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Fish Community Structure and Environmental Correlates in Nepal's Andhi Khola, Province No. 4, Syangja

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ABSTRACT

The study of correlations between fish diversity, environmental variables and fish habitat aspects at different space and time scales of Nepal's rivers and streams is scanty. This study investigated spatial and temporal patterns of fish assemblage structure in Nepal's Andhi Khola. The field survey was conducted from September 2018 to May 2019 and the fishes were sampled from three sites using a medium size cast net of mesh size ranging from 1.5 to 2.5 cm and gill net having 2-3 cm mesh size, 30-35 feet length and 3-4 feet width, with the help of local fisher man. A total of 907 individuals representing 15 species belonged to four orders, six families and 11 genera were recorded during the study time. To detect the feasible relationships between fish community structure and environmental variables, we executed a Canonical Correspondence Analysis (CCA). Based on similarity percentage (SIMPER) analysis, the major contributing species are *Barilius barila* (26.15%), *Barilius vagra* (20.48%), *Mastacembelus armatus* (8.04%), *Puntius terio* (6.64%), and *Barilius bendelisis* (5.94%). One-way analysis similarity (ANOSIM) tried out for both time and space variations in fish community structure suggested that there was a significant difference in temporal variation (R = 0.794, P = 0.0037) but no significant difference in spatial variation (R = -0.18, P = 0.923). Results from the Canonical Correspondence Analysis (CCA) vindicated that dissolved oxygen, free carbon-dioxide and total hardness were the principle physio-chemical correlates of fish assemblage structure. One-way analysis of similarity (ANOSIM) on the Non-metric Multidimensional Scaling (NMDS) showed significant difference between spring, autumn and winter season but no significant difference was found in spatial variation. The extraction and transportation of boulders, cobbles, pebbles, sand mining, haphazard ongoing road development and dam construction for the irrigation without fish ladders were found to be existing threats to the fish diversity of Andhi Khola.

Keywords: Fish assemblage, multivariate analysis, rivers, spatio-temporal, streams

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INTRODUCTION

The recognition of dissimilarity patterns in rivulet residence fish community down with possible contributory causes is a main issue in rivulet ecology (Matthews, 1998). Numerous research has shown that factors affecting fish community require the physiochemical environment, which is spatially diversified and seasonally differ, and living interactions such as competition and predation (Gorman, 1988; Harvey & Stewart, 1991; Grossman *et al.*, 1998). Among different environmental parameters, the dissolved oxygen (DO) and temperature are most important for fish and highly effect on fish diversity and distribution. The amount of dissolved oxygen and temperature are higher in summer than winter due to which fish diversity is higher in summer (Adoni, 1985). Along the headwater-downstream longitudinal gradient, fish community often incident increase in species richness and abundance, mostly evolving from an increase in habitat complication and diversity variation in the rate of fish migration and disappearance (Matthews, 1986). However, an asymptote or reduce in species richness is also noticed in the lower reaches of some creeks, which could be due to greater pollution levels (Oberdorff, 1993). Small brooks and creeks also indicate an ecological slope, along which upstream structures are relatively diversified but downstream assemblages are comparatively stable.

This is related with the common model of upstream environments being naturally variable and skeletally simple while downstream environments are the opposite. Riparian or creek fishes of the developing world have accepted to differentecological stresses affecting fish community structure of that habitat (Grossman, 1990). However, alliance between fishes and their environments play an imperative role for controlling and economizing of riparian species where any modification of it can lead to modify in their population (Kadye & Moyo, 2007). The goal of this study was to characterize fish community patterns in space and time and to investigate fish community structure relationships with the environmental parameters of Andhi Khola.

MATERIALS AND METHODS

Study Area

Andhi Khola (Figure 1) is a glacier-fed permanent river located in western region of Nepal. It is a main river of Syangja district and has its origin from Dahare hill, southeast from Karkineta. It is estimated to be 96 km long with a catchment area of 195 km^2, which finally drain to Kali Gandaki. It is located between 27°50'N and 28°10′N latitude, and 83°50′E and 84°50′E longitude with altitudes from 540 to 1020 m above sea level, covering an area of about 200 km^2. It flows southwards and joins Kaligandaki River near Mirmi, Syanjaa. The river basin is used for irrigation, drinking water supply, recreation and micro-hydropower generation.

Sampling Sites

For the present investigation, the field work was conducted from September 2018 to May 2019 with three seasons: autumn (September, October and November), winter (December, January and February), and spring (March, April and May). Fishes were sampled from three sites using a medium size cast net of mesh size ranging from 1.5 to 2.5cm and Gill net having 2-3 cm mesh size, 30-35 feet length and 3-4 feet width, with the help of local fisher man. These fishing gears were operated within 500 m area of each site for 2 hours in each station at 7-9 am. Total 40 hurls were made for cast net and 4 hauls for gill net to catch fishes.

For estimation of abundance of fishes, two pass removal methods (Seber, 1967) were used. Each removal pass includes moving first upstream/river then downstream/river within a pre-determined length (500 m) with equal effort 30 minutes for each pass at each site of the river. The number of fish species in the samples and the number of individuals in each species were counted and the local name of fish species was taken from local fishermen. The collected fish samples were photographed and were preserved in 10% formalin solution in plastic jars and brought to Central Department of Zoology (CDZ) lab, Tribhuvan University, Kirtipur, Kathmandu, Nepal for further identification. The identification was done by using standard taxonomic references (Talwar & Jhingran, 1991; Jayaram, 2010).

Analysis of Environmental Variables

Water samples of Andhi Khola were collected during morning time (7:00 am to 9:00 am) and analyzed once every three months during field visit. Water temperature was appraised with a mercury thermometer by putting it down in the water at a depth of one feet. The pH was measured by using a calibrated pH meter (HANNA Instrument, HI 98107). The dissolved oxygen was measured by the Winkler titra-metric method. The sample of water from every sampling site was collected in 300 ml BOD bottle without bubbling. Then, 2 ml of $MnSO_4$ and 2 ml of KI was poured gently from the side of the bottle, then this mixture was shaken well so as to complete the reaction and the sample was left half an hour for the settlement of the precipitates. About 2 ml of concentrated H_2SO_4 was added in the solution to dissolve the brown precipitate settled at the bottom. Sodium thiosulphate (0.025 N) was taken in the burette rinsed by the solution for titration. About 50ml of the mixture was taken on the conical flask and one or two drips of starch solution were added as indicator. Then, the titration was done against the sodium thiosulphate solution, till the solution become colourless. Total hardness (mg/l) was determined using EDTA titrametric method.

Data Analysis

The correlation between fish community structure

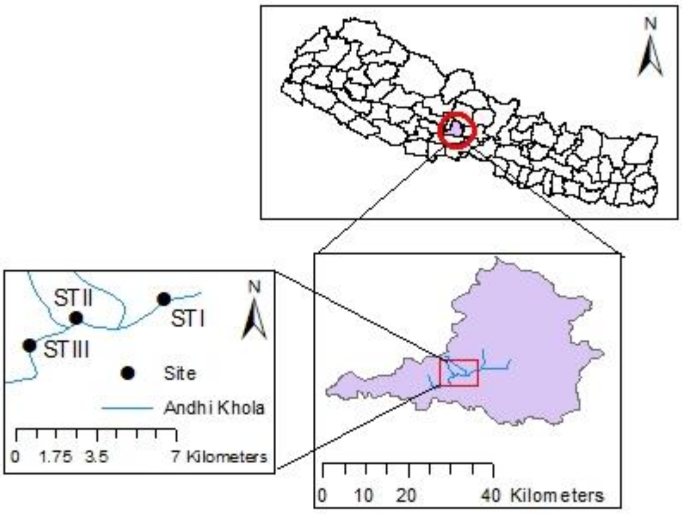

Figure 1. Map of study area showing Andhi Khola

and environmental variables was first done by selection of appropriate tests a Detrended correspondence analysis (DCA). The axis length and eigen value acquired from DCA suggested that the linear model of Canonical Correspondence Analaysis (CCA) was more applicable. Therefore, a direct multivariate ordination method (TerBraak, 1986) based on a linear response of species to environmental gradients was applied by using vegan library in R (Oksanen, 2015). One-way analysis of similarity (ANOSIM) was used to conclude the significance of space and time variation of fish community structure (Clarke, 1993). Similarity percentages analysis (SIMPER) was executed to notice the percentage of similarity in temporal scale (Clarke, 1993). The relationships amongst assemblages from each site and season are graphically represented using pvcluster analysis and non-metric Multi-Dimensional Scaling analysis (NMDS) (Clarke & Warwick, 2001).

RESULTS

A total of 907 individuals representing 15 species belonged to four orders, six families and 11 genera were recorded during the study period (Table 1). The order Cypriniformes was found to be dominated order which comprised 73.33% followed by Siluriformes 13.33%, Synbranchiformes 6.67% and Perciformes 6.67% (Figure 2). The Cyprinidae was the most species–rich family (5 genera and 8 species) followed by Cobitidae (2 genera and 3 species), Heteropneustidae, Sisoridae, Mastacembelidae and Channidae with single genus and species (Table 1, Figure 3).

The highest number of individuals was reported from station II and in spring season, whereas the lowest was found from station I and in autumn season (Table 2). Based on similarity percentage

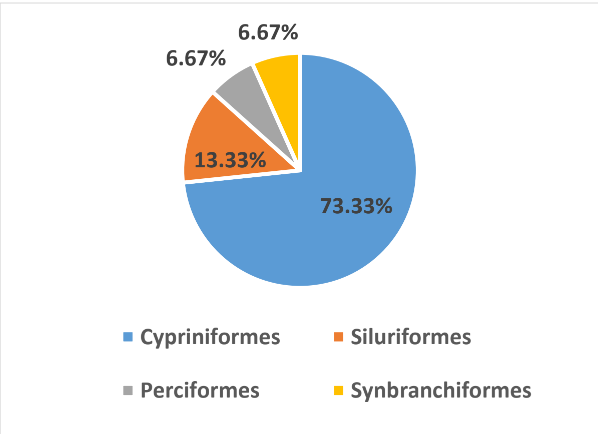

Figure 2. Order wise percentage compositions of fishes of Andhi Khola

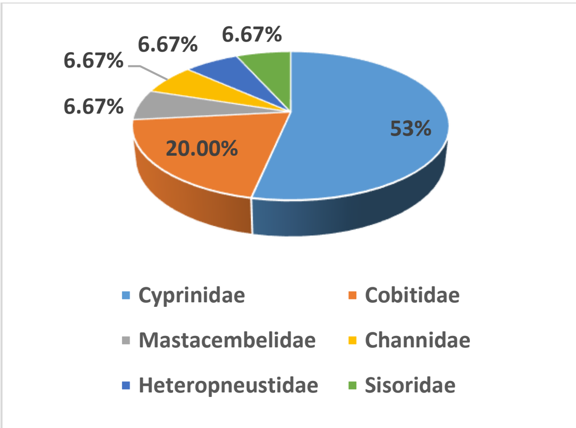

Figure 3. Family wise percentage compositions of fishes of Andhi Khola

Table 1. List of fishes collected from AndhiKhola

Order	**Family**	**Species**
Cypriniformes	Cyprinidae	*Barilius barila* (Hamilton-Buchanan, 1822)
		Barilius vagra (Hamilton-Buchanan, 1822)
		Barilius bendelisis (Hamilton-Buchanan, 1822)
		Neolissochilus hexagonolepis (MCclelland, 1839)
		Puntius terio (Hmailton, 1822)
		Schizothorax plagiostomus Hackel, 1838
		Garra mullya (Hamilton, 1822)
		Garra rupecula (MCclelland, 1839)
	Cobitidae	*Schistura horai* (Menon, 1952)
		Schistura sovana (Hamilton, 1822)
		Lepidocephalus guntea (Hmailton, 1822)
Siluriformes	Heteropneustidae	*Heteropneustes fossilis* (Bloch, 1794)
	Sisoridae	*Glyptothorax trilineatus* Blyth, 1860
Synbranchiformes	Mastacembelidae	*Mastacembelus armatus* (Lacepede, 1800)
Perciformes	Channidae	*Channa punctatus* (Bloch, 1793)

Table 2. Spatial and temporal species abundance and distribution

Code	Species	Total	Station I	Station II	StationIII	Aut	Win	Spr
C1	*Barilius barila*	320	87	133	100	58	119	143
C2	*Barilius vagra*	248	67	98	83	40	98	110
C3	*Barilius bendelisis*	33	13	14	6	0	15	18
C4	*Lepidocephalus guntea*	7	2	5	0	0	0	7
C5	*Garra rupecula*	32	11	14	7	6	5	21
C6	*Garra mullya*	36	16	13	7	11	8	17
C7	*Neolissochilus hexagonolepis*	26	12	9	5	0	11	15
C8	*Puntius terio*	32	5	16	11	3	2	27
C9	*Glyptothorax trilineatus*	25	7	13	5	1	10	14
C10	*Schistura horai*	21	8	9	4	10	3	8
C11	*Schistura savona*	25	7	10	8	6	3	16
C12	*Heteropneustes fossilis*	2	0	0	2	0	0	2
C13	*Channa puctatus*	13	0	5	8	13	0	0
C14	*Schizothorax plagiostomus*	22	5	12	5	6	16	0
C15	*Mastacembelus armatus*	65	14	29	22	11	13	41
Total		907	254	380	273	165	303	439

Table 3. Average similarity and discriminating fish using SIMPER analysis

Code	**Species**	**Contribution (%)**	**Code**	**Species**	**Contribution (%)**
C1	*Barilius barila*	26.15	C9	*Glyptothorax trilineatus*	4.007
C2	*Barilius vagra*	20.48	C13	*Channa puctatus*	4.004
C15	*Mastacembelus armatus*	8.04	C11	*Schistura savona*	3.502
C8	*Puntius terio*	6.645	C6	*Garra mullya*	2.96
C3	*Barilius bendelisis*	5.94	C10	*Schistura horai*	2.652
C7	*Neolissochilus hexagonolepis*	4.86	C4	*Lepidocephalus guntea*	1.651
C14	*Schizothorax plagiostomus*	4.29	C12	*Heteropneustes fossilis*	0.5432
C5	*Garra rupecula*	4.25			

(SIMPER) analysis the major contributing species were *Barilius barila* (26.15%), *Barilius vagra* (20.48%), *Mastacembelus armatus* (8.04%), *Puntius terio* (6.64%), and *Barilius bendelisis* (5.94%) (Table 3). One-way analysis similarity (ANOSIM) tried out for both space and time variations in fish community suggested that there was a significant difference in temporal variation ($R = 0.794$, $P = 0.0037$) but there was no significant difference in spatial variation (R = -0.18, P = 0.923).

Correlation between Fish Assemblage Structure and Environmental Variables

The Canonical correspondence (CCA) analysis showed that the fish species of *Barilius bendelisis* (C3), *Gara mullayu* (C6), *Neolissochilus hexagonolepis* (C7) and *Glyptothorax trilineatus* (C9) were positively linked to pH but negatively connected to total hardness. Fish species of *Barilius barila* (C1), *Barilius vagra* (C2) and *Schizothorax plagiostomus* (C14) showed positive

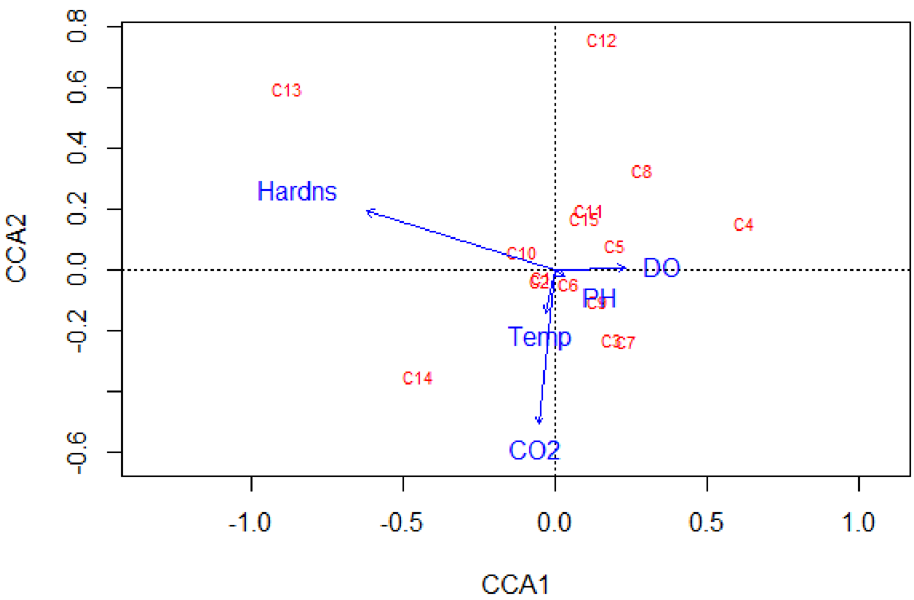

Figure 4. Canonical Correspondence Analysis (CCA) ordination of fish assemblages and environmental variables of AndhiKhola (Hardns= hardness, DO = dissolved oxygen, Temp = water temperature and CO_2 = free carbon-dioxide, for species code, please refer Table 1)

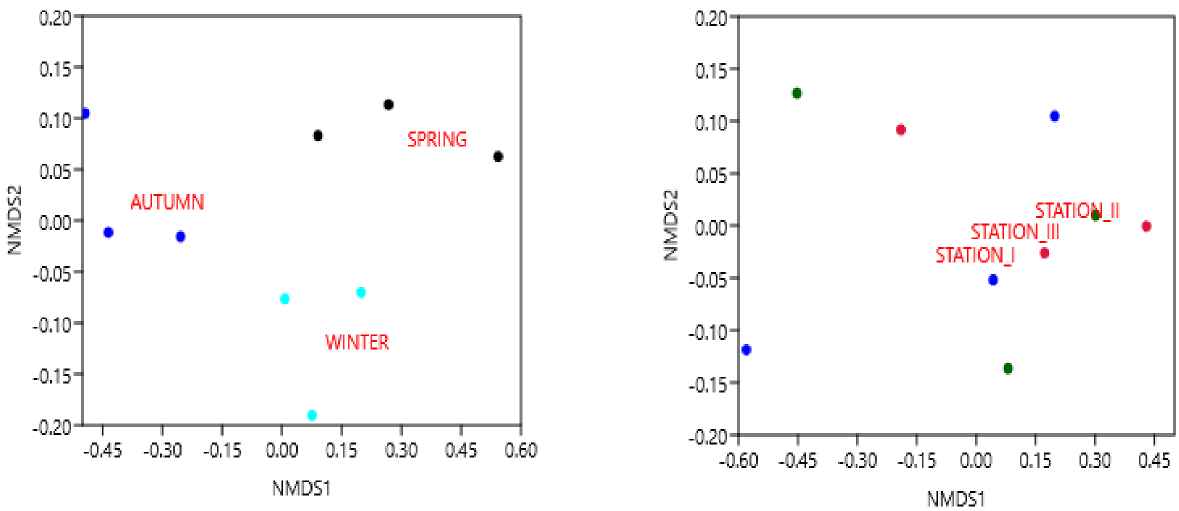

Figure 5. (a) NMDS ordination of temporal variation of fish assemblage in Andhi Khola, **(b)** NMDS ordination of spatial variation of fish assemblage in Andhi Khola

relation with the water temperature and free carbon-dioxide. Fish species of *Schistura horai* (C10) and *Channa punctatus* (C13) were positively related to total hardness but negatively related to pH. In contrast, fish species of *Lepidocephalus guntea* (C4), *Garra rupecula* (C5), *Puntius terio* (C8), *Schistura Savona* (C11), *Heteropneustes fossilis* (C12) and *Mastacembelus armatus* (C15) were positively associated with DO but negatively linked to water temperature and free carbon-dioxide (Figure 4). One-way analysis of similarity (ANOSIM) on the NMDS showed differences in species between spring, autumn and winter season but no significance difference was found in spatial variation (Figure 5a and b).

DISCUSSION

A total of 15 fish species were reported during the study period. Among them, similarity percentage (SIMPER) analysis indicated that, the fish species of *Barilius barila*, *Barilius vagra*, *Mastacembelus armatus*, *Puntius terio*, *Barilius bendelisis*, *Neolissochilus hexagonolepis*, *Schizothorax plagiostomus*, *Garra rupecula*, *Glyptothorax trilineatus*, *Channa puctatus*, *Schistura Savona*, *Garra mullya*, *Schistura horai*, and *Lepidocephalus guntea*, each contributing more than 1% of the total fish composition. From Andhi Khola, the Cypriniformes was recorded as a dominant order which comprised 73.33% of the total fish composition. This is closer to the findings of Edds (1986), Limbu and Gupta (2018), Limbu *et al.* (2018), Limbu *et al.* (2019a), Limbu *et al.* (2019b) and Limbu *et al.* (2019c). Nelson (2007) also indicated that the majority of the fishes from the river fall under the order Cypriniformes, this is the huge order of freshwater fishes which includes 2,422 species.

The Cyprinidae was recorded as a most species rich family with 8 species. The majority of the previous literature also showed, family Cyprinidae is the species rich family (Shrestha *et al.*, 2009; Limbu *et al.*, 2019c; Limbu *et al.*, 2018, Limbu *et al.*, 2019b and Subba *et al.*, 2017; Pokharel, 2018; Oli, 2016). One-way analysis similarity (ANOSIM) try out for both space and time variations in fish community structure suggested that there was a significant difference in temporal variation (R = 0.794, P = 0.0037) but no significant difference was observed in spatial variation (R = -0.18, P = 0.923) which is inconsistent with the

Effects of environmental parameters on species distributions were checked by Canonical Correspondence (CCA) analysis. Species detected close by the genesis either do not depict a powerful association to any of the parameters or are found at average values of environmental parameters (Marshall, 1998). In the present study, dissolved oxygen, hardness, and free carbon-dioxide were found to be an important parameter to shape the fish community structure of Andhi Khola. Water temperature (Kadye *et al*., 2007) and dissolved oxygen (Limbu *et al.*, 2019b) have already been shown to affect the fish community. Besides these, current velocity (Yu & Lee, 2002), depth (Vlach *et al.*, 2005; Kadye *et al*., 2007), width (Gerhard *et al.*, 2004), substrate (Vlach *et al.*, 2005), altitude (Magalhaes *et al.*, 2002), conductivity (Yu & Lee, 2002) and climate (Magalhaes *et al.*, 2002) have all been also shown to influence fish community structure. The fish species of *Barilius barila*, *Barilius vagra*, *Barilius bendelisis, and Mastacembelus armatus* were ubiquitously found whereas *Lepidocephalus guntae* and *Heteropneustes fossilis* were sporadically found. Moreover, *Channa sps*, *Schizothoraxsps*, *Lepidocephalus guntae* and *Heteropneustes fossilis* have been declining their number from Nepal's rivers and streams. According to local fishermen, fish species of *Channa stewartii*, *Macrognathus pancalus*, *Garra sps* and *Puntius sps* have been severely depleted from Andhi Khola and were not found in our collection too. It might be due to the anthropogenic impacts (habitat destruction, electro-fishing, ongoing hydro-project development, haphazard road development, urbanization and dam construction), and climate change. Sand mining and extraction of boulders, cobbles and pebbles are frequent in this river. So, we concluded that these activities are responsible for declining the fish diversity of Andhi Khola. Therefore, for the better monitor, management and conservation of Nepal's indigenous fish species, habitat rehabilitation, and construction of fish ladders/passage are necessarily needed. Moreover, extraction and transportation of boulders, cobbles, pebbles and sand mining are also stringently stopped in ordered to avoid the habitat destruction of aquatic fauna and flora including fish.

CONCLUSION

To conclude, the Canonical Correspondence Analysis (CCA) indicated that the total hardness, free carbon-dioxide and dissolved oxygen act as a driving variables to shape the fish community structure of Andhi Khola. The extraction and transportation of boulders, cobbles, pebbles, sand mining haphazard ongoing road development and dam construction for the irrigation without fish ladders were found to be existing threats to the fish diversity of Andhi Khola. *Lepidocephalus guntea*, and H*eteropneustes fossilis* were found to be at an alarming state for Andhi Khola. Moreover, population of *Channa stewartii*, *Macrognathus pancalus*, *Garra spp.* and *Puntius spp.* have been severely depleted and are not recorded in the present study from Andhi Khola. So, for the better protection and conservation of these species, habitat rehabilitation, and construction of fish ladders are necessarily needed. In addition, extraction and transportation of boulders, cobbles, pebbles and sand mining should also be stringently stopped in order to avoid the habitat destruction of aquatic fauna and flora including fish.

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Spatial and Temporal Variation of Fish Assemblages in Seti Gandaki River, Tanahu, Nepal

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ABSTRACT

The space and time variations of the fish community structure in hill streams of Nepal are poorly understood. This research aims at studying the space and time variation of fish community structure in the Seti Gandaki River, Tanahu, Nepal. The field survey was conducted from July 2017 to June 2018 and the fishes were sampled from six sites using a medium size cast net of mesh size ranging from 3 mm to 6 mm mesh size, 25-33 feet length and 3.5-5 feet width, with the help of local fisher man. A total of 1,440 individuals were caught representing 46 species belonging to three order, nine families and 23 genera. The analysis of similarity (ANOSIM) showed significant difference in space (R = 0.824, P = 0.001) but not in time (R = 0.135, P = 0.021). On the basis of similarity percentage (SIMPER) analysis, 85.43% similarity was found among the seasons and major contributing species were *Barilius bendelisis* (8.44%) followed by *B. vagra* (7.79%), *Tor putitora* (7.27%), *Garra gotyla* (7%), *Acanthocobotis botia* (6.7%), *Neolissochilus hexagonolepis* (6.64%), *Barilius shacra* (6%), *B. barila* (4.5%) and *Opsarius barna* (4.37%). On the other hand, 85.24% similarity was found among the sites and major contributing species were *B. bendelisis* (8.8%) followed by *B. vagra* (7.6%), *G. gotyla* (7.27%), *T. putitora* (7.17%), *A. botia* (6.97%), *N. hexagonolepis* (6.7%), *B. shacra* (6.34%), *B. barila* (4.7%) and *O. barna* (4.39%). Results from the Canonical Correspondence Analysis indicated that the environmental variables, such as pH, total hardness, alkalinity, dissolved oxygen and water temperature have shown to determine the fish community structure of Seti Gandaki River.

Keywords: Fish diversity, freshwater, habitat, spatio-temporal, stream

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INTRODUCTION

The physical-chemical environmental parameter influences the fish community structure, which are spatially different and temporally diverse, and biotic interactions such as competition and predation (Gorman, 1988; Harvey & Stewart, 1991; Grossman *et al.*, 1998). Habitat variables such as water temperature, depth (Kadye *et al.*, 2008), water velocity (Yu & Lee, 2002; Limbu & Prasad, 2020), stream width (Gerhard *et al.*, 2004), substrate, altitude, conductivity (Yu & Lee, 2002; Kadye *et al.*, 2008), dissolved oxygen, pH, free-carbon dioxide (Limbu *et al.*, 2019b) and climate (Magalhaes *et al.*, 2002) have all been shown to affect fish community. However, changing environmental parameters can affect biotic communities in multiple ways and function of ecosystems (McGill *et al.*, 2006; Conversi *et al*., 2015). Environmental variables are reported to shape the spatial distribution of species (Perry *et al*., 2005) and influence the temporal variation of communities (Rouyer *et al*., 2008).

The space and time variations of the fish community structure in the rivers and streams of Nepal are poorly understood (Limbu & Gupta, 2019). Some related studies done in the Nepal's rivers include Edds (1986), Shrestha *et al*. (2009), Shah (2016), Shrestha (2016). Subba *et al*., (2017), Limbu *et al*. (2018b; 2019a; 2019b). However, these studies did not mention, which factors (physio-chemical factors, current velocity, substrate composition, stream width, water temperature, water volume, etc.) contribute most to fish community variations. Some aspects of the fisheries and fish ecological studies such as their diversity, space and time distribution and abundance in rivers of Nepal are needed (Mishra & Baniya, 2016). To better monitor, manage and conserve, and to know their status of the fisheries, there is an urgent need to update the information on the spatial and temporal fish diversity, community structure and distribution patterns (Ngor *et al.*, 2018). Therefore, the present study was conducted to determine spatial and temporal variation of fish assemblages with environmental correlates in Seti Gandaki River, Tanahu, Nepal.

MATERIALS AND METHODS

Study Area

The present study area, the Seti Gandaki River is situated in Western Nepal, which rises from the base of the Annapurna massif and surges through south and south-east part of Pokhara and Damauli which finally joins with Trisuli River near Devghat.

To study fish and environmental parameters, six sampling sites of A, B, C, D, E and F (Figure 1) were allocated along the sampling stretch of the Seti Gandaki River. The sampling site A was selected at upstream of Bhimad Bazar (Changthandi confluence to 2 km upstream). The second sampling site B was chosen near confluence spot of Seti and Jidi Khola (downstream at Bhimad Bazar). The third sampling site C was selected at confluence spot of Seti and Phedi Khola. The sampling site D was selected at dam site of Seti Gandaki River. The sampling site E and site F were chosen at confluence spot of Seti and Madi Khola and downstream of proposed powerhouse in Seti River (Table 1).

Sampling

Sampling was conducted four times a year covering all seasons (winter in January, spring in April, summer in June and autumn in October) over one year of 2018. In this study, each sampling site was 200-250 m long and fish agglomeration was done approximately 2 hours by cast net at each sampling site. The fishes were sampled using cast net of mesh size ranging from 3 mm to 6 mm mesh size, 25-33 feet length and 3.5-5 feet width, with the help of local fisherman. Before preservation, collected fishes were photographed with Nikon Digital Camera (D5600, DX, 24.2 megapixels, Japan). After photography, about 10% collected fishes were

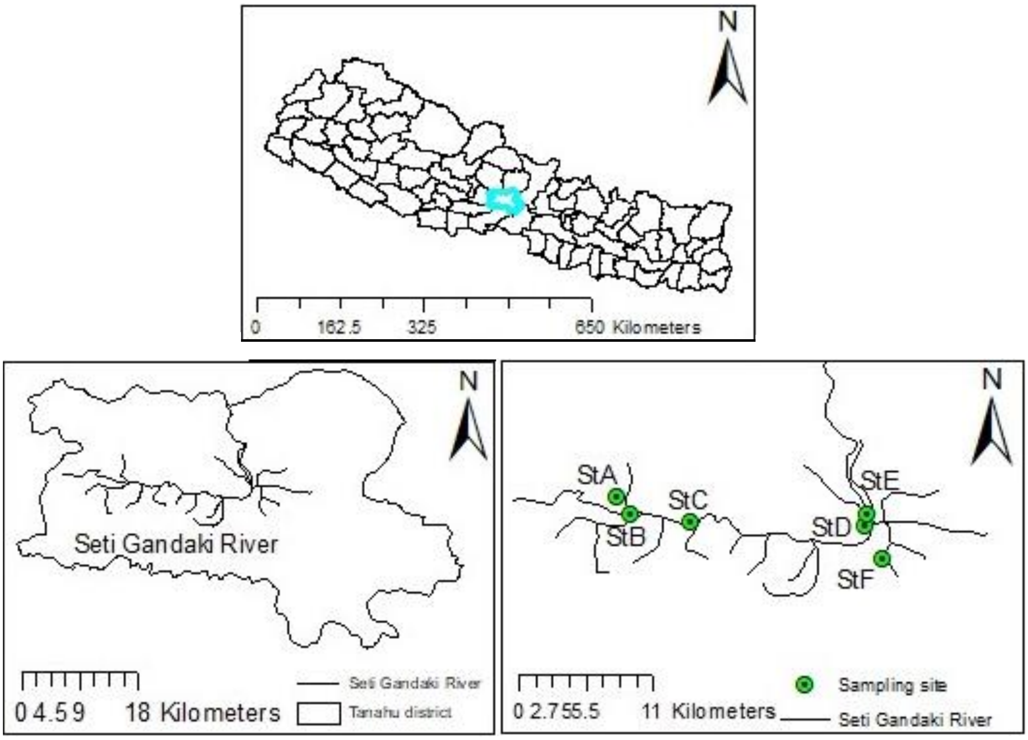

Figure 1. Sampling sites in Seti Gandaki River

Table 1. Information of different stations

Sites	Sampling spot	Location	GPS Location
A	Before Reservoir in Seti river	Myagde and Bhimad, Tanahun	27°59.9"N 84°04.56"E
B	Confluence of Seti and Jidi khola	Bhimad, Tanahun	27°58.26"N 84°5.34"E
C	Confluence of Seti and Phedi khola	Bhimad, Tanahun	27°58.3"N 84°8.10''E
D	Proposed Dam site in Seti River	Patan, Damauli	27°57.52''N 84°15.54''E
E	Confluence of Seti and Madi	Byas	27°58.25''N 84°15.57''E
F	Downstream of proposed Power house in Seti river	Damauli	27°56.25''N 84°16.40''E

preserved in 10% formaldehyde solution in plastic jar by making their head upside for the protection of their caudal fin and remaining samples were returned to their own natural habitat from where they were captured. Afterwards, preserved specimens were taken to the laboratory of the Central Department of Zoology (CDZ), Tribhuvan University, Institute of Science and Technology, Kirtipur, Kathmandu, Nepal. The identification was carried out with the help of standard taxonomic references (Talwar & Jhingran, 1991; Jayaram, 2010).

The following environmental variables were analyzed during each field visit: water temperature, dissolved oxygen (DO), pH, hardness, and alkalinity. Water temperature (ºC) was measured with a digital thermometer (Hanna, HI98501, UK) by placing it in the water at a depth of one feet. The DO (mg/l) was measured by the Winkler titra-metric method, while pH was measured using a pH meter (HI98107, Hanna Instrument, UK). Total hardness (mg/l) was determined using EDTA titrametric method. To determine alkalinity water sample of 10 ml was taken in a conical flask and one drop of phenolphthalein was added to it and mixed well. Bromocresol Green-methyl Red (1 packet) was added to it and stirred properly. It was then titrated with sulfuric acid and end point was recorded.

Data Analysis

The Shannon-Weiner diversity (Shannon & Weaver, 1963) was calculated by the following Eq. (1):

$$H = \sum_{i=1}^{S} Pi * logPi \quad (1)$$

Where S is the total number of species and Pi is the relative cover of i_{th} of species.

Margalef index (d) (Margalef, 1968) was used to measure species richness by using the following Eq. (2):

$$d = (S/1) = \log(N) \quad (2)$$

where S is the total species and N is total individuals.

The dominance index (Harper, 1999) was calculated by using the following Eq. (3):

$$D = \sum_i \left(\frac{ni}{n}\right)^2 \quad (3)$$

Where n_i is number of individuals of species i.

One-way analysis of similarities (ANOSIM) (Clarke, 1993) was used to test the significant difference among the space and time scales. To visualize the major contributing species both to space and time, similarity percentage (SIMPER) analysis was performed (Clarke, 1993). The correlation between fish community structure and environmental variables were first done using Detrended correspondence analysis (DCA). The axis length (≥ 2.5) and eigen value (≥ 0.5) acquired from DCA suggested that the linear model of Canonical Correspondence Analysis (CCA) was more applicable. Therefore, a direct multivariate ordination method (Ter Braak, 1986) based on a linear response of species to environmental gradients was applied by using vegan library in R (Oksanen, 2015).

RESULTS AND DISCUSSION

A total of 1,440 fish individuals were collected in the Seti Gandaki River, representing 46 fish species belonging to four orders, nine families and 23 genera (Table 2 and 3). Among the four orders, Cypriniformes was found to be the most dominating order with 33 species (71.7%), followed by Siluriformes with 10 species (21.7%), Perciformes with two species (4.34%) and Synbranchiformes with a single species (2.17%). Gautam *et al.* (2016) and Pokharel (2011) also reported that Cypriniformes as the dominant order in terms of both species composition, and individuals captured. Besides that, Jha (2006) reported 18 fish species, environment impact assessment of Upper Seti Hydropower Project (Nepal Electricity Authority/ Tanahu Hydropower Limited, 2012) reported 36 fish species, and Pokharel *et al*. (2018) reported 30 fish species, belonging to five orders, nine families and 24 genera from Seti Gandaki River.

The diversity in terms of number (46 species) observed in the present study was 16 species greater than Pokharel *et al*. (2018). It might be due to the limited study areas covered in the earlier report. Cypriniformes and Cyprinidae were the most abundant and species rich order and family, respectively. This is in consistent with the findings of previous studies reported from different rivers and streams of Nepal. For example, Shrestha *et al*. (2009), Shrestha (2016), Mishra and Baniya (2016), Subba *et al.* (2017), Limbu *et al.* (2018a), Limbu *et al.* (2019a, 2019b), and Limbu and Prasad (2020) from Tamor, Triyuga, Dewmai, Melamchi, Morang district, Damak, Ratuwa, Eastern Nepal and Nuwa River. Nelson (2007) also indicated that the majority of the fishes from the river fall under the order Cypriniformes, the huge order of freshwater fishes, which includes 2,422 species.

In terms of temporal variation of fish assemblages, fish species *Chagunius chagunio, Neolissochilus hexagonolepis, Tor putitora, Puntius sophore, Barilius barna, Barilius shacra, Garra gotyla, Acanthocobitis botia* and *Nemacheilus corica* were recorded from all four seasons (Table 2). The present finding is in consistent with previous studies (Limbu *et al.*, 2018b; Limbu *et al.*, 2019a; 2019b). Species like *Glyptothorax indicus* and *Nangra viridescens* were recorded from summer only. The highest individuals were recorded in summer and lowest in spring.

