants of other organisms as a feed source. It also needs relatively low oxygen levels, that can reduce competition with other organisms less tolerant to anaerobic conditions. According to Singh et al., (2010), Tubifex can live and survive in the water with physico-chemical properties as shown in Table 1.

Tubifex sp. can play a role as a bioindicator of ecosystem status, water quality, and polluted water, although its presence is low in density in oligotrophic ecosystems (Milbrink et al., 2002). Tubifex is often an important part of the aquatic food chain and can adapt to the various environmental conditions, including the ability to survive in anaerobic (anoxia) or aerobic conditions, in non- or slightly flowing water, in cool and stable temperature, in acidic to base pH value, in highly polluted or toxic environment

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

(Reynoldson, 1987; Kaeser & Sharpe, 2006; Lagauzère et al., 2009a; Singh et al., 2010; Łuszczek-Trojnar et al., 2014; Gusakov et al., 2023; Simangunsong et al., 2024). Tubifex's respiratory physiology can adapt to very low dissolved oxygen, so it can survive for a long time in an anaerobic environment (Aston, 1973; Simangunsong et al., 2024). Oxygen-poor conditions can also provide advantages for Tubifex because they can increase significant bioturbation compared to aerobic environments (Nie et al., 2011). Its ability to bioturbate places it as one of the important meiobenthic bioturbators or burrowing meiofauna in a habitat (de Lucas Pardo et al., 2013).

Table 1. The physico-chemical properties of water for Tubifex.

Parameters	Sampling Locations intake and residential wastewater	during culture period using pig dung	during culture period using dairy sludge	during culture period using poultry excreta
pH	6.9-7.2	7.01 ± 0.15 to 7.08 ± 0.26	6.87 ± 2.64 to 7.01 ± 2.34	6.5 ± 0.51 to 7.8 ± 0.54
Alkalinity (mg/l)	144-296	205.35 ± 6.92 to 211.42 ± 8.49	250.42 ± 2.00 to 259.14 ± 2.10	58.14 ± 1.14 to 128.75 ± 1.16
Salinity (ppt)	Nil			
Dissolved oxygen (ppm)	1.8-4.6	3.06 ± 1.15 to 3.28 ± 1.23	1.22 ± 0.007 to 1.42 ± 0.009	0.45 ± 0.006 to 1.4 ± 0.002
Free carbon dioxide (ppm)	6-16	3.00 ± 1.13 to 3.27 ± 1.23	2.25 ± 0.009 to 2.57 ± 0.010	0.7 ± 0.11 to 5.3 ± 0.19
Total dissolved solids (ppm)	298-431			
Total suspended solids (ppm)	30-400			
Biological oxygen demand (ppm)	25-310			
Chemical oxygen demand (ppm)	90-640			
Ammonia-nitrogen (NH_4^+-N) (ppm)	1.5-35	0.009 ± 0.001 to 0.012 ± 0.002	0.58 ± 0.008 to 0.69 ± 0.009	0.1 ± 0.001 to 0.3 ± 0.001
Nitrite-nitrogen (NO_2-N) (ppm)	0.30-2.73			
Nitrate-nitrogen (NO_3-N) (ppm)	1.12-5.35	0.30 ± 0.05 to 0.40 ± 0.09	0.58 ± 0.004 to 0.67 ± 0.005	0.32 ± 0.04 to 1.23 ± 0.10
Phosphate (PO_4-P) (ppm)	0.50-2.53	0.32 ± 0.04 to 0.51 ± 0.10	1.05 ± 0.10 to 1.7 ± 0.008	1.02 ± 0.10 to 2.48 ± 0.04
Chloride (ppm)	51-70			
Hardness (ppm)	230-260			

Source: Singh et al., (2010)

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

4. The Role of *Tubifex sp.* as Bioremediator

Tubifex sp. is a capable invertebrate for the study of the biological effects caused by a variety type of contaminants (Vytlačilová eet al., 2004) due to its tolerance and resistance to pollution (Lucan-Bouché et al., 1997; 1999a, b), thus, its presence can be an indicator of polluted water (Bustami et al., 2019). It can be marked as an essential aquatic riverine indicator species and can be used as a bioindicator organism for pollution monitoring and riverine health (Roy et al., 2022). Figure 10 showed harvesting activity in Tubifex's habitat for a study.

Figure 10. Harvesting *T. tubifex* in its habitat, i.e., drain (personal documentation).

In ecological engineering, many aquatic organisms, one of them is *T. Tubifex,* are considered for bioremeditation (Gifford et al., 2007). *T. tubifex* is able to reduce nutrients such as nitrogen and phosphorus (Kang et al., 2016) and organic matter (Yang et al., 2021); meanwhile, the presence of *T. tubifex* can also enhance the microbial decomposition rates (Kristensen, 2000). Furthermore, *T. tubifex* can improve water quality (Lou et al., 2009; Kang et al., 2018) and substrate (Arrate et al., 2004; Yang et al., 2021). The ability to reduce dependence on the concentration of pollution (Aston, 1973).

In the implementation, as a deposit feeder (Kaster & Wolff, 1982), *T. tubifex* has been used as a bioremediator in wastewater treatment to remediate the domestic and slaughterhouse wastewater which have a high content of organic material (Taufik & Warganegara, 2013), so it can also as decomposers and serve as nutrient-rich natural feed sources for various fish species and other aquatic organisms (Singh et al., 2010). *T. tubifex* is important for inorganic pollutants treatment, so its role in the context of ecological health is crucial (Sharma et al., 2024). The role of *T. tubifex* in the environment based on its capability to live in organic matter, or non-organic matter, and Table 2 shows some materials that are used for cultivation medium for Tubifex's growth.

The head-down deposit feeder can alterate substrate by burrowing into this substrate (Lopez & Levinton, 1987; Amon & Herndl, 1991; Clough & Lopez, 1993). This activity, bioturbation, impacts to water and materials exchange in the water column-substrate interface (Lagauzère et al., 2009a; Mao et al., 2020).

The bioturbation activity of tubificid worms (e.g., *Tubifex* sp.) plays an important key in processing or cycling of the organic materials and nutrients in the aquatic environment, including the involvement of microorganism processes occurring between the water column and substrate interfaces in aquatic ecosystem (Mermillod-Blondin et al., 2018) in both condition of aerobic and anaerobic (van de Bund et al., 1994; Mermillod-Blondin et al., 2001). This activity influences the modification of benthic microorganism communities' structure and diversity by influencing the biogeochemical cycle in the substrate. The bioturbation stimulates the biogeochemical processes at the water column-substrate interface and it has a significant effect on microbial communities' diversity and structures. The presence of *Tubifex* sp. and its bioturbation activity initiates to stimulate organic matter mineralization and bacterial species communities (Cariou et al., 2021).

Table 2. Studies about the ability of *Tubifex tubifex* to survive in a contaminated environment.

Medium	Cultivation periods of the research treatment	References
Tofu dregs, mustard greens, fine bran, and fish silage	33 days	Yazid et al., (2022)
Chicken manure, tofu dregs	21 days	Bonse et al., (2021)
Chicken manure	20 days	Pursetyo et al., (2011)
Soybean curd residue, chicken manure, and pig manure	21 days	Solang et al., (2014)
Feces of fish (catfish, carp, and tilapia)	45 days	Ardana et al., (2018)
Cow dung, raw fish and vegetables	145 days	Begum et al., (2014)
Palm kernel cake	50 days	Putri et al., (2018)
Soy bean and rice wine waste and chicken manure	45 days	Akbar et al., (2016)
Lemna minor and mud	50 days	Mahendra et al., (2019)
Hg^{2+}, Cu^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, and Mn^{2+}		Rathore & Khangarot (2002)
Cd, Ni, and P	24 hours	Gillis et al., (2004)

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Cu, Zn, Ni, and Pb	28 days	Şimşek et al., (2023)
Cd, Fe, Pb, Zn, and Cu	-	Singh et al., (2007)
Cd	-	Kaonga et al., (2010)
Cu, Co, Ni, Zn, and Pb	28 days	Méndez-Fernández et al., (2014)
Hexachlorocyclohexane (HCH) isomers	-	Di et al., (2016)
Hexachlorobenzene		Egeler et al., (2001)

T. tubifex' activities in the substrate can increase the biomass and photosynthesis rate of plants in the wetland ecosystem. The *T. tubifex* demonstrates a positive impact on plant production in the substrate by increasing the mineralization of nutrients, water, and oxygen in the rhizosphere area, hormonal effects, distribution of plant-stimulating microbial, and infectious microbial reduction in the roots (Blouin et al. 2005; Eisenhauer & Scheu 2008; Mermillod-Blondin & Lemoine, 2010).

