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***Research Article***

# MACROFUNGAL DIVERSITY OF BRAHAKSHETRA COMMUNITY FOREST, GHORAHI, DANG, NEPAL

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## ABSTRACT
Fungi are a diverse group of organisms ranging from microscopic to macroscopic mushrooms. Being a major group of decomposers, they are essential for the survival of other organisms in the ecosystem and important for the degradation of organic matter. The main aim of this study was to study the macrofungal diversity of Brahakshetra Community Forest, Ghorahi, Dang. The forest is mainly dominated by *Shorea robusta* and other associated species. The study was made from June to September 2020. The sampling was done by using a 10×10m quadrate in a line transect and each plot was divided into 5×5m quadrats and samples were collected during the rainy season from different habitats of the forest. Mushrooms were photographed in their natural habitat and preserved in dry and liquid preservation. The specimens were identified by examining their macroscopic and microscopic features, and also by using references from standard literature and websites. In the study, a total of 66 species were recorded under 30 genera belonging to 21 families, and 8 orders. Among them, 65 belong to basidiomycetes and 1 belongs to Ascomycetes. Agaricales was found as the largest order followed by Russulales and Boletales. The diversity indices, the Shannon Weiner index and Simpson diversity index were found to be 3.59 and 0.93 respectively. The result of this study concludes that the Brahakshetra Community Forest was rich in macrofungal diversity, especially ectomycorrhizal. There were positive trends between species richness of macrofungi and environmental variables, i.e., tree canopy, soil pH, soil moisture, and leaf litter which means species increased with increasing these environmental variables.

**Keywords:** Ectomycorrhizal, edible, environmental variable, positive trend

## INTRODUCTION
An important component of the forest ecosystem that has a broad range of functions is fungal communities (Song *et al.*, 2019). Most of the fungi are saprophytes that feed on dead and decaying materials and help to remove leaf litter and other debris. They are essential for the survival of other organisms in the ecosystem because they are a major group of decomposers (Hawksworth, 1991) which have an important role in nutrient cycling. Most land plants need to be associated with mycorrhizal fungi for efficient nutrient uptake, supply minerals, increase their productivity, and confer resistance to stress (Bonfante & Anca, 2009).

Fungi are a diverse group of organisms that are categorized according to their size into micro and macrofungi. They can be found in all types of environments and are commonly known as mushrooms, morels, truffles, molds, mildews, yeasts, earthstars, stinkhorns, rusts, smut, bracket, or shelf-fungi (Acharya & Parmar, 2016). The macrofungi family contains thousands of different species, and each one is exquisite in its own special way (Devi, 2017). Macro fungi can grow in soil, litter, wood, animal feces, and others. It does not have chlorophyll, so it cannot prepare its food (Putra *et al.*, 2019). They are higher fungi with distinctive hypogynous or epigeous fruiting bodies that are visible to the naked eye and can be manually harvested (Chang & Miles, 1992). They produce larger fruiting bodies, such as those found on gilled fungi, jelly fungi, coral fungi, stinkhorn fungi, bracket fungi, and bird's nest fungi (Hawksworth *et al.*, 1995). Based on the mode of adaptation they are parasitic, lignicolous, foliicolous, humicolous, coprophilous, termitophilous, saprobic, and mycorrhizal which form large fruiting bodies visible without the aid of a microscope.

From the taxonomic point of view, mainly Basidiomycetes and some species of Ascomycetes are macro fungi. Most of the ascomycetes contain cup fungi, morels, and truffles and the basidiomycetes comprise toad tools, bracket fungi, and puffballs. Ecologically, macrofungi can be classified into three groups: the saprophytes, the parasites, and the symbiotic (mycorrhizal) species. Most terrestrial macrofungi are saprobes or mycorrhizal symbionts, but some are pathogens of plants or fungi (Tang *et al.*, 2015). Fungi fruiting on a woody substrate is usually either saprobes or plant pathogens. Mushrooms have been classified as edible, inedible, poisonous, and medicinal. Edible mushrooms have been used as a source of food because they have rich nutritional value (Ukwuru *et al.*, 2018). Additionally, they have been employed in conventional medicine to treat a variety of ailments, including bacterial and viral infections, cancer, tumors, inflammation, and cardiovascular diseases (Iwalokum *et al.*, 2007).

Macrofungal diversity was studied in the community forest of Ghorahi 13, Dang. This study aimed to find the diversity and types of macrofungi found in the study area. This study also has contributed to mycoflora exploration in some areas which are still not explored.

As we know mushrooms have a great nutritional value so proper documentation and estimation are important.