In terms of spatial variation (Table 3), exotic fish, *Oreochromis niloticus* was recorded from site C and site E. According to the local fishermen, this fish was introduced in different lakes of Pokhara Valley (Fewa Lake and Begnas Lake) and have escaped from there and were found in river. Thapa (2018) also reported that 25.13% of *O. niloticus* in Diplang Lake. It is due to high tolerance capacity of this fish in adverse water quality conditions (Rao, 2017). According to local fishermen, there are large sized *Anguilla bengalensis* in the river, but during study it was not recorded. It may be due to decline in their number or obstruction in their regular migrating pathways due to constructions of different barriers (hydro dams) in the river.

The ANOSIM showed significance difference in space (R = 0.824, p = 0.001), but not in time (R = 0.135, p = 0.021), which is similar to the findings of Yan *et al.* (2010). According to SIMPER analysis, 85.43% similarity was found among the months with major contributing species, *Barilius bendelisis* (8.44%), *Barilius vagra* (7.79%), *T. putitora* (7.27%), *G. gotyla* (7%), *A. botia* (6.7%), *N. hexagonolepis* (6.64%), *B. shacra* (6%), *B. barila* (4.5%) and *Opsarius barna* (4.37%). An 85.24% similarity was found among the sites with major contributing species, *B. bendelisis* (8.8%), followed by *B. vagra* (7.6%), *G. gotyla* (7.27%), *T. putitora* (7.17%), *A. botia* (6.97%), *N. hexagonolepis* (6.93%), *B. shacra* (6.34%), *B. barila* (4.61%) and *O. barna* (4.39%) (Table 4).

Species Diversity

The Shannon-Weiner diversity index of temporal variation ranged in between 1.17 to 1.83. The highest diversity index was recorded during autumn and lowest in winter (Figure 2). In term of spatial variation of diversity index, highest diversity index was found to be at site C and lowest at site D (Figure 3). The evenness index was reported highest in autumn and lowest in winter (Figure 2), whereas highest evenness index was found to be at site D and lowest at site E (Figure 3). There is no significant difference ($p<0.05$) observed for temporal and spatial variation. The highest value of species richness was found in winter and lowest value was found in summer and spring respectively (Figure 2), whereas the highest richness value was reported from site D and lowest value was recorded at site A (Figure 3).

Table 2. Temporal variation of fish assemblages of Seti Gandaki River

Family	Species	Code	Summer	Autumn	Winter	Spring	Total individuals
Cyprinidae	*Chagunius chagunio*	C1	+	+	+	+	30
Cyprinidae	*Neolissochilus hexagonolepis*	C2	+	+	+	+	119
Cyprinidae	*Tor putitora*	C3	+	+	+	+	110
Cyprinidae	*Tor tor*	C4	+	+	-	+	31
Cyprinidae	*Puntius conchonius*	C5	+	+	-	-	14
Cyprinidae	*Puntius guganio*	C6	-	+	-	-	1
Cyprinidae	*Puntius sophore*	C7	+	+	+	+	31
Cyprinidae	*Puntius terio*	C8	+	+	+	-	19
Cyprinidae	*Puntius ticto*	C9	+	-	-	-	32
Cyprinidae	*Labeo dyocheilus*	C10	+	+	-	-	3
Cyprinidae	*Labeo dero*	C11	+	+	+	-	25
Cyprinidae	*Labeo pangusia*	C12	+	+	-	+	6
Cyprinidae	*Aspidoparia morar*	C13	+	-	+	+	18
Cyprinidae	*Barilius barila*	C14	+	+	-	+	61
Cyprinidae	*Opsarius barna*	C15	+	+	+	+	59
Cyprinidae	*Barilius bendelesis*	C16	+	+	+	+	137
Cyprinidae	*Barilius radiolatus*	C17	+	+	-	-	12
Cyprinidae	*Barilius shacra*	C18	+	+	+	+	79
Cyprinidae	*Barilius vagra*	C19	+	-	+	+	127
Cyprinidae	*Brachydanio rerio*	C20	-	+	-	-	1
Cyprinidae	*Esomus danricus*	C21	-	+	-	-	2
Cyprinidae	*Crossocheilus latius*	C22	+	-	+	+	7
Cyprinidae	*Garra annandalei*	C23	+	+	+	-	26
Cyprinidae	*Garra gotyla*	C24	+	+	+	+	108
Cyprinidae	*Garra lamta*	C25	+	+	-	-	29
Cyprinidae	*Garra mullya*	C26	+	+	-	-	14
Balitoridae	*Acanthocobitis botia*	C27	+	+	+	+	120
Cobitidae	*Nemacheilus corica*	C28	+	+	+	+	22
Cobitidae	*Schistura multifasciata*	C29	-	+	-	-	2
Cobitidae	*Schistura savona*	C30	-	+	-	-	3
Cobitidae	*Botia almorhae*	C31	+	+	-	-	10
Cobitidae	*Botia geto*	C32	-	+	-	+	9
Cobitidae	*Botia lohachata*	C33	-	-	-	+	4
Bagaridae	*Mystus bleekeri*	C34	-	+	-	+	3
Schilbeidae	*Clupisoma garua*	C35	+	+	+	-	16
Schilbeidae	*Clupisoma montana*	C36	-	-	-	+	2
Sisoridae	*Bagarius yarrelli*	C37	-	-	+	-	1
Sisoridae	*Glyptothorax alaknandi*	C38	+	-	-	+	19
Sisoridae	*Glyptothorax cavia*	C39	+	+	-	-	2

Table 2. Continue…

Family	Species	Code	Summer	Autumn	Winter	Spring	Total individuals
Sisoridae	*Glyptothorax indicus*	C40	+	-	-	-	1
Sisoridae	*Glyptothorax telchitta*	C41	+	-	-	+	11
Sisoridae	*Nangra viridescens*	C42	+	-	-	-	2
Sisoridaae	*Pseudecheneis sulcatus*	C43	-	+	-	+	9
Mastacembelidae	*Mastacembelus armatus*	C44	+	+	-	+	7
Cichlidae	*Oreochromis niloticus*	C45	+	+	-	-	76
Channidae	*Channa orientalis*	C46	+	+	-	-	20
	Total		**415**	**338**	**374**	**313**	**1,440**

Table 3. Spatial variation of fish assemblages of Seti Gandaki River

Family	Species	Sites						Total individuals
		A	B	C	D	E	F	
Cyprinidae	*Chagunius chagunio*	-	+	-	+	+	+	30
Cyprinidae	*Neolissochilus hexagonolepis*	+	+	+	-	+	+	119
Cyprinidae	*Tor putitora*	+	+	+	+	+	+	110
Cyprinidae	*Tor tor*	+	-	+	+	-	+	31
Cyprinidae	*Puntius conchonius*	+	-	+	-	-	-	14
Cyprinidae	*Puntius guganio*	-	-	+	-	-	-	1
Cyprinidae	*Puntius sophore*	+	+	+	-	-	-	31
Cyprinidae	*Puntius terio*	+	-	-	-	+	-	19
Cyprinidae	*Puntius ticto*	-	-	+	-	+	-	32
Cyprinidae	*Labeo dyocheilus*	-	+	-	+	-	+	3
Cyprinidae	*Labeo dero*	-	+	-	+	+	+	25
Cyprinidae	*Labeo pangusia*	-	+	-	+	+	-	6
Cyprinidae	*Aspidoparia morar*	+	+	-	+	-	-	18
Cyprinidae	*Barilius barila*	+	+	+	+	+	+	61
Cyprinidae	*Barilius barna*	+	+	+	+	+	+	59
Cyprinidae	*Barilius bendelesis*	+	+	+	+	+	+	137
Cyprinidae	*Barilius radiolatus*	-	-	+	-	-	-	12
Cyprinidae	*Barilius shacra*	+	+	+	-	+	+	79
Cyprinidae	*Barilius vagra*	+	+	+	+	_	+	127
Cyprinidae	*Brachydanio rerio*	_	+	_	_	_	_	1
Cyprinidae	*Esomus danricus*	_	_	+	_	_	_	2
Cyprinidae	*Crossocheilus latius*	_	+	_	_	+	+	7
Cyprinidae	*Garra annandalei*	+	+	+	_	_	+	26
Cyprinidae	*Garra gotyla*	+	+	+	+	+	+	108

Table 3. Continue…

Family	Species	Sites						Total individuals
		A	B	C	D	E	F	
Cyprinidae	*Garra lamta*	+	+	_	_	_	_	29
Cyprinidae	*Garra mullya*	_	+	_	_	+	+	14
Balitoridae	*Acanthocobitis botia*	+	+	+	+	+	_	120
Cobitidae	*Nemacheilus corica*	+	+	+	_	_	_	22
Cobitidae	*Schistura multifasciata*	_	_	+	_	_	_	2
Cobitidae	*Schistura savona*	_	_	+	_	_	_	3
Cobitidae	*Botia almorhae*	_	_	_	_	+	+	10
Cobitidae	*Botia geto*	_	_	+	_	+	+	9
Cobitidae	*Botia lohachata*	_	_	_	+	+	_	4
Bagaridae	*Mystus bleekeri*	+	_	_	_	_	_	3
Schilbeidae	*Clupisoma garua*	_	_	+	+	_	+	16
Schilbeidae	*Clupisoma montana*	_	_	_	_	+	+	2
Sisoridae	*Bagarius yarrelli*	_	_	+	_	_	_	1
Sisoridae	*Glyptothorax alaknandi*	+	+	+	_	+	+	19
Sisoridae	*Glyptothorax cavia*	_	_	_	+	_	_	2
Sisoridae	*Glyptothorax garhwali*	_	_	_	_	_	+	1
Sisoridae	*Glyptothorax indicus*	_	_	+	_	+	+	11
Sisoridae	*Nangra viridescens*	_	_	_	+	_	+	2
Sisoridae	*Pseudecheneis sulcatus*	_	_	+	+	_	_	9
Mastacembelidae	*Mastacembelus armatus*	_	+	+	_	+	+	7
Cichlidae	*Oreochromis niloticus*	_	_	+	_	+	_	76
Channidae	*Channa orientalis*	+	_	_	_	+	_	20
	Total	**290**	**167**	**568**	**90**	**170**	**155**	**1,440**

Table 4. Average similarity and discriminating fish in each month and site using SIMPER analysis

Month (85.43%)		**Site (85.24%)**	
Contributory species	%	Contributory species	%
Barilius bendelesis	8.39	*Barilius bendelesis*	8.72
Barilius vagra	7.69	*Barilius vagra*	7.60
Tor putitora	7.27	*Garra gotyla*	7.27
Garra gotyla	7.08	*Tor putitora*	7.17
Acanthocobotis botia	6.85	*Acanthocobitis botia*	6.97
Neolissochilus hexagonolepis	6.54	*Neolissochilus hexagonolepis*	6.93
Barilius shacra	6.08	*Barilius shacra*	6.34
Barilius barila	4.90	*Barilius barila*	4.61
Opsarius barna	4.37	*Opsarius barna*	4.39
Labeo dero	3.80	*Labeo dero*	3.59

Table 4. Continue…

Month (85.43%)		Site (85.24%)	
Contributory species	%	Contributory species	%
Barilius radiolatus	3.14	*Garra lamta*	2.98
Garra lamta	2.96	*Barilius radiolatus*	2.91
Chagunius chagunio	2.89	*Chagunius chagunio*	2.89
Nemacheilus corica	2.51	*Nemacheilus corica*	2.52
Tor tor	2.00	*Tor tor*	2.10
Clupisoma montana	1.92	*Clupisoma montana*	1.99
Aspidoparia morar	1.92	*Aspidoparia morar*	1.92
Pseudecheneis sulcatus	1.729	*Pseudecheneis sulcatus*	1.70
Garra mullya	1.67	*Garra mullya*	1.63
Garra annandalei	1.61	*Garra annandalei*	1.55
Glyptothorax indicus	1.56	*Puntius terio*	1.49
Puntius terio	1.51	*Glyptothorax indicus*	1.48
Botia lohachata	1.50	*Botia lohachata*	1.38
Puntius conchonius	1.24	*Puntius conchonius*	1.25

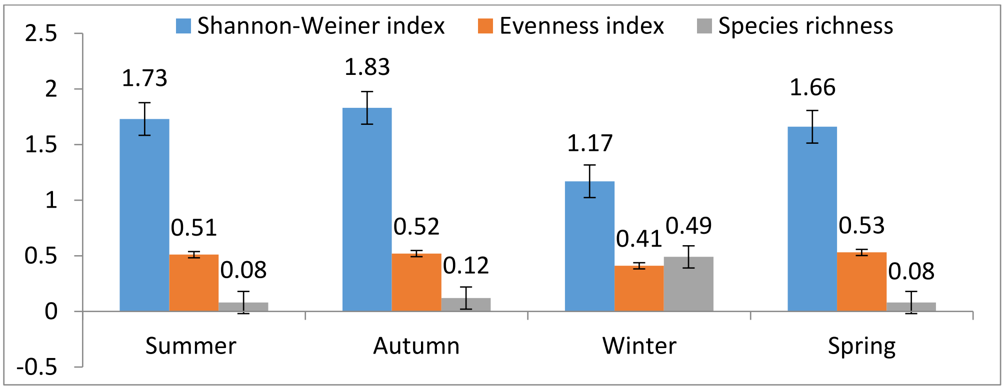

Figure 2. Temporal variation of species diversity index of Seti Gandaki River.

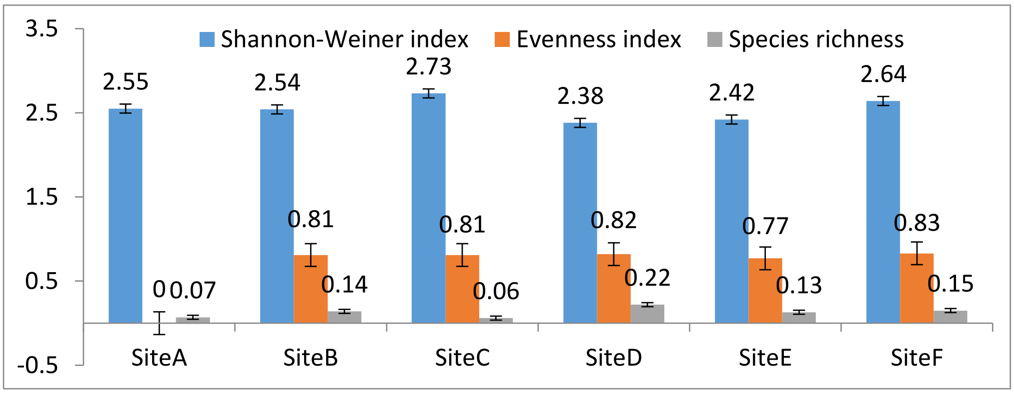

Figure 3. Spatial variation of species diversity index of Seti Gandaki River.

Canonical Correspondence Analysis

The result obtained after the CCA was plotted in Figure 4. The first and second axis of the CCA accounted for 42% and 30%, respectively. The CCA tri-plot indicated the correlation between species and environmental variables. The species of *T. putitora* (C3), *Tor tor* (C4), *P. sophore* (C7), *Puntius ticto* (C9), *Labeo dero* (C11), *Aspidoparia morar* (C13), *B. vagra* (C19), *Crossocheilus latius* (C22), *Botia lohachata* (C33), *Bagarius yarrelli* (C37), and *G. indicus* (C40) were positively related to pH but negatively related to hardness and DO. The fish species of *Chagunius chagunio* (C1), *Puntius terio* (C8), *Garra mullya* (C26), *A. botia* (C27), *Botia geto* (C32), *N. viridescens* (C42), *O. niloticus* (C45) and *Channa orientalis* (C46) were positively related to dissolved oxygen and hardness but negatively related to pH.

In contrast, fish species of *Puntius conchonius* (C5), *Labeo dyocheilus* (C10), *Labeo pangusia* (C12), *Barilius barila* (C14), *O. barna* (C15), *Brachydanio rerio* (C20), *Garra lamta* (C25), *Clupisoma montana* (C36), *Glyptothorax telchitta* (C41) and *Mastacembelus armatus* (C44) were positively related to alkalinity and water temperature. The fish species of *N. hexagonolepis* (C2), *B. bendelesis* (C16), *Barilius radiolatus* (C17), *B. shacra* (C18), *Garra annandalei* (C23), *G. gotyla* (C24), *N. corica* (C28), *Schistura multifasciata* (C29), *Schistura savona* (C30), *T. putitora* (C3) and *Glyptothorax cavia* (C39) were not related to any environmental parameters.

Physical and chemical characteristics are important determinants of the situation of fish community (Li *et al.*, 2012). Results from the CCA indicated that the environmental parameters, such as pH, total hardness, alkalinity, DO and water temperature have shown to determine the fish community structure of Seti Gandaki River. The previous studies, such as (Yu & Lee, 2002; Kadye *et al.*, 2008; Mishra & Baniya, 2016; Limbu *et al.*, 2019a). Limbu and Prasad (2020) have also mentioned that these variables play a crucial role in shaping the fish community structure.

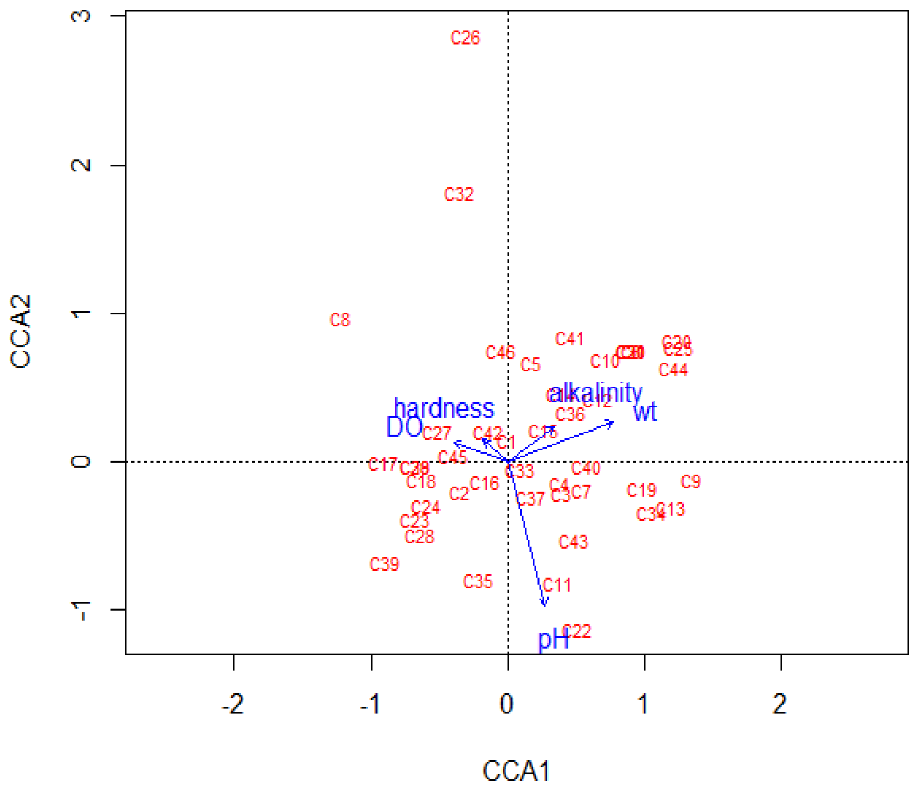

Figure 4. Canonical correspondence analysis (CCA) ordination showing fish species in relation to sites, seasons and environmental variables of Seti Gandaki River (DO = dissolved oxygen, Wt = water temperature) For species code, please refer Table 1.

CONCLUSION

In this study, 46 fish species were reported. Among them *B. bendelisis, followed by B. vagra*, *Tor putitora*, *Garra gotyla*, *Acanthocobotis botia*, *Neolisochilus hexagonolepis*, *B. shacra*, *B. barila and Opsarius barna* were the major contributory fish species reported from the Seti Gandaki River. Results from the Canonical Correspondence Analysis (CCA) ordination indicated that dissolved oxygen (DO), pH, hardness, and water temperature are the pivotal environmental parameters to determine fish community structure in the Seti Gandaki River, Tanahu, Nepal. River dam constructions were found to be major threats to long (for example, *Anguilla bengalensis*, *Bagarius* spp) and short (*Neolissochilus hexagonolepis*) distance migratory fishes. Besides, dynamiting, extraction and transportation of boulders, cobbles, pebbles, sand mining were also found to be existing threats to the fish diversity of Seti Gandaki River. So, for the better protection and conservation of the native species including migratory fish species, habitat rehabilitation, and construction of fish ladders are necessarily needed.

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Preparation and Characterization of Activated Carbon from *Pandanus candelabrum* Stem

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ABSTRACT

Pandanus candelabrum stem, a new precursor, was used to synthesise activate carbon. The effect of sodium hydroxide, NaOH, zinc (II) chloride, $ZnCl_2$ and phosphoric acid, H_3PO_4, different agents on prepared adsorbents was investigated. The adsorbents were prepared with chemical agents and carbonized at 400 ºC for 1 hour. Surface morphology, elemental composition and functional groups were analysed with scanning electron microscopy (SEM), energy dispersive X-ray (EDX), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR), respectively. The image analysis showed the presence of both micropores and mesopores in the adsorbents. The H_3PO_4 activated carbon had the maximum surface area (2648 m^2/g), pore volume (1.683 cm^3/g) and highest adsorption for iodine and methylene blue were 541 and 105 mg/g. *Pandanus candelabrum* stem is an alternative material that can be used to synthesis high porous adsorbent because is abundant, easy to access, inexpensive and readily available.

Keywords: Activated, adsorption, iodine number, methylene blue, *Pandanus candelabrum*, reagents

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INTRODUCTION

The most widely used adsorbent is activated carbon, which has high surface area, adsorptive capacity and is inexpensive (Sahira *et al*., 2013; Yorgun & Yildiz, 2015; Kumar & Jena, 2017). Biomass is a natural carbon source which is used for the preparation of green adsorbents, which have several advantages over non-renewable sources. This renewable biomass is abundant, cheap, environmentally friendly and renewable (Rai *et al*., 2016). The application of biomass wastes for the synthesis of solid adsorbent reduces the impact of environmental pollution (Cadavid *et al*., 2016). One of the popular methods employed in the preparation of activated carbon is the chemical activation process (Zibrik *et al*., 2017). This method entails doping of carbon source with activating reagent, followed by carbonization at chosen temperature values. This will have a significant effect on the carbon matrix morphology resulting to changes in the pore structure arrangement (Nafsun *et al.*, 2020). Which is primarily, due to interaction between carbon atoms and chemical agents such as strong acid (H_3PO_4), base (NaOH) or salts ($ZnCl_2$) (Gu & Wan, 2013; Shamsuddin *et al.*, 2016). The chemical method exhibits a very short time and low temperature during pyrolysis process (Giraldo *et al*., 2018). These advantages have resulted to the production of affordable and cheaper adsorbents using chemical activation method. Recently, many researchers have use different renewable resources for the preparation of adsorbent, such as mango tree bark (Dim, 2013), *Malva sylvestris* (Ramavandi & Asgari, 2018), fennel seed (Hussein & Jasim, 2019) and kaolinite clay (Dim *et al.*, 2020).

Pandanus stem is an agricultural by product which is mainly composed of cellulose, hemicellulose, and lignin. Therefore, *Pandanus* stem will be a suitable lignocellulosic biomass for preparation of porous active carbons. *Pandanus candelabrum* originated from *Pandanus* plant (Screw pine), which belongs to the family Pandanaceae which comprises about 700 species and is widely distributed in tropical and subtropical regions (Fillaeli *et al*., 2019). They are found in the regions of South-East Asia, Pacific islands, Madagascar, Indian Ocean islands, India and West Africa. It is a dioecious, small tree up to 12 m tall with branched trunk and aerial roots (Mario & Hiromatsu, 2018). *Pandanus* tree is a multi-purpose plant with different uses. It is known locally as screw pine. The leaves are used for making mats, baskets and fish traps, while the fibrous root is used for brushes (Akpabio & Akpakpan, 2012). In traditional medicine the leaves are used for the treatment of sore throat, and bark infusions are used to cure diarrhoea, dysentery, enteritis, antispasmodic, diuretic, and stimulant properties (Baba *et al.*, 2016). Despite of its various applications there is none for using *P. candelabrum* stem for synthesis of active adsorbent.

This work used *P. candelabrum* stem as carbon source to produce active carbon with H_3PO_4, $ZnCl_2$ and NaOH. Based on literature there is no study on activated carbons synthesized from *P. candelabrum* stem. The adsorbents were characterized in terms of functional group, elemental composition and morphology.

MATERIALS AND METHODS

Materials

Pandanus candelabrum stem, which was used as a precursor was obtained from Ogbunka, Anambra State, Nigeria. To remove impurities distilled water was used to wash the sample severally, after which it was dried for 6 h at 80 °C. Chemical agents used were H_3PO_4, $ZnCl_2$ and NaOH.

Preparation of Adsorbent

Accurately weighed 15 g of pulverized *Pandanus* stem was impregnated with activating agents in a ratio of 1:4 (w/w) H_3PO_4, (Yorgun and Yildiz, 2015) 1:3 (w/w) $ZnCl_2$ and 1:4 (w/w) NaOH (Kilic *et al*., 2012) (Precursor: Agent) by weight for 12 h. To ensure proper mixing and penetration of the agents into the internal structure of precursor the doping was done at 80 °C for 6 h. After impregnation, mixtures were carbonized at 400 °C (Yorgun & Yildiz, 2015) in nitrogen flow (200 cm^3/min) at heating rate of 10 °C/min, for 1 h in a horizontal tube furnace (model Y02PB, Thermocraft, Inc, USA), and was cool by open air. The resulting products were neutralized to pH of 7, by washing it several times with distilled water, and was oven dried at 105 °C for 12 h. The product was pulverized to sizes of 0.1 – 0.2 mm.

The modified adsorbents were labelled as sodium hydroxide activated carbon (SAC), zinc chloride activated carbon (ZAC) and phosphoric acid activated carbon (PAC) and stored in a tight container for further use. A control sample was prepared without impregnation and was labelled as control activated carbon (CAC) and stored for further used.

Characterization of Adsorbents

The physicochemical properties of the *P. candelabrum* stem as performed using the elemental analyzer and American Standard for Testing Materials, standard test methods (Yorgun & Yildiz, 2015, Shamsuddin *et al.*, 2016). Scanning electron microscope (SEM)-energy dispersive X-ray (EDX), (SEM-EDX, JEOLJSM 7600F) was used to determine the morphology and elemental composition. Thermo Electron Nicolet 4700 FTIR spectrometer recorded the spectra of the adsorbent from 4000 to 500 cm^{-1} resolution. The Brunauer-Emmett Teller (BET) method was used to determine specific surface area and pore volume using a micromeritics ASAP 2020. Methylene blue (MB) and iodine were used to test the adsorption capacities of adsorbents. For MB, about 250 mg of adsorbents were mixed with 25 mL of 1.5 g/L MB standard solution and shaken at 250 rpm in a thermostatic shaker at 25 °C for 24 h (Liu *et al.*, 2019)**.** While iodine value was tested by mixing 250 mg of adsorbents with 25 mL of 0.10N iodine solution, and was shaken for 30 min, and then iodine adsorption amount was determined by titration against standard $Na_2S_2O_3$ (Liu *et al.*, 2019). The MB and iodine adsorption amount of AC was determined according to Sahira *et al.*, (2013), Hassan *et al*., (2014) and Liu *et al*., (2019).

RESULTS & DISCUSSION

Characterization of Adsorbents

The result of characteristion of *P. candelabrum* stem are presented in Table 1. The stem was found to have moisture content of 4.99%, ash content of 6.32%, which was low, and volatile matter of 72.3%, which was high. As seen from Table 1, the

Table 1. The properties of *Pandanus candelabrum* stem

Proximate analysis (%)		Ultimate Analysis (%)	
Fixed carbon	12.00	C	45.04
Moisture content	4.99	H	6.72
Ash content	6.32	N	0.42
Volatile matter	72.30	O	43.85

Table 2. Physicochemical properties of adsorbent

Parameter	CAC	SAC	ZAC	PAC
Ash content (%)	11.663	17.622	11.554	13.095
Moisture Content (%)	7.784	4.382	0.994	0.994
Bulk density (g/cm^3)	0.354	0.444	0.259	0.316

high volatile matter and low ash content of *P. candelabrum* stem has made it suitable as an alternative starting material for production of activated carbon.

The results of adsorbent characterization are presented in Table 2. The activated carbon produced from *Pandanus* stem with different agents had different properties. The SAC had the highest ash content (17.62), bulk density (0.444 g/cm^3) and low pore volume (1.220), while ZAC has the lowest bulk density (0.259 g/cm^3) and ash content (11.554%). The increase in bulk density is because of the decrease in pore volume of the activated carbons which resulted to increase in weight of the absorbents. The presence of low volatile content may have contributed to the formation of large quantity of non-volatile matter such as ash (Kilic *et al.,* 2012). The properties of adsorbent obtained are in comparison with previous findings by some researchers (Ademuliyi *et al.,* 2016).

Figure 1 shows the surface areas, pore volumes and amount of adsorbed methylene blue and iodine for adsorbents activated with different chemical agents. The surface area is 2648 m^2/g for PAC, 1482.9 m^2/g for SAC, 1114.13 m^2/g for ZAC and 43.22 m^2/g for CAC. Pore volume is 1.683 cm^3/g for PAC, 1.448 cm^3/g for SAC, 1.220 cm^3/g for ZAC and 1.046 cm^3/g for CAC. In Figure 1, surface area (Figure 1a) and pore volume (Figure 1b), phosphoric had the highest surface area and pore volume, which is 2648 m^2/g and 1.683 cm^3/g respectively. The highest surface area of PAC may be due to H_3PO_4 interaction decomposed some component in the carbon source, such as aliphatic and aromatic compounds, and this resulted in easy gasification of volatile matters (Kilic *et al.*, 2012). The trends therefore, imply that the accessible area of adsorbent available for methylene blue adsorption follows the decreasing order, PAC > ZAC > SAC > CAC. The properties of adsorbents will favour adsorption.

From Figure 1c, the amount of iodine value and methylene blue adsorbed, is 541 and 105 mg/g for PAC, 175 and 36 mg/g for SAC, 128 and 16 mg/g for ZAC and 118 and 13 mg/g for CAC respectively. It can be observed that adsorption of iodine show increasing in adsorption capacity in the following decreasing order: PAC > SAC > ZAC > CAC. This shows capability of each adsorbent to adsorb small sized atom such as iodine (Nunes *et al.*, 2011). In addition, it can be seen from Figure 1c, PAC had the maximum iodine value and methylene blue (541 mg/g of carbon and 105 mg/g). H_3PO_4 aids in the degradation of carbon sources as well as in the creation of structures that exhibit cross-linking properties (Budinova *et al.*, 2006). And this will lead to the development of pores of micro and meso sizes in the adsorbent (Danish *et al.*, 2014, Yorgun & Yildiz, 2015).

The highest iodine value by PAC may be due to the presence of large micropores in the adsorbent (Moreno-Virgen *et al.*, 2012). This is followed by SAC which has iodine value of 175 mg/g. Here both micro porosity and mesoporosity were created through segregation and decomposition of graphite layers due to reduction and oxidative transformation. This aids volatilization, that disintegrates the matrix resulting to increased surface area available for adsorption (Foo &

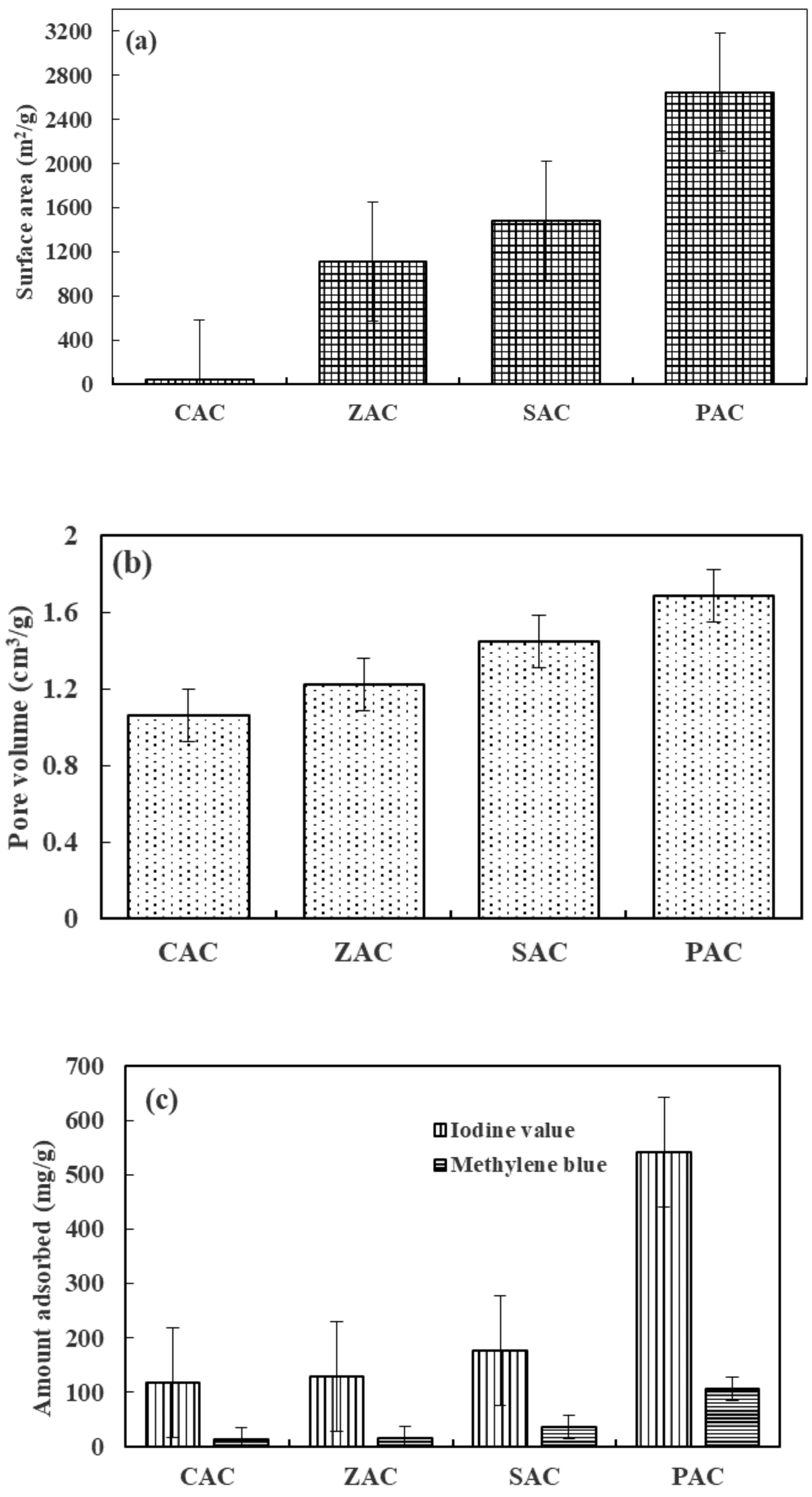

Figure 1. Surface areas (a), pore volumes (b) and amount of adsorbed methylene blue and iodine value (c) for carbon activated with different chemical agents

Hameed, 2012). While ZAC and CAC have iodine value of 128 and 118 mg/g of carbon respectively. In Figure 1c, it can also be observed that the adsorbents show the adsorption ability decreased in the following order: PAC > SAC > ZAC > CAC. In this context the ability to adsorbed methylene blue shows how each activated carbon will adsorb large molecules such as methylene blue which is much larger than iodine molecules (Cleiton *et al.*, 2011). PAC impregnated with H_3PO_4 has the highest adsorption value of 105 mg/g for methylene blue. This suggests it has well-developed mesopores and some portions of micropores (Shrestha *et al.*, 2012).

Scanning Electron Microscope (SEM) Analysis

The micrograph for unmodified carbon (CAC) (Figure 2a) shows broad surface which consists of few pores, aggregates of small particles, with irregular shape, relatively rough surface, and without cavities. The micrographs of modified carbon (Figures 2b-d) of adsorbent particles showed cavities, pores and rough surfaces. There is more presence of pores with openings in the adsorbents synthesized with H_3PO_4, NaOH and $ZnCl_2$ agents. The features on carbon surface revealed clearly the effect of activating agent on the textural properties. The pore openings are due to extraction of some materials from the surface by activating agents (Shamsuddin *et al*., 2015). The morphological features on the surface of adsorbent are the evidence of the effect of activating agents. PAC activated carbon impregnated with H_3PO_4 (Figure 2b) has more open pores and cavities. SAC and ZAC activated carbon (Figures 2c-d) also show presence of open pores. The various chemical agents lead to different reaction mechanisms which resulted to adsorbent with different pore structures. The mechanisms by which H_3PO_4 activates carbon source entails structural degradation and volatilization, whereas activation with NaOH and $ZnCl_2$ aids the creation of porosity by the expulsion of water molecules present in the carbon precursor (Kilic *et al.*, 2012).

Energy Dispersive X-ray Analysis

The unmodified carbon (CAC) and modified PAC, SAC and ZAC were evaluated using EDX (Table 3). The analysis showed that carbon content increased from 82.01 to 86.38 %, in the order of CAC < ZAC < SAC < PAC.

The decrease in oxygen content was from 4.06 % to 2.32 %, the trend was in the order of CAC < SAC < ZAC < PAC. But it was observed that SAC had the highest oxygen content among the modified activated carbon. This could suggest that NaOH-activated carbon has functional groups which contain more oxygen atom. SAC has the highest oxygen content of 3.79 % among all the chemical activated carbon. The high oxygen content of NaOH-activated carbon could be attributed to the oxidation of carbon precursor in alkaline environment (Huang & Zhao, 2015). From Table 3, it can also be seen that other elements present in CAC, SAC, ZAC and PAC are calcium, nitrogen, potassium, silicon, phosphorus, aluminium, sulphur, magnesium, sodium and chlorine.