The presence of *T. tubifex* leads to positive interactions between benthic microorganisms, animals, and plants, and furthermore accelerate habitat ameliorations (Bertness & Callaway 1994; Mermillod-Blondin & Lemoine, 2010). The bioturbation ameliorates oxygen conditions and actively increases the exchanges between high- and low-oxygen level in the water by bioirrigation mechanism. Hence, the presence of *T. tubifex* results oxygen availability in substrate and it contribute to plant growth (Morard & Silvestre 1996; Jackson & Colmer 2005; Mermillod-Blondin & Lemoine, 2010).

Bioturbation has been known as an important mechanism affecting biological processing and cycling in water ecosystems (Aller, 1983; Mermillod-Blondin & Lemoine, 2010; Cariou et al., 2021). The bioturbation impacts on thte dynamics of the organic matter at the water column-substrate interface in different ecosystems have been evaluated by Mermillod-Blondin (2011). Tubifex's bioturbating induces biological structure building and bioirrigation that have an impact on microorganism communities and the biogeochemical mechanisms (Kristensen et al., 2012; Deng et al., 2020). This activity significantly impacts microbial diversity structures by modification of biogeochemical mechanisms and the increase in the presence of organic matter such as mucus, minerals, and distribution of organic materials in substrate (Mermillod-Blondin & Rosenberg, 2006; Papaspyrou et al., 2005; Papaspyrou et al., 2006; Lukwambe et al., 2018; Aller, R. C., & Cochran, 2019; Gonzalez et al., 2019; Deng et al., 2020; Hou et al., 2021).

In principle, Oligochaeta have the ability to vermicompost organic and inorganic waste (Ratnasari et al., 2023). Soil contamination by organic and inorganic waste and the presence of heavy metals can also contribute to soil stability and decomposition process (Trentin et al., 2019; Wang et al., 2021). Consequently, this condition may occur in an aquatic environment, thus the approach of vermicomposting with earthworms may also be implemented by silkworms.

The vermicomposting process consists of two processes, i.e., (a) mechanical and physical, and (b) biochemistry and ecology. The first process involves the organic matter aeration, followed by mixing the organic matter with the worms. Meanwhile, the second process exhibits the interrelation of both the worms and microorganisms (Ndegwa et al., 2000; Ganti, 2018). Vermicomposting includes pre-composting, composting, and separation or harvesting of the product. Vermicomposting is considered to have been successfully applied to detoxification of industrial wastes or sludges, removal of metals or metal ions from solid waste and contaminated soil, wastewater sludge treatment, etc.

Vermicomposting involves microbes in the environment. The worms promote the growth of bacteria to decompose waste, and the worms also grind, crush, and degrade waste (Binet et al., 1998; Singleton et al., 2003). The vermicomposting is organic matter stabilization through the combined activity of worms and microorganisms. The microbes are responsible for organic matter degradation, meanwhile, the worm drives the process to alter the substrate and biological condition in the environment (Suthar, 2009). The vermicomposting provides mass reduction, shorter time in processing, and produces high concentration of humic acid (humus). Vermicompost also contains high-quality of humus and enzymes (Sinha et al., 2010a, b). Humus contains organic acids that can bind to metals to form stable metals in the environment (Swati & Hait, 2017). An organic compound which has a $COOH^-$ functional group can bind to ions H^+ or cations causing an increase in pH value (Kurniawan et al., 2022).

In addition, *T. tubifex* can also directly accumulate metals in the tissues (Gillis et al., 2004; Redeker & Blust, 2005; Singh et al., 2007). The ability of *Tubifex* sp. as a bioaccumulator have been tested in laboratory and naturally found in its habitats, thus Tubifex can be used as a standard substrate bioassay (Reynoldson et al., 1991; Martinez-Madrid et al., (1999); Maestre et al., 2007). In the contaminated environment, organisms develop their body's defense system for survival. Tubifex develops biochemical mechanisms to respond to environmental stress, and these metabolic mechanisms are activated in the early stage of xenobiotic contamination.

Therefore, Tubifex is able to accumulate metals, pharmaceutics, polycyclic aromatic hydrocarbons, or pesticides., Tubifex produces metabolic compounds, namely superoxide dismutase enzyme, catalase enzyme, carboxylesterase enzyme, and glutathione-S-transferase enzyme (Arendarczyk & Grabińska-Sota, 2020). Superoxide dismutase (SOD) is a metalloenzyme, a crucial antioxidant and detoxification enzyme in the cell. It is essential as a defense against oxygen toxicity or reactive oxygen species (ROS), and it uses an unstable free radical as its natural substrate. This enzyme catalyzes the intermediates of oxygen reduction and prevents the production, i.e., O_2^-, H_2O_2, and OH^- as the main factors which cause of oxygen toxicity, and then defends against this toxicity (Fridovich, 1981; Ighodaro & Akinloye, 2018). Catalase (CAT) is an antioxidant enzyme which is crucial for

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

reducing oxidative stress by breaking down cellular hydrogen peroxide into water and oxygen production (Nandi et al., 2019), and it is very efficient in breaking the chemical bonds of hydrogen peroxide molecules (Ighodaro & Akinloye, 2018). Glutathione-S-transferase (GST) is a group of enzymes which play an important role in preventing lipid peroxidation and detoxifying through their Se-independent glutathione-peroxidase activity (Singhal et al., 2015). Carboxylesterase (CarE) is responsible for detoxifying organic compounds, hydrolyzing ester bonds, and amide range. This enzyme is important in hydrolytic detoxification and phosphorylation of insecticides (Galloway et al., 2002; Satoh, 2005).

T. tubifex also shows the ability to produce metallothioneins (MTs) as a non-enzymatic protein that is responsible for metal ion detoxification (Mosleh et al., 2005; Mosleh et al., 2006) or heavy metals accumulation (absorption) (Widiastuti et al., 2019). Metallothionein has a high cysteine content, and the cysteine residues form thiol groups (-SH) that allow MTs to bind heavy metals. It has the ability to bind specific metals; each MT only binds one metal (e.g., Cd, Hg, and Pb with different MTs) (Astutik & Zulaika, 2015; Hertika et al., 2019; Hertika et al., 2023)

Thus, the presence of oligochaetes in an environment can increase organic matter (wastes) decomposition and reduction; furthermore, the active compound or functional group of organic wastes that plays an important role in increasing the pH value and binding cations including heavy metals. We presented an illustration about the role of *Tubifex* sp. In the pathway of organic matter decomposition, and heavy metals or chemical substances accumulations and detoxification (Figure 10).

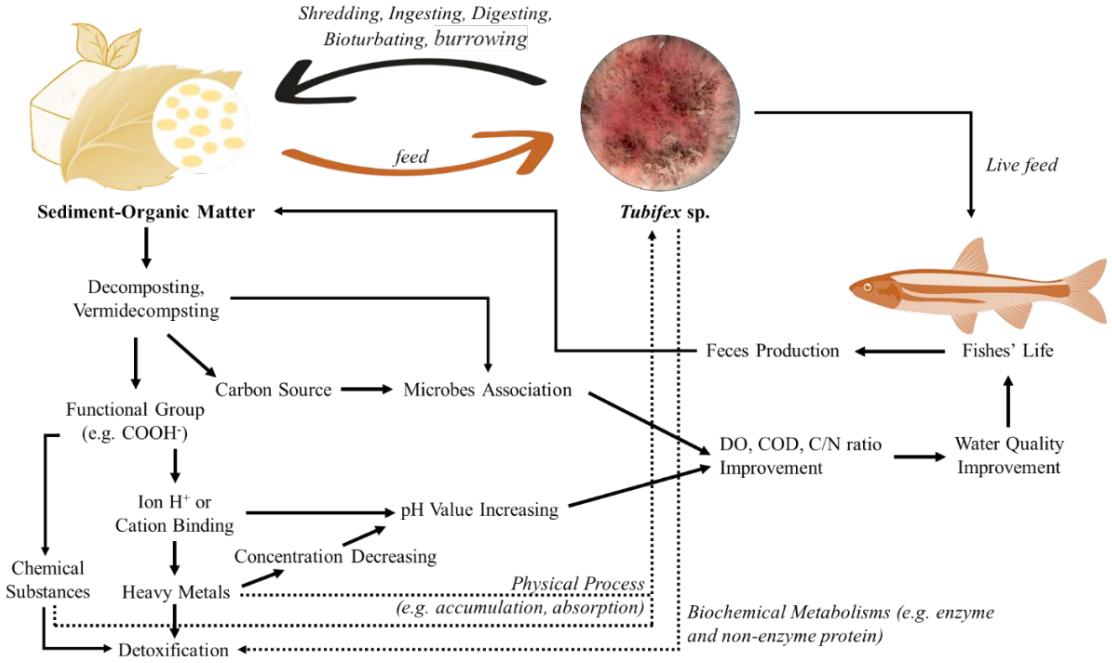


Figure 10. Schematic pathway of the role of *Tubifex* sp. in the freshwater ecosystem.

