

DIVERSITY, DISTRIBUTION AND ABUNDANCE OF SOIL MOLLUSCS IN UiTM NEGERI SEMBILAN FOREST RESERVE

Dayang Nor Shafiqah Awang Shukeran, Nursyazni Abdul Rahim*

*School of Biological Sciences, Faculty of Applied Sciences
Universiti Teknologi MARA (UiTM), Cawangan Negeri Sembilan, Kampus Kuala Pilah, 72000 Kuala Pilah, Negeri Sembilan, Malaysia*

*Corresponding author: syaznirahim@uitm.edu.my

Abstract

Soil molluscs have long been used as bioindicators due to their high distribution and functional importance in terrestrial ecosystems. However, their distribution is poorly documented and there is insufficient research about soil molluscs studies. Hence, a study was conducted to establish their current status. The present study aimed to identify the diversity and distribution of soil molluscs in UiTM Negeri Sembilan Forest Reserve. Live snails and dead shells were collected from sixteen 10m x 10m sampling plots of quadrats at three different sites to determine the abundance of this land snail. Sampling was carried out by combining visual observation and sorting-sieving soil and debris samples. The collected samples were preserved in 70% ethanol for later identification in the laboratory. A total of 16 individuals representing six species of three known families were recorded, namely Achatinidae, Ariophantidae, and Camaenidae. Achatinidae had dominated all of three sites with 56% of total samples collected. The statistical analysis showed site A (forest area) had the highest diversity index ($H' = 1.213$) and site B (forest margin) showed the highest evenness index ($E = 0.982$), whereas site C (water tank) had the lowest diversity index ($H' = 0.562$), evenness index ($E = 0.811$) and richness index ($R = 0.721$). One-way ANOVA was also performed on their morphometric variables which resulted in the existence of significant differences on the shell length and width ($p < 0.05$) while weight had no significant differences for all species at all sites ($p > 0.05$). The findings emphasized the importance for long-term studies and further research has to be done in order to gather additional information about the diversity of soil molluscs in this region.

Keywords: soil molluscs; diversity; abundance; bioindicator; UiTM

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Introduction

Phylum Mollusca is the phylum of invertebrate animals and the second largest phylum with over 100,000 species which make them one of the most diverse phyla in the animal kingdom, second only to arthropods in terms of species diversity, but with a significantly greater body plan (Kumar, 2020). There are four major groups within the phylum Mollusca which are Class Cephalopoda, Gastropoda, Bivalvia and Polyplacophora. Class Gastropoda, which is mainly known as terrestrial or soil molluscs, commonly snails and slugs, form the largest group of living molluscs, with over 35,000 species occurring in various environments (Waki, 2017).

Gastropods can be found almost everywhere in marine, terrestrial and freshwater areas (Baharuddin *et al.*, 2018). According to Kumar (2020), gastropods constitute one of the most varied types of animals, comprising more than half of all named molluscs and the most threatened by extinction (Belhiouani *et al.*, 2018). They are commonly found on the ground or in leaf litter, and frequently aggregated under plants (Brand *et al.*, 2020; Liew *et al.*, 2021). Apart from that, most terrestrial mollusc's species prefer a moist environment and are usually found in microhabitats such as under logs, rocks, coarse woody debris, and moist areas (Geiger *et al.*, 2017; Hong *et al.*, 2020).

Research on the diversity of terrestrial molluscs has increased in recent decades in which it interests researchers to study these animals as they play a vital role in forest ecosystems (Jerome *et al.*, 2019). A study by Parmar *et al.* (2016) stated that soil molluscs, particularly snails, act as a biological indicator for defining soil quality, determining the ecosystem’s damage and monitoring pollution. This is due to the fact that snails having the ability to pile up xenobiotics in their tissues, in which xenobiotic is a chemical compound that are foreign to animal life, such as plant constituents, pharmaceuticals, pesticides, cosmetics, flavorings, scents, food additives, industrial chemicals and environmental contaminants (Patterson *et al.*, 2010).