XRD Analysis

The diffractogram of SAC, ZAC and PAC in comparison with CAC are shown in Figure 3. Activating agents and carbonization caused changes in the crystal structure of the carbons. The CAC displayed peaks at approximately 26.6°, 31.2°, 32.1°and 49.5°, this indicates the presence of graphite (Wang *et al.*, 2009). In comparison with CAC, all the chemically activated carbons show peaks at about 26.7°, 33.1°, 34.2°, 36.6, 39.2°, 46.8°, 50.2°, 60.1° and 68.3° respectively. The peak intensity changed the activation and subsequent carbonization and this made the peaks look sharper and wider.

This suggests that activated carbons prepared with agents resulted in the formation of micro crystallites which are like graphite (Wang *et al.*, 2011). Therefore, the increase in pore size is because the pore walls are made of hexagonal graphitic microcrystalline which were disintegrated severely. The emergence of new diffractogram pattern shows the creation of new amorphous and crystalline planes (Huang & Zhao, 2015).

FTIR Surface Analysis

The surface chemical properties of the adsorbents were examined using FTIR analysis. Figure 4 shows the spectrum of the adsorbent obtained without chemical activation (CAC) and the spectra for those obtained with chemical activation (ZAC, SAC and PAC. Similarity as well as difference exist in the absorption pattern of the produced

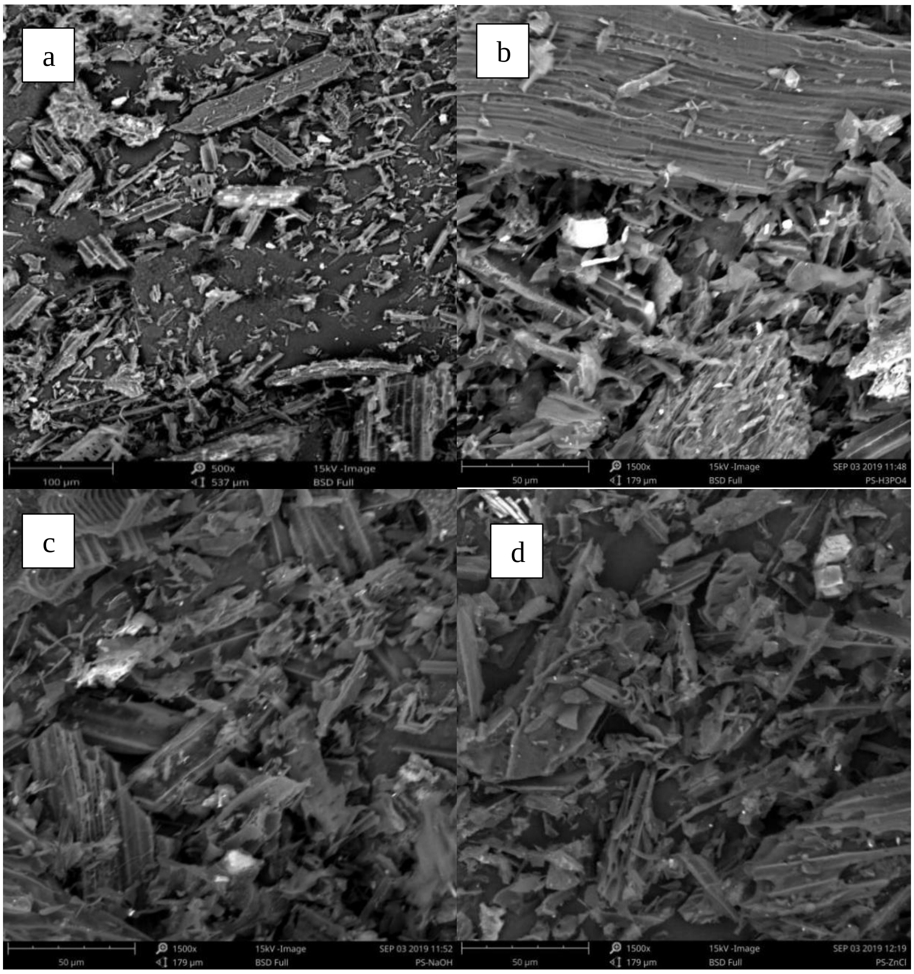

Figure 2. SEM micrograph for activated carbons, (a) Control activated carbon (CAC), (b) Zinc chloride activated carbon (ZAC), (c) sodium hydroxide activated carbon (SAC), (d) Phosphoric acid activated carbon (PAC)

Table 3. Elemental analysis of activated carbons

Element name	Element symbol	Proton number	CAC Wt (%)	ZAC Wt (%)	SAC Wt (%)	PAC Wt (%)
Carbon	C	6	82.01	83.10	84.42	86.38
Oxygen	O	8	4.06	2.79	3.79	2.32
Calcium	Ca	20	3.22	4.20	4.12	4.02
Nitrogen	N	7	1.85	0.20	1.45	1.01
Potassium	K	19	1.38	0.84	0.79	1.15
Silicon	Si	14	1.35	2.21	1.63	1.52
Phosphorus	P	15	1.16	1.20	1.56	2.03
Aluminium	Al	13	0.65	0.83	0.60	0.46
Sulphur	S	16	0.63	0.71	0.42	0.36
Magnesium	Mg	12	0.57	0.81	0.78	0.58
Sodium	Na	11	0.13	0.55	0.45	0.18
Chlorine	Cl	17	0.00	0.63	0.00	0.00

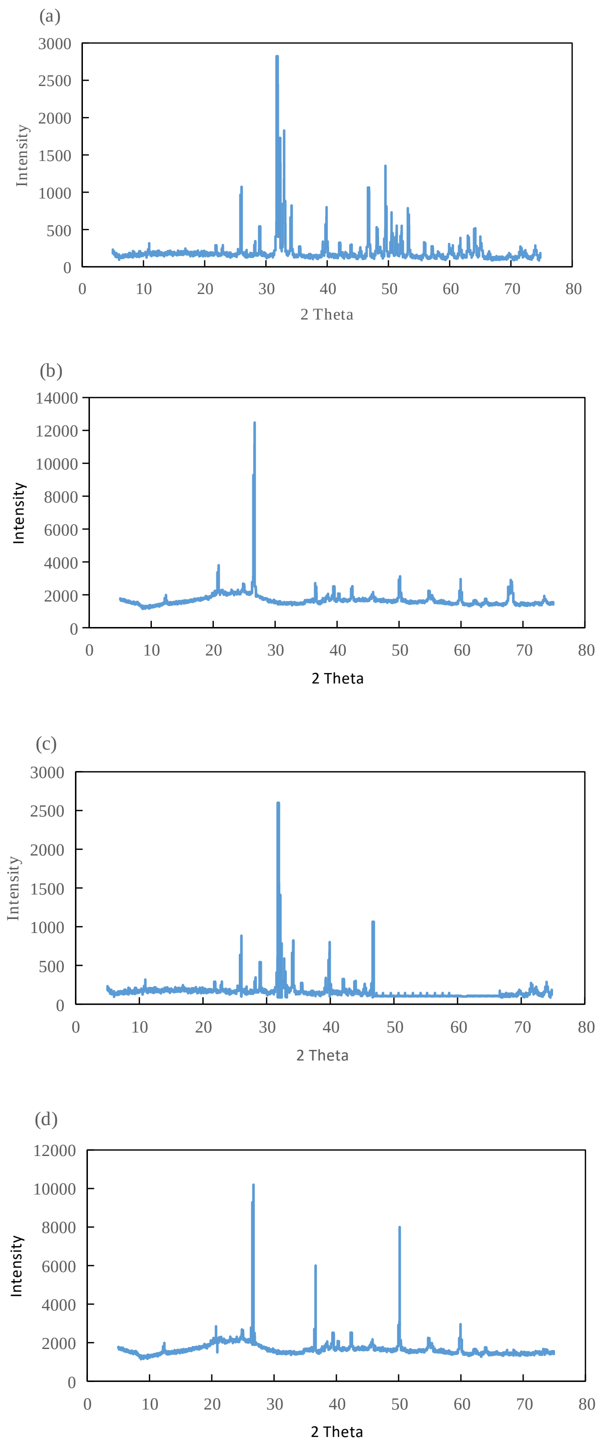

Figure 3. XRD for activated carbons: (a) Control activated carbon (CAC), (b) Zinc chloride activated carbon (ZAC), (c) sodium hydroxide activated carbon (SAC), (d) Phosphoric acid activated carbon (PAC)

adsorbents. The weak absorption band at 3900-3600 cm^{-1} are present in all the carbon spectra. This is assigned to hydroxyl bonds (Yakout & El-Deen, 2016). For CAC, the peak indicates the presence of O-H alcoholic group which was observed at the absorption around 3851 cm^{-1}. The absorption peak at 2923 and 2853 cm^{-1} corresponds to aliphatic stretch vibration and C-H functional group (Norouzi *et al.*, 2018). Those weak peaks observed around 2360.52 – 2343.44 cm^{-1} are assigned to the O-H group showing the presence of phenyl group. While the band observed at 1564 cm^{-1} indicates C=C bond showing the presence of carbon in aromatic structure (Kilic *et al.*, 2012). The peaks at 1462.40 and 1377.01cm^{-1}, correspond to methyl group. The little peak at 1154 - 1250 cm^{-1} could be ascribed to C-C and C-O stretched vibrations. These positions indicate the presence of acidic, alcohols, phenols, esters and ether group. The peaks at 668 and 721.61 cm^{-1} indicate the presence of aromatic structures (Wang *et al.*, 2014). This indicates that CAC contains methyl, aromatic and ester bonds which have the presence of oxygen species.

The spectra of PAC, SAC and ZAC indicates reduction in absorption peaks of functional groups. Irrespective of the activating agent used, the spectra were slightly different in the absorption patterns. The weak transmittance bands at 3851-3667 cm^{-1} exhibited by all the carbons, which is the presence of hydroxyl group. This weak intensity shows that the precursor was dried properly. The bands at 2853, 2923, 1462 and 1377 cm^{-1} are shrink or reduced for all the modified carbons. While the bands observed at 2343, 2360, 1154, 721 and 668 cm^{-1} disappeared for all the chemically activated carbon. This absorption illustrates the presence of active carbon in the prepared samples. Furthermore, in PAC, SAC and ZAC the elimination and reduction of some peaks suggests the decomposition and modification of surface chemistry by carbonization and chemical activation. These results suggest that concurrent carbonization and chemical activation caused the formation of new species and decomposition of many bonds that are weak in the functional group (Deng *et al.*, 2010).

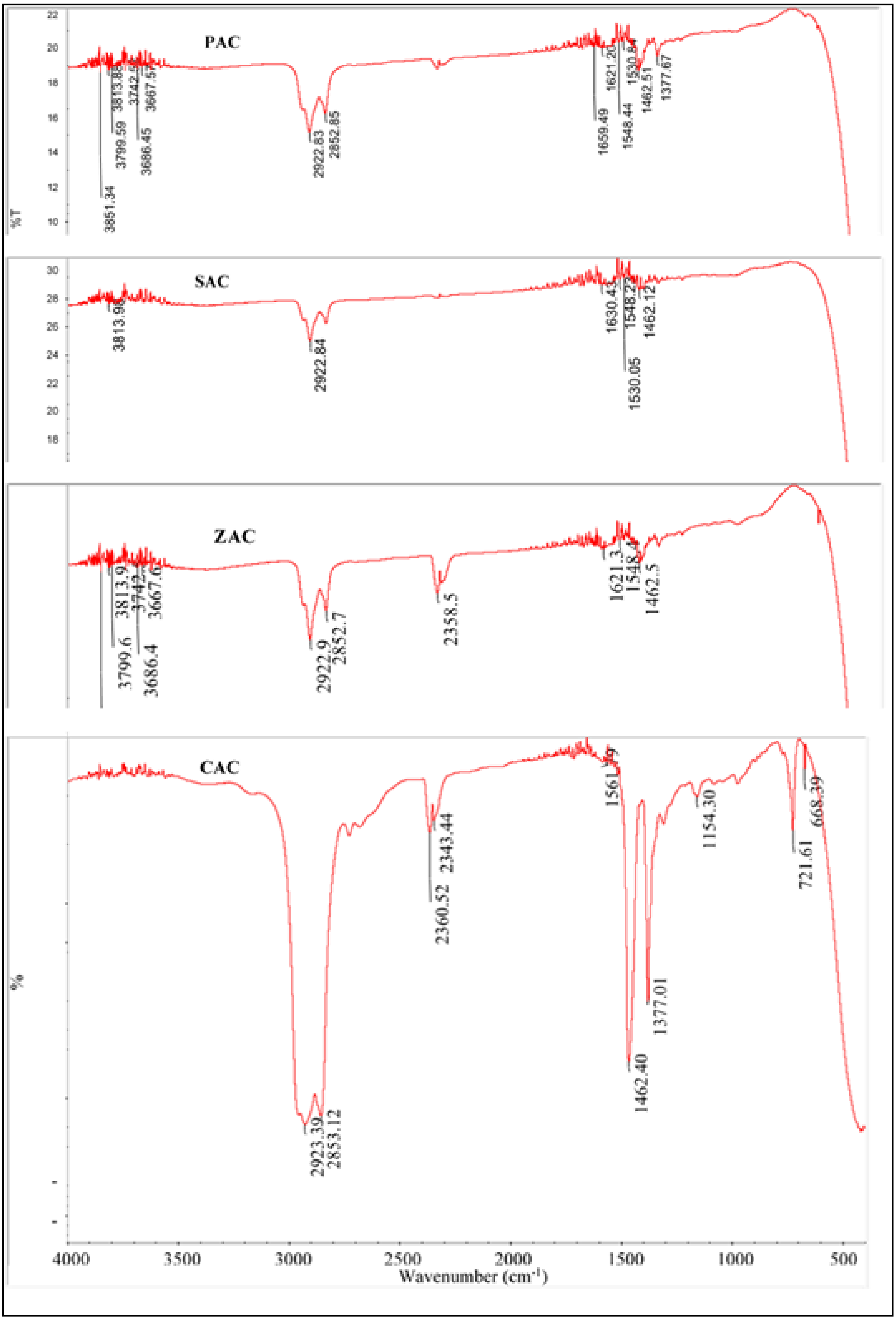

Figure 4. FTIR spectrum of CAC, ZAC, SAC and PAC

CONCLUSION

Pandanus candelabrum stem has been utilized to produce activated adsorbent using different chemical agents. The effect of agents on the proprieties of the adsorbent were investigated. FTIR results indicate that all the adsorbent prepared has some similarity and differences on the nature of their surface chemical properties. SEM micrograph of adsorbents impregnated with chemical agents showed well-developed pores structure compared to unmodified carbon. The study revealed that chemical agents had effect on the properties of the modified adsorbents. They showed increase in the adsorption of methylene blue in the order of CAC < ZAC < SAC < PAC, and iodine number is in the increasing order of CAC < ZAC < SAC < PAC. This shows that PAC (H_3PO_4) activated carbon had the highest iodine number and methylene blue (541 mg/g of carbon and 105 mg/g). The surface area is 2648 m^2/g for PAC, 1482.9 m^2/g for SAC, 1114.13 m^2/g for ZAC and 43.22 m^2/g for CAC. *Pandanus candelabrum* stem is an alternative lignocellulosic source that can be used to synthesize high porous adsorbent, which is abundant, easy to access, inexpensive and readily available.

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Microplastics Determination in the Rivers with Different Urbanisation Variances: A Case Study in Kuching City, Sarawak, Malaysia

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ABSTRACT

The presence of microplastics in aquatic systems is mainly due to the anthropogenic activities such as domestic waste dumping. Undeniably, rivers either in urban or suburban areas are always a waste dumpling sites from the surrounding residences. Thus, the purpose of this study was to determine the relationship between microplastic abundance and different degree of urbanization across Kuching in Sarawak. Three sampling locations with different degrees of urbanisation had been studied across Kuching. A total of 137 pieces of microplastics were collected along the study and analysed using stereoscopic microscope for the shape identification and FTIR spectrophotometer for functional groups present in the microplastics. Filament was the most abundant microplastics shape found, whereas the IR results showed that ethylenevinylacetate (9%), polyamides or nylon (15%), polypropylene (42%), poly(methylmethacrylate) (16%) and polystyrene (18%) were found in the study. The most abundant microplastics in the water samples was polypropylene (42%), whereas ethylenevinylacetate (9%) was the least. The degree of urbanisation does not directly relate to the microplastic present in the river system in Kuching City, but the anthropogenic activity is the main factor that affecting the microplastic abundance in the river.

Keywords: anthropologenic activity, FTIR, microplastics, polymer identification, urban, sub-urban,

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INTRODUCTION

The plastic manufacturing industry is one of the most dynamic and vibrant growth sectors in Malaysia. However, in line with the increase of plastic production, the plastic wastes have been recorded the third largest waste tonnage, after the putrescible waste and paper waste (Malaysian Department of Housing and Local Government, 2011).

As plastic particles can be broken into small particle called microplastics, this has prompted public concerns due to high possibility of microplastics flow into the food chain. The abundance of microplastics in aquatic environment as it is highly volatile in mobility (Ng & Obbard, 2006; Gall & Thompson, 2015; Kwon *et al*., 2017) and ingested by aquatic organisms and gets into food chain (Yusof *et al*., 2016; Avio *et al*., 2017; Yusof *et al*., 2017; Karami *et al*., 2017; Smith *et al*., 2018). Many researchers found that microplastics are more abundant in subtidal sediments and sea waters rather than on sandy beaches and in estuarine habitats (Thompson *et al*., 2004; Browne *et al*., 2011; Claessens *et al*., 2011; Peng *et al*., 2018; Peng *et al*., 2019). Thus, the sources of microplastics could origin from the rivers (Shazani *et al*., 2018) due to irresponsible domestic wastes dumping into the rivers by surrounding communities. Again, this could be also strongly related to the human population as well as the environmental awareness of the surrounding community. Hence, this study was designed to relate the source of microplastics to different degree of urbanization across Kuching City.

METHODOLOGY

Sampling Locations and Frequencies

The river water samples were collected from three areas in Kuching City namely Kuap River (**W1**) in sub-urban area, Maong River (**W2**) located in urban area and Sarawak River (**W3**) in Waterfront Kuching, which is an extra urban area. A brief description of the sampling locations with GPS coordinates is stated in Table 1. Figure 1 shows the geographical location of the three stations using Google Earth, Maxar Technologies (2019), whereas Figures 2 – 4 show closer views of each sampling location. Three samplings were carried out in October, November and December 2018. The water samples were collected using a water sampler Wildco, Wildco Alpha Bottle Kit 4.2 L) and each sample was then filtered using a membrane filter with 0.45 µm pore size.

Table 1. Descriptions of the sampling locations

Sampling points and GPS coordinates	Descriptions
W1 Kuap River 1°27'38.39"N 110°21'24.28"E	• Approximately 500 m from agricultural land and human settlement (Stakan Melayu Village) • Mixed forest zone along the riverbank, peat swamp area • Subjected to tidal effect. The river depth varies between high and low tide
W2 Maong River 1°30'43.60"N 110°20'25.44"E	• High residential density area • Disposal of grey water into drainages from surrounding housing area into the river • No tidal effect
W3 Sarawak River, Kuching Waterfront 1°33'30.78"N 110°20'52.60"E	• Very high density of human settlement, commercial and tourism activities • The river is not subjected to tidal effect

Figure 1. Sampling locations of **W1**, **W2** and **W3**. (Source: Google Earth, 2019 Maxar Technologies).

Figure 2. Environmental condition of **W1**.

Figure 3. Environmental condition of **W2.**

Figure 4. Environment condition of **W3.**

Wet Peroxide Digestion

Hydrogen peroxide (35%, 25 ml) and ferrous sulphate heptahydrate catalyst solution (0.05 M, 25 ml) were added into a beaker which contained the total solids (Zobkov & Esiukova, 2017). Then, the content in the beaker was heated up to 75 °C and removed from the hotplate when the gas bubbles were observed. The beaker was cooled in the fume hood and the solution was filtered by using 0.45 μm membrane filter in order to obtain the microplastics particles (Masura *et al*., 2015).

Microplastic Sorting and Identification

The membrane filter was viewed under a stereo microscope (Nikon, C-LEDS) at 40X magnification. The microplastics were sorted based on their maximum length, colour and shape (threadlike, fragment or spherical) based on the guidelines as given by Hidalgo-Ruz *et al*. (2012). The category of microplastic was identified based on the chemical functional groups present in the microplastic using Fourier transform infra-red (FTIR) spectrophotometer (Thermo Scientific, Nicolet^{TM} iS^{TM} 10) with attenuated total reflection (ATR).

RESULTS AND DISCUSSION

Morphology of the Sampling Sites

Kuap River was shaded by peat swamp vegetation, mainly mangrove trees, palms and aquatic shrubs along the riverbank. An irresponsible domestic waste dumping site is located at **W1**, just at the side of the riverbank (Figure 5). The colour of the river water was murky due to high sediment content that runoff from the riverbank as shown in Figure 2. The current and flow rate of the river were moderately slow and subjected to tidal. The samplings were usually carried out during the low tide due to accessibility to the river water. There is a village located about 1 km radius of the sampling point and estimated population in the village is about 2,500 – 3,000 people (Sarawak Government, 2020).

On the other hand, **W2** in Maong River is located at the middle of a densely-populated residential area with estimated population of 10,000-20,000 (Sarawak Government, 2020) (Figure 6). Maong River is not shaded by any vegetation and no riverbank erosion because the river bank surface is covered with concrete cement. The river serves the purpose of receiving grey and black waters discharge of the kitchens and toilets from urban-residential houses. The colour of the river water was cloudy and the flow was not subjected to tidal but dependent on the weather condition.

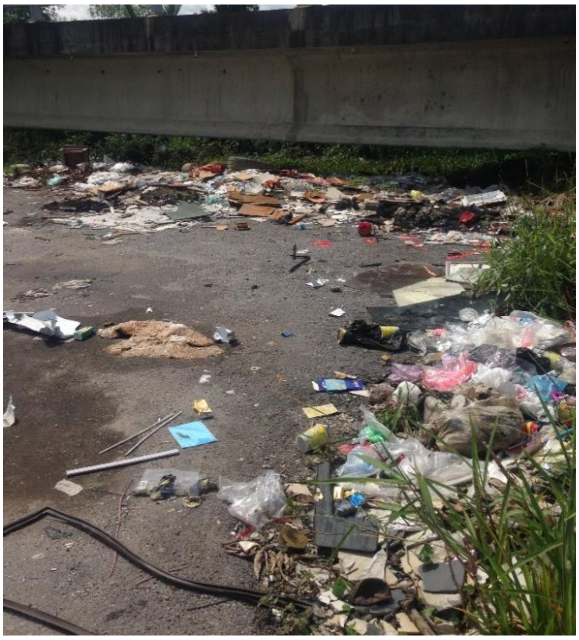

Figure 5. Irresponsible domestic waste dump site at Kuap riverbank.

The location of **W3** in Sarawak River is at the heart of Kuching City, which is mainly surrounded with commercial area and recreational park. Until today, Sarawak River is still an important river for Kuching City because the river does not only serve as a main water transportation system in the city but also as a drinking water source for the population living in Kuching City. The colour of the river water was brownish and not subjected to tidal effect due to the bund building at the downstream (Leete, 2008).

Microplastics Abundance

The total number of microplastics found in all samples were 137 pieces throughout the three-month study (Figure 7). The abundance levels varied depending on the sampling location. Station **W3** in Sarawak River recorded the highest microplastics number, that is 61 pieces consisting filaments (50 pieces), fragments and fibres. Meanwhile, the **W1** in Kuap River recorded the second most abundant microplastics found (49 pieces). There were 13 microbeads (Figure 8) identified and 25 filaments (Figure 9), followed by fragments (Figure 10) and the least are fibres.

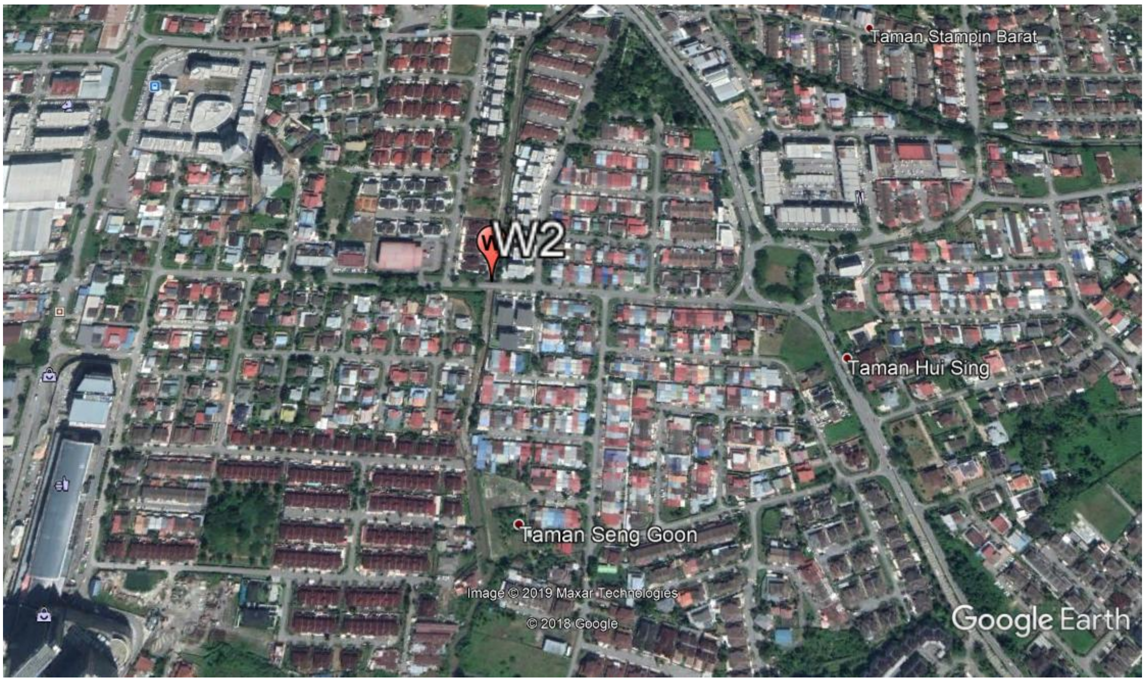

Figure 6. Surrounding condition of **W2.** (Source: Google Earth, 2019 Maxar Technologies)

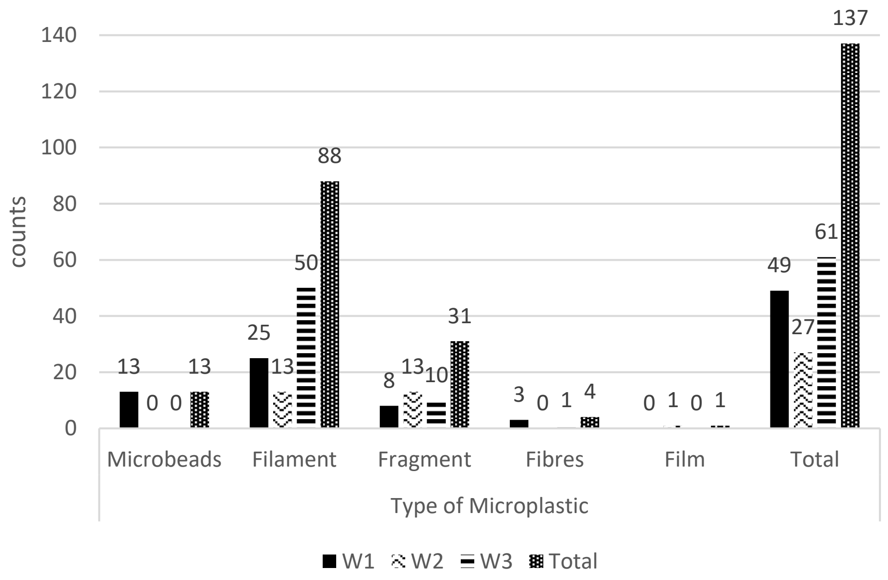

Figure 7. Total number of microplastics from three sampling stations.

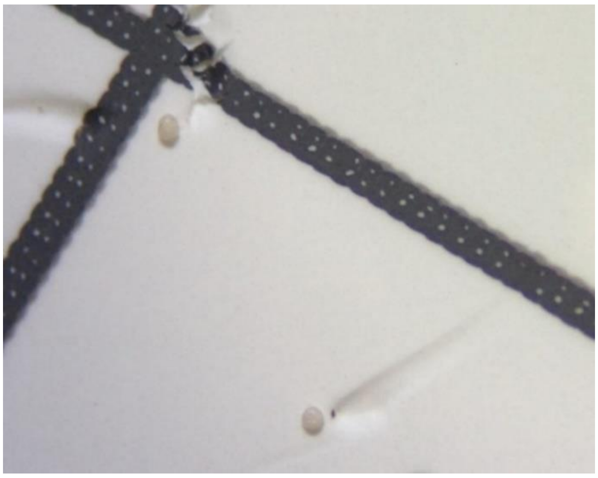

Figure 8. Microbeads found in the water samples from **W1** (at 40X magnification).

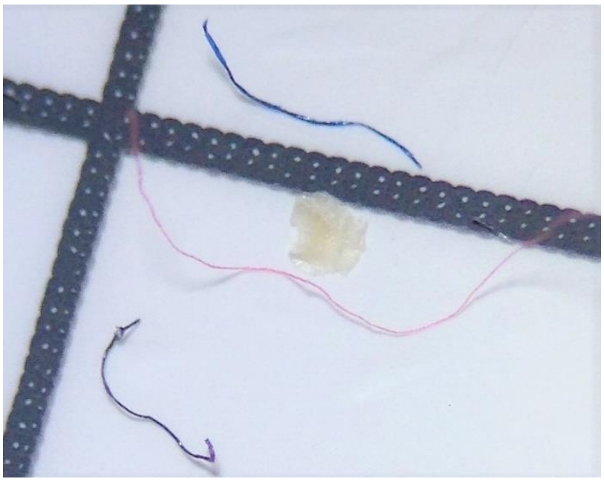

Figure 9. Filaments found in the water sample from **W1** (at 40X magnification).

Figure 10. Fragment found in the water sample from **W3** (at 40X magnification).

The type of microplastics present in the rivers is dependent on the anthropogenic activities (Kataoka *et al*., 2019) as well as the residences' environmental awareness. In fact, as shown in Figure 5, irresponsible solid wastes disposal at the river bank of **W1** was the main reason why the sample from **W1** contained more microplastic debris.

A higher number of filaments from fishing nets were found in Sarawak River (**W3**) due to the fishing activities along the riverside. For **W3**, solid waste disposal from commercial river cruises and fishing boats have contributed to the high amount of garbage stranded on the riverbank. As Sarawak River is one of the earliest settlement areas in Sarawak with high human population and industrial factories, it was expected that the anthropogenic impact in **W3** would be much greater than **W1** and **W2**. Lastly, **W2**, which is located in an urban housing area, had the lowest number of microplastics collected, in which the presence of microplastics in this area could be due to the discharge of grey waters from toilets and kitchens. In 2019, Dikareva and Simon found the variation in the quantity and type of plastic occurring in small streams. Similar to our study, their results showed that the most abundant microplastics were fragments and small particles (63-500 μm).

In general, it was observed that the sources of plastic flow into the river can be due to surrounding community' anthropogenic activities, the discharge of greywater from kitchen and irresponsible solid waste dumping at the river bank. These results were in line with the findings from Kataoka *et al.* (2019), where the group noticed that the anthropogenic activities near the river were the main contribution to the microplastics present in the river in Japan. However, in contrast to our findings, the group noticed that the degree of urbanization was significantly related to abundance of microplastics present in the river.

Microplastic classification

The classification of microplastics were performed using a FTIR spectrophotometer with an ATR adapter. The list of functional groups found in the IR spectra from the microplastic samples are tabulated in Table 2 and some examples of IR spectra for different plastic are shown in Figures 11-12.

Table 2. The list of functional groups with the respective IR frequencies (cm^{-1}) present in the microplastic samples

	Chemical functional groups in respective frequency				
Plastic type	υN-H	υC-H	υC=O	υC=C	υC-O
Ethylenevinylacetate (EVA)	-	3051	1774	-	1027
Polyamides (PA) / nylon	3696	2961	1642	-	-
Polypropylene (PP)	-	2951	-	-	-
Polymethylmethacrylate (PMMA)	-	2952	1711	-	1168
Polystyrene (PS)	-	2838	-	1601	-

Note: "-" means not available

Ethylenevinylacetate (EVA) was identified based on the strong IR peak located at 3051 cm^{-1} which is attributed to C-H vibration of alkane group. Meanwhile, the IR signals at 1774 and 1027 cm^{-1} are corresponding to the C=O and C-O stretching, respectively. For polyamides (PA), which is also well known as nylon, the weak peak at 3696 cm^{-1} is attributed to N-H vibration from the amine group [Figure 11(a)]. Meanwhile, the strong IR frequency at 2961 cm^{-1} is due to the C-H vibration and the band at 1642 cm^{-1} correspond to the C=O stretching mode (Figure 11a). It is worth to note that the C=O absorption of amides normally present at lower frequency than typical carbonyl group due to the resonance effect along the molecule.

For polypropylene (PP), since the molecule only contains long alkane chain, thus the only IR frequency can be observed is at 2951 cm^{-1} [Figure 11(b)]. Polypropylene is particularly prevalent in the environment due to their production in vast quantity for packaging applications such as candy wrappers, disposable food containers, and drinking water bottles.

The structure of PMMA can be characterised by the IR frequency at 1766 cm^{-1} (Figure 12) due to the C=O stretching of carboxyl group (Duan *et al*., 2008). A group of weak IR frequencies at the region of 1101 to 1167 cm^{-1} is attributed to the C–O stretching mode supporting the presence of O=C-O group in PMMA. Meanwhile, another two IR bands at 2951 and 2916 cm^{-1} are assigned to the C-H vibrations due to the alkane chain in the PMMA structure.

Another type of plastic, which has been widely used in food packing called polystyrene (PS) was also found in the samples. The micro size PS is the debris from a larger size PS due to natural weathering (Wu *et al*., 2017; Wirnkor *et al*., 2019). From the prospect of IR analysis, this type of plastic can be characterised by determining the aromatic C=C stretching at 1601 cm^{-1} as well as the C-H vibration at 2838 cm^{-1}.

Based on the numbers shown in Table 3, PP is the most abundant microplastics (42%) found in the samples, followed by PS (18%), whereas PMMA and PA accounting for 16 and 15%, respectively.

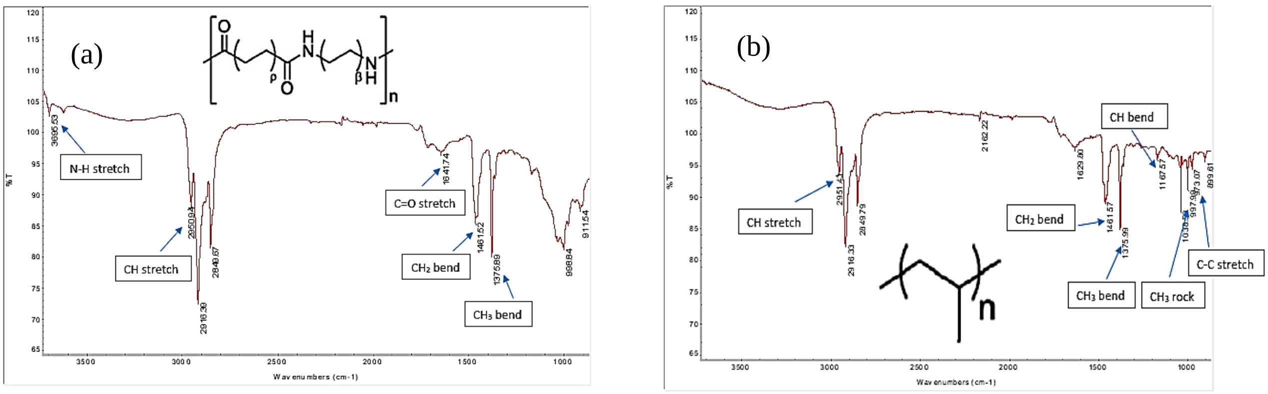

Figure 11. IR spectra of (a) polyamides (PA) or nylon, and (b) polypropylene (pp) that found in the water samples

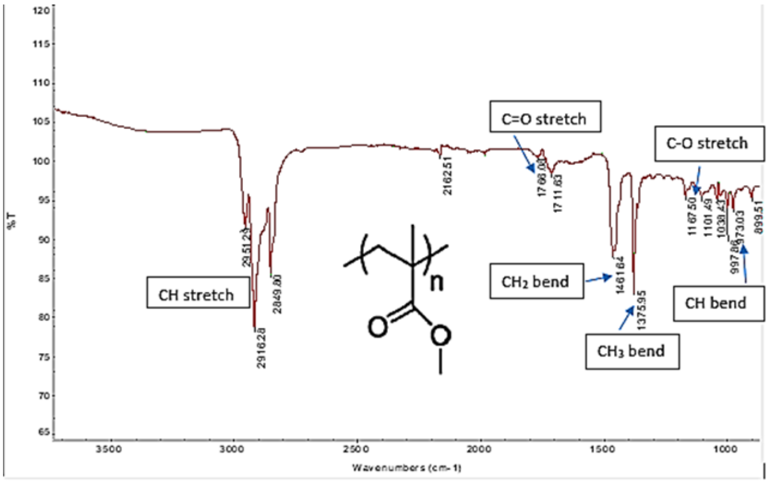

Figure 12. The IR spectrum of poly(methylmethacrylate), PMMA

Table 3. Distributions of microplastics (%) found in the three sampling locations

	W1	W2	W3	Abundance
EVA	28	0	0	9
PS	5	45	3	18
PA	10	23	13	15
PP	57	5	63	42
PMMA	0	27	21	16

Lastly, EVA was recorded as 9% abundance in overall collected samples. PP was recorded with the highest abundance from Sarawak River (**W3**). Due to light density nature of PP (0.90-0.91 g/cm^3), the plastic always floats on the water surface and exposed under the sun which promotes the breakdown of plastic into small debris. Due to this reason, the amount of this plastic found was dominant in the sample (Hidalgo-Ruz *et al*., 2012). The second most abundance plastic namely PMMA was found in Maong (**W2**) and Sarawak (**W3**) Rivers, whereas both PA and PS were found in three study sites especially **W2** (Table 3).