5. Conclusion

This paper has elaborated information on *Tubifex tubifex* comprehensively, about the biology as well as its role for bioremediator. *T. tubifex* has played an important role in bioremediation by physical activity (e.g., accumulation or absorption) and biochemical activity (e.g., enzyme and non-enzyme protein production). The presence of organic material in waters can be decomposed by Tubifex. Its bioturbation activity promoted exchange of materials between the water column-substrate interface. Decomposition of organic materials produces functional groups that are able to interact with inorganic materials through the binding of their ions. This can have a positive impact on reducing inorganic materials that pollute waters. Tubifex is a meiobenthic species of aquatic worm and an important key for organic matter decomposition, heavy metal, or chemical substances reduction and detoxification. It can perform vermicomposting on organic matter and also accumulate chemical substances by its metabolic compounds, such as superoxide dismutase enzyme, catalase enzyme, carboxylesterase enzyme, glutathione-S-transferase enzyme, and metallothionein enzyme. Reducing organic and inorganic pollution in water can indirectly improve water quality. Thus, Tubifex can play an important role in water bioremediation.

6. Acknowledgement

The authors would like to thank the Bangka Belitung University for funding of this research and publication by Hibah Unggulan Universitas Bangka Belitung 2024 with grant number: 425/UN50/L/PP/2024.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

7. References

Acosta V G M., Arellano-Carbajal F., Gillen K., Tweeten K A., Zattara E E. (2021). It cuts both ways: an annelid model system for the study of regeneration in the laboratory and in the classroom. *Frontiers in Cell and Developmental Biology* 9: 780422.

Akbar L O A., Muskita W H., Idris, M. (2016). Pengaruh substrat media terhadap biomassa cacing sutera (*Tubifex* sp.) yang dibudidayakan dengan sistem resirkulasi tertutup. *Media Akuatika*1: 175-185.

Alam MD A., Khan M A., Sarower-E-Mahfuj, MD., Ara Y., Parvez I., Amin M N. (2021). A model for tubificid worm (*Tubifex tubifex*) production and its effect on growth of three selected ornamental fish. *Bangladesh Journal of Fisheries* 33(2): 205-214.

Aller R C. (1983). The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry. *Journal of Marine Research* 41: 299-322.

Aller R C., Cochran J K. (2019). The critical role of bioturbation for particle dynamics, priming potential, and organic C remineralization in marine sediments: local and basin scales. *Frontiers in Earth Science* 7: 157.

Amon R M., Herndl G J. (1991). Deposit Feeding and Sediment: I. Interrelationship between *Holothuria tubulosa* (Holothurioida, Echinodermata) and the sediment microbial community. Marine Ecology 12(2): 163-174.

Anita P., Widiastuti I M. (2021). Biomass and nutritional content of silk worms (*Tubifex* sp.) on the substrate of germented chicken manure. *Jurnal Ilmiah AgriSains* 22(2): 106-113.

Anlauf K J., Moffitt C M. (2008). Models of stream habitat characteristics associated with tubificid populations in an intermountain watershed. *Hydrobiologia* 603: 147-158.

Ardana P P N., Abidin Z., Diniarti N. (2018). Pemanfaatan limbah budidaya ikan untuk peningkatan pertumbuhan biomassa cacing sutra (*Tubifex* sp.). *Jurnal Perikanan Unram* 8(1): 55-64.

Arrate J A., Rodriguez P., Martinez-Madrid M. (2004). Tubifex tubifex chronic toxicity test using artificial sediment: methodological issues. *Limnetica* 23(1-2): 025-36.

Aston R J. (1973). Tubificids and water quality: a review. *Environmental Pollution* 5(1): 1-10.

Astutik L W., Zulaika E. (2015). Medium yang mengandung logam Pb. *Jurnal Sains dan Seni ITS* 4(1): 1-4.

Balavoine G. (2014). Segment formation in Annelids: patterns, processes and evolution. *International Journal of Developmental Biology* 58: 469-483.

Baldo L., Ferraguti M. (2005). Mixed reproductive strategy in *Tubifex tubifex* (Oligochaeta, Tubificidae)?. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 303(2): 168-177.

Beauchamp K A., Kathman R D., McDowell T S., Hedrick R P. (2001). Molecular phylogeny of tubificid oligochaetes with special emphasis on *Tubifex tubifex* (Tubificidae). *Molecular Phylogenetics and Evolution* 19(2): 216-224.

Begum M., Noor P., Ahmed K N., Sultana N., Hasan Md R., Mohanta L C. (2014). Development of a culture techniques for Tubificid worm, under laboratory conditions. *Bangladesh Journal of Zoology* 42(1): 117-122.

Bely A E. (2006). Distribution of segment regeneration ability in the Annelida. *Integrative and Comparative Biology* 46(4): 508-518.

Bely A E. (2014). Early events in annelid regeneration: a cellular perspective. *American Zoologist* 54(4): 688-699.

Benbow M E. (2009) Annelida, Oligochaeta and Polychaeta. In: Gene E. Likens, (Editor) Encyclopedia of Inland Waters. volume 2, pp. 124-127 Oxford: Elsevier.

Bertness M D., Leonard G H. (1997). The role of positive interactions in communities: lessons from intertidal habitats. *Ecology* 78(7): 1976-1989.

Beukeboom L W., Vrijenhoek R C. (1998). Evolutionary genetics and ecology of sperm-dependent parthenogenesis. *Journal of Evolutionary Biology* 11(6): 755-782.

Binet F., Fayolle L., Pussard M., Crawford J J., Traina S J., Tuovinen O H. (1998). Significance of earthworms in stimulating soil microbial activity. *Biology and Fertility of Soils* 27: 79-84.

Blouin M., Zuily-Fodil Y., Pham-Thi A T., Laffray D., Reversat G., Pando A., Tondoh J., Lavelle P. (2005) Belowground organism activities affect plat aboveground phenotype, inducing plant tolerance to parasites. *Ecology Letters* 8: 202-208.

Bonse N., Hasim H., Tuiyo R. (2021). The effect of chicken manure and tofu dregs on biomass growth of silkworm. *Nike: Jurnal Ilmiah Perikanan dan Kelautan* 9(2): 33-37.

Bouché M L., Arnoult F., Vernet G. (2003). Caudal regeneration in *Tubifex tubifex* (Oligochaeta, Tubificidae) following copper exposure. *Invertebrate Biology* 122(1): 42-51.

Bouché M L., Biagianti-Risbourg S., Vernet G. (1999). A light and scanning electron microscope study of the morphology of the chaetae of *Tubifex tubifex* in a non-polluted medium. *Hydrobiologia* 411: 39-44.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Bouché M L., Habets F., Biagianti-Risbourg S., Vernet G. (2000). Toxic effects and bioaccumulation of cadmium in the aquatic oligochaete *Tubifex tubifex*. *Ecotoxicology and Environmental Safety* 46(3), 246-251.

Brinkhurst R O. (1964). Observations on the biology of the marine oligochaete *Tubifex costatus*. *Journal of the marine biological Association of the United Kingdom* 44(1): 11-16.

Bustami Y., Kurnia K., Supiandi M I. (2019). Diversity of annelids in the Kapuas and Melawi Rivers. *Jurnal Biota* 5(2): 55-63.

Cariou M., Francois C M., Voisin J., Pigneret M., Hervant F., Volatier L., Mermillod-Blondin F. (2021). Effects of bioturbation by tubificid worms on biogeochemical processes, bacterial community structure and diversity in heterotrophic wetland sediments. *Science of the Total Environment* 795: 148842.

Chipman A D. (2010). Parallel evolution of segmentation by co-option of ancestral gene regulatory networks. *BioEssays* 32(1): 60-70.

Christensen B., Theisen B F. (1998). Phylogenetic status of the family Naididae (Oligochaeta, Annelida) as inferred from DNA analyses. *Journal of Zoological Systematics and Evolutionary Research* 36(4): 169-172.

Clark R B. (1960). Habituation of the polychaete Nereis to sudden stimuli: II. Biological significance of habituation. *Animal Behaviour* 8: 92-103.

Clark R B., Tritton D J. (1970). Swimming mechanisms in nereidiform polychaetes. *Journal of Zoology* 161(2): 257-271.