In addition, knowledge in soil molluscs is poorly documented at UiTM Forest Reserve. In this area, there are insufficient data regarding these species, and no studies have been carried out to determine the species abundance and their diversity at a few different sites. Hence, this study is carried out to document the diversity and distribution of soil molluscs in UiTM Negeri Sembilan Forest Reserve. The objectives of this study are to identify the soil molluscs species based on morphological features and to determine their diversity and distribution at three different areas in UiTM Forest Reserve. This research will be beneficial for those who want to explore UiTM Negeri Sembilan Forest Reserve which may provide preliminary information towards conservation and preservation efforts on ecosystem health and forest resources as a need for sustainable development.

Methods

Study Area

The study of diversity, distribution and abundance of soil molluscs was conducted in UiTM Negeri Sembilan, Kuala Pilah Campus (UiTMKP) (Figure 1). Kuala Pilah Campus is located in Mukim Parit Tinggi, situated about eight kilometers from Kuala Pilah town and is known to have a humid climate (Noormi *et al.*, 2018) that may lead to the potential abundance of soil molluscs. Field surveys and samplings were carried out at three different sites based on the accessibility and suitability of soil molluscs (Figure 2).

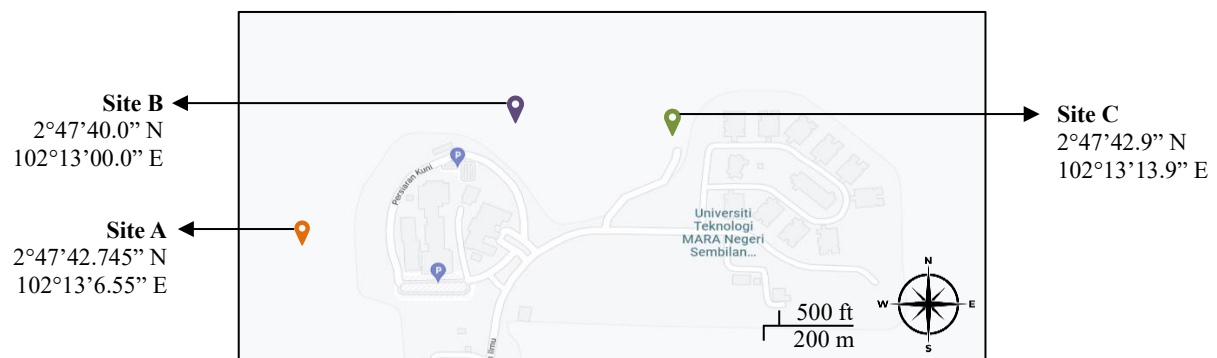


Figure 1. Plots of study area, which is labelled as A, B, and C (Google Map, 2022)



Figure 2. (a) Site A: Forest Area, (b) Site B: Forest Margin, (c) Site C: Water Tank

Sample collection, sorting-sieving, and preservation

The collection method involved direct visual searching, quadrat and bulk sampling. The samples from visual searching method were collected from natural different habitats such as dead snail shells and live snails and slugs that live on the tree trunks, on the vegetation, in crops, under the stone, within the litter, on the branches, and on fallen wood (Zaidi *et al.*, 2021). The quadrat technique was done by randomly projecting a grid onto a map of the study area of 10m x 10m sampling sites spaced at least two meters apart along a transect (Figure 3) following Durkan *et al.* (2013) with modifications. These were followed by bulk sampling by collecting three liters of leaf litter and one centimeter of topsoil within quadrats into a canvas or polythene bag (Utz *et al.*, 2018). Then, the litter samples underwent a sieving method particularly three sieves with mesh sizes of decreasing size (8 mm to 1 mm), for sorting into their respective species identification. The live snails and slugs were preserved in 70% ethanol in the laboratory, while the dead snail shells were kept in dry labelled vials (Wafula, 2019).