CONCLUSION

Most of the microplastic debris were found in **W3** located at Sarawak River with the type of filament, which has been identified as polypropylene (PP). As can be seen from the results of sampling points in **W1** and **W2**, the degree of urbanization was not directly related to the microplastics present in the rivers in Kuching City. The anthropogenic activity is the main factor that affects the microplastics abundance in the river. Nevertheless, we also highlight that the solid waste dumpling site next to the Kuap River has contributed significant the microplastic abundance to the river. The solid waste dumpling site is resulted from the low environmental awareness and irresponsible activity from the human.

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A Fluorescence Study on Surface Properties of Cationic Gemini Surfactant with Some Special Alcohols

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ABSTRACT

Alcohols are very important additives and played a measure role as co-surfactants in various industrial and research applications. While, Gemini surfactants are mainly used as effective emulsifiers, antifoaming agents, bactericidal agents, coating agents and corrosion inhibitors etc. Therefore, it is important to study about the variations in alkanol concentrations which affects the aggregation number and other related parameters. Surface properties of gemini surfactant butanediyl-*1*,*4*-bis(dimethyldodecylammonium bromide) (12-*4*-12) has been studied by using fluorescence method. This method has been used to calculate the aggregation number (N_{agg}) and the other related parameters like dielectric constant (D), Stern Volmer binding constant (K_{SV}) of mixed system. This method is also very important for the calculation of the micropolarity of the mixed system (gemini/alcohol). The micropolarity has been obtained with the help of the ratio of intensity of peaks (I_1/I_3) of the pyrene fluorescence emission spectrum. Cetylpridinium chloride and pyrene were used as quencher and probe, respectively.

Keywords: Gemini surfactant, special alcohols, aggregation number, micropolarity

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INTRODUCTION

Surfactants or detergents are the agents which have lyophilic and lyophobic parts in a molecule. These molecules show very interesting properties of both adsorptions on surface as well as interfaces at very low concentrations. The surface tension is being reduced when surfactants are used in low concentration.

The lyophilic part of the surfactants is soluble in water while lyophobic part is water insoluble. When water is used as a solvent then these parts are called hydrophilic and hydrophobic. The charge bearing portion is called hydrophilic and long chain (tail) is called hydrophobic. There are many examples of the conventional surfactants on which the surface properties have been studied very well. The surfactants have long carbon chain that may be linear or branched and the charge bearing part may be ionic. The hydrophilic part of the surfactants interacts strongly with the polar part of the water molecule.

Surfactants possess many applications both in everyday life as well as in the industrial field. Gemini surfactants showed more efficient properties as compared to conventional surfactants. These type of surfactants have two hydrophilic and two hydrophobic parts and their hydrophilic parts are being connected with a spacer. The examples include hexnediyl-*1,6*-bis(dimethylcetylammoniumbromide) (16-*6*-16), pentanediyl-*1,5*-bis (dimethylcetylammoniumbromide) (16-*5*-16), etc. (Ionescu & Fung, 1981; Warnheim & Jonsson, 1998; Jonstronger *et al*., 1990; Gharibi *et al*., 1992; Sjober *et al*., 1993).

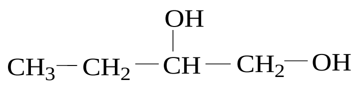
Gemini or dimeric surfactants have been first prepared by Mitsui Okahara and his colleagues (Okahara *et al*., 1988). These surfactants show more efficient wetting properties (Rosen, 1993) than conventional surfactants and possess very low critical micelle concentration (*CMC*). They show specific rheological and specific aggregation properties (Zana & Talmon, 1993; Frindi *et al*., 1994; Alami *et al*., 1993; Talmon & Binks, 1999).

A schematic presentation of Gemini surfactant as shown in Figure 1 contains the polar or ionic parts are connected through spacer. Due to excellent surface activity, Gemini surfactants possess many applications in various fields. They are mainly used in foaming, spreading aids and cleaning processes etc. These surfactants are used in gene therapy as well as bio-imaging because of their ability to interact with DNA (Ahmed *et al*., 2016). Gold, silver and gold-silver alloy nanoparticles can be prepared using Gemini surfactants with the help of seed mediated method (Tiwari *et al*., 2015). The purpose of selecting the present alcohols is to find out the effect of various special alcohols as additives in the Gemini surfactants, as these alcohols are expected to show advanced properties when compared to simple linear chain alcohols. Also, to the best of our knowledge, this is for the first time that the aggregation number of cationic Gemini surfactant in presence of special alcohols is reported. Mainly alcohols are used as co-surfactants with surfactants + oil systems to create micro-emulsion. The alcohol played very important role in micro-emulsion, as it decreases the binding modulus (Binks *et al*., 1989; Strey & Jonstromer, 1992) and increases the fluidity of the system (Lianos *et al*., 1982).

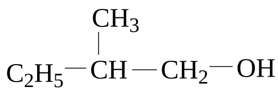
METHODOLOGY

The special alcohols 1,2-butanediol (≥98.2%, Sigma-Aldrich, Germany), 2-methyl-1-butanol (≥99.3%, Sigma-Aldrich, Germany), 2-ethyl-1-butanol (≥98.2%, Sigma-Aldrich, Germany), and 2-butene-1,4-diol (≥95.5%, Sigma-Aldrich, Germany) were used as received. These branched chain alcohols are very much effective in the formation of mixed micelle.

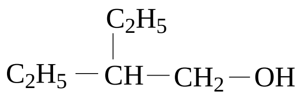
The chemical structures of special alcohols are given below.



1,2-butandiol



2-methyl-1-butanol



2-ethyl-1-butanol

$$HO-CH_2-CH=CH-CH_2-OH$$

2-butene-1,4-diol

The dimeric surfactant has been prepared by using 1,4-dibromobutane (≥98.3%, Merck, Germany), with *N*,*N*-dimethyldodecylamine (≥95.5%, Fluka, Germany) in dry alcohol at 80.15 °C for 48 h with stirring continuously. TLC technique was used to record the progress of the reaction. Recrystallization process has been used to remove solvent. The obtained values were in agreement with the reported literature data (Bhattachrya *et al*., 1996). Hitachi F-2500 fluorescence spectrometer has been used to record the spectra.

RESULTS AND DISCUSSION

The calculated values of mean aggregation number give an idea of structure and dimensions of the micelles formed by surfactant in the solution. The mean aggregation number is the amount of surfactant molecules that occupy together to form a spherical structure called micelle. Mainly their shape looks like a bubble. Force of attraction in the

Figure 1. Schematic representation of Gemini surfactant

micellar solution depends on hydrocarbon chain of the monomer. In view from the geometric considerations, the aggregation number (N_{agg}) increased rapidly in aqueous media if the length of the hydrophobic group (l_c) of the surfactant molecule is increased, while it decreased when there is an increase in the inter-section area (a_0) of the lyophilic group.

For calculating the micellar aggregation numbers (N_{agg}) of pure and mixed system, Fluorescence quenching is the best method. It is more competent method. All the spectra were recorded at the room temperature i.e., 298.15 K. N_2 stream was used to evaporate the solvent. By keeping pyrene concentration constant at $2x10^{-6}$ mol.L^{-1}, the surfactant solution has been added into the volumetric flask.

The Micellar aggregation numbers (N_{agg}) is calculated using Eq. (1) (Turro & Yekta, 1978).

$$\ln I_0 = \ln I_Q + \frac{N_{agg}[Q]}{[S]-cmc} \quad (1)$$

The [Q] and [S] represent the concentrations of quencher and total surfactant concentration respectively, while I_o represent the intensity of fluorescence when quencher is absent and I_Q represents the intensity of fluorescence when quencher is present.

Spectra have been recorded at different mole fractions of alcohols (data is given in the Table 1). High values of N_{agg} are obtained for mixtures as compared to pure solution (when no additive is added). The obtained data are in good agreement with the literature (Mohammad, 2019). The high concentration of the alcohols will decrease the repulsion among head groups so the compact micelles with higher aggregation number will be formed.

Table: 1 Aggregation number and other related parameter calculated by fluorescence measurements

α_1	N_{agg}	N_{gem}	$N_{alcohol}$	$K_{SV}/10^4$ (mol^{-1} dm^3)	I_1/I_3	D
System: 1,2-butandiol /12-4-12						
0.00	35	35	0	5.9	1.86	68
0.20	111	86	25	4.8	1.70	55
0.40	125	75	50	3.1	1.48	38
0.60	164	65	99	4.7	1.87	69
0.80	292	81	211	3.9	1.99	78
System: 2-methyl-1-butanol/12-4-12						
0.20	59	47	12	9.2	1.50	40
0.40	88	53	35	16.6	1.85	67
0.60	87	35	52	5.6	1.72	57
0.80	140	28	112	10.8	2.96	156
System: 2-ethyl-1-butanol /12-4-12						
0.20	48	38	10	5.3	1.64	50
0.40	54	34	20	3.5	1.84	66
0.60	76	31	45	5.9	1.86	68
0.80	109	22	87	8.5	2.34	107
System: 2-butene-1,4-diol/12-4-12						
0.20	49	39	10	5.6	1.82	65
0.40	52	31	21	4.4	1.55	43
0.60	65	26	39	7.1	1.73	58
0.80	81	16	65	13.4	1.95	75

α_1 = concentration of alcohols.

The intensity ratio of vibronic peaks, first (I_1) and third (I_3), represents the index of micro-polarity of the system in the presence of surfactant i.e. it provides a view on the microenvironment of the micelle. The smaller value of this ratio (<1) represents that pyrene have nonpolar surrounding, whereas greater value (>1) represents that the pyrene is having polar surrounding (Kalyanasundram & Thomas, 1977). Eq. (2) was used for calculating the apparent dielectric constant (Maeda, 1995).

$$\frac{I_1}{I_3} = 1.00461 + 0.01253\, D \qquad (2)$$

The local polarity is measured by calculating the values of *D* from Eq. (2) where the probe is present. If all the molecules are present into the regions of micelle it means that the size of the probe is large. The obtained D values in Table 1 are the mean values. This is due the complementary effect, which is created by the separation of the ionic head groups of Geminis. The dielectric constant on polar surfaces in water is reduced when the surface electrical potencia is increased; this is due to the orientation of water molecules by the electric field (Ferchmin, 1995; Lamm & Pack, 1997). The intercalation of alcohol species between the charges bearing part of the surfactant must increase the value of *D* with respect to that in the pure surfactant micelles.

Stern-Volmer binding constant, (K_{SV}) (Rohatgi-Mukherjee, 1992) has also been calculated by applying Eq. (3).

$$\frac{I_1}{I_3} = 1 + K_{SV}[Q] \qquad (3)$$

Here K_{SV} is referred as the ratio of the bimolecular quenching constant to the unimolecular decay constant. If one got the higher value of K_{SV} values, it means that the pyrene having longer lifetime in micellar solutions and quenching is more efficient.

CONCLUSION

As the concentration of alcohols increase the values of N_{gem} and $N_{alcohol}$ also increases. This indicates the strong synergism between Gemini surfactant and additives (alcohols). When micelles are formed from more than one chemical species, then there is a formation of mixed micelle. Mixed micelles have very important applications in technical, pharmaceutical, and biological fields because they work better than pure micelles (Holland & Rubingh, 1992). The aim of this study is to find the effect of different alcohols on Gemini surfactant, furthermore they can be used again for drug encapsulation and in delivery. Also alcohols are very good co-surfactants and they can be used in micro-emulsion formulation (Lianos *et al*., 1984) There are very few studies using amines as co-surfactants so they are also potential candidates for such formulation (Wormuth & Kaler, 1987).

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A Comparative Study on Biomass Fuel Consumption, Collection and Preference Patterns by Rural Households of Forest and Non-Forest Areas in Northern Bangladesh

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ABSTRACT

Biomass fuel is the most important form of renewable energy in many parts of the world including Bangladesh. Its extraction is considered as a leading cause of forest degradation of developing countries like Bangladesh. Its consumption, collection and preference patterns are thus very important indicators of overexploitation of forest. On the other hand, forests are meager in mainly northern region of Bangladesh. Reliable data and information are scanty on biomass fuel in Bangladesh, particularly in formulating its proper management plan. The aim of this study was to conduct a comparative study on the aforesaid patterns in forest and non-forest areas of northern region of Bangladesh. The study was carried out by adaptive multistage random sampling technique. A total of 90 households (45 from forest area, 45 from non-forest area) were selected randomly and based on the monthly income the households were categorized into rich, medium, poor groups. The consumption of biomass fuel was found to be differed significantly between forest (2.10 kg/capita/day) and non-forest (1.71 kg/capita/day) area. Forest, market, agriculture, homestead and roadside plantation were identified as sources of biomass fuel, and the contribution of each sources varied significantly between the areas except market. In forest area, maximum amount of biomass fuel was collected from nearby forests (44%) and poor households collected 78% of biomass fuels from the same sources. In non-forest area, roadside plantation (31%) and homesteads (24%) were the major sources of biomass fuel, and poor households collected biomass fuel mainly from roadside plantations (75%). Stems, branches, leaves, agricultural residues and cow dung were used as biomass fuel in both areas but the consumption of each biomass fuel types varied significantly except leaves. Women were identified as major biomass fuel collector and most of the biomass fuel was found to be collected during morning to noon in both areas. Most commonly used fuelwood species was *Shorea robusta* in forest area and *Eucalyptus camaldulensis* in non-forest area. The findings of this study will help policymakers to take steps in halting deforestation as well as meeting the villager's needs for biomass fuel.

Keywords: Bangladesh, biomass fuel, forest degradation, fuelwood, homesteads, natural forests

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INTRODUCTION

Biomass, the total of non-fossil organic materials that have intrinsic chemical energy content, derived from carbon based materials and mainly comprised of agricultural residues, animal manures, agro-industrial residues, municipal solid wastes and harvests from forests (Balat & Ayar, 2005; Hossain & Charpentier, 2015). Biomass energy is the largest source of renewable energy representing 77.78% of renewable global energy supply and 10% of global primary energy supply (WEC, 2016). Biomass fuel is the most potential indigenous source of energy and provides almost 35% of primary energy demand in developing countries (Balat, 2006; Demirbas, 2006). In some developing countries, it accounts for more than 90% of total rural energy source (Demirbas & Demirbas, 2007). It is still the main energy source in many developing countries (e.g. Nepal 97%, Bhutan 86%, Africa 39%) and mainly fuelwood is used as bio-energy in those countries for cooking and heating (Hoogwijk *et al.*, 2005).

Bangladesh is one of the most densely populated country in the world with 161.38 million people (WPP, 2019). Traditional biomass fuels are predominant sources of rural energy to meet cooking, commercial, and industrial needs in Bangladesh, mainly in the form of agricultural residues (46%), wood wastes (34%), and animal dung (20%) (Rahman *et al.*, 2013; Huda *et al.*, 2014; Islam *et al.*, 2014). Homestead, agriculture and plantation are main sources of biomass fuel and about 76% of rural fuel demand is supplied by biomass of which 74% are collected from agriculture and homestead (Akther *et al.*, 2010). The collection of biomass fuel is unsustainable (Hassan *et al.*, 2012) and overexploitation of natural and homestead forests is potentially sharing deforestation and day by day the shortage intensity is being increased across the country (Akther *et al.*, 2010).

The extraction and utilization of biomass fuel depend on demographic and socio-economic factors of households and varies from village to village, region to region and country to country. In Bangladesh, different aspects of biomass fuel have been studied by various researchers. Kennes *et al.* (1984) assessed the quantitative description of biomass energy situation by various socio-economic groups of Bangladesh. Bari *et al.* (1998) studied the biomass energy supply and use at village levels using three methods in Mymensingh and Kishoreganj district. Miah *et al.* (2003) assessed biomass fuel used by the rural households in Chittagong region. Jashimuddin *et al.* (2006) investigated the consumption of biomass fuel and preference pattern in disregarded villages of Sandwip and Noakhali Sadar upazila. Miah *et al.* (2010) investigated the consumption of energy by rural households in disregarded villages of Chandanaish upazila of Chittagong district. Miah *et al.* (2011) compared domestic energy use pattern of rural and semi-urban area of Noakhali district. Chowdhury *et al.* (2011) described biomass fuel use and burning techniques by forest user groups of Rema kalenga Wildlife Sanctuary. Halder *et al.* (2014) conducted research on the resources of biomass energy and practices of related technologies in Bangladesh. Baul *et al.* (2018) compared energy consumption and related emission from renewable (biomass) and non-renewable sources in Bangladesh. Alam *et al* (2019) investigated biomass fuel consumption pattern at household level in northern region of Bangladesh. But no comparative study on biomass fuel situation of forest and non-forest sources were carried out. There is a lack of reliable information on biomass fuel to meet up its demand-supply gap. Considering these view-points, the study was undertaken to compare per capita biomass fuel consumption by rural households of forest and non-forest area in the northern region of Bangladesh. The study also explored the types and sources of biomass fuel, and figured out the collection pattern of biomass fuel in both study areas.

MATERIALS AND METHODS

Two study areas were selected purposively, one for representing the forest area and another for the non-forest area. Birganj upazila (sub-district) of Dinajpur district and Ulipur upazila (sub-district) of Kurigram district were selected as forest area and non-forest area respectively (Figure 1). Birganj National Park and Singra National Park are located in Birganj upazila, while no forest is located in Ulipur upazila. Birganj Upazila is located in between 25°48' and 26°04' N latitudes and in between 88°29' and 88°44' E longitudes (Banglapedia, 2012a). Ulipur Upazila is located in between 25°33' and 25°49' N latitudes and in between 89°29' and 89°51' E longitudes (Banglapedia, 2012b). In Bangladesh a district has some upazilas, an upazila is composed of some unions, a union is composed of some Villages. Birganj upazila covering an area of 413.11 sq km which consists of 11 unions, 187 Villages. The total number of households is 73,895 and average household size is 4.27. The total population of Birganj upazila is 317,253 and the rate of literacy is 48.05 (BBS, 2011). Ulipur upazila covering an area of 458.48 sq km which consists of 13 unions, 354 villages. Total number of households is 103,061 and an average size of the household is 3.83. The total population of Ulipur upazila is 395,707 populations and the rate of literacy is 45.6 (BBS, 2011).

An adaptive multistage random sampling technique was applied to locate the villages and households where upazila was considered as primary sampling unit and households as ultimate sampling unit. The sequence of selection for this study was upazila to union, union to village, village to household. Vognagar union of Birganj upazila and Buraburi union of Ulipur upazilla were selected randomly. Adibashipara, Atharopaika

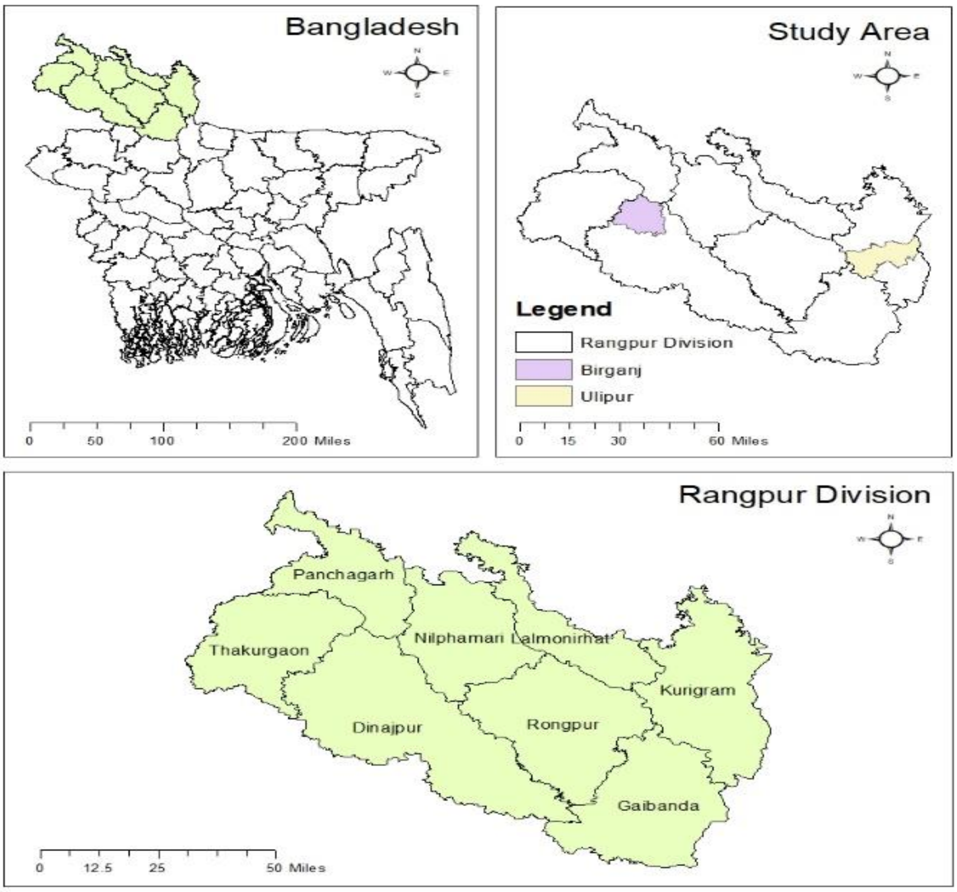

Figure 1. Map of the study areas

were selected randomly among the villages of Vognagar union and Buraburi union, respectively.

The survey was done by a semi-structured questionnaire after completion of a reconnaissance survey. According to preliminary survey the households of both study areas were divided into 3 categories based on the monthly income of the households: poor (less than 10000 BDT), medium (10000-15000 BDT), rich (more than 15000 BDT). A total of 90 households (15 from each category of each area) were selected randomly. The quantity of collection and consumption of biomass fuel was recorded daily basis in local units, later it was converted to kilogram. The survey was conducted from October-December (2019) and data were collected from last ten days of first months, second ten days of second month and first ten days of last month. A paired ranking exercise was also conducted after the interview to find out the fuelwood species were preferred by the respondents. Among the respondents 74% were female, 26% were male in forest area while 66% were female, 34% were male in non-forest area. A total of 63% respondents were illiterate and another 37% were literate in forest area on the other hand 46% of the respondents were illiterate and 54% were literate in non-forest area. The respondents age classes also varied, with 49% were above 50 years, 31% were 30-50 years, 20% were below 30 years in forest area while in non-forest area, 34% of the respondents were above 50 years, 37% were 30-50 years, 29% were below 30 years. Finally, all the data were cross checked in a group meeting at each village involving the people of various level.

The data was analyzed using Microsoft Excel (2013) and SPSS (23.0). ArcGis (10.8) was used for creating the map of the study area. One way analysis of variance (ANOVA) was used for examining significant difference of the variables between forest and non-forest area. ANOVA was also carried out for determining the significant difference of variables between the income groups of each area. Duncan's Multiple Range Test (DMRT) was conducted for identifying significant difference between variables within the forest and non-forest area.

RESULTS

Consumption of Biomass Fuel

Different income groups were found to be consumed various amount of biomass and per capita biomass fuel consumption differed significantly between forest and non-forest area. (Table 1) The average biomass fuel consumption of forest area was found to be 2.10 kg/capita/day. ANOVA indicated biomass fuel consumption of forest area varied significantly ($F=9.69$, $P<0.001$) between the income groups and DMRT revealed only the consumption of poor income group significantly ($p<0.05$) differed from medium and rich income group (Table 1). In non-forest area, average consumption of biomass fuel was 1.71 (kg/capita/day). The consumption between the income groups of non-forest area differed significantly ($F=3.25$, $P<0.05$) and DMRT determined biomass fuel consumption between poor and rich income groups varied significantly ($p<0.05$) while the consumption of medium income group did not differ significantly either with poor or rich income group (Table 1). The maximum amount of biomass fuel was consumed by poor households both in forest (2.28 kg/capita/day) and non-forest (1.81 kg/capita/day) area while less amount of biomass fuel was consumed by rich households both in forest area (1.89 kg/capita/day) and non-forest area (1.57 kg/capita/day) (Table 1).

Types of Biomass Fuel Used

The respondents of the study areas were asked to report on different types of biomass fuel for household use only. The stems, branches, leaves, agricultural residues, cow dung were the different types of biomass fuel used. The overall consumption of these types of biomass fuel varied significantly between forest area and non-forest area except leaves (Table 2). ANOVA determined that consumption of different types of biomass fuel significantly ($P<0.001$) varied between income groups of both forest and non-forest area and DMRT revealed the consumption of all these types of biomass fuel differed significantly ($p<0.05$) among the income groups of both areas (Table 3). Stems of trees were used as a major type of biomass fuel in both forest area (43%) and non-forest area (33%) (Table 3). In the forest area, branches of trees (23%) occupied the second-largest position as a type of biomass fuel used followed by leaves of trees (21%), agricultural residues (7%), cow dung (6%) while in non-forest area, agricultural residues (21%) obtained the second largest position followed by leaves of trees (17%), branches of trees (15%) and cow dung (14%) (Table 3). Rich households of forest area mainly consumed stems (72%), branches (15%), while medium households consumed stems (45%), branches (21%) and poor households consumed leaves (41%), branches (33%) (Table 3). Whereas in non-forest area, rich households mainly met biomass fuel needs as stems

Table 1. Average biomass fuel consumption by the households of the study areas

Income Groups	Biomass Fuel Consumption (kg/capita/day)	Average (kg/capita/day)
	Forest Area	
Poor	2.28 (±0.20) a	
Medium	2.14 (±0.17) b	2.10 (±0.30)***
Rich	1.89 (±0.33) b	
	Non-forest Area	
Poor	1.81 (±0.25) a	
Medium	1.74 (±0.26) ab	1.71 (±0.29)*
Rich	1.57 (±0.31) b	

Note: Same letter(s) in the same column were not significantly ($p<0.05$) different according to DMRT. Asterisks indicated significant (*$p<0.05$, ** $p< 0.01$, *** $p< 0.001$) difference of the variable(s) between the income groups according to ANOVA. Value inside the first bracket indicated standard deviation.

Table 2. Analysis of variance (ANOVA) of the variables between forest and non-forest area

Variables	F Value	P value
Biomass fuel consumption (kg/capita/day)	41.56	<0.001
Sources of biomass fuel		
Forest	112.83	<0.001
Market	0.001	0.973
Agriculture	45.24	<0.001
Homestead	10.53	0.002
Roadside plantation	20.97	<0.001
Types of biomass fuel		
Stems	4.15	0.045
Branches	13.97	<0.001
Leaves	1.72	0.192
Agricultural residues	43.97	<0.001
Cow dung	8.37	0.005

(60%), agricultural residues (23%), medium households as stems (35%), agricultural residues (30%) and poor households as cow dung (34%), leaves (33%) (Table 3).

Sources of Biomass Fuel

Total five sources of biomass fuel were identified in both study areas, as forest, roadside plantation, homestead, market and agriculture. Among the sources, overall contribution of forest, agriculture, homestead and roadside plantation significantly differed between forest and non-forest area except market (Table 2). ANOVA revealed the contribution of all sources varied significantly (p<0.001) between the income groups of both areas and DMRT determined the significant (p<0.05) difference of the contribution of each sources between the income groups of both forest and non-forest areas (Table 4). In forest area, the maximum amount of biomass fuel was collected from forest (44%) while less amount was collected from agriculture (5%). In non-forest area, maximum amount of biomass fuel was contributed by roadside plantation (31%) followed by market (28%), homestead (24%) and agriculture (17%) (Table 4). Poor households of forest area collected biomass fuel mainly from forest (78%) while poor households of non-forest area mainly collected biomass fuel from roadside plantation (75%) (Table 4). Market contributed the maximum amount of biomass fuel for rich households of both forest area (59%) and non-forest area (48%) while medium households of both areas collected

Table 3. Different types of biomass fuel (%) used by the households of the study areas

Income Groups	Stems	Branches	Leaves	Agricultural residues	Cow dung
Forest Area					
Poor	13 (±7.17) a	33 (±10.39) a	41 (±7.34) a	2 (±2.67) a	11 (±7.65) a
Medium	45 (±11.21) b	21 (±8.91) b	14 (±6.90) b	16 (±6.91) b	4 (±4.70) b
Rich	72 (±21.05) c	15 (±6.40) b	8 (±6.56) c	3 (±3.09) a	2 (±3.03) b
Average	43 (±28.07)***	23 (±11.39)***	21 (±16.02)***	7 (±7.88)***	6 (±6.67)***
Non-forest Area					
Poor	3 (±2.72) a	20 (±5.60) a	33 (±4.51) a	10 (±5.38) a	34 (±17.25) a
Medium	35 (±6.23) b	15 (±5.63) b	12 (±5.38) b	30 (±5.08) b	8 (±6.22) b
Rich	60 (±12.21) c	11 (±4.30) c	6 (±4.79) c	23 (±5.86) b	0 (±0.00) c
Average	33 (±25.24)***	15 (±6.67)***	17 (±12.65)***	21 (±7.85)***	14 (±17.95)***

Note: Same letter(s) in the same column were not significantly (p<0.05) different according to DMRT. Asterisks indicated significant (*p<0.05, ** p< 0.01, *** p< 0.001) difference of the variable(s) between the income groups according to ANOVA. Value inside the first bracket indicated standard deviation.

Table 4. Sources (%) of biomass fuel for the households of the study areas

Income Groups	Forest	Market	Agriculture	Homestead	Roadside Plantation
Forest Area					
Poor	78 (±11.20) a	0 (±0.00) a	2 (±1.92) a	8 (±5.01) a	12 (±5.81) a
Medium	39 (±8.85 b	25 (±11.82) b	11 (±7.96) b	17 (±8.72) b	8 (±6.80) b
Rich	15 (±8.51) c	59(±35.14) c	2 (±1.81) a	24 (±9.70) b	0 (±0.00) c
Average	44 (±27.95)***	28 (±32.27)***	5 (±6.38)***	16 (±10.12)***	7 (±7.13)***
Non-forest Area					
Poor	0 (±0.00)	0 (±0.00) a	10 (±6.72) a	15 (±9.21) a	75 (±16.33) a
Medium	0 (±0.00)	37 (±13.94) b	26 (±9.25) b	20 (±8.01) a	17 (±11.64) b
Rich	0 (±0.00)	48 (±33.10) b	16 (±8.72) a	36 (±7.02) b	0 (±0.00) c
Average	0 (±0.00)	28 (±29.01)***	17 (±10.5)***	24 (±10.94)***	31 (±34.42)***

Note: Same letter(s) in the same column were not significantly ($p<0.05$) different according to DMRT. Asterisks indicated significant (*$p<0.05$, ** $p< 0.01$, *** $p< 0.001$) difference of the variable(s) between the income groups according to ANOVA. Value inside the first bracket indicated standard deviation.

biomass fuel from all sources on average (Table 4).

Collection of Biomass Fuel

Female was identified as predominant collectors of biomass fuel from forest, roadside plantation, homestead and agriculture in both areas. 52% of biomass fuel of forest area and 65% of biomass fuel of non-forest area were collected by the female (Figure 2a). In forest area, male (35%) obtained the second major biomass fuel collector followed by children (13%), while in non-forest area, children (22%) occupied the second position followed by the male (13%) (Figure 2a). Most of the biomass fuel was collected during morning and noon in both areas. In the forest area, 61% of biomass fuel was collected in the noon followed by morning (22%), afternoon (17%) while in non-forest area, 51% of biomass fuel was collected in the morning followed by noon (40%), afternoon (9%) (Figure 2b).

Species Used as Biomass Fuel

A list of 39 species (tree and shrub) was identified as various parts of these were mostly used as biomass fuel in the study areas (Table 5). In forest area, *Shorea robusta* (90%) was most commonly used species followed by *Acacia auriculiformis* (78%), *Eucalyptus camaldulensis* (63%), *Bambusa* sp. (58%), *Melia azedarach* (53%), *Mangifera indica* (45%), *Samanea saman* (40%), *Litchi chinensis* (28%), *Artocarpus heterophyllus* (20%), *Swietenia macrophylla* (15%) (Figure c). In non-forest area, *Eucalyptus camaldulensis* (80%) was most used species as biomass fuel followed by *Lannea coromandelica* (70%), *Trewia nudiflora* (65%), *Bambusa* sp. (58%), *Acacia auriculiformis* (45%), *Mangifera indica* (45%), *Albizia procera* (35%), *Artocarpus heterophyllus* (30%), *Swietenia macrophylla* (23%), *Neolamarckia cadamba* (13%) (Figure 3).

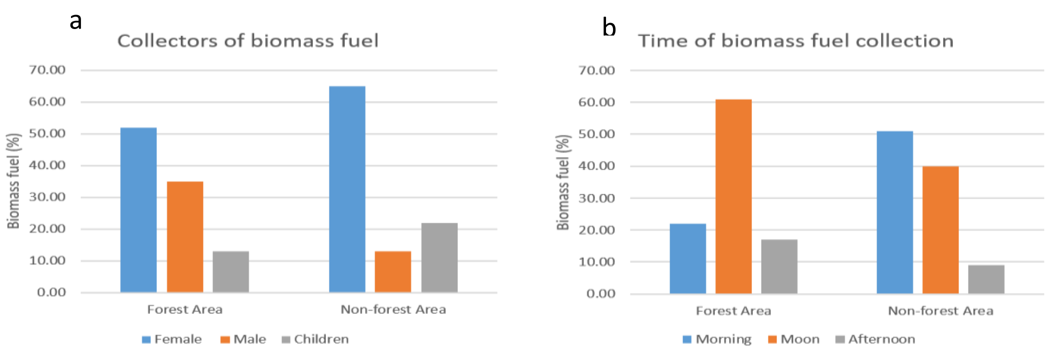

Figure 2. Collectors of biomass fuel (a) and time of biomass fuel collection (b) in the study areas

Table 5. Species used as biomass fuel in the study areas

Forest Area		Non-forest Area	
Local Name	**Scientific Name**	**Local Name**	**Scientific Name**
Akashmoni	*Acacia auriculiformis*	Akashmoni	*Acacia auriculiformis*
Am	*Mangifera indica*	Am	*Mangifera indica*
Amloki	*Phyllanthus emblica*	Bas	*Bambusa* sp.
Bahera	*Terminalia bellirica*	Boroi	*Ziziphus maurutiana*
Bas	*Bambusa* sp.	Chalta	*Dillenia indica*
Chapalish	*Artocarpus chaplasha*	Dogli/Pitali	*Trewia nudiflora*
Dumur	*Ficus hispida*	Eucalyptus	*Eucalyptus camaldulensis*
Eucalyptus	*Eucalyptus camaldulensis*	Henda	*Ricinus communis*
Gamar	*Gmelina arborea*	Jam	*Syzygium cumini*
Ghora-neem	*Melia azedarach*	Jhiga	*Lannea coromandelica*
Guti-jam	*Syzygium jambos*	Kadam	*Neolamarckia cadamba*
Jarul	*Lagerstroemia speciosa*	Kash	*Saccharum spontaneum*
Jolpai	*Elaeocarpus serratus*	Kathal	*Artocarpus heterophyllus*
Kadam	*Neolamarckia cadamba*	Kodbel	*Limonia acidissima*
Kathal	*Artocarpus heterophyllus*	Silkoroi	*Albizia procera*
Lichu	*Litchi chinensis*	Mehogoni	*Swietenia macrophyll*
Mangium	*Acacia mangium*	Neem	*Azadirachta indica*
Mehogoni	*Swietenia macrophyll*	Pakor	*Ficus rumphii*
Minjiri	*Senna siamea*	Peyara	*Psidium guajava*
Raintree	*Samanea saman*	Raintree	*Samanea saman*
Sal	*Shorea robusta*	Segun	*Tectona grandis*
Segun	*Tectona grandis*	Shimul	*Bombax ceiba*
Shimul	*Bombax ceiba*	Sissoo	*Dalberzia sissoo*
Silkoroi	*Albizia procera*	Sonalu	*Cassia fistula*
Sonalu	*Cassia fistula*	Supari	*Areca catechu*
Supari	*Areca catechu*	Tetul	*Tamarindus indica*

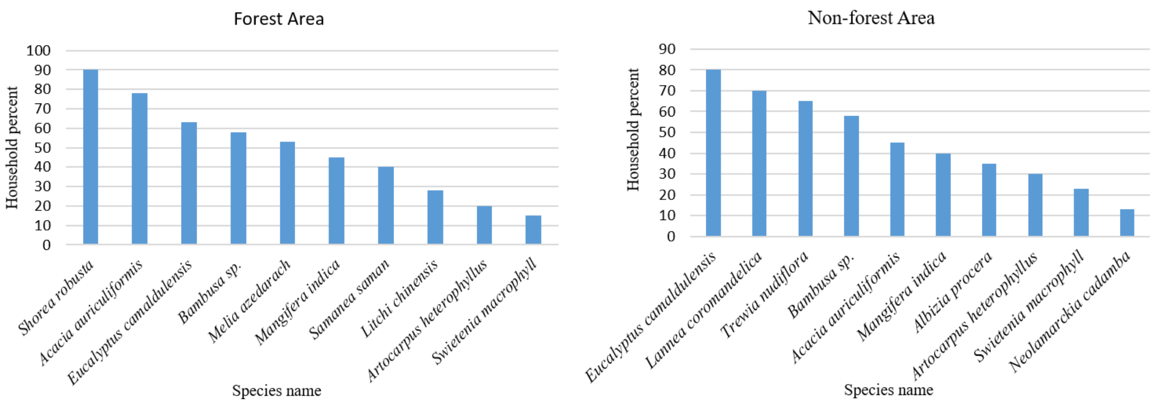

Figure 3. Preference of species for biomass fuel in the study area

DISCUSSION

The present study provided a comparative overview of biomass fuel consumption, collection and preference patterns of forest area and non-forest areas in the rural northern region of Bangladesh. The method of this study was similar to previous studies (Akther *et al.*, 2010; Hassan *et al.*, 2012; Alam *et al.*, 2019). Income is a determinant of social class and it enforces the way of life and biomass fuel consumer behavior, hence household income was the main consideration rather than other demographic profiles for grouping the households into various categories following Miah *et al.* (2003). The moisture content of biomass fuel was not always considered, therefore, the result of this study is an approximation and may not always be accurate. The present study revealed significant difference of per capita biomass fuel and the mean biomass fuel consumption was 2.10 kg/capita/day and 1.71 (kg/capita/day) in forest area and non-forest area, respectively. Bhatt and Sachan (2004) reported maximum and minimum biomass fuel consumption 2.80 kg/capita/day and 1.07 kg/capita/day respectively for households of different mountain villages of India while Kandel *et al.* (2016) reported 1.70 kg/capita/day for the rural households of community forest user groups of Nepal and the finding of the present study is corroborated with these previous studies. Rich households of both areas were found using LPG gas, electricity for cooking and heating while poor households have no such kind of options. Besides few rich households were also found using developed stove but all medium and poor households were found using traditional stoves only. Therefore, rich households of both areas consumed less amount of biomass fuel compared to poor and medium households.