Clough L M., Lopez G R. (1993). Potential carbon sources for the head-down deposit-feeding polychaete *Heteromastus filiformis*. *Journal of Marine Research* 51: 595-616.

Conceição L E., Yúfera M., Makridis P., Morais S., Dinis M T. (2010). Live feeds for early stages of fish rearing. *Aquaculture Research* 41(5): 613-640.

de Lucas Pardo M A., Bakker M., van Kessel T., Cozzoli F., Winterwerp J C. (2013). Erodibility of soft freshwater sediments in Markermeer: the role of bioturbation by meiobenthic fauna. *Ocean Dynamics* 63: 1137-1150.

Deng L., Bölsterli D., Kristensen E., Meile C., Su C C., Bernasconi S M., Seidenkrantzf M., Glombitza C., Lagostina L., Hana X., Jørgenseng B B., Røyg H., Lever M A. (2020). Macrofaunal control of microbial community structure in continental margin sediments. *Proceedings of the National Academy of Sciences* 117(27): 15911-15922.

Di S., Huang L., Diao J., Zhou Z. (2016). Selective bioaccumulation and elimination of hexachlorocyclohexane isomers in *Tubifex tubifex* (Oligochaeta, Tubificidae). *Environmental Science and Pollution Research* 23: 6990-6998.

Dumnicka E., Poznańska M. (2006). Novel polish recordings of rare aquatic Oligochaeta species. *Oceanological and Hydrobiological Studies* 35(2): 111-120.

Egeler P., Meller M., Roembke J., Spoerlein P., Streit B., Nagel R. (2001). *Tubifex tubifex* as a link in food chain transfer of hexachlorobenzene from contaminated sediment to fish. In *Aquatic Oligochaete Biology VIII: Proceedings of the 8th International Symposium on Aquatic Oligochaeta, held in Bilbao, Spain 18-22 July 2000* (pp. 171-184). Springer Netherlands.

Eisenhauer N., Scheu S. (2008). Earthworms as drivers of the competition between grasses and legumes. *Soil Biology and Biochemistry* 40(10): 2650-2659.

Erséus C., Gustavsson L. (2002). A proposal to regard the former family Naididae as a subfamily within Tubificidae (Annelida, Clitellata). *Hydrobiologia* 485: 253-256.

Erséus C., Källersjö M. (2004). 18S rDNA phylogeny of Clitellata (Annelida). *Zoologica Scripta* 33(2): 187-196.

Erséus C., Källersjö M., Ekman M., Hovmöller R. (2002). 18S rDNA phylogeny of the Tubificidae (Clitellata) and its constituent taxa: dismissal of the Naididae. *Molecular Phylogenetics and Evolution 22*(3): 414-422.

Fedonkin M. A. (2003). The origin of the Metazoa in the light of the Proterozoic fossil records. *Paleontological Research* 7: 9-41.

Fowler D J., Goodnight C J. (1965). The Effect of Environmental Factors on the Respiration of Tubifex. *American Midland Naturalist* 74(2): 418-428.

Foxon G E H. (1936). XL.—Observations on the locomotion of some Arthropods and annelids. *Journal of Natural History* 18(106): 403-419.

Fridovich I. (1981). Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry* 64: 97-112.

Galloway T S., Millward N., Browne M A., Depledge M H. (2002). Rapid assessment of organophosphorous/carbamate exposure in the bivalve mollusc *Mytilus edulis* using combined esterase activities as biomarkers. *Aquatic Toxicology* 61(3-4): 169-180.

Ganti S. (2018). Vermicomposting. International Journalof Waste Resources 8(2): 1000342.

Gifford S., Dunstan R H., O'Connor W., Koller C E., MacFarlane G R. (2007). Aquatic zooremediation: deploying animals to remediate contaminated aquatic environments. *Trends in Biotechnology* 25(2): 60-65.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Gillis P L., Dixon D G., Borgmann U., Reynoldson T B. (2004). Uptake and depuration of cadmium, nickel, and lead in laboratory-exposed *Tubifex tubifex* and corresponding changes in the concentration of a metallothionein-like protein. *Environmental Toxicology and Chemistry: An International Journal* 23(1): 76-85.

Gline S E., Nakamoto A., Cho S J., Chi C., Weisblat D A. (2011). Lineage analysis of micromere 4d, a super-phylotypic cell for Lophotrochozoa, in the leech Helobdella and the sludgeworm Tubifex. *Developmental Biology* 353(1): 120-133.

Gonzalez S V., Johnston E., Gribben P E., Dafforn K. (2019). The application of bioturbators for aquatic bioremediation: review and meta-analysis. *Environmental Pollution*: 250: 426-436.

Goto A., Kitamura K., Shimizu T. (1999). Cell lineage analysis of pattern formation in the Tubifex embryo. I. Segmentation in the mesoderm. *The International Journal of Developmental Biology* 43(4): 317-327.

Goto A., Kitamura K., Shimizu T. (1999). Cell lineage analysis of pattern formation in the Tubifex embryo. I. Segmentation in the mesoderm. *The International Journal of Developmental Biology* 43(4): 317-327.

Govedich F R., Bain B A., Moser W E., Gelder S R., Davies R W., Brinkhurst R O. (2010). Annelida (clitellata): Oligochaeta, branchiobdellida, hirudinida, and acanthobdellida. In *Ecology and classification of North American freshwater invertebrates* (pp. 385-436). Academic Press.

Gray J. (1939). Studies in animal locomotion: VIII. The kinetics of locomotion of Nereis diversicolor. *Journal of Experimental Biology* 16(1): 9-17.

Gusakov V., Dien T D., Tran H Q., Thanh N T H., Huan P T., Ha V T., Dinh C N. (2023). An Annotated Checklist of the Main Representatives of Meiobenthos from Inland Water Bodies of Central and Southern Vietnam—II—Annelid Worms (Oligochaeta and Aeolosomatidae). *Water* 15(12): 2273.

Gustus R M., Cloney R A. (1973). Ultrastructure of the larval compound setae of the polychaete Nereis vexillosa Grube. *Journal of Morphology* 140(3): 355-366.

Hallett S L., Atkinson S D., Bartholomew J L. (2005). Countering morphological ambiguities: development of a PCR assay to assist the identification of *Tubifex tubifex* oligochaetes. *Hydrobiologia* 543(2005): 305-309.

Hannibal R L., Patel N H. (2013). What is a segment?. *EvoDevo* 4: 1-10.

Haque M I M., Alam A R U., Akter N., Siddique M A., Sultana M., Hossain M A., Hasan M. (2020). Molecular characterization of 'tubifex worms' based on 16S rRNA and cytochrome c oxidase subunit I. *Aquaculture Reports* 16: 100292.

Hare L., Tessier A., Warren L. (2001). Cadmium accumulation by invertebrates living at the sediment-water interface. *Environmental Toxicology and Chemistry: An International Journal* 20(4): 880-889.

Hausen H. (2005). Chaetae and chaetogenesis in polychaetes (Annelida). *Hydrobiologia* 535-536(1):37-52.

Herawati V E., Hutabarat J., Radjasa K O. (2020). Growth performance of tilapia (*Oreochromis niloticus* Linnaeus, 1758) larvae with feeding *Tubifex tubifex* (Müller, 1774) from different fermentation of animal manures. *Iranian Journal of Fisheries Sciences* 19(4): 2039-2052.

Hertika A M S., Arfiati D., Lusiana E D., Putra R B. (2023). Performance of metallothionein biomarker from *Sulcospira testudinaria* to assess heavy metal pollution in the Brantas River Watershed, Indonesia. *Journal of Ecological Engineering* 24(3): 276–286.

Hertika A M S., Kusriani K., Indrayani E., Yona D., Putra R B D S. (2019). Metallothionein expression on oysters (*Crassostrea cuculata* and *Crassostrea glomerata*) from the southern coastal region of East Java. *F1000Research* 8: 56.

Hesselberg T., Vincent J F V. (2006). A comparative study of the functional morphology of parapodia and setae in nereids (Polychaeta: Nereididae). *Animal Biology* 56(1): 103-120.

Hill S D., Saglam N., Shain D H. (2018). Reproduction in the Annelida. *Encyclopedia of Reproduction* 6: 526-532.

Hirao Y. (1965). Cocoon formation inTubifex, with its relation to the activity of the clitellar epithelium. *Journal of the Faculty of Science* 15: 625-632.

Hou Y., Li B., Feng G., Zhang C., He J., Li H., Zhu J. (2021). Responses of bacterial communities and organic matter degradation in surface sediment to *Macrobrachium nipponense* bioturbation. *Science of the Total Environment* 759: 143534.

Hurley R R., Woodward J C., Rothwell J J. (2017). Ingestion of microplastics by freshwater tubifex worms. *Environmental Science & Technology* 51(21): 12844-12851.