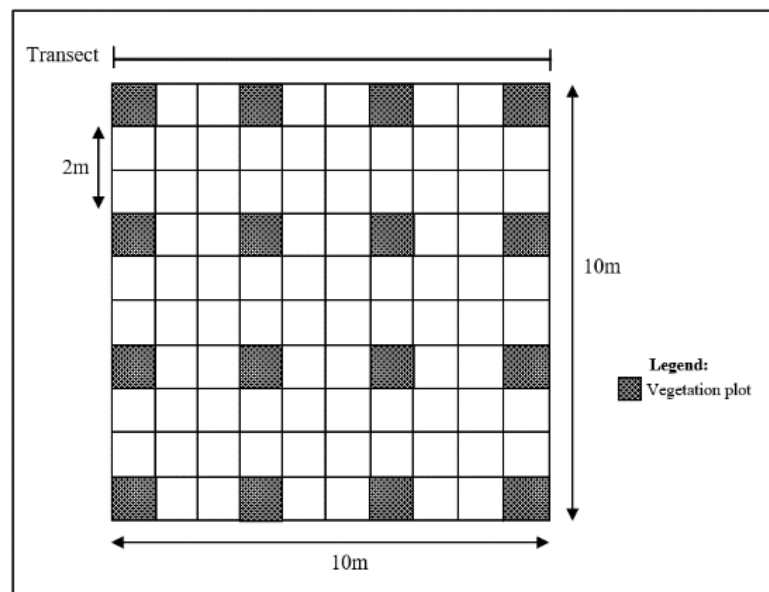


Figure 3. Transects and locations of vegetation sampling (Area: 10m²)

Species identification

The samples were identified up to the species level and all data obtained were recorded. All individuals were identified to their lowest taxonomic level based on the method and key features as previously published by Oke (2013), Carpenter & De Angelis (2016), Nurinsiyah & Hausdorf (2017), and Nurinsiyah *et al.* (2019). The identification key features were done through biometric measurements, such as body weight of individuals, diameter and height, number of spatial arrangements, their umbilicus whether it is perforate, imperforate, rimate or implicate, colour, teeth, spiral bands, and genital tracts (Douafer & Soltani, 2014; Okeke *et al.*, 2016). The species of soil molluscs that have been identified were recorded and used for statistical analysis.

Data analysis

The data were recorded and analyzed using Shannon Diversity Index, Shannon Evenness Index, Margalef Richness Index, and Analysis of Variance. Shannon Diversity Index (H) was used to indicate the degree of species composition for each site whereas Shannon Evenness Index (E) was used to determine the pattern of distribution of species in a relationship to other species in a sample per unit area (Halim *et al.*, 2019). Margalef Richness Index (R) was used to indicate the number of species in a sample or abundance of the species per unit area (Zaidi *et al.*, 2021) while one-way ANOVA was used for their morphometric measure to compare means of different samples in terms of the shell length, width and weight.

Result and Discussion

Composition of soil molluscs

The sampling of soil molluscs has been done three times a week for a month. The sampling was done from 8.00 a.m. until 11 a.m. In this study, a total number of 16 individuals were collected and identified from three different sites (Table 1). Taxonomic composition showed three families that represent four genera and six species including *Achatina fulica*, *Hemiplecta cymatium*, *Hemiplecta buettikoferi*, *Bradybaena similaris*, *Macrochlamys malaccana* and one unidentified species by observing the morphology. The highest species found from three different sites was *A. fulica* with a total number of 9 individuals and the lowest species found were *B. similaris*, followed by *H. cymatium* and unidentified species with a total number of one individual, respectively. Among all three sites, site A yielded the highest number of individuals, followed by site C and site B. The distribution of soil molluscs species based on sites is presented in Table 1 while the images of species found in the entire plot is presented in Figure 4.

Table 1. Sites distribution of soil molluscs

Site	Family	Species	No. of individuals
A	Achatinidae	<i>Achatina fulica</i>	4
	Ariophantidae	<i>Macrochlamys malaccana</i>	2
		<i>Hemiplecta buettikoferi</i>	1
		<i>Hemiplecta cymatium</i>	1
B	Achatinidae	<i>Achatina fulica</i>	2
	Camaenidae	<i>Bradybaena similaris</i>	1
	unID	-	1
C	Achatinidae	<i>Achatina fulica</i>	3
	Ariophantidae	<i>Hemiplecta buettikoferi</i>	1
Total			16