The households of both forest and non-forest area used stems, branches, leaves, agricultural residues, cow dung as biomass fuel that consisted with the findings of Jashimuddin *et al.* (2006), Asaduzzaman *et al.* (2010) and Miah *et al.* (2010). Though the households of both areas used same components of biomass fuel, the present study revealed significant different patterns of biomass fuel consumption among the income groups of both areas. All the respondents opined that mainly household income, availability and accessibility to different types of biomass fuel brought about different consumption pattern. Maximum amount of stems and branches alone (87% in forest area, 71% in non-forest area) consumed by rich households while maximum of amount of leaves, agricultural residues, cow dung alone (54% in forest area, 77% in non-forest area) used by poor households and medium households consumed all types of biomass fuel on average. The finding of the present study is abrogated with Miah *et al.* (2003) that rich households consumed maximum amount of stems and branches alone (94%) while medium households consumed branches and leaves alone (77%) and poor households consumed leaves and agricultural residues alone (54%) in Chittagong region of Bangladesh. The households of forest area depended on the forest for biomass fuel and average 44% of total biomass fuel was contributed by forest while in non-forest-area the contribution of the forest was quenched by agriculture (17%) and roadside plantation (31%). The contribution of market was significantly same on average (28%) for both areas and homestead contributed average 8% more in non-forest area than forest area. All income groups did not have the same access to all sources of biomass fuel. Rich households had the ability to buy biomass fuel and almost all of them had a large homestead, but poor groups were mostly landless and did not have ability to buy. The rich households collected maximum amount of biomass fuel from market (59% in forest area, 48% in non-forest area) and homestead (24% in forest area, 36% in non-forest area). Poor households of forest area collected 78% of biomass fuel from forest while poor households of non-forest area collected 75% of biomass fuel from a roadside plantation. Medium households collected biomass fuel from all sources on average. Miah *et al.* (2003) reported market, homestead, agriculture, plantation contributed average 28%, 33%, 15%, 25% respectively and poor households collected 74% biomass fuel from the plantation, rich households collected 47% biomass fuel from market medium households collected from all sources averagely in rural areas of Chittagong (Bangladesh) which is almost similar to the present study.

Traditionally, women are mainly responsible for the domestic activities in the rural northern region of Bangladesh, therefore, women were found as major biomass fuel collector for both areas though, other family members helped them in biomass fuel collection. Most of the biomass fuel was collected during morning to noon. It is conceded as convenient time for biomass fuel collection by the respondents. Female collected biomass fuel after completing the domestic work in the morning, while male collected when only they got free from professional work. Similar type of gender disproportion in biomass fuel collection by rural households of Bangladesh was also reported by Hassan *et al*. (2013). The present study identified 39 species (tree, shrub) mostly used as biomass fuel in both study areas. *Shorea robusta*, *Eucalyptus camaldulensis* the most commonly used tree species in forest area and non-forest area respectively. The forests of northern Bangladesh is under the classification of tropical moist deciduous forest that mainly composed of *Shorea robusta* (Khan *et al.*, 2007) and *Eucalyptus* sp. were planted extensively in the degraded lands and roadsides of northern Bangladesh for diminishing fuel demand since 1977 (Hossain, 2016). The maximal use is possessed due to the availability of these species in the respective area. Alam *et al*. (2019) identified almost similar species used as biomass fuel in the northern region of Bangladesh and reported *Azadirachta indica* as most preferred followed by *Eucalyptus globules*, *Bambusa* sp., *Artocarpus heterophyllus*, *Mangifera indica*, and *Swietenia mahagoni*.

CONCLUSION

Despite the phenomenal economic condition of the study areas, the poor and medium households have become a pervasive feature of the biomass fuel consumption. The consumption, collection and preference patterns of biomass fuel significantly varied between forest and non-forest area of rural northern Bangladesh. It is aggravating the current situation of a huge amount of biomass fuel collection and creating a continuous pressure on natural forests and agricultural residues, which are very important components of mainly environmental stability and soil fertility respectively. The preferred tree species for biomass fuel sources indicates the high dependency on both natural forests and homesteads and it is alarming for the green future. The consumption of agricultural residues is also alarming in future agricultural food production. Therefore, immediate actions should be undertaken by the respective authorities to increase roadside plantations in forest areas and keep continuing the present plantation activities in non-forest areas for ensuring rural energy security and promoting sustainable energy supply.

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The Control of Panicle Blight Bacterial Pathogen on Rice Seeds Through *In Vitro* Treatments

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ABSTRACT

Burkholderia glumae is a seed-borne pathogen of rice known to cause bacterial panicle blight disease. The lack of effective control methods makes seed treatment the alternative management approach. The aim of this research was to determine an effective seed treatments technique, using liquid smoke, clove oil, hot water and copper hydroxide fungicide treatment against bacteria *B. glumae*. The experiment used a complete randomized design with five treatments and three replications, including control, liquid smoke, clove oil, hot water, and copper hydroxide fungicide. The results showed the propensity for all treatments to reduce bacterial populations on rice seeds, while liquid smoke, clove oil, and fungicide did not reduce vigour and viability. Application of copper hydroxide fungicide 77% at concentration of 5% was recommended as the best treatment to control the bacterial pathogen.

Keywords: *Burkholderia glumae*, clove oil, copper hydroxide fungicide, hot water, liquid smoke, rice

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INTRODUCTION

Rice has been an important staple food crop for over half of the world's population, including Indonesia (Ishaq *et al*., 2017). However, extreme weather conditions in the form of floods, droughts and the outbreak of pests and diseases have been affecting the crop productivity (Lamichhane *et al.*, 2015). In Indonesia, *Burkholderia glumae* Urakami *et al*. (1994) is the important causal bacterial pathogen of rice panicle blight disease or bacterial grain rot disease, causing severe infection, and reducing the rice yield up to 75% (Trung *et al*. 1993). Recently, the occurrence of panicle blight disease has been reported in several locations, including West, Central and East Java (Wiyono *et al*., 2017), as well as South Sulawesi (Baharuddin *et al*., 2017) and North Sumatra (Hasibuan *et al*., 2018). According to the Minister of Agriculture, Republic of Indonesia, *B. glumae* is categorized as an A2 quarantine plant pest, which is impossible to remove from the seeds through quarantine treatments (Quarantine Agriculture, 2015). Since the potential for seed-borne pathogens dispersal via seed trade is high, the use of clean seed is recommended as an initial management approach to control the rice panicle blight disease (Suryani, 2017). For example, dipping in liquid smoke (a natural product made from condensation or pyrolysis of materials containing lignin, cellulose, hemicellulose and other carbon compounds), clove oil as well as hot water treatment, in order to reduce or eliminate the pathogens (Taylor & Dye, 1976; Zagory & Parmeter, 1984; Milus, 1997; Light & van Staden, 2004; Situmeang, 2013; Belmar *et al.*, 2014; Achrom, 2015; Spadoro *et al*., 2017; Aisyah *et al*., 2018).

This study was aimed at testing the potential of several seed-treatment methods, including liquid smoke, clove oil, and hot water, to control *B. glumae* on rice seeds.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Plant Disease, Faculty of Agriculture, Universitas Sumatera Utara, Indonesia from May to October 2018.

Bacterial isolate of *B. glumae* (IRC PRC) was obtained from the collection at Laboratory of Plant Disease, Faculty of Agriculture, Universitas

Sumatera Utara, which was confirmed by the previous study (Hasibuan *et al*., 2018), cultured on King's B medium, and incubated at 37 °C for 48 h. The bacterial suspension in sterile distilled water was measured using a spectrophotometer in order to attain 10^8 cfu/ml (OD 0.5, λ = 600 nm). Conversely, the rice seeds were surface sterilized using NaOCl for 5 minutes, washed with sterile distilled water, and air-dried on sterile filter papers, followed by introduction into the bacterial suspension (10^8 cfu/ml) and agitation for 4 h at room temperature. Then, the inoculated seeds were air-dried in a laminar air flow cabinet for 45 minutes and left overnight (a minimum of 15 hours) before the treatments.

All inoculated seeds (100 seeds per treatment) were treated with the following treatments for 10 minutes:

- Sterile distilled water as control,
- liquid smoke [5% and 7%, (v/v)],
- clove oil [2% and 5%, (v/v)],
- hot water (50 °C and 60 °C),
- Copper hydroxide fungicide 77% [2% and 5%, (v/v)].

The concentration and temperature for each treatment were selected on the basis of previous studies (Tung & Serrano, 2011; Situmeang, 2013; Achrom, 2015; Hoerussalam *et al.*, 2017)

The bacterial count was subsequently determined by dipping the seeds in phosphate-buffered saline (PBS) solution, and ground to attain 80% crushed samples. Then, the samples were suspended in 20 ml NaCl for 1 h at room temperature and agitated for 2 h. Next, the suspensions were subjected to a 10-fold dilution series, plated on King's B medium, and incubated at 37 °C for 48 h. The bacterial colonies formed were counted.

After the seeds were treated, the seeds were germinated and assessed for seed vigour and viability. The seed vigour was assessed by evaluating the percentage of healthy seedlings grown five days after planting according to Eq. (1).

$$\mathrm{VI} = \frac{\Sigma\,\mathrm{NS\ 5\ dap}}{\Sigma\,\mathrm{seeds\ planted}} \times 100\% \qquad (1)$$

Note:
VI : Vigour Index; NS: normal seedlings; dap: day after planting

Seed viability assessment involved the evaluation of total healthy seedlings percentage 5 day- and 14 day-after planting according to Eq. (2).

$$\mathrm{VI} = \frac{\Sigma\,\mathrm{NS\ 5\ dap} + \Sigma\,\mathrm{NS\ 14\ dap}}{\Sigma\,\mathrm{seeds\ planted}} \times 100\% \qquad (2)$$

Note:
VI : Vigour Index; NS: normal seedlings; dap: day after planting

(Sutopo, 2004)

The data obtained were evaluated using Analysis of Variance for non-factorial Completed Block Design with three replications and Duncan's Multiple Range Test with SPSS Software to ascertain the possibility of treatments significantly affecting other parameters.

RESULTS AND DISCUSSION

The bacterial count were reduced after the treatments compared to the initial values recorded (10^8 cfu/ml), after a 48 h incubation (Table 1). All treatments were able to reduce bacterial population by up to 99%, as compared to the initial bacterial suspension concentration. Furthermore, all treatments significantly reduced the *B. glumae* bacterial count when compared to the control treatment. The higher concentration in all treatments resulted lesser bacterial count as compared to the lower concentrations. Therefore, the higher concentrations of each treatment was associated with the visibly reduced bacterial colonies, where fungicide at the concentration of 5%, together with the two concentrations of liquid smoke demonstrated the most significant effect.

The best seed vigour (95.57% and 94.53%) was observed with fungicide, at the respective concentration of 2% and 5%, which was not different from the control and as well as from the liquid smoke treatments (Table 2). Table 2 shows the outcome for clove oil and hot water treatments, which was not good, as 2% and 5% clove oil resulted in <80% of seeds capable of normal growth, based on the Indonesian standard quality requirements, which is more than 90% (Directorate General of Food Crop, 1991). Furthermore, none of the seeds were germinated in hot water treatment (Table 2).

Although there was no significant difference with the conrol treatment, rice seed germination rate was as high as control treatment (>80%) for the liquid smoke and fungicide treatments. Conversely, the germination rate was at 75.43% and 56.66%, when treated with clove oil at the respective concentration of 5% and 2%. The use of selected concentrations of clove oil were on the basis of previous studies (Kishore & Pande, 2007; van der Wolf *et al*., 2008; Hoerussalam., 2017), which applied not more than 5% of clove oil concentration as seed treatment for various crops. However, the rice seeds became toxic and could not germinate when the seeds were treated by clove oil at concentration of 2%, 3% and 4%. Also, 50 °C and 60 °C hot treatments were unsuitable alternatives, because almost all seeds were not germinated (Table 2). The rice seed germination with treatments as seen in Figure 1.

Copper hydroxide fungicide and liquid smoke were able to reduce the population of *B. glumae in vitro*. This was possibly due to the presence of active ingredients in the form of phenol compounds, organic acids, and also copper, which particularly inhibits bacterial growth by binding to and hindering the synthesis of proteins in bacterial cells (Aisyah *et al*., 2018). Therefore, errors are generated while reading the genetic codes, ultimately leading to bacterial cell death (Hikichi & Egami, 1998). The liquid smoke treatment is applied to suppress several plant diseases, based on the effective antimicrobial (Lingbeck *et al*., 2014) antioxidant and antibacterial activities (Yang *et al*., 2016). The major antibacterial compounds detected in liquid smoke include 2,6-dimethoxyphenol (syringol, 29.54%), 2-methoxyphenol (guaiacol, 12.36%), and 3,5-dimethoxy-4-hydroxytoluene (11.07%) (Yang *et al*., 2016). In addition, there are also a great potential for application as an organic pesticide and herbicide (Payamara, 2011).

Liquid smoke confers a positive effect in seed germination and seedlings vigor on a wide range of plant species (Abdollahi, 2012; Flematti *et al*., 2011). These activities are possibly due to the chemical composition, including catechol, which has previously been used for promoting *Nicotiana attenuata* root growth (Wang *et al*., 2017).

This current study showed a bad performance for hot water as a seed treatment to promote germination, as the heat generated does not break dormancy, but causes damages, especially in the embryo, which inhibits seeds growth. The hot water treatment at 54 °C for 10 minutes caused retardation and reduction in wheat seed germination due to seed coat breakage (Tapke, 1923). Meanwhile, the introduction of hot water at 55 °C for 25 minutes on tomato seeds infected by *Clavibacter michiganensis. michiganensis* (Smith) lead to a low germination rate of 52.22% (Nalis, 2015). This also occured after introducing corn seeds infected by *Pantoea stewartii subsp. Stewartii* to temperatures above 50 °C (Nalis, 2015).

Table 1. Bacterial population on rice seeds infected by *B. glumae* after different treatments

Treatments	Bacterial population (cfu/ml)
A0 (Control)	5.04×10^{8} a
A1 (Liquid smoke 5%)	3.26×10^{3} e
A2 (Liquid smoke 7%)	3.16×10^{3} e
A3 (Clove oil 2%)	4.69×10^{3} b
A4 (Clove oil 5%)	4.32×10^{3} c
A5 (Hot water 50ºC)	4.70×10^{3} b
A6 (Hot water 60ºC)	4.32×10^{3} c
A7 (Fungicide 2%)	3.89×10^{3} d
A8 (Fungicide 5%)	3.10×10^{3} e

Table 2. Post-treatment vigour and viability of rice seeds infected with *B. glumae*

Treatments	Seed vigour (%)	Seed viability (%)
A0 (Control)	89.35 a	93.40 ab
A1 (Liquid smoke 5%)	90.86 a	95.88 ab
A2 (Liquid smoke 7%)	87.63 a	88.21 b
A3 (clove oil 2%)	30.52 c	56.66 d
A4 (Clove oil 5%)	58.33 b	75.43 c
A5 (Hot water 50 ºC)	0.00 d	0.00 e
A6 (Hot water 60 ºC)	0.56 d	0.57 e
A7 (Fungicide 2%)	95.57 a	96.96 ab
A8 (Fungicide 5%)	94.53 a	99.14 a

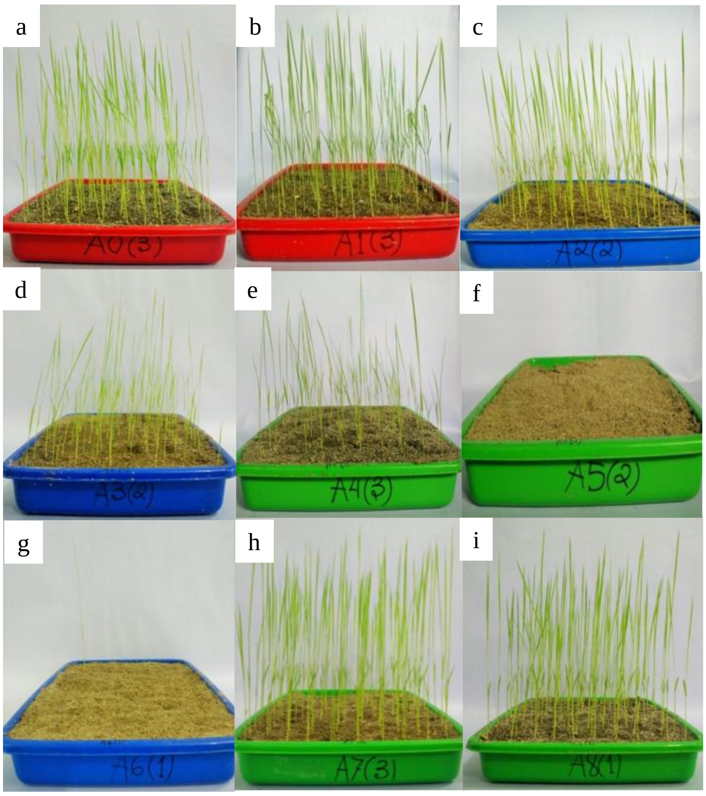

Figure 1. The germination of infected rice seeds with *B. glumae* after treated in the following treatments a) control; b) 5% liquid smoke; c) 7% liquid smoke; d) 2% clove oil; e) 5% clove oil; f) hot water at 50 ºC; g) hot water at 60 ºC; h) 2% fungicide; i) 5% fungicide

CONCLUSION

The application 5% copper hydroxide fungicide 77% could control *B. glumae* population on rice seeds. This treatment was confirmed to confer a positive influence on the vigour and viability of seeds.

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Diversity of Airborne Fungi at Pepper Plantation Lembah Bidong, Kuala Terengganu

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ABSTRACT

Piper nigrum L. is well-known as the king of spices and widely used in various field such as food and medicines. In Malaysia, 98% of pepper production comes from the state of Sarawak. The National Commodity Policy (2011-2020) targets to increase the pepper plantation area from the current 16,331 ha to 20,110 ha by year 2020. However, pepper diseases remain as a major challenge in the pepper industry. A great number of airborne fungi pathogen may contribute to a significant economic loss in pepper production. Therefore, this study aims to morphologically identify the diversity of fungi obtained from air-borne samples in a pepper planation that are capable of causing pepper plant diseases. This experiment was conducted at a pepper plantation near Lembah Bidong, Kuala Terengganu. An Andersen spore sampler was used to collect the fungi spores. Culture based identification were then made. The study resulted in the identification of four genus of fungi such as *Fusarium* sp, *Fusarium semictectum Fusarium oxysporum*, *Curvularia* sp., *Penicillium* sp. and *Trichoderma* sp. (Ascomycetes). Further molecular identification will confirm the species of fungal pathogens and more understanding of their population as well as severity.

Keywords: Pepper, *Piper nigrum* L., air-borne, fungi, Andersen spore sampler

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INTRODUCTION

In Malaysia, pepper plant is identified as one of the national commodities (Chen *et al.*, 2010). Malaysia is the fifth largest pepper producing country in the world with 98% of the country's annual production coming from the State of Sarawak (Adam *et al.*, 2018). Domestic pepper consumption increased from 12,000 tons in 2014 by 11% to 13,500 tons in year 2015 as reported by International Pepper Community (IPC) (2014). However, the production of black pepper started falling due to pest and disease occurrence since the early 1980s and it is the main problem faced by growers in Malaysia (Akinsanmi & Drenth, 2009).

Crop loss due to pests and diseases have resulted in a yearly reduction of about 2% of the total pepper area (Adam *et al.*, 2018). Several listed diseases of pepper plant such as anthracnose, *Phytophthora* foot rot, stem rot, fruit rot, mosaic viruses and and *Fusarium* wilt have been reported and are known to cause economic losses (Shahnazi *et al*., 2012; Farhana *et al*., 2013; Farith *et al*., 2015). In India, *Phytophthora* foot rot also known as quick wilt is recognized as one of the major causes of low productivity (Thomas, 2017). Additionally, the number of newly described *Phytophthora* species causing diseases in pepper plants have increased and *P. palmivora* has been identified as pathogen causing foot rot pepper vines in Malaysia (Brasier, 2008; Farhana *et al*., 2013; Farith *et al*., 2015; Habetewold *et.al*, 2017).

Some fungal pathogens such as *Fusarium*, *Penicillium* and *Aspergillus* which are known to cause stem rot, fruit rot, and wilt can be transferred by air-borne spores or survive in crop debris (Rivka, 2001; Shahnazi *et al*., 2012). Fungi of the genera *Cladosporium*, and *Penicillium* have the ability to produce a lot of spores that they can be found in virtually every cubic meter of air (Wyatt *et al.*, 2013).

Dispersal in air is one of many mechanisms by which plant pathogens can spread to new susceptible plants either within the same field or even in a completely different continent (Pady & Kapica, 2007; West & Kimber, 2015). Studies available on air-borne fungi pathogen sampling and identification in Malaysia and other Asian countries have mainly been carried out using dust collection methods (Cai *et al*., 2011; Norbäck *et al*., 2014), settle plate method (Shams-Ghahfarokhi *et al*., 2014) and the use of the single-stage viable cascade air sampler (SKC) (Er *et al*., 2015).

However, the single-stage thermo Scientific Andersen N6 Microbial Sampler used in this study has been reported to be very effective in trapping viable fungi pathogens in polluted air aerosol onto a 100 x 15 mm petri dish with agar because of the precision-drilled orifices in its impactor stage, its adjustable stage and the relatively higher flow rate of its pump (Gentry *et al.*, 2012). This study was thus set up to use the Andersen N6 microbial sampler to trap air-borne fungi spore in a pepper plantation near Lembah Bidong, Kuala Terengganu to determine the diversity of air-borne fungi that may cause diseases in the pepper plantation.

MATERIALS AND METHODS

Field Sampling

This study was conducted on 6th November 2019 from 3 pm to 6 pm at the only commercial pepper plantation in Lembah Bidong, Terengganu. The plantation follows a strict two weeks fungicide application scheduled hence sampling was carried out one week after fungicide application. A plot of 1.4 hectares was selected for the air-borne fungi sampling to be carried out. The zigzag method of point selection was chosen and in total of fifteen points were sampled (Figure 1). The blocks of pepper plants selected for sampling were 16 m apart and each block was 220 m long.

An Andersen N6 Microbial Sampler (Andersen Instruments Inc., USA) was used for the fungi spore sampling. The single stage was adjusted to a height of 1.5 m and potato dextrose agar (PDA) was exposed on the metal stage. Three sampling replicates were collected at each point and the pump of the sampler was turned on for three minutes.

Isolation of Fungi

After the air sampling, all the agar plates were incubated at room temperature for 2 to 7 days (27±2 °C). Different morphology from the fungi colonies such as mycelia formation and pigmentation were isolated. Then spores suspension was prepared and adjusted to concentration of 10^6 by using hemocytometer spores counting. The pure cultures were obtained by growing the single colony of the fungi isolated from the spore suspension prepared. Only pure single colony of fungi were selected for identification (Siti Nordahliawate *et al.,* 2012).

Identification of Fungi

After 7 to 10 days of incubation, morphological characteristics such as pigmentation and colony formation as well as microscopic characteristics such as conidia spores were observed under the microscope (Klich, 2002; Leslie & Summerell, 2006; Ellis, 1971). For the microscopic identification, slides were prepared and some small pieces of the pure cultures were cut as well and, observed at 100 x 10 magnification using Olympus CX22 (Olympus Corp., Japan) compound microscope.

Diversity of fungi

Colony-forming units (CFU) from the pure cultures were counted after which fungi diversity was determined. Fungi species diversity was calculated using the Shannon-Weiner Index as shown in Eq. (1) (Spellerberg, 2008).

$$H' = -\sum_{i=1}^{s} Pi \text{ In } Pi\binom{n}{k} \quad (1)$$

Where: ∑ refers to "the sum of" there are s species in the community. H' is the value of Shannon-Weiner Index. *Pi* is the relative abundance (proportion) of the i species in the community and ln is the natural log.

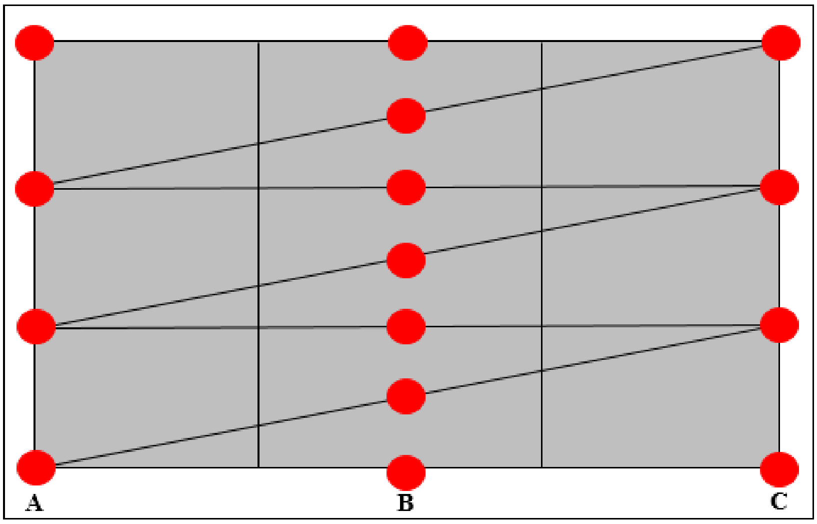

Figure 1. The dots show the zig-zag sampling points where the Anderson spore sampler was located for air sampling in a 1.4 hectares of pepper plantation

RESULTS

Fungi Identification

The results showed that a total of four fungi genus of the Ascomycota phylum were identified morphologically. They were *Fusarium* sp. *Curvularia* sp. *Penicillium* sp. and *Trichoderma* sp. All isolates were easily distinguished by the pigmentation and growth of the pure cultures through visual observation (Figure 2). Isolates identity were confirmed after microscopic observation especially shapes of conidia and other criteria such as conidiophore, phialides and chlamydospore (Figure 3).

We observed that the *Fusarium* sp. rapidly grow on PDA medium and produced robust woolly mycelium than others (Figure 2a). Microconidia was more common on hyphae growing on the agar. The mode of formation of microconidia, i.e. monophialides or polyphialides as well as the presence of microconidia chains and chlamydospores were observed for *Fusarium* morphology characteristics identification (Leslie and Summerell, 2006) (Figure 3a). Most of the macroconidia showed a distinct basal foot cell and whereas the microconidia were formed on simple chain with or without branches (Figure 3a). However, some species were observed showing some differences of macroconidia shapes such as *F. oxysporum* showed short with a thin walled and *F. semitectum* showed slender with a curved dorsal surface. *Fusarium* species are known to cause *Fusarium* wilt disease of black pepper and in China, it had caused a major decline in pepper production (Xiong *et al.*, 2015). In addition, Shahnazi *et al.* (2012) had reported that *Fusarium* species also giving yellowing disease that impact in economic losses at pepper plantations in Malaysia.

The genus *Curvularia* consists of more than 40 species and its taxonomy changed many times to accommodate species formerly classified as *Bipolaris* spp. (Zhang *et al.*, 2004; Kusai *et al.*, 2015). The cottony mycelial produced a border shape (regular or irregular) with pigmentation of colony ranges from black, moss green and sometime grey (Figure 2b). Conidia are ellipsoidal (Zhang *et al.*, 2004) (Figure 3b) that could easily be distinguished from the other fungi species such as *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. The *Curvularia* species are well known to cause leaf spot and leaf blight symptoms in maize, rice, beans and cowpea (Liu *et al.*, 2010; Al-Jaradi *et al.*, 2018). Although, little is known about it effects on black pepper nevertheless, there is a potential when conditions are favourable for foliar diseases to develop.

Both *Trichoderma* sp. and *Penicillium* sp. were easily distinguished morphologically from their pigmentation and formation on the PDA. The pure culture visually classified as *Trichoderma* sp.

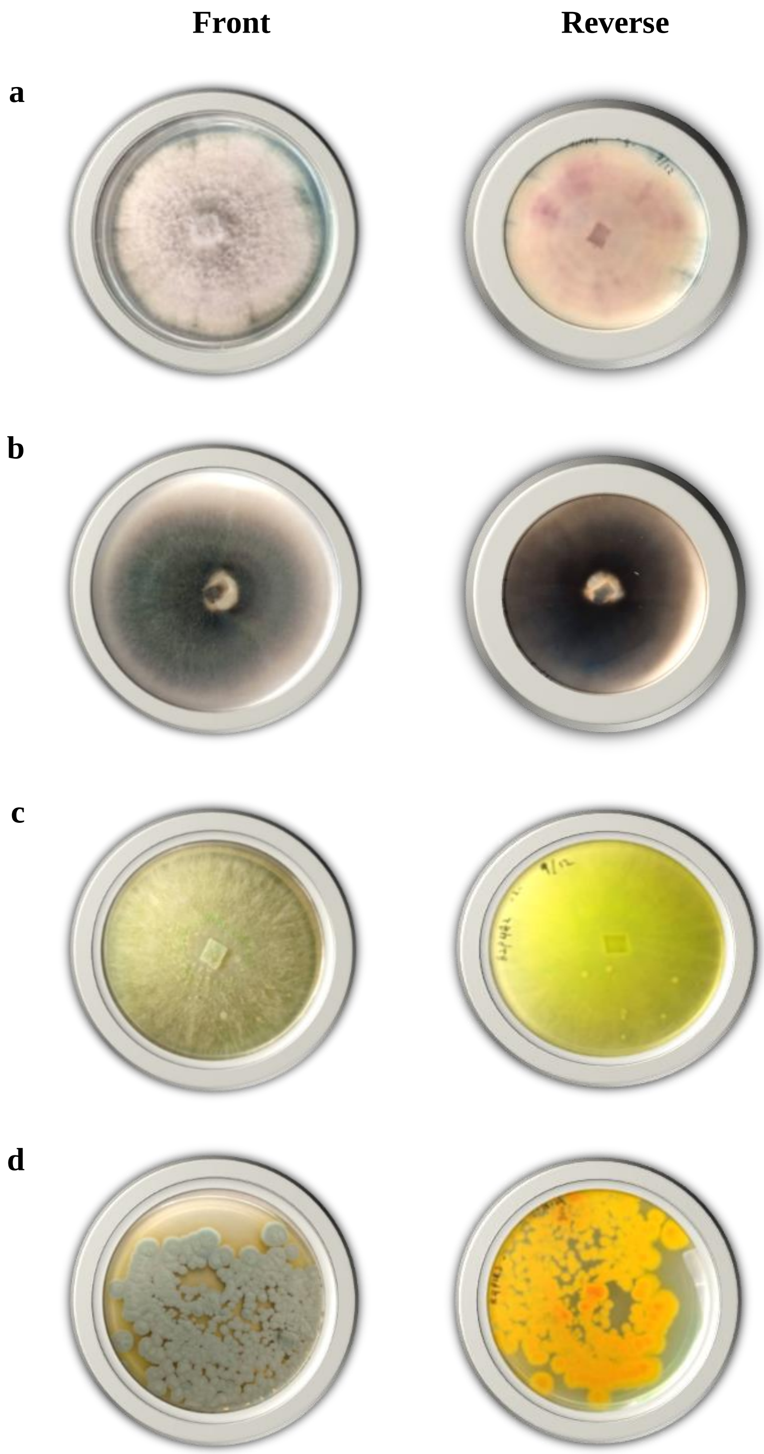

Figure 2. The variation of pigmentation and morphology of the fungal colonies on Potato Dextrose Agar (PDA) medium a) *Fusarium* sp., b) *Curvularia* sp., c) *Trichoderma* sp. and d) *Penicillium* sp.

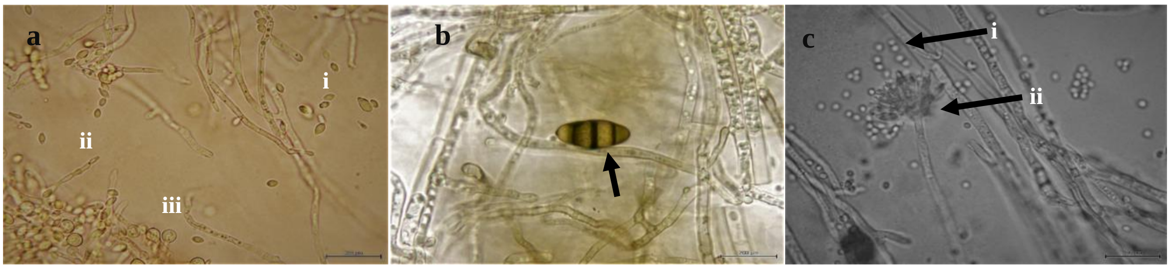

Figure 3. Microscopic characteristics of different fungi under 100 x 10 magnification. a. *Fusarium* sp. i: microconidia, ii: microconidia chain, iii: chlamydospore b. *Curvularia* sp. conidia (arrow) and c. *Penicillium* sp. i: conidia, ii: conidiophores

showed yellowish-light green pigmentation while the pure culture classified as *Penicillium* showed green pigmentation with white zone on PDA (Figure 2). For *Penicillium*, the colonies were rapidly growing, filamentous and cottony in texture (Figure 2d) that produced septate hyaline hyphae, branched conidiophores, phialides, and conidia (Figure 3c). *Penicillium* sp. is well known and also one of the most common fungi appearing in a diverse range of habitats, from soil to vegetation to air, indoor environments and various food product (Frisvad *et al.*, 2004). All the fungi identified in this study are well known plant pathogens whereas *Fusarium* and *Penicillium* are known to produce mycotoxins (Agrios, 2005; Perrone & Susca, 2017; Ji *et al.*, 2019). *Trichoderma* sp. may cause diseases in other plants but have also been reported to have the ability to reduce the foot rot pathogen *Phytophthora capsici* in pepper plants (Rajan *et al*., 2002).

Diversity of Fungi

The CFU was used to calculate the diversity of the six species that based on the Shannon-Weiner Index. Results showed that *Fusarium* sp. was the greatest (H' = 0.44) compared to other fungi species (Table 1). Thus, the black pepper plantation area is expected to be infected with *Fusarium* species when spores are abundance to invade the plant. Several factors may cause the spores abundance such as favourable conditions (weather and humidity) and the susceptible host (Agrios, 2005; Lacey & West, 2006).

Table 1. Diversity of fungi species at pepper plantation area isolated from air sampling

No.	Species (i)	Number in sample (CFU)	Species diversity (H')
1	*Fusarium* sp.	2,071	0.44
2	*Fusarium oxysporum*	102	0.14
3	*Fusarium semitectum*	7	0.02
4	*Curvularia* sp.	3	0.00
5	*Trichoderma* sp.	106	0.14
6	*Penicillium* sp.	85	0.12

CONCLUSION

This study proved that *Fusarium* sp. was the dominant fungi species identified compared to other fungi pathogens at the black pepper Lembah Bidong, Terengganu. Several Fusarium species may appear at one area such as in this study, three *Fusarium* species were identified with distinct morphological characteristics (*Fusarium oxysporum* and *F. semitectum*). However, there is limitation in morphological identification when most of the *Fusarium* species produced similar banana-shaped macroconidia (Leslie & Summerell, 2006).

Although *Fusarium* species are well-known soil-borne fungi, leaves infection will produce a massive microconidia and/or macroconidia. Consequently, could be dispersed throughout the area by air (Leslie & Summerell, 2006; West & Kimber, 2015; Lucas, 2020). All the species of *Fusarium* identified are known to cause diseases in pepper plants. *Fusarium oxysporium* causes *Fusarium* wilt in pepper plants and can cause great economic damage while *Fusarium semitectum* is reported to cause root rot. Some species of *Fusarium* are also known to cause leaf yellowing (Shahnazi *et al*., 2012).