## MATERIALS AND METHOD

### Study area

The study was done in Brahakshetra Community Forest, Gorahi-13, Dang. It lies in Lumbini Province in the Mid-Western part of Nepal which is 413 km southwest of Nepal's capital Kathmandu (Fig. 1). This place is known for its landscape and a slightly milder climate. Its geographical location is 082.18648°E longitude and 27.59254°N latitude within an altitudinal range of 710-1240m above sea level. It occupies nearly 491.18 hectares of land in Ghorahi Sub-Metropolitan ward number 13. Planted forest occupies 5.86 hectares. Brahakshetra Community Forest was established in 2052 B.S. The forest is mainly dominated by *Shorea robusta* and other main associated species are *Dalbergia Sissoo, Diploknema butyracea, Syzygium cumini, Pinus roxburghii.* The common shrubs are *Woodfordia fruticose, Lantana camera,* and *Justicia adhatoda.*

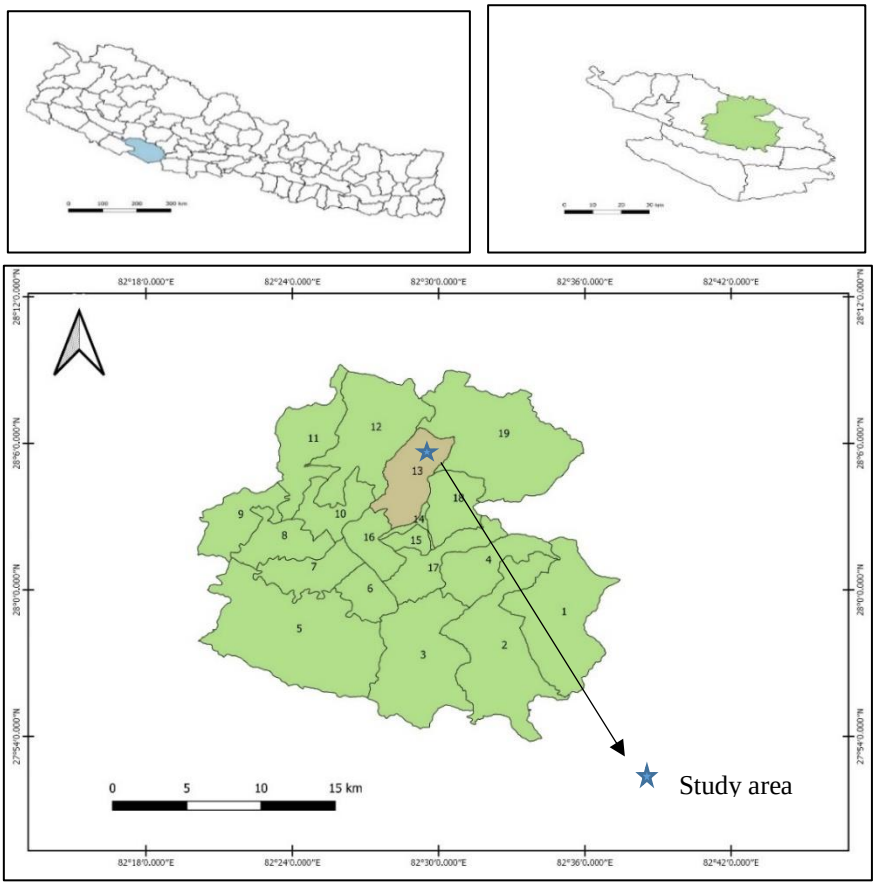


**Figure 1. Map showing Brahakshetra Community Forest, Dang.**

### Climatic data

The climatic data of the nearest metrological station were obtained from the Department of Hydrology and Meteorology, Government of Nepal for the period from 2011 to 2021 (Fig. 2). The climatic data were recorded from a weather station i.e., Ghorahi, Dang.

### Sampling process

Field sampling was conducted from 15 June to 30 September 2020. For the sampling of macrofungi, the quadrat method of 10×10m was used (Shah *et al.*, 2020; Baral *et al.*, 2015). Each plot was further divided into four 5×5m quadrats (Fig. 3). The difference in elevation between each transect was about 100 meters. The distance between two quadrats in a line transect was approximately 50 m, and stratified random sampling was used to locate 10 quadrats in each transect. All macrofungi species were collected and photographed in each plot (Photo plates 1-29). The altitude, latitude, and longitude of each quadrat were obtained from Global Positioning System (GPS) and the slope was measured by using a clinometer. Litter cover (%) and canopy cover (%) were estimated visually from the center of each quadrat (Baral *et al.*, 2000). The Soil sample was taken from the four corners of the quadrat and one from the center about 15 cm deep (Zobel *et al.*, 1987).