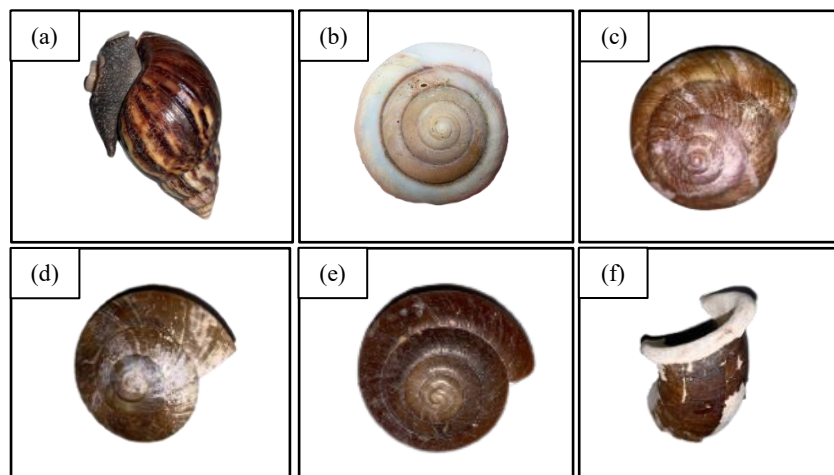


Figure 4. Collected soil molluscs species such as (a) *Achatina fulica*, (b) *Bradybaena similaris*, (c) *Hemiplecta buettikoferi*, (d) *Hemiplecta cymatium*, (e) *Macrochlamys malaccana*, (f) unID

Based on the findings, site A recorded the most diverse site with family Achatinidae dominating the most individuals, in which in the forest area, it is the least disturbed area and there were no anthropogenic activities found that can harm the environment (Nurhayati *et al.*, 2021). The species identified at site A were *Achatina fulica*, *Macrochlamys malaccana*, *Hemiplecta buettikoferi* and *Hemiplecta cymatium*. Among all sites, *A. fulica* dominated the most and represented about 56% of the total species abundance (Figure 5).

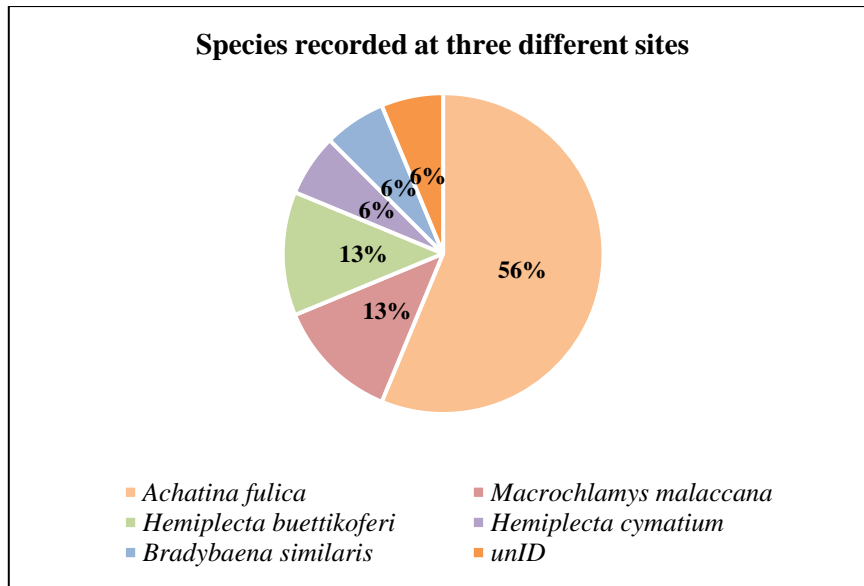


Figure 5. Percentages of total species recorded at three different sites

Similar findings were collected by Jerome *et al.* (2019) on the diversity of the terrestrial molluscs of an Urban Secondary Forest in which in which family Achatinidae dominated the most individuals and the most abundant family with 61.57% of their specimens, consisted of *Achatina fulica*, *Archachatina ventricosa* and *Limnicolaria flammea*. The abundance of these species can be explained in part by the fact that the forest provides them with ideal conditions for reproduction (Karamoko *et al.*, 2009). In addition, Jerome *et al.* (2019) also stated that human activities such as sightseeing and educational activities in the forest did not seem to disturb these species since they have been adapted to human presence and even fed on rejected foods by humans (Karamoko *et al.*, 2009). According to a study done by Albuquerque *et al.* (2019), he found out that *A. fulica* is a moisture dependent animal, in which they favour a damp habitat, such as forests, considering UiTM Forest Reserve has a moist environment (Noormi *et al.*, 2018). These such factors contributed to them being the most abundant family in this study.