The sampling date and season had favourable conditions for pathogen germination. This support the disease triangle concept which sates the importance of host, environment and pathogen for a disease to appear consequently resulting in disease epidemic (Agrios, 2005; Lucas, 2020). The mechanism of spores disperses such as tap and hail, will increase fungal pathogens infection at the field (Magyar *et al,* 2016). Therefore, by knowing the number of spores and species of fungi in the air will contribute to control measures instituted by the plantation. Moreover, it will help in decision making of the plantation especially in chemical control such as fungicides. A study by Siti Nordahliawate *et. al*, (2012) showed monitoring of air-borne spores and weather conditions can accurately predict when fungicide application maybe necessary.

At the field, air-borne spores contain several different fungi species that could be easily disseminated by wind blowing. Therefore, molecular approach could confirm the species when morphology identification shows a high degree of similarity and may cause misidentification. We believe that this study will benefit the pepper plantation to further monitor the air-borne fungi surrounding the field that may cause economically important diseases.

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An Ethnobotanical Study of Indigenous Leafy Vegetables Among Local Communities in Bintulu, Sarawak, Malaysia

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ABSTRACT

The consumption of indigenous leafy vegetables (ILV) is a common practice among local people in Bintulu. It serves as an important food resource for local communities in rural areas. However, these traditional practices were declining in urban areas and among younger generations. Therefore, the study aimed to record the ILV consumed by the local people, and mode of consumption of the ILV. A field survey was carried out with two phases; phase one was the distribution of structured questionnaires and the second phase was the interview session with 20 respondents from each of the three native markets located in Bintulu Division; Bintulu, Tatau, and Sebauh market. The study has identified 20 species of ILV from 18 different families: Agavaceae, Anacardiaceae, Athyriaceae, Blechnaceae, Brassicaceae, Compositae, Euphorbiaceae, Flacourtiaceae, Gnetaceae, Leguminosae, Limnocharitaceae, Menispermaceae, Myrtaceae, Olacaceae, Ophioglossaceae, Piperaceae, Smilacaceae and Verbenaceae. Identified ILV were consumed in many ways by the natives. The plant parts used also differ from one species to another and had different taste. A further study should be carried out to analyse the plant nutritional values and agronomy factors for commercialization of the potential ILV.

Keywords: Bintulu, consumption method, ethnobotany, ethnobotanical study, indigenous leafy vegetables

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INTRODUCTION

Leafy vegetables represent a group of edible plants in which the young shoots, leaves, and sometimes tender stems were included as foods. Utilization of indigenous plants that grow wildly in the jungle and its fringe, abandoned areas, along the stream, paddy fields, and swamp as leafy vegetables happened since decades ago and passed from generation to generation. Although the indigenous leafy vegetables (ILV) were often despising and lesser known compared to commercialised vegetables, their importance as food resources were comparable (Ainul Asyira *et al*., 2016; Akhtar *et al*., 2012; van Rensburg *et al*., 2004). In some countries such as Nigeria and other African countries, the uses of ILV were established as an approach to improve food security and have been described in many studies (van Rensburg *et al*., 2004; Aworh, 2015). The importance of ILV in reducing food scarcity and malnutrition could not be neglected (Aworh, 2015).

Generally, ILV were believed to have medicinal values and was included in daily diets to improve health. The old folks use the ILV as traditional medicine to reduce illness such as stomachache, diabetes, hypertension, proper functioning of kidney, treating intestinal worm problems and other illness (Kulip, 2003). The consumption of ILV is also believed to maintain skin health and preserving a youthful complexion (Samy *et al*., 2014). The high fibre content present in the leafy vegetables has been reported with the potential of preventing the occurrence of constipation (Dhingra *et al*., 2012). Besides, the intake of leafy vegetables also has been suggested to reduce obesity and cardiovascular disease (Hassan & Umar, 2006). Therefore, it is recommended to consume 400 g of vegetables per day (World Health Organization, 2012). Nowadays, people around the world practice the modern lifestyle, which includes the intake of higher processed food compared to natural foods (Hartley, 2015). This situation results in an increment of chronic diseases such as cardiovascular diseases, diabetes and hypertension among Malaysians.

In Malaysia, the ILV were consumed in various ways either eaten raw, cooked, fermented, blanched, or boiled and commonly included in a meal with rice, a staple food. Some of the ILV are very well known in the preparation of various traditional dishes. A proper processing method is commonly adopted to reduce antinutrient content in the ILV. Any ILV with high hydrogen cyanide content requires meticulous preparation such as soaking in running water and boiling to remove the poisonous content (Voon & Kueh, 1999). During dish preparation, other ingredients such as anchovies, shrimp paste, dried shrimp, and coconut milk are commonly included (Rukayah, 2000). Dish preparation is varied and influenced by the individual's preferences. According to Vorster and van Rensburg (2005), the cooking dishes are depending on the cultural groups and are commonly homogenized within the groups.

The utilization of ILV in Sarawak has been practised over generations as food sources (Shaffiq *et al.* 2013). However, due to rapid development in agriculture and food production, massive urbanization and modernization have caused ILV to be neglected and lesser-known compared to other commercial vegetables (Chai & Kho, 2000). Therefore, this study was conducted to determine the ILV consumed in Bintulu, Sarawak and their mode of consumption by local communities.

The information gathered from this study will enhance the awareness on the traditional knowledge of ILV among the communities.

MATERIALS & METHODS

Study Area

Bintulu was established as one of the divisions in Sarawak in January 1987 estimated at 12,166 km^2. It is in the Northern part of Sarawak and neighbouring other divisions namely Sibu, Miri, Kapit, and Mukah (Figure 1a). The area was divided into three districts namely Bintulu, Tatau, and Sebauh (Bintulu Development Authority, 2017) (Figure 1b). The study was conducted at three native markets in Bintulu, Tatau, and Sebauh where the locals commonly trading their harvested ILV (Figure 2). The native market is locally known as 'Pasar Tamu', which offered a wide variety of fresh vegetables from wild and jungle products (Bintulu Development Authority, 2017).

Data Collection

A field survey was conducted in the weekends from March to July 2015. The distribution of questionnaires was done from 7 am to 12 noon due to the high presence of native market visitors. The study aimed to determine the profile and behaviour of the consumers on ILV, therefore the participants were randomly selected.

According to the formula as described by Collins (1986), a sample size of respondent was determined as 120 respondents, which include the sellers and buyers at the markets. The questionnaire consists of two distinct parts, which are Part A and Part B. Part A was consisted of the demographic profile such as gender, age, living area and ethnic. Part B was designed to obtain the detailed background of the ILV such as type of vegetables consumed, growth form, habitat, and availability factors. The questionnaires were written in dual language, Malay and English to be understandable and user-friendly.

The second phase of the qualitative method involved a face-to-face interview, based on the availability of 20 most listed ILV from the questionnaires. A non-probability technique or snowball sampling method was used for selecting the respondents to identify potential samples and evaluated further information from the respondents. Upon identification of the ILV species, a further interview was conducted with 20 respondents of each market to get further information related to the ILV. For each identified plant species, the respondents were asked about the (i) local name (ii) habitat (iii) uses (iv) plant parts used (v) taste (vi) mode of consumption, and (vii) processing methods in dish preparation. The identified ILV available at the market were photographed. The availability of the ILV at the market was observed to study the probability of the market supply for all species.

Statistical Analysis

The statistical analysis was carried out using Statistical Package for Social Science (SPSS) of IBM SPSS Statistics V22.0 Software to compute frequency and descriptive analysis of the questionnaires. The descriptive analysis was used to find the mean score, median, and standard deviation of the demographic characteristics; ethnic, gender, age, and living area of the respondents. The qualitative data gained from respondents in the interview session were documented in table form.

RESULTS AND DISCUSSION

Role of Demographic on Consumption in ILV

This study was participated by various ethnics with the highest percentage recorded by Iban communities (58.30%), followed by Malay (12.50%), Chinese (7.50%), Melanau (4.20%), Kenyah (5.80%), Bidayuh (4.20%), and other ethnics (7.50%), with the mean and median 3.34 and 3.00, respectively (Table 1). Traditionally, Iban communities residing in the longhouse at the rural areas and alongside the riverbank, hence they practice unconventional farming such as hunting and gathering wild plants. As the modernization occurs rapidly, some of the communities shifted and reside in urban areas and urban outskirts in Bintulu, Sarawak for better education and employment (Ichikawa, 2019).

Female respondents involved were 68.30%, approximately two times higher than male respondents (31.70%). This study emphasized the role of the female in sustaining the practice of ILV consumption over generations. According to Howard (2003), indigenous vegetables are considered to be "female crops" in many parts of Africa because they are mostly grown or collected by women. Women also tend to utilise indigenous vegetables for domestic consumption or marketing. A previous study by van Rensburg *et al*. (2007) documented the responsibility of women in sustaining traditional knowledge among Africans. According to the study, women are dominant in collecting wild leafy vegetables and dishes preparation. Whereas men involved directly once the wild leafy vegetables were cultivated as food crops and domesticated (van Rensburg *et al*., 2007). Thus, the majority of studies done showed that women are more dominant and involved in ILV compared to men.

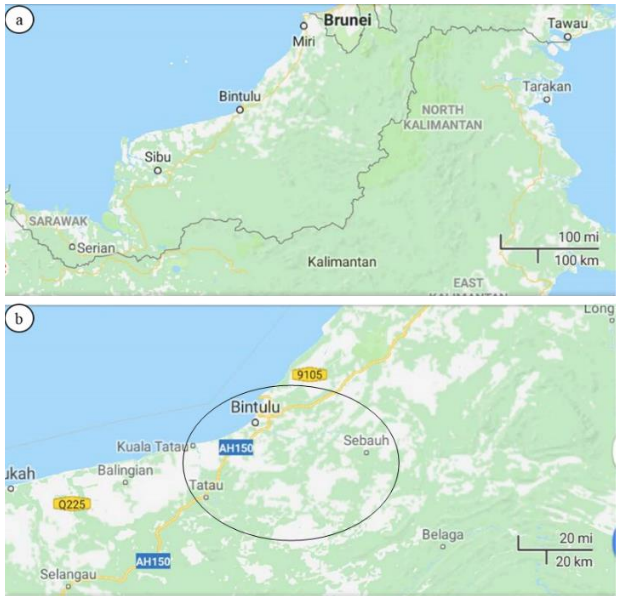

Figure 1. Map showing the location of (a) Bintulu Division in Sarawak and (b) location of Bintulu, Tatau and Sebauh native markets.

Figure 2. Studied areas: Bintulu market, Tatau market and Sebauh market.

Table 1: The demographic characteristics of the respondents

Characteristics	F (n=120)	%	Std. dev	Mean	Median
Ethnic			1.78	3.34	3.00
Iban	70	58.30			
Malay	15	12.50			
Chinese	9	7.50			
Melanau	5	4.20			
Kenyah	7	5.80			
Bidayuh	5	4.20			
Others	9	7.50			
Gender			0.47	1.68	2.00
Male	38	31.70			
Female	82	68.30			
Age			1.05	2.66	3.00
15-20	22	18.30			
21-30	26	21.70			
31-40	44	36.70			
41-50	27	22.50			
51-60	1	0.80			
Living area			0.67	2.10	2.00
Rural	66	55.00			
Suburban	33	27.50			
Town	21	17.50			

The consumptions of ILV were prevalent among middle-age people in the range of 31 - 40 years old with a percentage of 36.70%. The respondents among the younger generation with the age range from 15 - 20 and 21 - 30 years old recorded the percentage of 18.30% and 21.70%, respectively. From the interview session, it was identified that the younger generation especially those residing in the urban areas have less knowledge and practice on the ILV consumption since some of them migrated to town areas. Other study documented by Aban *et al*. (2009) showed similar results where the middle age people dominant in purchasing and consumption of ILV. Agboola *et al.* (2015) mention that the knowledge on ILV will be soon gone as the older generation passed away. Migrated people were also mostly influenced to study with what they had seen or knew outside their native (Agboola *et al.*, 2015).

The result revealed that the respondents involved in the study mainly residing in rural areas followed by suburban areas and town areas with a percentage of 55%, 27.5%, and 17.5%, respectively. Commonly, local people residing in the rural areas have easy access to the forest and have been supplying these vegetables to the people residing at the suburban and town areas (Samy *et al.*, 2014). In contrast, people living in the suburban and town areas who have limited access to the forest and wild areas tend to purchase these vegetables from the nearest markets. This showed the existence of a mutual role between people residing in rural areas, suburban and town areas. This factor influences the availability and purchasing rate of these vegetables in the market.

The Diversity of ILV Consumed by Local People

A total of twenty species of ILV that were commonly consumed by the locals were identified (Table 2 and Figure 3). Among the identified plants, there are 18 families and 20 genera. Euphorbiaceae was dominant with three species, while other families such as Agavaceae, Anacardiaceae, Athyriaceae, Blechnaceae, Brassicaceae, Compositae, Flacourtiaceae, Gnetaceae, Leguminosae, Limnocharitaceae, Menispermaceae, Myrtaceae, Olacaceae, Ophiglossaceae, Piperaceae, Smilacaceae and Verbenaceae represented by only one species. According to Rahman and Akter (2013), the Euphorbiaceae family is distributed abundantly in the tropical region including Indo-Malayan. Hence, the diversity of ILV species consumed by the local people in Bintulu from the Euphorbiaceae family higher than other families.

Six categories of growth form of the species were identified, which were listed by the respondents: trees, shrubs, herbs, ferns, aquatic plants, and creeping plants. Six species were shrub, followed by five tree species, herbs and ferns with three species, respectively, two aquatic plants species and lastly one creeping plant species. The previous ethnobotanical study by Chai and Kho (2000) revealed that the species of plants used by the locals residing at Ulu Skrang and Ulu Kanowit, Sarawak also dominated by trees and shrub. Other studies by Saupi *et al.* (2009; 2015) also recorded the consumption of aquatic plants as leafy vegetables. Hence, the growth forms of ILV at Bintulu Division market were mostly shrubs and trees however, other growth forms also can be found at the market.

Habitat of the ILV

Generally, 17 species of the ILV were collected from the wild and only three species have been cultivated for consumption (Figure 4). Five habitats were identified for the ILV collected from the wild: secondary forest, bushes, riverbank, swamp, and water channel. Out of the 17 species of wild vegetables, the majority of ILV were collected from the secondary forest with seven species, five species from bushes, two aquatic species available from the water channel areas, and two species of ferns from the swampy areas. Only one ILV species was collected from the riverbank (Figure 5). The findings from Chai and Kho (2000) in another division of Sarawak also recognised several species of wild edible plants that were used as vegetables. The wild plants are commonly sought as an alternative food source because of their abundant availability around housing areas especially for the locals residing in rural areas. Other studies in different countries also revealed the higher number of wild plant species that have been utilized as leafy vegetables compared to cultivated species (Irawan *et al*., 2006; Salinitro *et al*., 2017). Thus, natives tend to utilize ILV for daily consumption as the source can easily be accessed and collected from the wild or nearby areas.

Availability of the ILV at Native Markets

The availability of the ILV species was more frequent at the Bintulu market when compared to Tatau and Sebauh market (Table 3). The location of Bintulu market, which is located at the hub of Bintulu town may influence the frequency rate at this market. It became one of the attractions to the people from outside and inside of Bintulu. In addition, the activity of logistics such as plantation factory, cement manufacturing, oil and gas industry, and port activities invited many outsiders to stay and live at Bintulu town, which allows the marketing and demand of ILV to increase. Hence, the locals tend to supply the ILV at this market than other markets.

From the observations at these native markets, six species; *Diplazium esculentum*, *Dracaena elliptica*, *Gnetum gnemon*, *Pangium edule*, *Scorodocarpus borneensis* and *Stenochlaena palustris* were most frequently available. These vegetables were continuously available in every month observed, hence it indicated that these vegetables get higher demands by the locals. Voon and Kueh (1999) reported that these vegetables also preferred and consumed by people throughout Sarawak. Other vegetables such as *Mangifera pajang*, *Pterococcus corniculatus*, *Neptunia oleraceae* and *Limnocharis flava* are among the least frequently available, with 2-3 times within the observation periods.

Table 2. The description of the ILV such as family, local name, growth form and habitat

Local name	Family	Species	Growth form	Habitat
Sepang	Euphorbiaceae	*Acalypha caturus* Blume	Shrub	Cultivated
Ensabi Iban	Brassicaceae	*Brassica juncea* (L.) Czern var. Ensabi	Herb	Cultivated
Mandei	Euphorbiaceae	*Claoxylon longifolium* (Blume) Endl.	Shrub	Bushes
Paku	Athyriaceae	*Diplazium esculentum* (Retz.) Sw.	Fern	Swamp
Sabong kekura	Agavaceae	*Dracaena elliptica* Thumb	Shrub	Riverbank
Anak mambong	Compositae	*Erechtites valerianifolia* (Link ex Spreng) DC.	Herb	Bushes
Sabong	Gnetaceae	*Gnetum gnemon* L.	Tree	Forest
Tongkat langit	Ophiglossaceae	*Helminthostachys zeylanica* (L.) Hook	Fern	Forest
Jinjir	Limnocharitaceae	*Limnocharis flava* (L.) Buchenau	Aquatic	Ditch
Mawang	Anacardiaceae	*Mangifera pajang* Konsterman	Tree	Forest
Tangki	Leguminosae	*Neptunia oleraceae* Lour.	Aquatic	Waterway
Kepayang	Flacourtiaceae	*Pangium edule* Reinw.	Tree	Forest
Burung q	Piperaceae	*Piper umbellatum* (L.) Mi	Shrub	Forest
Singkil	Verbenaceae	*Premna cordifolia* Roxb.	Shrub	Bushes
Daun nga	Euphorbiaceae	*Pterococcus corniculatus* (Sm.) Pax & H. Hoffm	Herb	Cultivated
Tubu	Menispermaceae	*Pycnarrhena tumefacta* Miers	Tree	Forest
Kesinduk	Olacaceae	*Scorodocarpus borneenssis* Becc.	Tree	Forest
Kemudang	Smilacaceae	*Smilax odoratissima* Blume	Creeping	Bushes
Midin	Blechnaceae	*Stenochlaena palustris* (Burm.f.) Bedd.	Fern	Swamp
Bungkang	Myrtaceae	*Syzgium polyanthum* (Wight) Walp.	Shrub	Bushes

Figure 3. ILV consumed by local peoples in Bintulu Sarawak

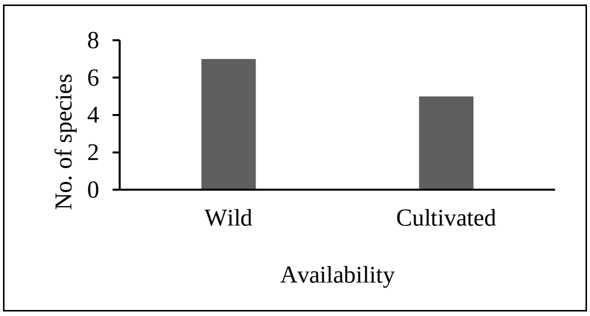

Figure 4. The availability of the ILV from the wild and cultivated

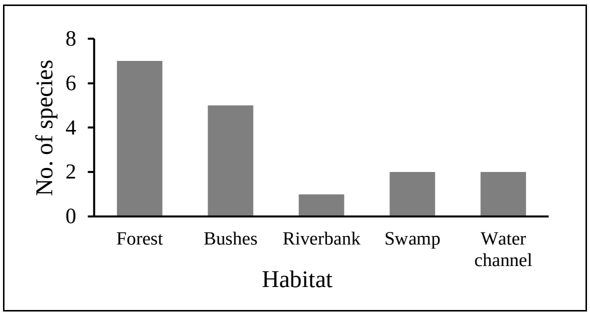

Figure 5. The number of ILV species obtained from various habitat

Factors Influencing the Availability of ILV at Native Markets

The availability of ILV at these native markets was influenced by the factors such as consumer demand (46.70%), the habitat of the ILV (21.70%), weather (20.80%), supply (6.70%), and price (4.20%) (Figure 6). The ILV were commonly used in the preparation of various traditional delicacies served in the daily diet and during festival or celebration. Therefore, ILV are commonly at high demand. The seller always ensures the continuous supply of the ILV on their shelves to fulfil this demand (Man *et al*., 2009). However, ensuring a continuous supply of the wild species has been a challenge especially during the rainy season. Price was the least factor that influenced the availability of the ILV, since they were commonly sold at lower price compared to commercialized vegetables.

Table 3. Availability of the ILV at the native market observed in March until July

ILV	Bintulu					Tatau					Sebauh				
	Mh	**Ap**	**My**	**Jn**	**Jl**	**Mh**	**Ap**	**My**	**Jn**	**Jl**	**Mh**	**Ap**	**My**	**Jn**	**Jl**
A. caturus		/	/	/	/		/	/				/	/		
B. juncea	/	/	/	/					/	/	/	/	/		
C. longifolium	/	/	/	/	/				/			/	/	/	
D. esculentum	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
D. elliptica	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
E. valerianifolia	/	/	/	/	/			/		/				/	/
G. gnemon	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
H. zeylanica			/	/	/					/	/	/	/	/	
L. flava	/	/													
M. pajang						/	/	/				/			
N. oleraceae		/													
P. edule	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
P. umbellatum	/	/	/	/		/	/								/
P. cordifolia	/	/	/	/	/	/	/				/	/	/		
P. corniculatus					/					/					
P. tumefacta		/	/	/	/	/	/				/	/			
S. borneenssis	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
S. odoratissima		/	/	/			/	/	/		/	/	/		
S. palustris	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
S. polyanthum		/	/	/	/	/	/				/	/	/		
Total number	12	17	16	16	14	11	13	10	9	10	12	15	13	9	8

Mode of Consumption

The study revealed that ILV were used in different modes of consumption (Table 4). There were 14 species that are cooked as stir-fried vegetables, three species are used as seasoning, two species are fermented, and one species being eaten raw (Figure 7). The stir-fried vegetables commonly include other ingredients to enhance the taste such as anchovies, coconut milk, shrimp paste, black pepper, or mixed with other vegetables according to the consumer's preference (Rukayah, 2000).

The *P. edule* and *Brassica juncea* are very well known in the preparation of fermented food called 'kasam' by the locals and *B. juncea* is known as 'kimchi iban'. Several ILV that have a strong taste are also mainly used as seasoning. This includes *S. borneensis*, *Syzgium polyanthum* and *Pycnarrhena tumefacta* which are used to add flavour in the preparation of fish or meat dishes. *Scorodocarpus borneensis* possesses strong and pungent smell like garlic, hence is sometimes used by the locals to replace garlic (Chai & Kho, 2000). The dried leaves of *P. tumefacta* are pounded and used as seasoning similar to monosodium glutamate (Det *et al*., 2013). Whereas *Erechtites valeriani-folia* are eaten raw or commonly dipped into a special sauce made up of shrimp paste and chili pepper known locally as 'sambal belacan'.

The locals commonly use the soft parts of the plant such as young leaves, shoots, buds, petioles, and tender stem in the dish preparation. The larger leaves will be chopped or crushed into a smaller portion to be easily cooked and chewed. Normally, the seller at the market already packed the edible part of the different vegetables for customer's easy use. The leaves of *Acalypha caturus, B. juncea, Claoxylon longifo-lium, D. esculentum, E. valeria-nifolia, G. gnemon, Helminthostachys zeylanica, L. flava, N. oleraceae, Piper umbellatum, P. tume-facta, Smilax odoratis-sima, S. palustris* and *S. polyanthum* have a sweet taste, hence they are favoured by the locals. The leaves with hairy structures at the adaxial and abaxial surface will cause sensory deterrent during consumption, therefore the hairy structure of *P. umbellatum* leaves was always discarded.

Table 4. The preparation methods of ILV.

Local name	Family	Species	Part Used	Taste	Processing methods
Sepang	Euphorbiaceae	*Acalypha caturus* Blume	Shoots, leaves	Sweet	The leaves used as fried vegetables together with anchovies
Ensabi Iban	Brassicaceae	*Brassica juncea* (L.) Czern var. Ensabi	Leaves, petioles	Sweet	The leaves and petioles were fermented and serve as 'kimchi'
Mandei	Euphorbiaceae	*Claoxylon longifolium* (Blume) Endl.	Leaves, bud	Sweet	The young leaves chopped and cooked with coconut milk and anchovies
Paku	Athyriaceae	*Diplazium esculentum* (Retz.) Sw.	Shoots, tender stem	Sweet	The leaves and stem chopped and fried together with anchovies and shrimp paste
Sabong kekura	Agavaceae	*Dracaena elliptica* Thumb	Young leaves	Bitter	Stir-fried together with anchovies and shrimp paste
Anak mambong	Compositae	*Erechtites valerianifolia* (Link ex Spreng) DC.	Young leaves	Sweet	Eaten raw as 'salad' or blanched like cassava leaves
Sabong	Gnetaceae	*Gnetum gnemon* L.	Leaves, petioles	Sweet	Fried and mixed with other vegetables
Tongkat langit	Ophiglossaceae	*Helminthostachys zeylanica* (L.) Hook	Leaves, petiole, tender stem	Sweet	Blanched and prepared as dishes called 'kerabu' and fried with shrimp paste
Jinjir	Limnocharitaceae	*Limnocharis flava* (L.) Buchenau	Leaves, shoot, petiole	Sweet	The leaves and petiole were chopped and fried with anchovies
Mawang	Anacardiaceae	*Mangifera pajang* Konsterman	Young leaves	Sour	The leaves were chopped and fried together with shrimp paste, chilli and anchovies
Tangki	Leguminosae	*Neptunia oleraceae* Lour.	Shoots, tender stem	Sweet	Leaves and stem were chopped and cooked together with coconut milk
Kepayang	Flacourtiaceae	*Pangium edule* Reinw.	Leaves	Bitter	The leaves soaked in the water a few days, boiled and fermented as 'kasam'
Burung	Piperaceae	*Piper umbellatum* (L.) Mi	Shoots, leaves	Sweet	The leaves are cut and cooked as soup together with fish. The hair removed by crushed the leaves with hand in running water
Singkil	Verbenaceae	*Premna cordifolia* Roxb.	Leaves	Sweet & foul smell	Leaves are stir-fried together with bamboo shoots
Daun nga	Euphorbiaceae	*Pterococcus corniculatus* (Sm.) Pax & H. Hoffm	Leaves, petiole	Sweet	The leaves were fried with shrimp paste and other vegetables
Tubu	Menispermaceae	*Pycnarrhena tumefacta* Miers	Leaves	Sweet	The leaves are added in the preparation of meat and fish dishes
Kesinduk	Olacaceae	*Scorodocarpus borneenssis* Becc.	Leaves	Sweet & foul smell	The leaves used as a seasoning like garlic or fried with other vegetables
Kemudang	Smilacaceae	*Smilax odoratissima* Blume	Shoots, leaves	Sweet	Leaves are chopped and fried with other vegetables
Midin	Blechnaceae	*Stenochlaena palustris* (Burm.f.) Bedd.	Shoots, tender stem	Sweet	Stir-fried with anchovies and shrimp paste
Bungkang	Myrtaceae	*Syzgium polyanthum* (Wight) Walp.	Leaves	Sweet	The fresh leaves used as ingredients in meat dishes and dried leaves used as a seasoning

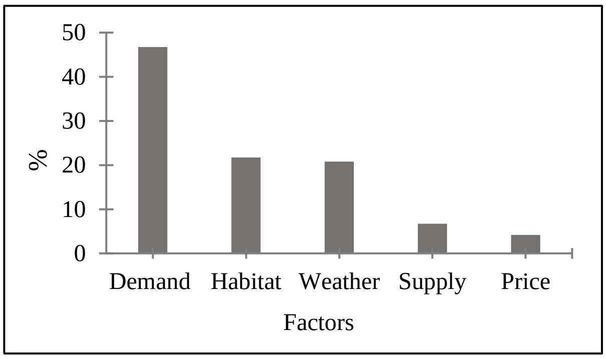

Figure 6. The factors that influenced the availability of ILV at the markets

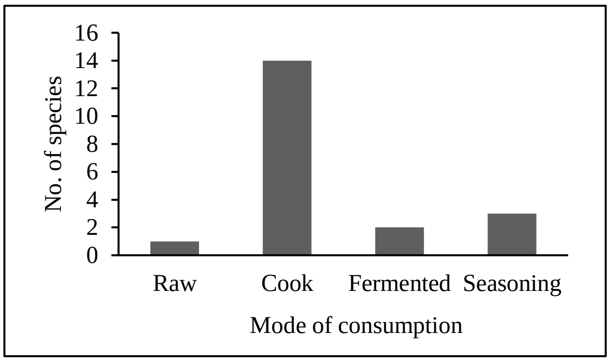

Figure 7. Mode of consumption of the ILV

CONCLUSION

Ethnobotanical information from this study had allowed the identification of twenty ILV species that were commonly consumed by the local communities in Bintulu, Sarawak. ILV can be consumed in various preparation methods which can help to overcome the hunger in the community. The traditional knowledge is diminishing among the younger generation. Therefore, more studies should be conducted to document the uses of diverse wild plants in Bintulu. This study provides reference information for science such as agriculture development, by providing valuable information on ILV, which may have agriculture value such as new food crops.

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Termite Fauna of Gunung Telapak Buruk, Berembun Forest Reserve, Negeri Sembilan, Malaysia

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ABSTRACT

Termites have great importance in a tropical terrestrial ecosystem, especially in the decomposition process, mediate ecosystem processes and facilitate in improving the structure and quality of the soil. Termite survey was conducted at Gunung Telapak Buruk, Berembun Forest Reserve, Negeri Sembilan, during a scientific expedition from 30th March to 1st April 2019. This study aims to provide the first checklist of termite fauna in Gunung Telapak Buruk. Termites were collected using the casual collection method at selected trails around Gunung Telapak Buruk. A total of 21 termite species were recorded in this study. The termite assemblage comprises two families, namely Rhinotermitidae and Termitidae. Family Termitidae dominated the termite assemblage with 90.5% (19 species). The collected termite species in this study comprises 12% of recorded termite species of Peninsular Malaysia by Tho in 1992. The genus *Odontotermes* from the family Termitidae dominated the assemblage with five species. Four types of feeding groups were identified in this study. The wood feeders recorded highest number species with 66.7%, followed by soil feeders (14.3%), epiphyte feeder (9.52%), and wood-litter feeders (9.52%). All the recorded species are new record for this forest reserve as no previous record was available for this area.

Keywords: Diversity, forest, highland, mountain, termite fauna

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INTRODUCTION

Systematic study on termites in Malaysia has started since 1980s, whereby two main termite identification guide books for Malaysia were published in the year 1981 by Thapa and 1992 by Tho. Since then, termite research in Malaysia have covered the area of fundamental and application studies (Eggleton *et al*., 1997; Jones & Brendell, 1998; Jones *et al*., 1998; Homathevi *et al*., 2002; Lee, 2004; Abdul Hafiz *et al*., 2007; Abdul Hafiz & Abu Hassan, 2009; Hanis *et al*., 2014; Nivaarani & Homathevi, 2015; Kori & Arumugam, 2017; Rahman *et al*., 2018; Saputra *et al*., 2018; Visheentha *et al*., 2018; Alia Diyana *et al*., 2019; Arumugam *et al*., 2019). However, the pest problem caused by termites and the development in science and technology has reduced the focus on forest termite diversity study, especially in Peninsular Malaysia. There is a high necessity for termite diversity study to update the number of termite species of Malaysia, where the final collection list was made by Tho (1992). There are still many unexplored places in Peninsular Malaysia. Hence, this study was focused on Gunung Telapak Buruk (GTB), Berembun Forest Reserve. This study is the first scientific termite study conducted at GTB to identify the termite species composition.

Termites are classified under the order Blattodea (Inward *et al*., 2007). From the listed 3106 living and fossil termites, 175 termite species have been recorded in Peninsular Malaysia (Tho, 1992; Krishna, 2013). They are one of the significant organisms that contribute to the decomposition process in the forest (Wood & Johnson, 1986). Termites also provide other ecosystem services in the forest such as improvement of soil structure and quality, facilitation of nutrient cycles (carbon and nitrogen cycle), and continuation of the food chain (Wood & Johnson, 1986; Bignell & Eggleton, 2000; McGavin, 2001; Inoue *et al*., 2006). The termite community also can be used as an environmental bioindicator to observe changes in land use (Pribadi *et al*., 2011). However, 10% of the total termite species are documented as pests in the world (Kirton, 2005). The termites have caused severe structural damage and, consequently, high economic losses in many countries (Adams & Raj, 2005; Gibb & Oseto, 2006).

The eusocial termite's colony comprises four main castes, which are king, queen, soldiers, and workers (Gibb & Oseto, 2006). King and queen are the winged reproductive and responsible for the reproduction in the colony. Soldiers are the caste protecting the colony. Soldiers can be differentiated from other castes with the appearance of either mandible or rostrum on their head. Workers are the sterile individuals in the colony that take care of the egg-laying queen, provide food for the colony and construct new tunnels and chambers (Higashi *et al*., 2000; Gibb & Oseto, 2006). Termites can be classified into different groups according to their feeding and nesting behaviour. Termites were grouped based on the classification system proposed by Eggleton *et al*. (1997) as follows: wood-feeders, litter forages, micro-epiphyte feeder, soil-feeders, and soil-wood interface-feeders. The nesting group comprises wood nesters, arboreal nesters, epigeal nesters, and hypogeal or subterranean nester (Bignell & Eggleton, 2000).

MATERIALS AND METHODS

Termite collection was done at GTB during Gunung Telapak Buruk Scientific Expedition 2019 that was organised by Negeri Sembilan Forestry Department. Gunung Telapak Buruk located at Berembun Forest Reserve in Negeri Sembilan state (2°49'59.8332"N, 102°2'39.2748"E). The data collection was performed from 30th March until 1st April 2019 using casual collection method. Termites were collected at four sampling sites that were surrounded by primary forest, namely Track CP2, Track CP4, Track Transmitter, and main road around the campsite. The elevation of the sampling sites is about 900 m above sea level. Termites (five worker and soldier castes) were collected along the trails manually using forceps. Microhabitats such as mound, nest, mud tubes on a tree trunk, leaf litter, and deadwood were searched for termites (Nivaarani & Homathevi, 2015; Arumugam *et al*., 2019). Collected termites were preserved in 80% ethanol. Further identification was done at Natural Resources Laboratory, Faculty of Earth Science (FSB), Universiti Malaysia Kelantan (UMK), Jeli Campus, with the aid of Thapa (1981) and Tho (1992) using a stereomicroscope. Collected termites were deposited at Natural Resources Museum of FSB, UMK, Jeli Campus for future reference.

RESULTS AND DISCUSSION

Termite survey at GTB recorded a total of 21 termite species. The assemblage comprises two families (Rhinotermitidae and Termitidae) and 13 genera (Table 1). Termitidae recorded the highest number of species (19 species) at GTB, as documented in other termite studies across Malaysia (Homathevi *et al*., 2002; Nivaarani & Homathevi, 2015; Kori & Arumugam, 2017; Arumugam *et al*., 2019). This is because Termitidae is well known as the largest termite family in the world where the family comprises 75% to 80% of the termite species (Tho, 1992; Brandl *et al*., 2007). Termitidae also dominated the assemblage with three subfamilies (Macrotermitinae, Nasutitermitinae, and Termitinae) while Rhinotermitidae recorded only one subfamily, Rhinotermitinae. Subfamily Nasutitermitinae and genus *Odontotermes* from the family Termitidae documented the highest number of species with 11 species and five species, respectively (Figure 1).

The feeding group and nesting group of the identified termites were analysed based on Eggleton *et al*. (1997) and Bignell and Eggleton (2000). Wood feeders dominated the assemblage with 66.7%, followed by soil feeders with 14.3%, epiphyte feeders (9.52%), and wood (litter) feeders (9.52%) as shown in Figure 2. The termite assemblage of the study site also comprises hypogeal nesters (52.4%), arboreal nesters (28.6%), and wood nesters (19.05%). However, this result may be due to the sampling strategy conducted in this study. Factors such as collection method and inadequate sampling effort in soil sample of forest floor influence the type of feeders collected at GTB. This may result to the highest number of wood feeders recorded in this study. Standardized line transect method is recommended for future termite study at GTB.