Ighodaro O M., Akinloye O A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine* 54(4): 287-293.

Isaeva V V., Kasyanov N V. (2021). Symmetry transformations in metazoan evolution and development. *Symmetry* 13(2): 160.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Iyer R G., Rogers D V., Levine M., Winchell C J., Weisblat D A. (2019). Reproductive differences among species, and between individuals and cohorts, in the leech genus Helobdella (Lophotrochozoa; Annelida; Clitellata; Hirudinida; Glossiphoniidae), with implications for reproductive resource allocation in hermaphrodites. *PLoS One* 14(4): e0214581.

Jackson M B., Colmer T D. (2005) Response and adaptation by plants to flooding stress. *Annals of Botany* 96: 501-505.

Kaeser A J., Sharpe W E. (2006). Patterns of distribution and abundance of *Tubifex tubifex* and other aquatic oligochaetes in *Myxobolus cerebralis* enzootic areas in Pennsylvania. *Journal of Aquatic Animal Health* 18(1): 64-78.

Kang Y., Xie H., Zhang J., Zhao C., Wang W., Guo Y., Guo Z. (2018). Intensified nutrients removal in constructed wetlands by integrated *Tubifex tubifex* and mussels: Performance and mechanisms. *Ecotoxicology and Environmental Safety* 162: 446-453.

Kang Y., Zhang J., Xie H., Guo Z., Li P., Cheng C., Lv L. (2016). Enhancement of the performance of constructed wetlands for wastewater treatment in winter: the effect of *Tubifex tubifex*. *RSC Advances* 6(41): 34841-34848.

Kang Y., Zhang J., Xie H., Guo Z., Ngo H H., Guo W., Liang S. (2017). Enhanced nutrient removal and mechanisms study in benthic fauna added surface-flow constructed wetlands: The role of *Tubifex tubifex*. *Bioresource Technology* 224: 157-165.

Kaonga C C., Kumwenda J., Mapoma H T. (2010). Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms; *Tubifex tubifex* in sewage sludge. *International Journal of Environmental Science & Technology* 7: 119-126.

Kaster J L. (1980). The reproductive biology of *Tubifex tubifex* Muller (Annelida: Tubificidae). *American Midland Naturalist* 104(2): 364-366.

Kaster J L., Wolff R. J. (1982). A convoluted respiratory exchange surface in *Tubifex tubifex* (Tubificidae). *Transactions of the American Microscopical Society* 101(1): 91-95.

Kautsar A., Marzuki M., Scrabra A R. (2022). The effect of additional silk worm (*Tubifex* sp.) on artificial feed on the number of larva Guppy fish (*Poecilia reticulata*). *Indonesian Journal of Tropical Aquatic 5*(1): 33-42.

Kelly D A., Moore B C. (2016). The morphological diversity of intromittent organs. *Integrative and Comparative Biology* 56(4): 630-634.

Kharche S D., Birade H S. (2013). Parthenogenesis and activation of mammalian oocytes for in vitro embryo production: A review. *Advances in Bioscience and Biotechnology* 4: 170-182

Klingenberg C P. (2015). Analyzing fluctuating asymmetry with geometric morphometrics: Concepts, methods, and applications. *Symmetry* 7: 843-934.

Knight-Jones P., Fordy M R. (1979). Setal structure, functions and interrelationships in Spirorbidae (Polychaeta, Sedentaria). *Zoologica Scripta* 8(1-4): 119-138.

Kono T. (2008). Genetic modification for bimaternal embryo development. *Reproduction, Fertility and Development* 21(1): 31-36.

Kostyuchenko R P., Kozin V V. (2021). Comparative aspects of annelid regeneration: Towards understanding the mechanisms of regeneration. *Genes* 12(8): 1148.

Kostyuchenko R P., Kozin V V., Kupriashova E E. (2016). Regeneration and asexual reproduction in annelids: cells, genes, and evolution. *Biology Bulletin* 43: 185-194.

Kristensen E., Penha-Lopes G., Delefosse M., Valdemarsen T., Quintana C O., Banta G T. (2012). What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine Ecology Progress Series* 446: 285-302.

Kruczkowska W., Kciuk M., Pasieka Z., Kłosiński K., Płuciennik E., Elmer J., Waszczykowska K., Kołat D., Kałuzińska-Kołat Ż. (2023). The artificial oxygen carrier erythrocruorin—characteristics and potential significance in medicine. *Journal of Molecular Medicine* 101(8): 961-972.

Kuo D H. (2017). The polychaete-to-clitellate transition: An EvoDevo perspective. *Developmental Biology* 427(2): 230-240.

Kurniawan A., Kurniawan A., Robin R. (2023). Interaction of organisms in abandoned tin mining pits: Perspective of life in acid mine drainage environment. *Jurnal Ilmu Lingkungan* 21(1): 159-171.

Kurniawan A., Adibrata S., Lingga R., Setiadi J., Wulandari U A., Hidayah R S N. (2024). Growth performances of lobster juvenile (*Cherax quadricarinatus*) fed with silkworm and rice flour. *Journal of Aquatropica Asia* 9(1): 45-50.

Lagauzère S., Terrail R., Bonzom J M. (2009a). Ecotoxicity of uranium to *Tubifex tubifex* worms (Annelida, Clitellata, Tubificidae) exposed to contaminated sediment. *Ecotoxicology and Environmental Safety* 72(2): 527-537.

Lagauzère S., Boyer P., Stora G., Bonzom J M. (2009b). Effects of uranium-contaminated sediments on the bioturbation activity of *Chironomus riparius* larvae (Insecta, Diptera) and *Tubifex tubifex* worms (Annelida, Tubificidae). *Chemosphere* 76(3): 324-334.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Launay C., Félix M A., Dieng J., Delattre M. (2020). Diversification and hybrid incompatibility in auto-pseudogamous species of *Mesorhabditis nematodes*. *BMC Evolutionary Biology* 20: 1-15.

Lazim M N., Learner M A. (1986). The life-cycle and productivity of *Tibifex tubifex* (Oligochaeta; Tibificidae) in the Moat-Feeder Stream, Cardiff, South Wales. *Ecography* 9(3): 185-192.

Learner M A., Lochhead G., Hughes B D. (1978). A review of the biology of British Naididae (Oligochaeta) with emphasis on the lotic environment. *Freshwater Biology* 8(4): 357-375.

Lopez G R., Levinton J S. (1987). Ecology of deposit-feeding animals in marine sediments. *The Quarterly Review of Biology* 62(3): 235-260.

Lou J Q., Guo M X., Sun P D., Wu G., Song Y Q. (2009). Full-scale experiments of municipal sewage treated by symbiotic system consisting of tubifex and microbes. *Huan Jing ke Xue = Huanjing Kexue* 30(12): 3602-3608.

Lucan-Bouché M L., Arsac F., Biagianti-Risbourg S., Habets F., Vernet G. (1997). Experimental study of the lethal effects induced by copper and lead on the Oligochaeta *Tubifex tubifex*. *Bulletin de la Société zoologique de France* 122(4): 389-392.

Lucan-Bouché M L., Biagianti-Risbourg S., Arsac F., Vernet G. (1999a). An original decontamination process developed by the aquatic oligochaete *Tubifex tubifex* exposed to copper and lead. *Aquatic Toxicology* 45(1): 9-17.

Lucan-Bouché M L., Biagianti-Risbourg S., Arsac F., Vernet G. (1999b). Autotomy as a mechanism of decontamination used by the oligochaete *Tubifex tubifex*. *Bulletin de la Société zoologique de France* 124(4):383-387.

Lukwambe B., Yang W., Zheng Y., Nicholaus R., Zhu J., Zheng Z. (2018). Bioturbation by the razor clam (Sinonovacula constricta) on the microbial community and enzymatic activities in the sediment of an ecological aquaculture wastewater treatment system. *Science of the Total Environment* 643: 1098-1107.

Lüscher A., Milinski M. (2003). Simultaneous hermaphrodites reproducing in pairs self-fertilize some of their eggs: an experimental test of predictions of mixed-mating and Hermaphrodite's Dilemma theory. *Journal of Evolutionary Biology* 16(5): 1030-1037.

Łuszczek-Trojnar E., Sroka K., Klaczak A., Nowak M., Popek W. (2014). Bioaccumulation and purification of cadmium in *Tubifex tubifex*. *Turkish Journal of Fisheries and Aquatic Sciences* 14(4): 939-946.