Statistical analysis of soil molluscs

Shannon Diversity Index, Shannon Evenness Index and Margalef Richness Index were applied to analyze the diversity of the soil molluscs in UiTM Negeri Sembilan Forest Reserve from the different sites. The diversity index was performed to determine the value of taxon richness (Lim *et al.*, 2018). Based on Table 2, the highest diversity index value was found in site A ($H' = 1.213$), followed by site B ($H' = 1.079$) and site C ($H' = 0.562$). Site A shows a low to moderate diversity value and it has the greatest number in terms of diversity compared to those two because it has the largest number of individuals collected since a large number of species can increase diversity. The higher the value of H' , the higher the diversity of species in a particular community (Halim *et al.*, 2019). As for site C, it has a quite high number of individuals and low number of species which could be an indicator of the low diversity in the area (Nurhayati *et al.*, 2021). This is supported by the value of Shannon's diversity index of 0.562.

Less canopy cover and more human activities in site C are likely to contribute to this species diversity difference (Nurhayati *et al.*, 2021). Unlike site A and site B, it is an area that is covered with deciduous forest and is not too exposed to sunlight. The only difference between these two sites is site B is close to the pond and marshes which make the environment a bit warm (Phoong *et al.*, 2018). Meanwhile, site C includes many short trees and vegetation such as shrubs and various flowering plants that are exposed to sunlight. According to Baharuddin *et al.* (2018), gastropods that are exposed to direct sunlight in a longer period may lead to desiccation that could harm them. As a consequence, site C area has less canopy coverage compared to site A. In addition, site C is located near to the college and faculty buildings area that could lead to habitat disturbance which could influence the abundance of soil

molluscs around the area (Zaidi *et al.*, 2021).

Table 2. Diversity (H'), Evenness (E) and Richness (R) Index of soil molluscs at three sites

Site	Total Number of Individuals	Shannon Diversity Index (H')	Shannon Evenness Index, (E)	Margalef Richness Index (R)
A (Forest Area)	8	1.213	0.875	1.443
B (Forest Margin)	4	1.079	0.982	1.443
C (Water Tank)	4	0.562	0.811	0.721

The evenness index was performed to assess the degree of species equality in a community (Belhiouani *et al.*, 2018). As for E value as shown in Table 2, site B has the highest evenness index (E=0.982), followed by site A (E=0.875) and site C (0.811). Site B has a high index that indicates a value closest to 1, in which 1 represents complete evenness (Baharuddin *et al.*, 2018). As for site A and site C, the distribution is less even compared to site B due to the omnipresence of the dominant species *A. fulica*, the most abundant species in those areas with rates of 62.5% and 75.0%, respectively (Belhiouani *et al.*, 2018). However, their values are somewhat evenly numbered within sampling quadrats as both values are closer to 1. Site B with the diversity index of H'=1.079, and has high evenness implies the lack of dominance by specific gastropod taxa (Jahid & Singh, 2018). Similar findings were collected through a study done by Belhiouani *et al.* (2018) on terrestrial gastropod diversity on the Northeast Algeria, in which the omnipresence of the most abundant species, *Ms. vermiculata* lead to the unevenness at their two sampling sites, which are Ibn Badis and Djebel Hamimat. Indeed, the presence or absence of gastropods that lead to the evenness could be related with several environmental conditions such as climate parameters and soil properties (Liew *et al.*, 2021).

As for R value in Table 2, site A and site B have the same richness index of R=1.443 as compared to site C (R=0.721). Lower species found in site C indicates lower species richness as they are correlated with one another (Halim *et al.*, 2019). This is also correlated with the soil physicochemical characteristics (Douafer & Soltani, 2014), plant communities (Zaidi *et al.*, 2021), climatic factors (Ameur *et al.*, 2019), anthropogenic disturbances and vegetation (Belhiouani *et al.*, 2018) that determine the richness of soil molluscs in the sampling sites. Apart from the effect on species richness, the type of substrates also influences the abundance of gastropods (Suwannatrai *et al.*, 2011). Among all sites, they have slight differences in soil type. Site C has the lowest richness index as this site has the sandy type of soil, composed of a very coarse sand. Low density of soil molluscs in sand substrate is due to their inability to attach in the substrate (Zala *et al.*, 2018). The substrate condition influences the development of biotic communities, where sediment with a little clay is a desirable substrate for gastropods (Kabir *et al.*, 2014). As compared to site A and site B, they comprise of the soil with fine particles and wetter soils. Site A and B are also near to rock bases in which the suitability of rocks as habitats is primarily attributed to their complexity such as overhangs and crevices that provide shelter as well as food for the diverse gastropod species (Madin *et al.*, 2021). Apart from that, site A and site B have widespread of leaf litter composition that affect the snail abundance and abundance of logs which also have a positive effect on species richness (Gheoca *et al.*, 2021) as they provide an important source of calcium which benefits them for their shell formation (Dong *et al.*, 2020).