Table 1. Termite species recorded at Gunung Telapak Buruk: Feeding groups, w = wood feeders, s = soil feeders, epy = epiphyte feeders, l = litter feeders, f = fungus growers. Nesting groups, a = arboreal nesters, w = wood nesters, h = hypogeal nesters

	Feeding Group	Nesting Group
Family: Rhinotermitidae		
Subfamily: Rhinotermitinae		
Schedorhinotermes medioobscurus (Holmgren)	w	w
Parrhinotermes aequalis (Haviland)	w	w
Family: Termitidae		
Subfamily: Macrotermitinae		
Macrotermes carbonarius (Hagen)	w/l	h
Macrotermes malaccensis (Haviland)	w/l	h
Odontotermes neodenticulatus Thapa	w(f)	h
Odontotermes oblongatus Holmgren	w(f)	h
Odontotermes prodives Thapa	w(f)	h
Odontotermes sarawakensis Holmgren	w(f)	h
Odontotermes sp.	w(f)	h
Subfamily: Nasutitermitinae		
Bulbitermes flavicans (Holmgren)	w	a
Bulbitermes sp. 1	w	a
Bulbitermes sp. 2	w	a
Havilanditermes sp.	w	w
Hirtitermes hirtiventris (Holmgren)	w	w
Hospitalitermes hospitalis (Haviland)	epy	a
Hospitalitermes umbrinus (Haviland)	epy	a
Leucopitermes sp.	s	h
Longipeditermes longipes (Haviland)	w	h
Nasutitermes regularis (Haviland)	w	a
Subulioditermes major (Thapa)	s	h
Subfamily: Termitinae		
Coxocapritermes sp.	s	h
Total number of species = 21		

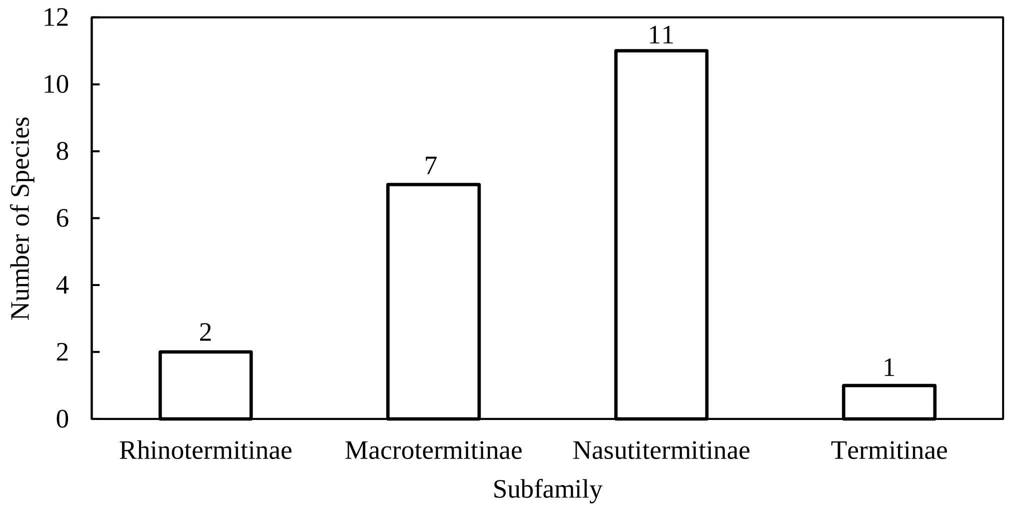

Figure 1. Termite species according to subfamilies at Gunung Telapak Buruk

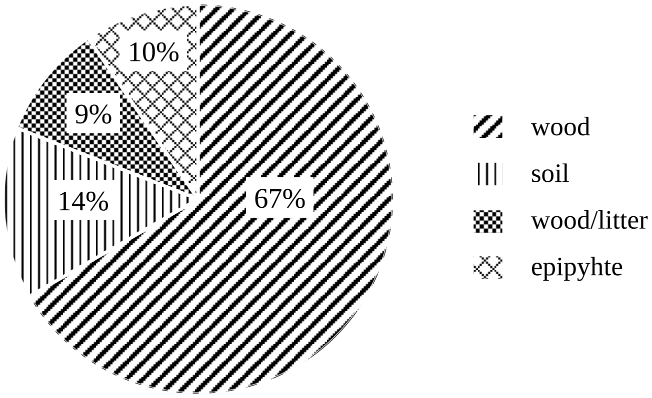

Figure 2. Percentage of termite feeding groups identified at Gunung Telapak Buruk

There are still limited studies on termite in the forest of Negeri Sembilan. Published data shows that a termite study was conducted at Pasoh Forest Reserve (PFR) in Negeri Sembilan by Jones and Brendell in 1998. The site consists of 80 termite species. Study at PFR was conducted at an elevation from 90 m to 150 m above sea level while the current study was conducted at the area above 900 m above sea level. The difference in altitude affects the number of termite species recorded in the forest. Previous studies proved that the number of termite species decreased with the increase of elevation (Gathorne-Hardy *et al*., 2001; Pratiknyo *et al*., 2018). PFR recorded higher termite species due to the lower elevation, while GTB recorded a low number of species due to higher elevation.

Approximately 10% of the world termite species act as pests either in the forest, urban, or agricultural land (Kirton, 2005). In forests, termite pests can be observed inside living trees, where this may endanger trees in the future. In this study, forest termite pest is not reported. All the identified termites provide beneficial ecosystem services to the forest as decomposers and soil engineers. This observation contrasts with the study at PFR as the latter recorded genera that are well known as pests in forests, such as *Coptotermes* and *Microcerotermes* (Cowie *et al*., 1989; Jones & Brendell, 1998). Species from these genera always build their nest on living trees and also attack the tree (Nivaarani & Homathevi, 2015; Kori *et al*., 2017). In this study, all the termites that were identified in the wood were collected either from dead wood or decaying wood on the forest floor. Termites observed during this study were not found to attack any living tree.

CONCLUSION

In conclusion, Gunung Telapak Buruk recorded 21 termite species from two families and 13 genera. The collected termite species in this study comprises 12% of recorded termite species of Peninsular Malaysia. Genus *Odontotermes* and subfamily Nasutitermitinae from family Termitidae dominated the assemblage with five species and 11 species, respectively. All the recorded species in this study are a new record for Gunung Telapak Buruk as no previous record was available for this area. A comprehensive and longer study period covering the area would definitely increase the number of termite species found in Gunung Telapak Buruk.

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Plant Association of Lanternflies (Hemiptera: Fulgoridae) from Malaysian Borneo

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ABSTRACT

The family Fulgoridae is known for their distinct morphological structures and striking colouration. Despite so, comprehensive documentation of insect-plant interaction from this charismatic family is greatly scarce. Presented here are records of plant association across four species of Fulgoridae from Malaysian Borneo. The current study was based on voucher specimens and field samplings from selected localities in Sarawak and Sabah, Malaysian Borneo. A total of 11 species of plants belonging to 11 genera and nine families were recorded. Three fulgorid species namely *Penthicodes quadrimaculata*, *Pyrops intricatus* and *Py. sultanus* shares the same host plant being the mata kucing fruit tree (*Dimocarpus longan* ssp. *malesianus*). The most speciose insect-plant association belongs to *Pe. farinosa* and *Py. sultanus* with six species documented. This is the first record of host plants reported for *Py. intricatus*, *Pe. farinosa* and *Pe. quadrimaculata* in Malaysian Borneo.

Keywords: Fulgoridae, host plant, insect-plant association, Malaysian Borneo, Sabah, Sarawak

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INTRODUCTION

The Fulgoridae or lanternflies is a family of homopteran bugs known for their striking colouration and head ornamentation. Despite these charismatic traits, little is known of their biology particularly on insect-plant associations. The group was assumed to feed on dicotyledonous trees in tropical forests (Wilson & Wheeler, 1992). Nagai and Porion (1996) stated that very few Fulgoridae have been reported to be of any economic importance, *Rhabdocephala brunnea* on *Baccharis sarothroides*, *Phrictus diadema* on *Theobroma cacao* (Malvaceae), *Phrictus quinquepartitus* on *Terminalia oblonga* (Combretaceae), *Enchophora sanguinea* on *Simarouba amara* (Simaroubaceae), *Fulgora laternaria* on *Hymenaea courbaril* (Fabaceae), *Amycle pinyonae* on *Pinus monophylla* (Pinaceae) and *Pyrops candelaria* on *Dimocarpus longan* (Sapindaceae) and *Mangifera indica* (Anacardiaceae).

The family Fulgoridae lacks documentation on records of host plants. Very little is known on the possible hosts of Fulgoridae as they are rarely seen feeding on trees (Goemans, 2006). Most species do not seem to be host plant specific but obtain nourishment from several vegetable species (Nagai & Porion, 1996). Eastop (1972) stated that tropical sap feeding insects are ought to be polyphagous. Johnson and Foster (1986) on the other hand found that some fulgorid species might be monophagous and are very selective of tree species even in a diverse forest. There are few known host plants associated with Fulgoridae, mostly from Sapindales (Bourgoin, 2020). Until recently, *Pyrops sultanus* was only known to feed on wild rambutans (*Nephelium* spp.) and tarap (*Artocarpus odoratissimus*) (Bosuang *et al*., 2017). The aim of this study is to provide new host records for Fulgoridae from Malaysian Borneo. Presented here are records of plant associations for the species *Penthicodes farinosa*, *Pe. quadrimaculata*, *Py.*

intricatus and *Py. sultanus* based on voucher specimens from Malaysian Borneo.

MATERIALS AND METHODS

The present study was based on voucher specimens from the following institutions: 1) UNIMAS Insect Reference Collection, Sarawak (UIRC); 2) Research, Development and Innovation Division of Forest Department Sarawak, Sarawak (RDID); 3) Sabah Parks Natural History Museum, Sabah (SPM); 4) Institute for Tropical Biology and Conservation, Sabah (ITBC); and 5) Sabah Museum, Sabah (SM). Field samplings were conducted in selected localities in Sarawak. The methods used for collecting fulgorids include aerial net and modified Pennsylvanian light trap. Species identification was done using Nagai and Porion (1996) and Bosuang *et al*. (2017). Distribution map was generated using SimpleMappr (Shorthouse, 2010) based on their local distributional range.

RESULTS

A total of 27 (5.93%) out of 455 individuals of fulgorid specimens were noted with host records. The Research, Development and Innovation Division of Forest Department Sarawak (RDID) was the only institution in Malaysian Borneo that provides information on host plants for the sampled specimens. Four species of Fulgoridae from two genera were documented with host plant records consisting of nine families, 11 genera and 11 species (Table 1).

Pyrops intricatus (Walker, 1857)

(Figure 1)

Materials examined. MALAYSIA: Sarawak: Niah, Kpg. Tg. Berlipat, 20 May 1980, Mercer and Suhaili, *Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree), 1♀ (FN80-0640), 1♂ (FN80-0641) (RDID); Samunsam, Wildlife Sanctuary, 21 September 1982, Ento Team, *Nephelium maingayi* (Serait fruit tree), 1♂ (FN82-0460) (RDID).

Plant associations. *Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree) and *Nephelium maingayi* (Serait fruit tree).

Figure 1. Lateral view of *Pyrops intricatus* perching on an unidentified tree (Photograph by: Marcellinus Isaac Stia Dominic)

Table 1. List of fulgorid species and plant associations of Malaysian Borneo

Species	Host family	Host plant	Number of individuals
Penthicodes farinosa	Fabaceae	*Acacia mangium*	7
		Albizzia falcataria	6
	Arecaceae	*Cocos nucifera* (Coconut tree)	2
	Simaroubaceae	*Quassia borneensis* (Medang pahit tree)	1
	Ebenaceae	*Diospyros maingayi* (Merpinang Daun Besar tree)	1
	Lamiaceae	*Premna foetida* (Singkil tree)	3
Penthicodes quadrimaculata	Sapindaceae	*Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree)	1
Pyrops intricatus	Sapindaceae	*Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree)	1
		Nephelium maingayi (Serait fruit tree)	1
Pyrops sultanus	Meliaceae	*Aglaia tomentosa* (Langsat hutan fruit tree)	1
	Moraceae	*Artocarpus integer* (Temedak fruit tree)	1
		Artocarpus odoratissimus (Tarap fruit tree)*	-
	Dipterocarpaceae	*Shorea* sp. (Meranti tree)	1
	Sapindaceae	*Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree)	1
		Nephelium spp. (wild rambutans fruit tree)*	-

Note: * Information obtained from Bosuang *et al*. (2017)

Distribution. Endemic to Borneo (Kalimantan and Sarawak only).

Local distribution. Sarawak (Niah, Samunsam, Serian, Semengoh, Tatau, Julau, Lambir Hills National Park, Balul, Batu Keling, Batu Kalo, Long Murum, Julau Nanga Jepiu, Ulu Katibas, Kota Samarahan, Bau, Sri Aman and Gunung Gading National Park) (Figure 5).

***Penthicodes farinosa* (Weber, 1801)**
(Figure 2)

Materials examined. MALAYSIA: Sarawak: Sibu, Oya Road Nursery, 9 December 1976, A. A. Hamid, *Acacia mangium* (Akasia tree), 1♀ (FN00319) (RDID); Sabal FR, 17 May 1984, Isa Sait, *Acacia mangium* (Akasia tree), 2♂, 1♀ (RDID); Sabal FR, 17 May 1984, Manis Lintang, *Acacia mangium* (Akasia tree), 1♂ (RDID); Sabal FR, 20 May 1984, A. A. Hamid, *Acacia mangium* (Akasia tree), 1♀ (RDID); Sabal FR, 21 May 1984, Manis Lintang, *Acacia mangium* (Akasia tree), 1♀ (RDID); Sibu, Oya Road Nursery, 3 June 1979, C. W. L. Mercer, *Albizzia falcataria* (Batai tree), 2♂ (FN.79-0160, FN.79-0163), 2♀ (FN.79-0159, FN.79-0161) (RDID); Sibu, Oya Road Nursery, Butterfly Garden, 4 July 1979, A. A. Hamid, Handpick, *Albizzia falcataria* (Batai tree), 2♀ (FN79-52) (RDID); Semengoh, Agriculture Research Centre, 24 December 1979, Suhaili, *Albizzia falcataria* (Batai tree), 1♀ (FN79-0914) (RDID); Kpg. Spaoh, Coconut Garden, 6 October 1980, Mercer, Lippa and Rahman, *Cocos nucifera* (Coconut tree), 1♂ (FN.80-1764), 1♀ (FN80-1765) (RDID); Simunjan Kanan, Sg. Jitak, 29 September 1980, Mercer, Lippa and Rahman, *Quassia borneensis* (Medang pahit tree), 1♂ (FN.80-1481) (RDID); Tatau, Sg. Selitut, 11 May 1981, Mercer, Lippa and Suhaili, *Diospyros maingayi* (Merpinang Daun Besar tree), 1♀ (FN81-0244) (RDID); Kpg. Spaoh, 6 October 1980, Mercer, Lippa and Rahman, *Premna foetida* (Singkil tree), 3♀ (FN.80-1766-FN.80-1768) (RDID).

Plant associations. *Acacia mangium* (Akasia tree), *Albizzia falcataria* (Batai tree), *Cocos nucifera* (Coconut tree), *Quassia borneensis* (Medang pahit tree), *Diospyros maingayi* (Merpinang Daun Besar tree) and *Premna foetida* (Singkil tree).

Figure 2. Dorsal view of *Penthicodes farinosa* on the ground preparing for flight (Photograph by: Marcellinus Isaac Stia Dominic)

Distribution. India, Myanmar, Peninsular Malaysia, Java, Sumatra, Sulawesi, the Philippines and Borneo.

Local distribution. Sarawak (Kota Samarahan, Sri Aman, Batu Kawah, Ranchan Recreational Park, Matang, Santubong, Kpg. Tanah Putih, Bau, Kota Samarahan, Sibu, Bako National Park, Semengoh Agriculture Research Centre, Simunjan Kanan, Kpg. Spaoh, Sibu, Tatau, Mukah, Niah, Sabal Forest Reserve, Sampadi Forest Reserve, Bintulu, Sebuyau, Kuching, Sarikei, Kelingkang, Lambir Hills National Park, Jelalong, Tanjung Datu, Sabal, and Gedong) and Sabah (Sorinsim, Sipitang, Hutan Epulun, Kota Kinabalu, Klias Peat Swamp Forest Field Centre Beaufort, Maliau Basin and Beluran) (Figure 5).

***Pyrops sultanus* (Adam and White, 1847)**
(Figure 3)

Materials examined. MALAYSIA: Sarawak: Niah, Sg. Sekaloh, 17 May 1980, Mercer and Suhaili, *Aglaia tomentosa* (Langsat hutan fruit tree), Handpick, 2♀ (FN80-0588, FN80-0589) (RDID); Niah, 18 May 1980, Mercer and Suhaili, *Artocarpus integer* (Temedak fruit tree), 1♀ (FN80-0611) (RDID); Niah, Gunung Sabis, 15 May 1980, Mercer and Suhaili, *Shorea* sp. (Meranti tree), 1♀ (FN80-0588) (RDID); Niah, Kpg. Tanjong Berlipat, 23 May 1980, Mercer and Suhaili, *Dimocarpus longan* ssp. *Malesianus* (Mata kucing fruit tree), 2♀ (FN80-0719, FN80-0720) (RDID).

Figure 3. Dorsal view of *Pyrops sultanus*, ♀. Scale = 10 mm

Plant associations. *Aglaia tomentosa* (Langsat hutan fruit tree), *Artocarpus integer* (Temedak fruit tree), *Shorea* sp. (Meranti tree), *Nephelium* sp. (wild rambutan fruit tree), *Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree) and *Artocarpus odoratissimus* (Tarap fruit tree).

Distribution. Endemic to Borneo.

Local distribution. Sarawak (Gunung Serapi, Gunung Sabis, Niah, Kuching, Mulu National Park, Hose Mountain, Kota Samarahan, Tanjung Datu National Park, Gunung Pueh and Tama Abu) and Sabah (Papar, Crocker Range National Park, Ranau, Kinabalu National Park, Tawau Hills Park, Kota Belud, Poring Hot Spring, Kota Belud, Kota Marudu, Sembulan, Keningau, and Inanam) (Figure 5).

Penthicodes quadrimaculata Lallemand, 1963
(Figure 4)

Material examined. MALAYSIA: Sarawak: Niah, Kpg. Tanjong Berlipat, 20 May 1980, Mercer and Suhaili, *Dimocarpus longan* ssp. *Malesianus* (Mata kucing fruit tree), Handpick, 1♀ (FN. 80-0642) (RDID).

Plant association. *Dimocarpus longan* ssp. *malesianus* (Mata kucing fruit tree).

Distribution. Java, Sumatra and Borneo.

Local distribution. Sarawak (Niah and Kota Samarahan) and Sabah (Kinabalu Park, Crocker Range National Park, Kota Marudu, Sipitang and Mount Trus Madi) (Figure 5).

Figure 4. Dorsal view of *Penthicodes quadrimaculata*, ♀. Scale = 10 mm

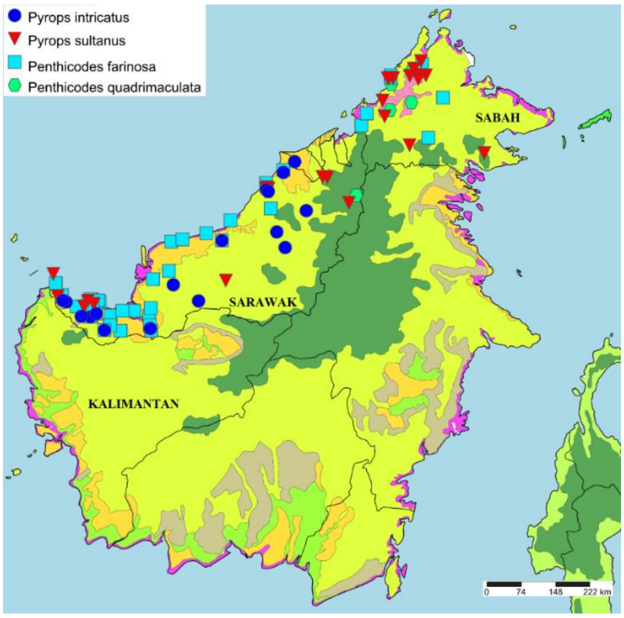

Figure 5. Distribution map of *Py. intricatus*, *Py. sultanus*, *Pe. farinosa* and *Pe. quadrimaculata* in Malaysian Borneo with background colours indicating ecoregions

DISCUSSION

Only one host plant was recorded for the fulgorid species *Pe. quadrimaculata*. Three species namely *Pe. quadrimaculata*, *Py. intricatus* and *Py. sultanus* share the same host plant being the mata kucing fruit tree (*Dimocarpus longan* ssp. *malesianus*). The fulgorid species with the most abundant host plants recorded includes *Pe. farinosa* and *Py. sultanus,* with six species documented, followed by *Py. intricatus* with two species, and *Pe. quadrimaculata* with only one species. It must be noted that out of all five institutions sampled in Malaysian Borneo, only RDID recorded the specimens with host plants labelling. RDID has a Biodiversity (Flora) Section with a dedicated herbarium that allows identification of plants to be conducted. Fulgorid specimens collected after 1984 did not contain any host plants on the label. This may be due to its limited economic importance (Goemans, 2006; Bosuang *et al*., 2017), leading to shortfall in the investigation of host plants from this family.

A preliminary survey of insects at Libiki Bamboo Resort, Sarawak, in March 2019 revealed two individuals of *Py. intricatus*, sitting at an unidentified tree trunk at a height of approximately 165 cm from the ground. When approached, they would move to the lateral side of the tree and will only fly away to the nearest vegetation when direct contact is made. This behaviour is not unique to Fulgoridae and has also been observed in the grasshopper species *Valanga nigricornis* (Orthoptera: Acrididae). After a while, the same lanternflies were found to perch again at the same area of the tree throughout the survey. Our finding is supported by Goemans (2006) and O'Brien (2002) in which lanternflies tend to stay for several weeks on the same 'fulgorid tree' and comes back year after year. Some fulgorid species were found to be strongly associated with certain tree species and fidelity towards a particular tree of a given species (Hogue, 1984; Johnson & Foster, 1986; O'Brien, 2002; Goemans, 2006). Hence, this gives the opportunity for researchers to go back and retry to capture the lanternflies when they failed on the first attempt.

There is a significant paucity in voucher specimens of Fulgoridae, particularly in Sabah. Many specimens are represented as singletons. Identifying these hosts can help in sampling as they are notoriously known to be difficult to locate in the wild. Collections and descriptions of fulgorid nymphs of Malaysian Borneo is also scarce. RDID holds only one specimen of fulgorid nymph which is *Zanna nobilis*. In addition, *Z. nobilis* was the only species of fulgorid nymph illustrated in guidebooks dealing with the Bornean fauna (e.g. Nagai & Porion, 1996; 2002; 2004; Bosuang *et al*., 2017). Knowledge on hosts can also be beneficial to the ecotourism industry. Similar to the firefly watching tourism, guides can bring visitors to the identified hosts and spectate lanternflies perching on the tree, in which guides need only to find the fulgorid tree once (O'Brien, 2002). Guides in Gunung Mulu National Park, Sarawak, for instance would know locations of these trees and include them in tours (Mason *et al*., 2020).

Despite not being recognised as an indicator of well-preserved forests, the presence of lanternflies may be linked with long lasting uninterrupted big or old trees (Constant & Alisto, 2015), which is congruent with the current study. Libiki Bamboo Resort can be categorised as a secondary forest and is predominantly dominated by bamboos. The *Py. intricatus* sampled were found perching on a tall tree that is standing in the middle of a clearing. The fact that these trees are tall makes leaf collection for tree identification impossible without the appropriate equipment. This is one of the reasons why many insect specimens lack host plant documentations. To rectify this issue, collaboration with botanists is greatly essential.

According to Bosuang *et al*. (2017), *Pe. farinosa* is a common species in Borneo. This statement agrees with the current study as *Pe. farinosa* was found to be the most abundant species (204 individuals) in Malaysian Borneo. In this study, this species was noted to be present in urban areas, particularly in Universiti Malaysia Sarawak, seemingly scattered all around the campus. This is likely due to their wide range of host species, classifying them as a generalist. The availability of a wider range of niche allows generalist to thrive in a range of habitats as opposed to a specialist (McPeek, 1996). Commonness of host plants can also determine phytophagous insects as common or rare (Barbosa *et al*., 2000; Hopkins *et al*., 2002).

CONCLUSION

Host plants for *Py. intricatus*, *Pe. farinosa* and *Pe. quadrimaculata* are reported here for the first time. Results from this study indicate that 27 out of 455 voucher specimens examined are documented with host plants records. When based solely on voucher specimens, the hosts comprise nine families, namely Fabaceae (two species), Arecaceae (one species), Simaroubaceae (one species), Ebenaceae (one species), Lamiaceae (one species), Sapindaceae (two species), Meliaceae (one species), Moraceae (one species) and Dipterocarpaceae (one species), making a total of 11 genera and 11 species. *Penthicodes farinosa* and *Py. sultanus* was found to have the most speciose hosts, with six species documented. There is also a huge lack of fulgorid nymphs' specimens kept in all five repositories visited. Voucher specimens provide permanent, physical documentation of species and evidence of their occurrences at a specific point in time (Abang & Hill, 2007). More sampling is necessary to uncover insect-plant relationship within this group. Biology, life history, behaviour, and phenology of all Fulgoridae is still undocumented and should be investigated at haste (Yap *et al*., 2017), considering Borneo is known for rapid deforestation due to urbanisation. With this notion in mind, future research will be plan out to further identify the hosts for this charismatic yet enigmatic family.

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The Influence of Perch Height Selection on Vocalization of Pied Triller, *Lalage nigra* (Aves: Campephagidae) in Suburban Landscapes, Sarawak

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ABSTRACT

Understanding how birds utilised songs and calls to communicate among conspecifics is crucial for their survival, yet it remains an understudied area in bird ecology. We studied the influence of perch height selection on the vocalisation of Pied Triller, a common garden bird, in two suburban landscapes namely Pustaka Negeri Sarawak Recreational Park, Kuching and Universiti Malaysia Sarawak campus, Kota Samarahan from October 2018 to February 2019. Using a Marantz recorder connected to a parabolic reflector, we recorded calls and songs during morning (0630-1030 hr) and late afternoon (1600-1830 hr). Perch object, tree species, roost substrate and perch height from the ground were identified and measured. Clear and good quality spectrograms were used directly to describe song and call types. Photos and video recordings were analysed to describe perch behaviour. The vocal output representatives were then matched to the corresponding behaviour displayed. Our results revealed that Pied Trillers emit four different vocal outputs namely (i) song type, (ii) call type A, (iii) call type B and (iv) call type C. A total of nine behaviours were observed, of which the perch-hop behaviour was observed the most from both male and female Pied Trillers (33.71%), followed by perching (29.21%), foraging (20.22%), preening (7.78%), defecating (2.25%), eating (2.25%), roosting (2.25%), bill wipe (1.12%) and flight (1.12%). They prefer to roost on high perch to emit calls compared to songs. This probably suggest that Pied Triller prioritised vocal transmission and signalling to avoid being masked by surrounding anthropogenic noises and to avoid being conspicuous to potential predators. The most frequently visited perch object was the Weeping fig tree, *Ficus benjamina*.

Keywords: Call, perch height, Pied Triller, song, suburban landscapes

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INTRODUCTION

Pied Triller, *Lalage nigra* (Family: Campephagidae) is a common resident in the lowlands of Borneo and a familiar garden bird (Smythies, 1999). This cuckoo-shrike is also often seen foraging for insects in foliage of small trees of open habitats, urban gardens, coastal Casuarinas, and mangroves (MacKinnon & Phillipps, 1993; Phillipps & Phillipps, 2014). They are sexually dimorphic with males having black upperparts with white wing bar and white edges to wing converts and outer tail feathers, broad white eyebrow with black eye-stripe and grey rump (MacKinnon & Phillipps, 1993). Comparatively, females are similar but brown instead of black and the breast are finely barred with black.

There are two types of bird vocalisations which are calls and songs (Welty & Baptista, 1988). Calls tend to be shorter, simpler, and produced by both sexes throughout the year (Catchpole & Slater, 2008). They are also less spontaneous and often related to specific function such as flight, threat, and alarm. A song on the other hand is more complex than a call and occurs spontaneously (Welty & Baptista, 1988). Songs also constitute a group of notes separated from another group of notes by a pause longer than the pauses between the notes themselves (Welty & Baptista, 1988). When illustrating a song, sonograph is used to analyse, measure, classify and recognise the different sounds produced by the birds (Catchpole & Slater, 2008). Therefore, discrimination between different species, population, individuals, song type within individuals and renditions of the same song type from an individual bird was possible (Catchpole & Slater, 2008).

A Pied Triller's call can vary from a distinctive double syllable *chwee whuk* (Phillipps & Phillipps, 2014) to a double croak *chook-chook* or a rolling, descending *tre-tre-tre-tre*, more musical than the rich, vigorous, metallic whistle of a White-shouldered Triller (MacKinnon & Phillipps, 1993). Smythies (1999) described the call as double note similar to the croak of a frog, while Vowles and Vowles (1997) described the call as a harsh grating chat.

There has been a dearth of studies that focused on ecological roles and behavioural adaptation of bird vocalisation. Such is shown by the limited vocal recordings available for Pied Triller from Borneo in online bird call archives such as Xeno-Canto Foundation and Macaulay Library at the Cornell Lab of Ornithology. Only two song recordings were available in Xeno-Canto Foundation (2013) recorded from Mantanani Resort, Pulau Mantanani Besar, Sabah and another song recorded from a paddy field in Penampang, Sabah. Previous studies reported that city birds in general tend to choose higher perch height due to anthropogenic noise in order to efficiently transmit sexual and territorial vocal displays at the same time minimising risk of predation due to avian and mammalian predators (Møller, 2011). The primary objective of this study is to determine the influence of perch height selection on vocalisation of Pied Triller in suburban landscapes. This study hypothesised that perch height selection could influence the vocalisation of Pied Triller. A higher perch height will be chosen to emit calls over songs based on the six vocal output parameters. Urban noise will influence a higher perch height preference by the bird.

MATERIALS AND METHODS

Study Site

The study was conducted in two suburban landscapes. The first study site was in Pustaka Negeri Sarawak (N1.58˚, E110.35˚, E: 12 m asl) (Figure 1). This public library is located adjacent to the Sarawak State Mosque and the Sarawak Golf Club in Petra Jaya, Kuching Division. The 37.23 ha recreational park consists mixture of landscape mainly a lake, grassland, wetlands, open and dense forest and some formal gardens (Pharo *et al.*, 2015). The mixture of vegetation in the recreational park hosts about 4741 ornamental shrubs and flowering plants, 878 local tree species, 336 fruit trees, 275 palm species and 50 different herbs (Pharo *et al.*, 2015).

The second study site was conducted in the West Campus of Universiti Malaysia Sarawak (UNIMAS) (N1.46˚, E110.43˚, E: 17 m asl) in Kota Samarahan Division (Figure 1). The campus is approximately 2000 ha in size, surrounded by secondary mix peat swamp forests with a thin strip of mangrove vegetation along the river (Voon *et al.*, 2014). The campus has diverse types of vegetation consisting of mangrove plants (such as *Avicennia* spp., *Sonneratia* spp., *Nypa fructicans*, and *Rhizophora* spp.), ornamental urban trees, Macarthur palms, Fig trees, and Acacias. The five most common tree species planted in UNIMAS are Fern tree, *Filicium decipiens*, Indonesian bay leaf tree, *Syzygium polyantha*, Florida royal palm, *Roystonea regia*, wild Cinnamon tree, *Cinnamomum iners*, and Spanish cherry tree,

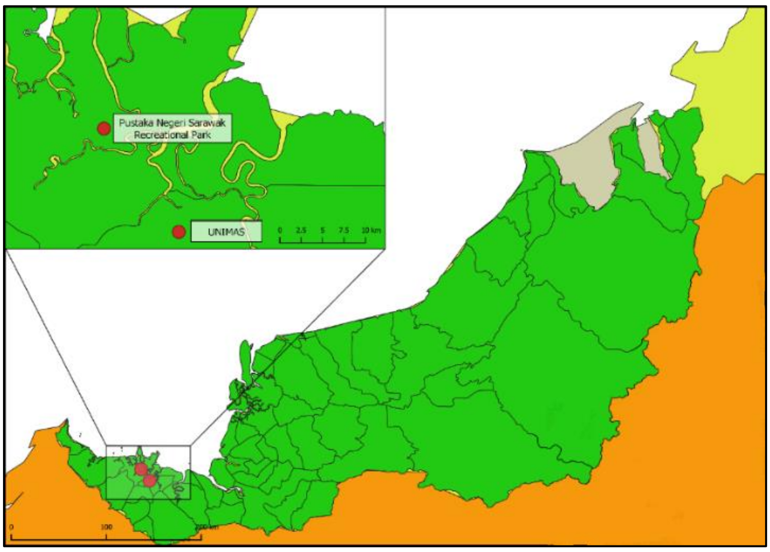

Figure 1. Map showing location of sampling sites in Kuching and Kota Samarahan (Modified after QGIS 3.6.2)

Mimusops elengi (Zainudin *et al.*, 2012).

Sampling Method

Active sampling method was conducted in this study by walking a predetermined 100 m transect lines while simultaneously recording bird calls and songs using (i) a digital recorder (Barker & Mennill, 2009) (Marantz PMD-671, sample frequency 44.1 kHz, resolution 16 bit) that was connected to (ii) a Senheisser MKH 20 P48 omnidirectional microphone, (iii) a Sony Dynamics Stereo headphone MDR-7506 and (iv) a parabolic reflector. A laser rangefinder (Nikon Forestry Pro) was used to measure the height (m) of the bird perch from the ground. Four assumptions were made when recording includes (i) all individuals along the route were detected, (ii) birds do not move before detection, (iii) distances were measured accurately, (iv) individual birds were counted only once (Bibby *et al.*, 1992).

Audio recordings were carried out two to three times a week from October 2018 to February 2019. Bird calls and songs were recorded in the morning (0630 to 1030 hr) and late afternoon (1600-1830 hr). Individuals seen perching were photographed and captured in video to record roosting and call behaviour using a digital compact camera (Nikon Coolpix P900). For each observation, the perch height of the singing individual and the height of the tree was also measured (Krams, 2001). The sex of the individual, the presence of other individual, and the description of perch object were also recorded. Environment parameters that were recorded include temperature (˚C), humidity (%) and light intensity (Lux) of surrounding by using an Extech 45170CM 5-in-1 Environmental meter while cloud cover (%) was recorded using an AccuWeather Application.

Data Analysis

To screen new vocal outputs for background noise, Audacity (version 2.1.2) was used to filter about 15 dB of background noise before visualising the spectrogram for each vocal output using Sound Ruler Acoustic Analysis (version 0.9.6.0). The good quality spectrograms were used directly by analysing the six vocal parameters including (i) dominant frequency, kHz, (ii) minimum frequency, kHz, (iii) maximum frequency, kHz, (iv) duration of vocal, s, (v) relative amplitude, dB and the (vi) number of syllables of the vocal output, n.

Photos and video recordings of the perch observations were determined and analysed to describe the perch behaviour displayed. The vocal output representatives were then matched to the corresponding behaviour displayed. A multivariate linear regression in PAST software (version 3.14) (Hammer *et al*., 2001) was used to calculate the significant difference between perch height selection by the birds towards the six song output parameters. A vocal ethogram of the vocal bird was constructed based on Stanton (2016).

RESULTS

A total of 89 perch height observations were recorded consisting of 70 observations of male Pied Trillers (78.65%), 16 females (17.98%) and three male and female observed together (3.37%). From this, 59 observations included songs and calls (66.29%) while 30 observations were without songs and calls (33.71%). Table 1 shows the total number of perch height observations.

Vocal and Behavioural Analysis

From the total observation, nine behaviours were

Table 1. Total number of perch height observations

	Number of perch height observations			
	Include songs and calls		Excluding songs and calls	
Individual	Number of perch height (n)	Percentage (%)	Number of perch height (n)	Percentage (%)
Male	50	56.18	20	22.47
Female	6	6.74	10	11.24
Male and Female	3	3.37	0	0
Total observations	59	66.29	30	33.71

observed during the study. Perch-hop was the most observed behaviour from both male and female Pied Trillers (33.71%), followed by perching (29.21%), foraging (20.22%), preening (7.78%), defecating (2.25%), eating (2.25%), roosting (2.25%), bill wipe (1.12%) and flight (1.12%). Figure 2 shows the percentage of the behaviour displayed by both sexes of the species.

Perch-hop - The bird hops actively from one branch to another with frequent short flights. They often pause in between to sing and call.

Perching - This behaviour is described when the bird is seen resting on a natural or artificial perch while looking around with its tarsus and toes firmly standing and gripping on the perch object. They occasionally emit songs and calls from a perch.

Foraging - This behaviour appears almost similar to that of a perch hop but the bird hops slower from one branch to another with less frequent short flights. Pied Triller typically spend more time gleaning the branches and leaves for insects in between hops or flights.

Preening - This behaviour is described as the bird naturally cleans and rearranged its feathers using its bill. They frequently sing in between preening.

Defecating - The bird expelled faeces through the anus from a perch. The bird normally sings after defecating.

Eating - This behaviour involves swallowing of food and intermittent movement of oesophagus. It does not accompany with songs and calls emitted by the bird.

Roosting - This behaviour describes the bird resting in a stationary state with its legs bend and less actively looking around. They do not emit songs and calls during a roost.

Bill wipe - This behaviour is described as the bird cleans its bill by wiping the sides on a perch. It does not accompany with songs and calls.

Flight - The bird takes off from a perch and flap its wings in irregular intervals. The bird has been seen singing during take-offs.

Four types of vocalisations were identified, consisting of song type, call type A, call type B and call type C. Figure 3 shows the spectrograms of all four vocalisation types with their respective description as follows:

Song type - This song type consists of a two note nasal tones varying from a descending *tre-tre-tre-tre* to a constant *tre-tre-tre-tre*. It can vary from seven to 24 syllables between 1.13 to 3.52 s. Perch-hop was the most observed behaviour that was displayed while emitting this song type.

Call type A - This call type also consists of a two note nasal tones which gives a *pee-chit* sound. It was emitted with two or four syllables between 3.6 to 9.6 s. The most observed behaviour that was displayed while emitting this call type was perching.

Call type B - This call type varies from a *chit-chee-dit-tre-tre-tre*, *chit-chee-dit-chit*, and *chit-chee-dit* nasal tone sounds with duration between 0.52 to 2.6 s. The observed behaviours displayed while emitting this call type were perching and perch-hopping.

Call type C - This call type is similar to song type but a syllable was only emitted giving a *tre* sound. It also consists of two note nasal tones sound with duration of 0.07 s. Perching behaviour was observed while emitting this call type.