Maestre Z., Martinez-Madrid M., Rodriguez P., Reynoldson T. (2007). Ecotoxicity assessment of river sediments and a critical evaluation of some of the procedures used in the aquatic oligochaete *Tubifex tubifex* chronic bioassay. *Archives of Environmental Contamination and Toxicology* 53: 559-570.

Mahendra D D., Nufus M., Putri V R. (2019). Giving Lemna minor and mud to the growth of *Tubifex* sp. *Budapest International Research in Exact Sciences Journal* 1(3): 23-27.

Mandal R N., Kar S., Chakrabarti P P., Chattopadhyay D N., Paul B N., Adhikari S., Maity J., Pillai B R. (2018). Production of Tubifex-a new dimension of aquaculture in feeding juvenile fish. *Aquaculture Asia* 22: 20-24.

Mandal R N., Kar S., Chattopadhyay D N., Maity J., Paul B N., Chakrabarti P P., Jayasankar P. (2016). Tubifex production using agro-industrial wastes and raw cattle dung. *Journal of Applied Aquaculture* 28(2): 70-75.

Mao R., Wu J., Qin X., Ma C., Song J., Cheng D., Sun H., Li M. (2020). The effect of tubificid bioturbation on vertical water exchange across the sediment–water interface. *Water* 12(12): 3467.

Mariom M., Liza S N., Mollah M F A. (2016). Identification of genera of tubificid worms in Bangladesh through morphological study. *Asian Journal of Medical and Biological Research* 2(1): 27-32.

Mariom M., Mollah Md F A. (2012). Development of a suitable culture medium for the production of tubificid worms. *Asian Fisheries Science* 25(2012): 40-51.

Marotta R., Crottini A., Prada V., Ferraguti M. (2009). A morphological reappraisal of *Tubifex blanchardi* Vejdovský, 1891 (Clitellata: Tubificidae). *Acta Zoologica* 90(2): 179-188.

Marotta R., Crottini A., Raimondi E., Fondello C., Ferraguti M. (2014). Alike but different: the evolution of the Tubifex tubifex species complex (Annelida, Clitellata) through polyploidization. *BMC Evolutionary Biology* 14: 1-14.

Martinez-Madrid M., Rodriguez P., Perez-Iglesias J I., Navarro E. (1999). Sediment toxicity bioassays for assessment of contaminated sites in the Nervion River (Northern Spain). 2. *Tubifex tubifex* reproduction sediment bioassay. *Ecotoxicology* 8: 111-124.

Masciandaro G., Macci C., Peruzzi E., Ceccanti B., Doni S. (2013). Organic matter–microorganism-plant in soil bioremediation: a synergic approach. *Reviews in Environmental Science and Bio/Technology* 12: 399-419.

Mashudi F., Thaib A., Nurhayati. (2023). Pertumbuhan populasi cacing sutra (*Tubifex* sp.) pada suhu rata-rata 27°C. *Jurnal Tilapia* 4(2): 32-38.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Mc Loughlin S., Bomfleur B., Mörs T., Reguero M A. (2016). Fossil clitellate annelid cocoons and their microbiological inclusions from the Eocene of Seymour Island, Antarctica. *Palaeontologia Electronica* 19(1.11A): 1-27

Méndez-Fernández L., De Jonge M., Bervoets L. (2014). Influences of sediment geochemistry on metal accumulation rates and toxicity in the aquatic oligochaete *Tubifex tubifex*. *Aquatic Toxicology* 157: 109-119.

Mermillod-Blondin, F. (2011). The functional significance of bioturbation and biodeposition on biogeochemical processes at the water-sediment interface in freshwater and marine ecosystems. *Journal of the North American Benthological Society* 30(3): 770-778.

Mermillod-Blondin F., Bouvarot M., Déjollat Y., Adrien J., Maire E., Lemoine D G., Marmonier P., Volatier L. (2018). Influence of tubificid worms on sediment structure, benthic biofilm and fauna in wetlands: A field enclosure experiment. *Freshwater Biology* 63(11): 1420-1432.

Mermillod-Blondin F., Foulquier A., Gilbert F., Navel S., Montuelle B., Bellvert F., Comte G., Grossi V., Fourel F., Lecuyer C., Sinom L. (2013). Benzo(a)pyrene inhibits the role of the bioturbator *Tubifex tubifex* in river sediment biogeochemistry. *Science of the Total Environment* 15(450-451): 230-241.

Mermillod-Blondin F., Gérino M., Degrange V., Lensi R., Chassé J L., Rard M., Châtelliers M C D. (2001). Testing the functional redundancy of Limnodrilus and Tubifex (Oligochaeta, Tubificidae) in hyporheic sediments: an experimental study in microcosms. *Canadian Journal of Fisheries and Aquatic Sciences* 58(9): 1747-1759.

Mermillod-Blondin F., Lemoine D G. (2010). Ecosystem engineering by tubificid worms stimulates macrophyte growth in poorly oxygenated wetland sediments. *Functional Ecology* 24(2): 444-453.

Mermillod-Blondin F., Nogaro G., Datry T., Malard F., Gibert J. (2005). Do tubificid worms influence the fate of organic matter and pollutants in stormwater sediments?. *Environmental Pollution* 134(1): 57-69.

Mermillod-Blondin F., Rosenberg R. (2006). Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. *Aquatic Sciences* 68: 434-442.

Merz R A., Edwards D R. (1998). Jointed setae–their role in locomotion and gait transitions in polychaete worms. *Journal of Experimental Marine Biology and Ecology* 228(2): 273-290.

Meshcheryakov V N. (1990). The Sludgeworm Tubifex. T. A. Dettlaff et al. (eds.). Animal Species for Developmental Studies: Volume 1 Invertebrates (pp. 41-67). Boston, MA: Springer US.

Mewekani S., Tampobulon I. (2019). Analisis perkembangbiakan cacing rambut (*Tubifex* sp.) pada berbagai media tumbuh. *Jurnal Tabura Perikanan dan Kelautan* 1(1): 64-74.

Milbrink G., Timm T., Lundberg S. (2002). Indicative profundal oligochaete assemblages in selected small Swedish lakes. *Hydrobiologia* 468: 53-61.

Minelli A., Fusco G. (2004). Evo-devo perspectives on segmentation: model organisms, and beyond. *Trends in Ecology & Evolution* 19(8): 423-429.

Morard P., Silvestre J. (1996). Plant injury due to oxygen deficiency in the root environment of soilless culture: a review. *Plant and Soil* 184: 243-254.

Mosleh Y Y., Paris-Palacios S., Biagianti-Risbourg S. (2006). Metallothioneins induction and antioxidative response in aquatic worms *Tubifex tubifex* (Oligochaeta, Tubificidae) exposed to copper. *Chemosphere* 64(1): 121-128.

Nakadera Y., Koene J M. (2013). Reproductive strategies in hermaphroditic gastropods: conceptual and empirical approaches. *Canadian Journal of Zoology* 91(6): 367-381.

Nakamoto A., Arai A., Shimizu T. (2000). Cell lineage analysis of pattern formation in the Tubifex embryo. II. Segmentation in the ectoderm. *International Journal of Developmental Biology* 44(7): 797-806.

Nakamoto A., Nagy L M., Shimizu T. (2011). Secondary embryonic axis formation by transplantation of D quadrant micromeres in an oligochaete annelid. *Development* 138(2): 283-290.

Nakamura T., Shiomi I., Shimizu T. (2017). Embryonic expression of festina lente (fel), a novel maternal gene, in the oligochaete annelid *Tubifex tubifex*. *Gene Expression Patterns* 25: 29-35.

Nandi A., Yan L J., Jana C K., Das N. (2019). Role of catalase in oxidative stress-and age-associated degenerative diseases. *Oxidative Medicine and Cellular Longevity* 2019(1): 9613090.

Ndegwa P M., Thompson S A., Das K C. (2000). Effects of stocking density and feeding rate on vermicomposting of biosolids. *Bioresource Technology* 71(1): 5-12.

Negredo C., Dillane E., Mulcahy M F. (2003). Small subunit ribosomal DNA characterization of an unidentified aurantiactinomyxon form and its oligochaete host *Tubifex ignotus*. *Diseases of Aquatic Organisms* 54(3): 229-241.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Nie X B., Li Z H., Long Y N., He P P., Xu C. (2017). Chlorine inactivation of *Tubifex tubifex* in drinking water and the synergistic effect of sequential inactivation with UV irradiation and chlorine. *Chemosphere* 177: 7-14.

Nie X., Wu S., Wu F., Sun S., Jiang C., Tan W., Wan J L. (2011). Effects of the bioturbation activity of *Tubifex tubifex* on nitrogen release from sediments. *Acta Scientiae Circumstantiae* 31: 107-113.