According to Gheoca *et al.* (2021), habitat characteristics affect both snail diversity, abundance, and species richness. The presence of leaf litter, logs and deadwood are important factors in determining their diversity. Based on a study conducted by Lim *et al.* (2018), most of their specimens are found on the forest floor, hidden within the leaf litter or up on the trees, and on the underside of leaves.

Morphometric analysis of soil molluscs

One-way Analysis of Variance (ANOVA) test was applied in order to compare means of sizes such as shell length, shell width and weight for all species of different study sites. The threshold value for the statistical significance was taken as $p < 0.05$. The statistical test was done using SPSS Version 20. Table 3 below shows the summary for one-way analysis of variance for morphometric variables.

Table 3. Summary of one-way analysis of variance for morphometric variables

Morphology variables		Sum of Squares	df	Mean Square	F	Sig.
Shell Length	Between Groups	93.600	5	18.720	7.752	.003
	Within Groups	24.149	10	2.415		
	Total	117.749	15			
Shell Width	Between Groups	15.354	5	3.071	17.598	.000
	Within Groups	1.745	10	.174		
	Total	17.099	15			
Weight	Between Groups	3851.440	5	770.288	3.035	.064
	Within Groups	2537.946	10	253.795		
	Total	6389.386	15			

Source: SPSS Version 20 (IBM, USA).

Based on Table 3, the measured morphologies differed significantly among all species at the three sampling sites for gastropods shell length and width as their significance value is less than the threshold value ($p < 0.05$), which means the null hypothesis (H_0), which is denoted as no difference in means (Kim, 2017) is rejected and the test is statistically significant. Hence, there was a significant difference in the morphology of all species for shell length and width among the three sampling sites. However, gastropod weight had no significant differences between the species of all sites since the significance value is greater than the threshold value ($p > 0.05$), which means the null hypothesis is accepted and the test is not statistically significant. Hence, there was no significant difference in their weight at all sites.

According to a study done by Matos *et al.* (2020), the shell width appears to be related with their foot size, in which larger shell openings will allow for the maintenance of the foot that results in greater adhesion to the substrate (Ito *et al.*, 2002). Larger shells tend to increase the soil molluscs capacity for water reservation and allow for more resistance to desiccation (Matos *et al.*, 2020). Apart from that, their sizes could be directly influenced by the latitude (Linse *et al.*, 2006) and this is also associated with body temperature (Chapperon *et al.*, 2013; Seuront & Ng, 2016), density-dependent intraspecific competition and food availability (Matos *et al.*, 2020).

Indeed, shell shapes and sizes vary greatly between sites and habitats in response to environmental factors such as temperature in order for them to suit themselves to inhabit the habitat (Matos *et al.*, 2020). Their variation of sizes within species tend to increase in body size with increasing latitude or elevation (Olson *et al.*, 2009).

Conclusion

In conclusion, the soil molluscs diversity, distribution, and abundance in UiTM Negeri Sembilan Forest Reserve were successfully identified and recorded at three different study sites which were site A (forest area), site B (forest margin) and site C (water tank). A total of 16 individuals which represent three known families and five known species and one unknown species of soil molluscs were found throughout this study. Site A represents the highest number of individuals with eight individuals followed by site B and site C with four individuals collected, respectively. Overall, site A shows the highest diversity index and site B shows the highest evenness index. Meanwhile, site C has the lowest diversity index, evenness index and richness index. As for ANOVA analysis, there were significant differences in their means between the shell length and width of gastropods at all sites while weight had no significant differences in their means. Lastly, it is recommended to expand the area of study for in depth and breadth of the soil mollusc's distribution and population dynamic which will be helpful to reflect the composition and abundance of soil molluscs in UiTM Negeri Sembilan Forest Reserve more realistically.

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Author Contribution

Shukeran, D. N. S. A. - collecting data, data processing and analysis, manuscript writing; Rahim, N. A. - supervision, manuscript writing, review and editing.

Conflict of Interest

Authors declare no conflict of interest.

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