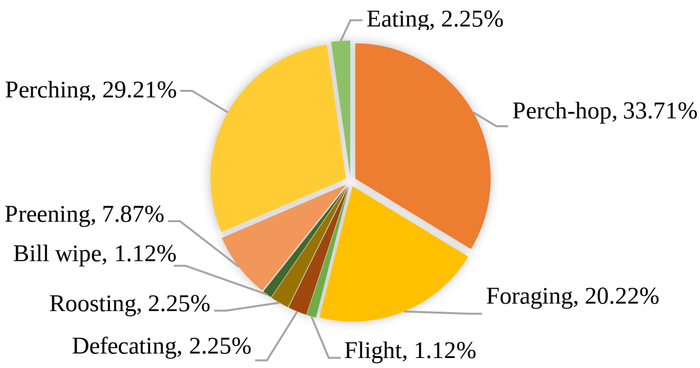

Figure 2. Pie chart showing the percentage of the behaviour displayed by both sexes of Pied Triller

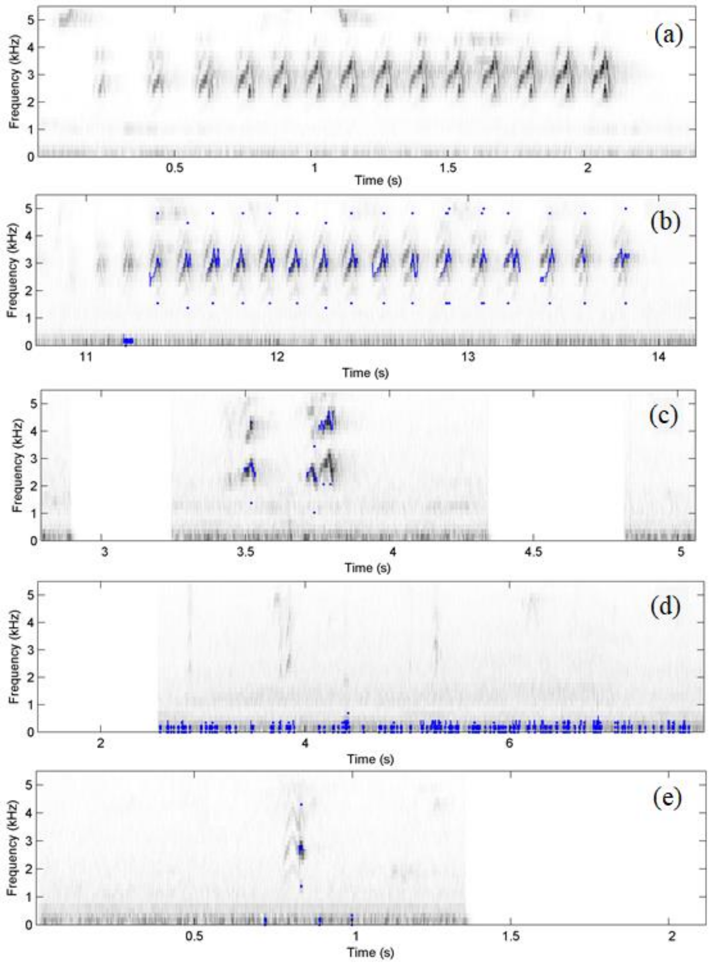

Figure 3. Spectrograms of song and call types of Pied Triller depicting (a) Song type - a descending 14 syllables, (b) Song type - 19 repeated syllables, (c) Call type A - a four syllables structure, (d) Call type B - varying nasal tone sounds with brief duration and (e) Call type C - a *tre* sound with two note nasal tones sound

Perch Height and Perch Object Description

A total of 87 perch height observations were recorded on tree structures (97.75%) while two observations were on public parking signage (2.25%) (Figure 4). Eight different tree species were identified including five ornamental trees and three timber trees (Table 2). Out of the five ornamental trees, four were fruiting trees while the other was a leguminous tree. The three timber trees consist of a fruiting tree and two leguminous tree. Public parking signage was least preferred as perching object by the bird.

Table 2. Percentage of perch tree in both sites

	Ornamental tree	Percentage (%)	Timber tree	Percentage (%)
Fruiting	Fern tree (*Filicium decipiens*)	2.25	Cheese tree (*Glochidion* sp.)	3.37
	Weeping fig tree (*Ficus benjamina*)	29.21		
	Weeping paperbark tree (*Melaleuca leucadendra*)	1.12		
	Blackboard tree (*Alstonia scholaris*)	23.6		
Leguminous	Rain tree (*Samanea saman*)	16.85	Moluccan albizia tree (*Albizia falcafacia*)	3.37
			Acacia tree (*Acacia* sp.)	17.98

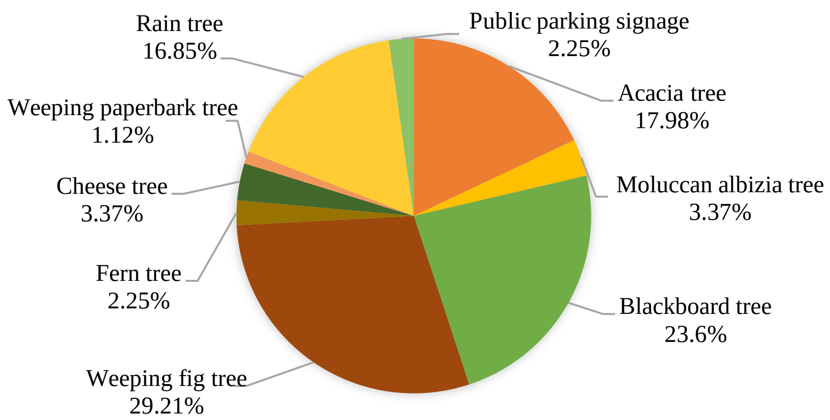

Figure 4. Pie chart showing the percentage of the perch objects

The highest tree perch height was at 21.6 m on a Weeping paperbark tree, *Melaleuca leucadendra*. This is followed by Cheese tree, *Glochidion* sp. (8.13 m), Acacia tree, *Acacia* sp. (7.56 m), Weeping fig tree, *Ficus benjamina* (6.55 m), Rain tree, *Samanea saman* (5.73 m), Blackboard tree, *Alstonia sholaris* (5.67 m), Moluccan albizia tree, *Albizia falcafacia* (4.20 m) and Fern tree, *Filicium decipiens* (2.40 m). Perch observation for artificial structure was only at the height of 0.8 m on a public parking signage (Table 3).

Out of 59 song perch observations containing songs and calls (66.29%), 32 clear vocal outputs (54.23%) were used directly as sample size without filtering background noise. Songs produced by the Pied Triller were higher in terms of minF, domF, amp, duration of vocal and the number of syllables compared to the three call types. Call type C has the highest maxF compared to the other vocal type. The six parameters analysed for each vocal outputs are summarised in Table 4.

Table 3. Perch height and perch object description

Perch object	Range height (m)	Average perch height (m)	Optimum perch height (m)
Acacia tree	4.20 - 12.50	7.56 ± 3.19	12.56 ± 4.54
Moluccan albizia tree	3.80 - 4.40	4.20 ± 0.28	7.40
Blackboard tree	2.80 - 7.20	5.67 ± 1.38	7.65 ± 0.59
Weeping fig tree	3.20 - 13.80	6.55 ± 2.21	10.76 ± 2.19
Fern tree	2.40	2.40	4.40
Cheese tree	2.40 - 11.40	8.13 ± 4.07	11.53 ± 3.19
Weeping paperbark tree	21.60	21.60	22.60
Rain tree	4.00 - 6.60	5.73 ± 1.12	10.07 ± 0.10
Public parking signage	0.80	0.80	0.80

Table 4. Average values of vocal parameters for four vocal types

	MinF (kHz)	MaxF (kHz)	DomF (kHz)	Amp (dB)	Duration (s)	Number of syllables (n)
Song type	1533.48 ± 92.55	4738.17± 186.47	3134.61 ± 164.01	33.33 ± 40.56	2.27 ± 0.69	14.30 ± 4.49
Call type A	1397.88 ± 300.89	4844.00 ± 280.23	2863.88 ± 605.63	3.64 ± 1.01	0.43 ± 0.29	2.50 ± 0.87
Call type B	-	-	-	-	-	-
Call type C	1317.00	5240.00	2842.00	5.46	0.07	1.00

Notes: (-) indicates vocal analysis was not available

The Relationship Between Perch Height and Vocalisation

The following test shows the relationship between perch height with song type and call type A. Call type B and C was not further analysed because the vocal output recorded for call type B was of poorer quality while the number of recordings for call type C was insufficient to be considered a minimum sample size.

Song type - All parameters show low negative correlation against perch height ((b): maxF: r = -0.16; (d): amp: r = -0.20; (e): duration: r = -0.32; (f): number of syllables: r = -0.21)) except minF and domF where they show low positive correlation toward perch height ((a): minF: r = 0.17; (c): domF: r = 0.25) (Figure 5).

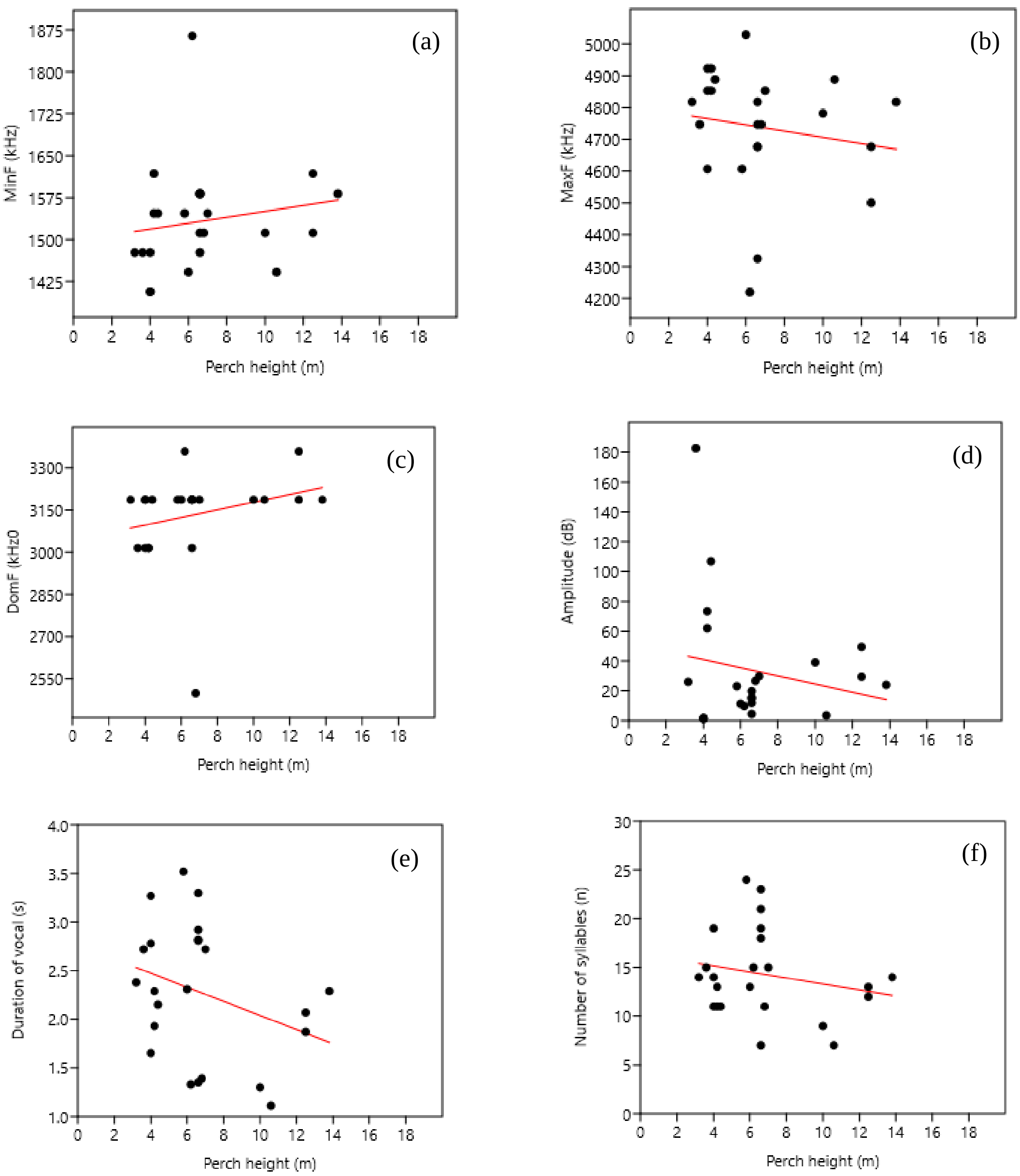

Figure 5. Relation between (a) minF, (b) maxF, (c) domF, (d) amp, (e) duration of vocal and (f) number of syllables of song type towards perch height

Call type A - All parameters show low positive correlation against perch height ((a): minF: r = 0.45; (b): maxF: r = 0.31; (c): domF: r = 0.05; (d): amp: r = 0.37; (f): number of syllables: r = 0.16)) except duration of vocal as it shows low negative correlation toward perch height ((e): duration: r = -0.26) (Figure 6).

DISCUSSION

Pied Triller was observed to have preference to perch on tree structures (97.75%) compared to artificial structures (2.25%). Passerine birds such as Pied Triller have anisodactyl foot (Proctor & Lynch, 1993) evolved to best fit around tree branches (Mench & Blatchford, 2014), stems or twigs as their toe segments are the right lengths to allow their toes to bend comfortably around them (Welty & Baptista, 1988). This suggests the reason for such preference for tree perch as they have better grip on natural structures. The public parking signage is made of metal therefore it is more difficult to perch on. They were also observed among foliage of medium trees such as Cheese tree, Fern tree and Weeping fig tree, and on large trees such as Acacia tree, Blackboard tree, Moluccan albizia tree, Rain tree and Weeping paperbark tree. Weeping fig tree, *Ficus benjamina* was the most

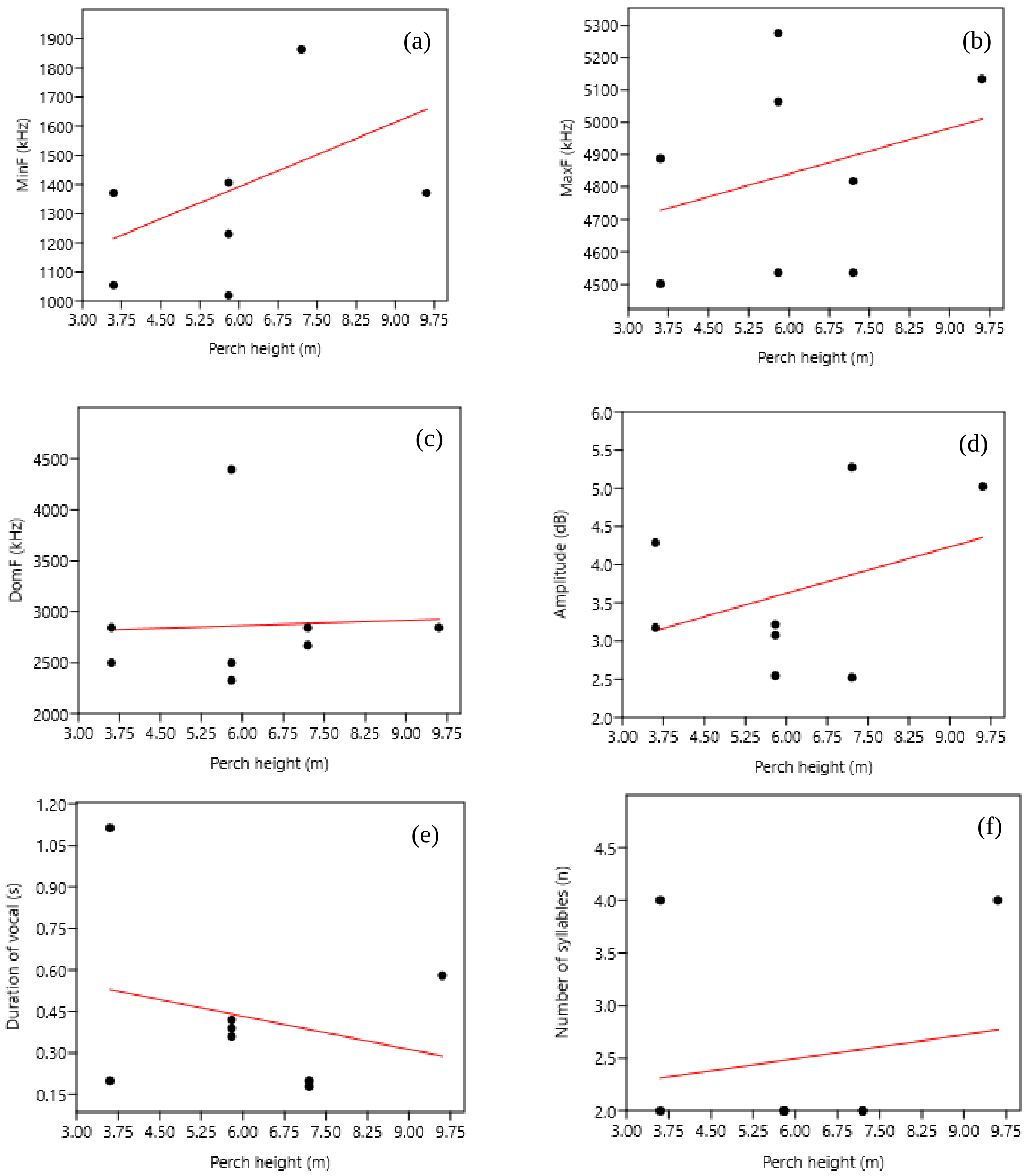

Figure 6. Relation between (a) minF, (b) maxF, (c) domF, (d) amp, (e) duration of vocal and (f) number of syllables of call type A towards perch height

frequent tree perch by Pied Triller. Possible reason why Pied Trillers prefer this tree species compared to the other fruiting tree species to forage for insects (Phillipps & Phillipps, 2014) maybe due to the availability of Fig wasps, *Eupristina koningsbergeri* the pollinators of the Weeping fig tree (Zhang & Yang, 2017).

The most observed behaviour by the Pied Triller was perch-hop (33.71%) while flight behaviour was the least observed behaviour display (1.12%). Perch-hop is described as the bird moving from one place to another by propelling itself continuously with its feet (Smith & Wassmer, 2016) with occasional short flights to the nearest branch in between. Small and light garden bird prefers to hop rather than running or flying as it is the quickest and most effective way to get about in a tree (Welty & Baptista, 1988). Flight requires the bird to flap therefore it is energetically more costly than running (Butler, 2016) and hopping. Perch-hopping may also be the best locomotion for trade-off between energy costs and gains when foraging (Scott, 2005). Pied Trillers were often observed perch-hopping from branch to branch among the foliage with occasional short flights to the nearest branch and pausing in between to emit songs and calls.

Based on perch height selection, song type shows a negative correlation for all parameters except minF and domF. This may suggest that song type is not necessarily used for communication between individuals of the species as the perch height increases. Song type may function significantly for male Pied Trillers to defend their territory and to attract females (Catchpole & Slater, 2008). Songs with higher minimum and dominant frequency are more favourable as they help to project the song louder and further away (Luther & Baptista, 2010; Magalhães Tolentino *et al*., 2018). Furthermore, emitting songs tend to make the bird to be more conspicuous (Beck & George, 2000) as the duration of vocal output is longer compared to calls. Therefore, emitting songs increases the probability of predation by cats (Møller, 2011) or raptors (Beck & George, 2000).

Call type A in this study however shows a positive correlation for all parameters except duration of vocal output. Calls emitted by the bird tend to increase in minF, maxF, domF, amp and number of syllables with the increase in perch height as to improve interactions with conspecifics (Beck & George, 2000).

Another factor that may influence the perch height selection on vocalisation is due to urbanisation. Birds exposed to urbanisation for a long time tend to sing at higher position in the vegetation (Møller, 2011). Since anthropogenic noise in urban areas is noisy and has higher minimum frequency (Hu & Cardoso, 2009), birds of an open habitat tend to have higher pitch songs (Welty & Baptista, 1988) with higher sound level and higher low frequencies (Møller, 2011). Birds will try to mitigate communication impairments that masks their songs with the background noise to maintain song output distance in territory defense and mate attraction (Brumm, 2004). Birds often incorporate regular rhythms such as trills and pure sounds such as whistles to make the sound more out stand from the background (Catchpole & Slater, 2008). Pied Triller emits a distinctive nasal sound as a technique to unmask their vocal output by the background noise of urban surrounding.

CONCLUSION

Pied Triller produced song output with lower maximum frequency, amplitude, duration and number of syllables but higher minimum and dominant frequency. This vocalisation strategy could probably benefit the species to remain inconspicuous from potential predators like raptors and cats while maintaining song distance. In contrast, call output has higher minimum frequency, maximum frequency, dominant frequency, amplitude and number of syllables as perch height increases to improve interactions with conspecifics. The preference of high perch height when calling over singing probably suggested that Pied Triller prioritised vocal transmission and call sign. This garden bird species preferred tree structures to perch over artificial structures due to their anisodactyl foot ideal for perching on trees. The most frequently visited perch object was the Weeping fig tree, *Ficus benjamina*.

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SHORT COMMUNICATION

Species Composition and Ecological Distribution of the Subfamily Cicindelinae Latreille, 1801 (Coleoptera: Carabidae) Based on Voucher Specimens in Sarawak

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ABSTRACT

The subfamily Cicindelinae which belongs to the family Carabidae are commonly known as tiger beetles. Despite the recent studies conducted on this taxon, current ecological knowledge on tiger beetles is limited. Thus, this study aimed to provide a current checklist of tiger beetles as well as their species distribution, abundances and habitat preferences in Sarawak based on voucher specimens from Universiti Malaysia Sarawak Insect Reference Collection (UIRC). A total of 76 specimens of tiger beetles were examined in which comprising of seven genera from eight species. The genus *Neocollyris* was represented with two species, namely *Neocollyris* (s. str.) *ermaginata* and *Neocollyris* (*Stenocollyris*) *sarawakensis macrodera*, while the other genera were represented by only one species, respectively. The most abundant species with 64 individuals (84.21%) were represented by *Cosmodela aurulenta*, followed by *Abroscelis tenuipes araneipes* and *Therates labiatus* with three individuals (3.95%) and *N. ermaginata* with two individuals (2.63%). In this study, there were eight ecological habitats being identified, namely heath forest, limestone forest, littoral forest, mixed dipterocarp forest, oil palm plantation, peat swamp forest, riverine forest and urban area. The data indicates that *C. aurulenta* being recorded at seven ecological habitats suggesting this species to have a wide distribution capability while species such as *A. tenuipes araneipes*, *Callytron doriai*, *Cicindela* sp., *Myriochila* (s. str.) *specularis brevipennis*, *N. ermaginata* and *N. s. macrodera* suggesting these species to have a very narrow habitat specialisation. The results of this preliminary study provide evidence on the need to conduct further studies on the ecological aspects of tiger beetles in Sarawak.

Keywords: Cicindelinae, ecological distribution, Sarawak, tiger beetles, voucher specimens

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The subfamily Cicindelinae or commonly known as tiger beetles were formerly assigned to a separate family Cicindelidae (Carter, 1989; Pearson & Cassola, 2001; Pearson, 2007) and is now classified as a subfamily within Carabidae (Hill & Abang, 2010; Bosuang *et al.*, 2017; Gough *et al.*, 2018). The distinctive characteristics of these beetles can be observed from their large eyes, long narrow legs, and acutely toothed mandibles. Adult tiger beetles are known to be brightly coloured usually with iridescent or metallic and often with distinct colour pattern (Triplehorn & Johnson, 2005; Hill & Abang, 2010). When disturbed, they fly rapidly or run swiftly in short distances making them a challenge to capture. Moreover, most species are diurnal, usually found active in daylight in open habitats such as water edges, grasslands, woodland paths, and roads, but a few are nocturnal (Knisley, 1984). These beetles specifically, are ground surface predators and can be seen visually hunting with their sickle-like mandibles on a variety of smaller insects in which they feed on (Knisley, 1984). Area with enough sun light with the presence of bare soil are a necessary habitat requirement for the tiger beetle's predatory activities. Besides that, recent studies from different regions emphasise that most tiger beetles have very narrow habitat specialization and as a consequence, the cicindelid beetles have become a crucial global flagship group for beetle and insect conservation, especially as a biological indicator of habitat health (Pearson & Vogler, 2001; Jaskula, 2011).

Notwithstanding the abundance and evident dominance of tiger beetles in habitats of Borneo, these beetles have little ecological study being properly understood, except for some recent work on the subfamily Cicindelinae in new descriptions of species (Wiesner, 2016) and notes on Bruneian tiger beetles (Damken *et al.*, 2017). Other than these, recent studies and the substantial amount of distribution information and notes from collectors published in Brunei, the habitat preference of tiger beetles in Borneo is poorly known especially in Sarawak. Hence, the objectives of this study were to identify tiger beetle species in Sarawak as well as their distribution, abundances and habitat based on specimens deposited in Universiti Malaysia Sarawak (UNIMAS) Insect Reference Collection (UIRC).

This study examined on voucher specimens of Cicindelinae deposited in the UIRC dated from 1994 to 2019. Collecting data such as scientific name, collecting date, locality and collector's name were recorded. Based on the specimen's locality data, their ecological habitats were identified and distribution map was generated. Specimens with insufficient information of georeferenced localities were excluded from the analysis. Specimens were then sorted according to the morphological characteristics for identification into respective genus, species and subspecies, whichever possible. Species identification follows available references by Chung (2003), Hill and Abang (2010) and Bosuang *et al.* (2017) for comparison and species confirmation. Relative abundance of each tiger beetle species recorded from UIRC was then calculated by a percentage of dividing the number of individuals of each species with the total number of individuals recorded for all species.

A total of 76 specimens of Cicindelinae beetles were recorded in this study representing eight species and seven genera, namely, *Abroscelis*, *Callytron*, *Cicindela*, *Cosmodela*, *Myriochila*, *Neocollyris* and *Therates*. Table 1 shows the current checklist of Cicindelinae documented in Sarawak based on specimens deposited in UIRC. *Neocollyris* was represented by two species, namely, *Neocollyris* (s. str.) *ermaginata* and *Neocollyris* (*Stenocollyris*) *sarawakensis macrodera*, while the other genera were represented by only one species, respectively.

Cosmodela aurulenta or commonly known as the golden-spotted tiger beetle was the most abundant species with 64 individuals (84.21%), followed by *Abroscelis tenuipes araneipes* and *Therates labiatus* with three individuals (3.95%), whereas *N. ermaginata* with two individuals (2.63%) respectively (Table 2). *Cosmodela aurulenta* is among the largest and the most common tiger beetle in Borneo (Hill & Abang, 2010; Bosuang *et al.*, 2017). Specimens of this species are widely available as voucher specimens in both private and public collections (López-López *et al.*, 2015; Damken *et al.*, 2017). In addition, *C. aurulenta* has conspicuous and bold colour pattern in which can easily attract the attention of naturalist as well as amateur collectors.

Three new distributional records of tiger beetles were documented in the current study, namely, *Cicindela* sp., *N. s. macrodera* and *T. labiatus* that were not listed in previous study by Damken *et al.* (2017) (Figure 1). There are eight types of ecological habitats documented in this study, namely, heath forest (HF), limestone forest (LMF), littoral forest (LTF), mixed dipterocarp forest (MDF), oil palm plantation (OP), peat swamp forest (PSF), riverine forest (RF) and urban area (UB) (Figure 2). Table 3 shows the tiger beetles in Sarawak and their ecological distribution. *C. aurulenta* was widely distributed in seven habitats except littoral forest, followed by *T. labiatus* with three ecological habitats, namely limestone forest, oil palm plantation and peat swamp forest. *N. emarginata* can be found in mixed dipterocarp forest and oil palm plantation. Five species of Cicindelinae were documented in only one ecological habitat: *A. t. araneipes* (LTF), *Callytron doriai* (PSF), *Cicindela* sp. (OP), *N. s. macrodera* (OP) and *Myriochila* (s. str.) *specularis brevipennis* (UB).

This study suggests that most tiger beetle species (six species) showed very narrow habitat specialisation and restricted to one or at most two ecological habitats. This finding is in accordance to Jaskula (2015) who studied tiger beetles in Maghreb, the Mediterranean region, where these tiger beetles were found to be restricted to only a very small part within a specific habitat. Moreover, some other studies also indicated that narrow

Table 1. A checklist of Cicindelinae species documented from Universiti Malaysia Sarawak Insect Reference Collection (UIRC)

Genera	**Species**
Abroscelis	*Abroscelis tenuipes araneipes* (Schaum, 1863)
Callytron	*Callytron doriai* (Horn, 1897)
Cicindela	*Cicindela* sp.
Cosmodela	*Cosmodela aurulenta* (Fabricius, 1801)
Myriochila	*Myriochila* (s. str.) *specularis brevipennis* (Horn, 1897)
Neocollyris	*Neocollyris* (s. str.) *ermarginata* (Dejean, 1825)
	Neocollyris (*Stenocollyris*) *sarawakensis macrodera* (Chaudoir, 1864)
Therates	*Therates labiatus* (Fabricius, 1801)

Table 2. The relative abundance of Cicindelinae species deposited in Universiti Malaysia Sarawak Insect Reference Collection (UIRC)

Genera	**Species**	**Number of Individuals**	**Relative Abundance (%)**
Abroscelis	*Abroscelis tenuipes araneipes* (Schaum, 1863)	3	3.95
Callytron	*Callytron doriai* (Horn, 1897)	1	1.32
Cicindela	*Cicindela* sp.	1	1.32
Cosmodela	*Cosmodela aurulenta* (Fabricius, 1801)	64	84.21
Myriochila	*Myriochila* (s. str.) *specularis brevipennis* (Horn, 1897)	1	1.32
Neocollyris	*Neocollyris* (s. str.) *emarginata* (Dejean, 1825)	2	2.63
	Neocollyris (*Stenocollyris*) *sarawakensis macrodera* (Chaudoir, 1864)	1	1.32
Therates	*Therates labiatus* (Fabricius, 1801)	3	3.95
Total	**8**	**76**	**100**

Table 3. Tiger beetles of Sarawak and their ecological distribution: HF – heath forest, LMF – limestone forest, LTF – littoral forest, MDF – mixed dipterocarp forest, OP – oil palm plantation, PSF – peat swamp forest, RF – riparian forest and UB – urban area.

Species	**Ecological Distribution of Cicindelinae in Sarawak**							
	HF	**LMF**	**LTF**	**MDF**	**OP**	**PSF**	**RF**	**UB**
Abroscelis tenuipes araneipes (Schaum, 1863)			/					
Callytron doriai (Horn, 1897)						/		
Cicindela sp.					/			
Cosmodela aurulenta (Fabricius, 1801)	/	/		/	/	/	/	/
Myriochila (s. str.) *specularis brevipennis* (Horn, 1897)								/
Neocollyris (s. str.) *emarginata* (Dejean, 1825)					/			
Neocollyris (*Stenocollyris*) *sarawakensis macrodera* (Chaudoir, 1864)					/			
Therates labiatus (Fabricius, 1801)		/			/	/		

Figure 1. Habitus images. (a) *Cicindela* sp.; (b) *Neocollyris* (*Stenocollyris*) *sarawakensis macrodera* (Chaudoir, 1864); (c) *Therates labiatus* (Fabricius, 1801). Scale bar= 1 mm

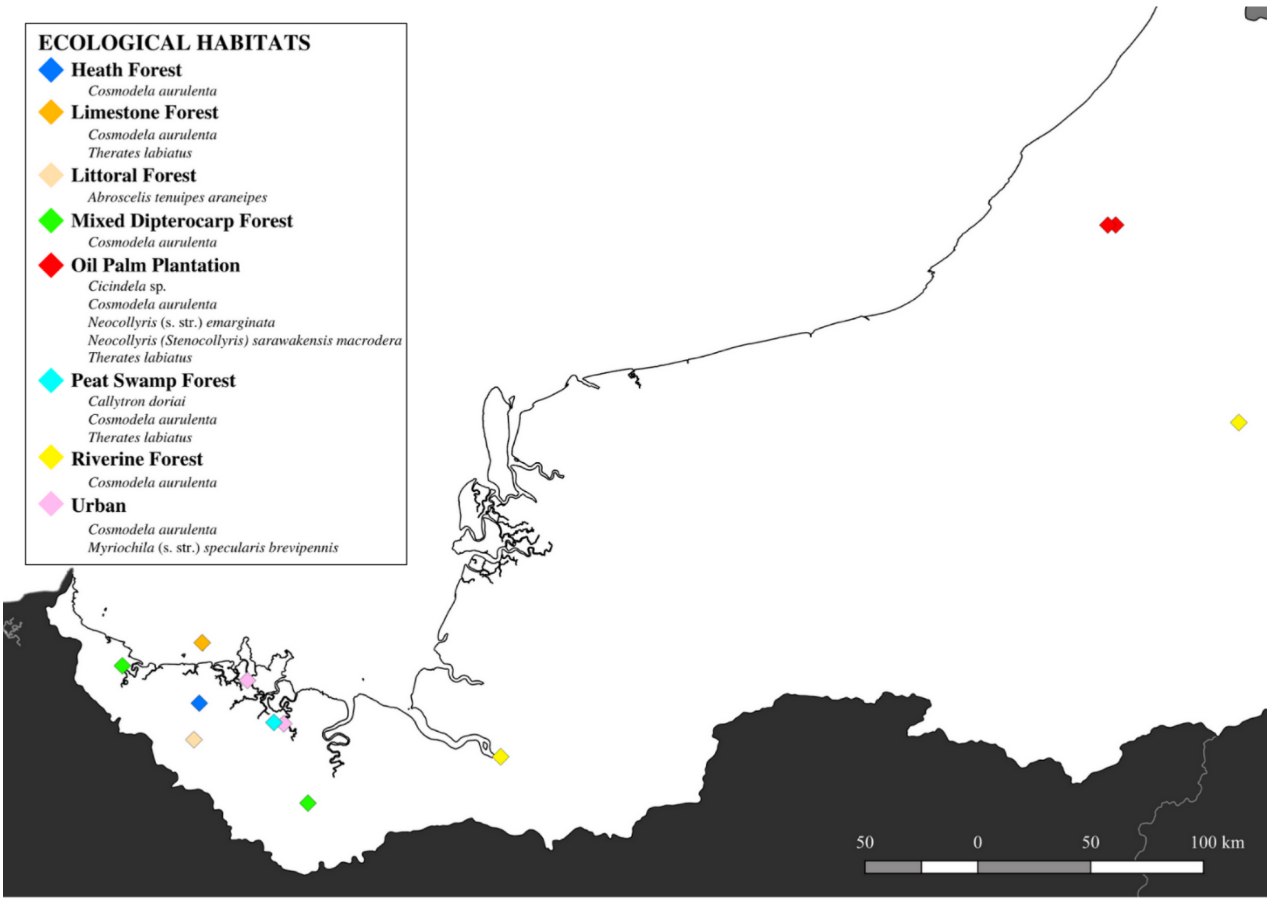

Figure 2. Ecological habitats of tiger beetle in Sarawak based on specimens deposited in UIRC

habitat specialisation were resulted from morphological, physiological or behavioural adaptations in both adult and larval stages (Pearson & Lederhouse, 1987; Hadley *et al.*, 1990; Dangelle *et al.*, 2013).

Meanwhile, *T. labiatus* and *C. aurulenta* can be encountered at three or more ecological habitats suggesting a wider distribution capability. The crucial role of some tiger beetles portraying an opportunistic feeding behavior helps in colonization at different habitat types. This can be observed especially in *C. aurulenta*, which is a typical predatory beetle and habitat generalists (Bosuang *et al.*, 2017).

Hence, this widely distributed species may be useful as the potential indicator to human disturbance effect especially in varying habitat types of Sarawak (Arndt *et al.*, 2005). A similar finding by Aydin *et al*. (2005) also suggested that *Megacephala euphratica euphratica*, *Lophyridia concolor*, *L. littoralis winkleri*, and *Myriochile melancholica melancholica* which were widely distributed in Çukurova Delta of Southern Turkey as potential indicator species.

In addition, the use of voucher specimens serves as an important study material as they are maintained to provide the permanent, physical documentation of species and as evidence of their presence at a particular point in time (Abang & Hill, 2007). Therefore, this study on tiger beetles benefited from the knowledge on voucher specimens for an effective communicating information on science, conservation of biodiversity as well as a potential biological indicator of habitat health. Hence, a data on distribution of these tiger beetles species documented from voucher specimens in UIRC is one step towards the direction of providing knowledge on the subfamily Cicindelinae as potential biological indicator in Sarawak.

Knowledge on tiger beetles' species distribution and abundances is a preliminary step towards providing more information on the subfamily Cicindelinae as a potential biological indicator. In this study, two species, *C. aurulenta* and *T. labiatus*, being found in most habitat types suggests that this group has a wide distribution capability, therefore a habitat generalist. Habitat generalist allows survival in a larger environmental gradient due to their broader environmental tolerances.

Meanwhile, a majority of tiger beetles species from this study, namely *A. t. araneipes*, *C. doriai*, *Cicindela* sp., *M. s. brevipennis*, *N. emarginata* and *N. s. macrodera*, were found in one or at most two habitats suggesting them having a narrow habitat specialisation, therefore habitat specialist. These habitat specialists live in only particular habitats since they are more sensitive towards environmental changes.

In addition, the role of voucher specimens deposited in depositories like UIRC not only bring advantages on systematic, faunistic and ecological studies but also when voucher specimens made physically available in collections, they are beneficial for future studies regarding a certain taxon. A detailed ecological distribution on tiger beetles and its faunistic composition should be done in future to further evaluate on the potential of tiger beetles as bio indicator species in Sarawak as well to improve understanding of these beetles for a better conservation plan on this subfamily.

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