Nikanorova D D., Kupriashova E E., Kostyuchenko R P. (2020). Regeneration in annelids: Cell sources, tissue remodeling, and differential gene expression. *Russian Journal of Developmental Biology* 51: 148-161.

Nuraisyah A., Rendi R., Abror M., Mukti R C. (2023). Teknologi budidaya cacing sutra (*Tubifex sp.*) di Balai Besar Perikanan Budidaya Air Tawar (BBPBAT) Sukabumi, Jawa Barat. *Jurnal Sumberdaya Akuatik Indopasifik* 7(2): 153-160.

Oplinger R W., Bartley M., Wagner E J. (2011). Culture of *Tubifex tubifex*: effect of feed type, ration, temperature and density on juvenile recruitment, production and adult survival. *North American Journal of Aquaculture* 73(1): 68-75.

Palmer M. F. (1968). Aspects of the respiratory physiology of *Tubifex tubifex* in relation to its ecology. *Journal of Zoology* 154(4), 463-473.

Papaspyrou S., Gregersen T., Cox R P., Thessalou-Legaki M., Kristensen E. (2005). Sediment properties and bacterial community in burrows of the ghost shrimp *Pestarella tyrrhena* (Decapoda: Thalassinidea). *Aquatic Microbial Ecology* 38(2): 181-190.

Papaspyrou S., Gregersen T., Kristensen E., Christensen B., Cox R P. (2006). Microbial reaction rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes (*Nereis diversicolor* and *N. virens*). *Marine Biology* 148: 541-550.

Parish J. (1981). Reproductive ecology of Naididae (Oligochaeta). *Hydrobiologia* 83(1): 115-123.

Parker H M., McDaniel, C D. (2009). Parthenogenesis in unfertilized eggs of *Coturnix chinensis*, the Chinese painted quail, and the effect of egg clutch position on embryonic development. *Poultry Science* 88(4): 784-790.

Paul S., Balakrishnan S., Arumugaperumal A., Lathakumari S., Syamala S S., Vijayan V., Durairaj S C J., Arumugaswami V., Sivasubramaniam S. (2022). Importance of clitellar tissue in the regeneration ability of earthworm *Eudrilus eugeniae*. *Functional & Integrative Genomics* 22(4): 1-32.

Pelegri S P., Blackburn T H. (1995). Effects of *Tubifex tubifex* (Oligochaeta: Tubificidae) on N-mineralization in freshwater sediments, measured with 15N isotopes. *Aquatic Microbial Ecology* 9(3): 289-294.

Peng Y., Wang H., Cui Y. (2014). Two species of Naididae (Annelida, Clitellata) from Southern Tibet, China. *ZooKeys* 444: 59-68.

Peng Y., Wang H., Cui Y. (2017). Four species of Tubifex Lamarck (Annelida: Oligochaeta: Naididae) from Tibet, China. *Zootaxa* 4320(2): 366-378.

Przeslawski R., Zhu Q., Aller R. (2009). Effects of abiotic stressors on infaunal burrowing and associated sediment characteristics. *Marine Ecology Progress Series* 392: 33-42.

Pursetyo K T., Satyantini W H., Mubarak A S. (2011). The effect of remanuring dry chicken manure in Tubifex tubifex population. *Jurnal Ilmiah Perikanan dan Kelautan* 3(2): 177-182.

Putri B., Hudaidah S., Kesuma W I. (2018). Pemanfaatan bungkil inti sawit sebagai media pertumbuhan cacing sutra (*Tubifex* sp.). *E-Jurnal Rekayasa Dan Teknologi Budidaya Perairan* 6(2): 729-738.

Ragi M S., Jaya D S. (2014). Distribution and diversity of oligochaetes in selected ponds of Thiruvananthapuram district, Kerala, South India. *Advances in Ecology* 1: 138360.

Ramachandran R., McDaniel C D. (2018). Parthenogenesis in birds: a review. *Reproduction* 155(6): R245-R257.

Rathore R S., Khangarot B S. (2002). Effects of temperature on the sensitivity of sludge worm *Tubifex tubifex* Müller to selected heavy metals. *Ecotoxicology and Environmental Safety* 53(1): 27-36.

Ratnasari A., Syafiuddin A., Mehmood M A., Boopathy R. (2023). A review of the vermicomposting process of organic and inorganic waste in soils: Additives effects, bioconversion process, and recommendations. *Bioresource Technology Reports* 21: 101332.

Rech K C., Guereschi R M., de Oliveira Nuner A P., Sticca S C. (2013). Density and organic matter influence on egg-laying and hatching of *Branchiura sowerbyi* (Oligochaeta). *Journal of the World Aquaculture Society* 44(2): 267-272.

Redeker E S., Blust, R. (2004). Accumulation and toxicity of cadmium in the aquatic oligochaete *Tubifex tubifex*: a kinetic modeling approach. *Environmental Science & Technology* 38(2): 537-543.

Reynoldson T B. (1987). The role of environmental factors in the ecology of tubificid oligochaetes-an experimental study. *Ecography* 10(4): 241-248.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Reynoldson T B., Thompson S P., Bamsey J L. (1991). A sediment bioassay using the tubificid oligochaete worm *Tubifex tubifex*. *Environmental Toxicology and Chemistry: An International Journal* 10(8): 1061-1072.

Rodriguez P., Fend S V. (2018). On spermatophore-producing aquatic microdrile oligochaetes (Annelida: Clitellata). *Zootaxa* 4497(1): 41-60.

Rodriguez P., Martinez-Madrid M., Arrate J A., Navarro E. (2001). Selective feeding by the aquatic oligochaete *Tubifex tubifex* (Tubificidae, Clitellata). *Hydrobiologia* 463: 133-140.

Rossi A M., Saidel W M., Marotta R., Saglam N., Shain D H. (2013). Operculum ultrastructure in leech cocoons. *Journal of Morphology* 274(8): 940-946.

Roy S., Johnson C., Bayen S., Mohanty T R., Ray A., Bhor M., Swain H S., Das B K. (2022). Macrobenthic pollution bioindicator for ecological monitoring in riverine ecosystem. *International Journal Environment Science & Natural Resources* 29(5): 556273.

Royer W E., Sharma H., Strand K., Knapp J E., Bhyravbhatla B. (2006). *Lumbricus erythrocruorin* at 3.5 Å resolution: architecture of a megadalton respiratory complex. *Structure* 14(7): 1167-1177.

Şahin S K., Yıldız S. (2011). Species distribution of oligochaetes related to environmental parameters in Lake Sapanca (Marmara Region, Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* 11(3): 359-366.

Satoh T. (2005). Toxicological implications of esterases-from molecular structures to functions. *Toxicology and Applied Pharmacology* 207(2): 11-18.

Schlupp I. (2005). The evolutionary ecology of gynogenesis. *Annual Review of Ecology Evolution and Systematics* 36(1): 399-417.

Schmelz R M., Collado R. (2010). A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). *Soil Organisms* 82(1): 1-176.

Scopetani C., Esterhuizen M., Cincinelli A., Pflugmacher S. (2020). Microplastics exposure causes negligible effects on the oxidative response enzymes glutathione reductase and peroxidase in the oligochaete *Tubifex tubifex. Toxics* 8(1): 14.

Sharma P., Chukwuka A V., Chatterjee S., Bhowmick S., Mistri T K., Saha N C. (2024). Fluorene-induced stress in the benthic oligochaete *Tubifex tubifex*: A multi-biomarker assessment of toxicological pathways and mechanisms under acute and subchronic exposures. *Chemosphere* 352: 141412.

Shimizu T. (2020). Oviposition behaviour in the clitellate annelid *Tubifex tubifex*: video recordings of mature worms under laboratory culture conditions. *Invertebrate Reproduction & Development* 64(3): 188-200.

Shimizu T., Nakamoto A. (2001). Segmentation in annelids: cellular and molecular basis for metameric body plan. *Zoological Science* 18(3): 285-298.

Simangunsong T., Anjaini J., Situmorang N., Liu, C H. (2023). The latest application of Tubifex as live feed in aquaculture. *Journal of Environmental Engineering and Sustainable Technology* 10(2): 112-121.

Simangunsong T., Anjaini J., Soedibya P H T., Liu C H. (2024). Utilization of Tubifex worms as natural feed for growth and development of fish larvae. *Journal of Environmental Engineering and Sustainable Technology* 11(1): 33-43.

Şimşek A., Teuchies J., Haghnazar H., Blust R., Bakan G. (2023). Evaluation of bioaccumulation and toxicity of *Tubifex tubifex* exposed to contaminated river sediment by potentially toxic elements-A case study of the Middle Black Sea, Turkey. *Journal of Geochemical Exploration* 252: 107263.

Singh R K., Chavan S L., Sapkale P H. (2007). Heavy metal concentrations in water, sediments and body tissues of red worm (*Tubifex* spp.) collected from natural habitats in Mumbai, India. *Environmental Monitoring and Assessment* 129(1): 471-481.

Singh R K., Vartak V R., Chavan S L., Desai A S., Khandagale P., Sawant B., Sapkale P. (2010). Management of waste organic matters and residential used water for culture and biomass production of red worm (*Tubifex tubifex*). *International Journal of Environment and Waste Management* 5(1-2): 140-151.

Singhal S S., Singh S P., Singhal P., Horne D., Singhal J., Awasthi S. (2015). Antioxidant role of glutathione S-transferases: 4-Hydroxynonenal, a key molecule in stress-mediated signaling. *Toxicology and Applied Pharmacology* 289(3): 361-370.

Singleton D R., Hendrix P F., Coleman D C., Whitman W B. (2003). Identification of uncultured bacteria tightly associated with the intestine of the earthworm *Lumbricus rubellus* (Lumbricidae; Oligochaeta). *Soil Biology and Biochemistry* 35(12): 1547-1555.

Sinha R K., Agarwal S., Chauhan K., Valani D. (2010a). The wonders of earthworms & its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers. *Agricultural Sciences* 1(2): 76.

Sinha R K., Herat S., Bharambe G., Brahambhatt A. (2010b). Vermistabilization of sewage sludge (biosolids) by earthworms: converting a potential biohazard destined for landfill disposal

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

into a pathogen-free, nutritive and safe biofertilizer for farms. *Waste Management & Research* 28(10): 872-881.

Solang J., Pangkey H., Wullur S., Lantu S. (2014). Ratio of C: N in culture media of silk worm, *Tubifex* sp. *Aquatic Science & Management* 2(1): 19-23.

Starunov V V., Dray N., Belikova E V., Kerner P., Vervoort M., Balavoine G. (2015). A metameric origin for the annelid pygidium?. *BMC Evolutionary Biology* 15: 1-17.

Suthar S. (2009). Potential of *Allolobophora parva* (Oligochaeta) in vermicomposting. *Bioresource Technology* 100(24): 6422-6427.

Swati A., Hait S. (2017). Fate and bioavailability of heavy metals during vermicomposting of various organic wastes-A review. *Process Safety and Environmental Protection* 109: 30-45.

Takahashi H., Shimizu T., Aruga J. (2008). Expression pattern of annelid Zic in embryonic development of the oligochaete *Tubifex tubifex*. *Development Genes and Evolution* 218: 553-560.

Taufik A., Warganegara N. (2013). Bioremediasi cacing merah (*Tubifex tubifex*) dalam pengolahan air limbah. *Jurnal Riset Kesehatan Poltekkes Depkes Bandung* 6(1): 61-66.

Thit A., Banta G T., Palmqvist A., Selck H. (2020). Effects of sediment-associated Cu on *Tubifex tubifex*–Insights gained by standard ecotoxicological and novel, but simple, bioturbation endpoints. *Environmental Pollution* 266: 115251.

Tilic E., Bartolomaeus T. (2016). Structure, function and cell dynamics during chaetogenesis of abdominal uncini in *Sabellaria alveolata* (Sabellariidae, Annelida). *Zoological Letters* 2(1): 1-18.

Timm T. (2012). Life forms in Oligochaeta: a literature review. *Zoology in the Middle East* 58(4): 71-82.

Trentin E., Facco D B., Hammerschmitt R K., Ferreira P A A., Morsch L., Belles S W., Ricachenevsky F K., Nicoloso F T., Ceretta C A., Tiecher T L., Tarouco C P., Berghetti A L P., Toselli M., Brunetto, G. (2019). Potential of vermicompost and limestone in reducing copper toxicity in young grapevines grown in Cu-contaminated vineyard soil. *Chemosphere* 226: 421-430.

Trevor J H. (1977). The burrowing of *Nereis diversicolor* O.F Müller, together with some observations on *Arenicola marina* (L.) (Annelida: Polychaeta). *Journal of Experimental Marine Biology and Ecology* 30(2): 129-145.

Urbisz A Z., Chajec Ł., Świątek P. (2015). The ovary of *Tubifex tubifex* (Clitellata, Naididae, Tubificinae) is composed of one, huge germ-line cyst that is enriched with cytoskeletal components. *PLoS One* 10(5): e0126173.

van de Bund W J., Goedkoop W., Johnson R K. (1994). Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. *Journal of the North American Benthological Society* 13(4): 532-539.

Vijverberg K., Ozias-Akins P., Schranz M E. (2019). Identifying and engineering genes for parthenogenesis in plants. *Frontiers in Plant Science* 10: 128.

Vytlačilová J., Chobot V., Jahodář L., Laakso I., Vuorela P. (2004). *Tubifex tubifex* Müll-photosensitive organism. *Central European Journal of Public Health* 12: S89-S93.

Walczyńska K S., Zhu L., Liang Y. (2023). Insights into the role of the Wnt signaling pathway in the regeneration of animal model systems. *The International Journal of Developmental Biology* 67(3): 65-78.

Wang H., Erséus C. (2003). Marine species of Ainudrilus and Heterodrilus (Oligochaeta: Tubificidae: Rhyacodrilinae) from Hainan Island in southern China. *New Zealand Journal of Marine and Freshwater Research* 37(1): 205-217.

Wang F., Zhang W., Miao L., Ji T., Wang Y., Zhang H., Ding Y., Zhu W. (2021). The effects of vermicompost and shell powder addition on Cd bioavailability, enzyme activity and bacterial community in Cd-contaminated soil: A field study. *Ecotoxicology and Environmental Safety* 215: 112163.

Weber R E., Vinogradov S N. (2001). Nonvertebrate hemoglobins: functions and molecular adaptations. *Physiological Reviews* 81: 569-628.

Welch P S. (1921). Bifurcation in the embryos of Tubifex. *The Biological Bulletin* 41(4): 188-202.

Widiastuti I M., Hertika A M S., Musa M., Arfiati D. (2019). Mercury absorption in *Tubifex* sp. worm contaminated with metal washing waste. *Pollution Research* 38(3): 575-583.

Woodin S A., Merz R A. (1987). Holding on by their hooks: anchors for worms. *Evolution* 41(2): 427-432.

Yanar M., Yanar Y., Genç M A. (2003). *Tubifex tubifex* Müller, 1774 (Annelidae)'in Besin Kompozisyonu. *Ege Journal of Fisheries and Aquatic Sciences* 20(1-2): 103-110.

Yang J., Li Q., An Y., Zhang M., Du J., Chen C., Zhao R, Zhao D, An S. (2020a). The improvement of pollutant removal efficiency in saturated vertical flow constructed wetlands by *Tubifex tubifex. Bioresource Technology* 318: 124202.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)

Yang J., Wan Y., Cao Z., Zhang M., Zheng F., Leng X., Zhao D., An S. (2020b). Enhanced organic matter decomposition in sediment by *Tubifex tubifex* and its pathway. *Journal of Environmental Management* 275: 111281.

Yang J., Wan Y., Zhang M., Cao Z., Leng X., Zhao D., An S. (2021). Accelerated nitrogen consumption in sediment by *Tubifex tubifex* and its significance in eutrophic sediment remediation. *Environmental Pollution* 272: 115925.

Yang J., Zhang M., Chen C., Zhao D., Chen Y., An S. (2023). Effect of *Tubifex tubifex* on the purification function of saturated vertical flow constructed wetlands for effluents with varying C/N ratios. *Chemosphere* 340: 139872.

Yazid M., Djunaidah I S., Nurhudah M. (2022). Growth performance of silkworms (*Tubifex* sp.) cultivated using a dry substrate. *Jurnal Perikanan Universitas Gadjah Mada* 24(2): 101-107.

Yıldız S., Ustaoğlu M R., Balik S. (2007). The Oligochaeta (Annelida) Fauna of Yuvarlak Stream (Köyceğiz-Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* 7(1): 1-6.

Zattara E E., Bely A E. (2015). Fine taxonomic sampling of nervous systems within Naididae (Annelida: Clitellata) reveals evolutionary lability and revised homologies of annelid neural components. *Frontiers in Zoology* 12: 1-21.

Zhang P., Selck H., Tangaa S R., Pang C., Zhao B. (2017). Bioaccumulation and effects of sediment-associated gold-and graphene oxide nanoparticles on *Tubifex tubifex*. *Journal of Environmental Sciences* 51: 138-145.

DOI:https//doi.org/10.22452/mjs.vol45no1.9
Malaysian Journal of Science 45(1): 103-127 (March 2026)