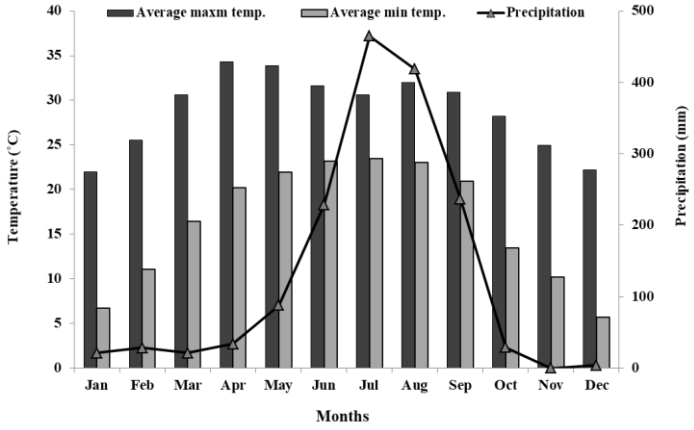


**Figure 2. Climatic data of average minimum and maximum temperature, and precipitation**

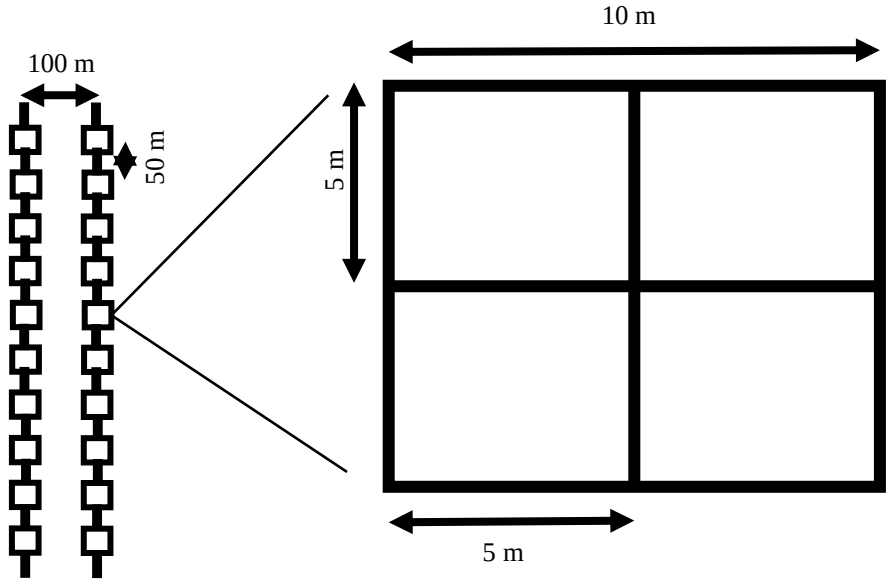


**Figure 3. Sampling design**

**Collection and preservation of mushroom**

The collection was done in June, July, and August. The collection was based on their sexual reproduction structure. Before being collected, the mushroom was captured in its natural habitat. The damaged, decayed, and insect-eaten species were thrown away. With the help of a sharp knife, the basidiocarps were carefully dug up (Shah *et al.*, 2020). Each species was cleaned with the help of a brush and kept in an individual paper bag to avoid the mixing of spores. The collected species were examined for morphological characteristics like color, size, shape, odor, and texture. Field labels were tagged to the specimens with the collected data which includes collection number, date, and location, and other features were noted down in the field notebook.

Mushrooms are very delicate and possess a maximum amount of moisture, they undergo rapid decay. So, the delicate mushrooms were preserved in a liquid solution in the ratio of 70:25:5 (distilled water, alcohol, and formalin) (Ainsworth, 1971) while strong mushrooms were preserved in dry preservation (Sun drying or air drying). The spore print was taken in white paper for black or brown spore and black paper for white spores.

**Microscopic studies in laboratory**

The small pieces of spores was taken from the spore print using cello tape and placed on a slide. Before placing we used lactophenol cotton blue for mounting and then observed under a compound microscope. We used immersion oil to see the spore in a magnified view and saw the detailed structure of the spores. The length and width of spores of each species were measured in the lab and their mean value was provided using a high-power microscope (Olympus CX22, magnification 40× and 100×).

**Mushroom identification**

The specimens were identified by examining their macroscopic and microscopic features: microscopic features (spore shape and size), macroscopic features (cap shape, size, and color; stipe shape and size, volva and annulus present or absent; gill/pore shape and color, attachment) using references from standard literature (Pacioni, 1985; Singer, 1986; Thind, 1961; Philips, 1981; Corner, 1970; Adhikari, 2000; Adhikari, 2014).

**Data analysis**

The frequency and density of macrofungal species were calculated (Daubenmire, 1959). The parameters used to assess macrofungal diversity in the study area are listed below.

$$\text{Frequency (\%)}: \frac{\text{No. of plots with species}}{\text{Total no. of plots}} \times 100\%$$

$$\text{Relative frequency (RF)}: \frac{\text{Frequency of the species}}{\text{The total frequency of all species}} \times 100\%$$

$$\text{Density}: \frac{\text{Total no.of individual of particular species in all quadtrats}}{\text{Area of one quadrat} \times \text{Total number of quadrats}}$$

$$\text{Relative density (RD \%)}: \frac{\text{No.of species A counted}}{\text{No.of all species collected}} \times 100\%$$

Data was normal ($p>0.05$). Regression analysis was carried out to reveal the impact of independent variables on dependent variables. For the analysis of abundance and site-based present-absent data, Canonical Correspondence Analysis (CCA) was done by using R 4.2.1 software. CCA was performed to study the effect of an environmental variable on species composition.

Species diversity is the species richness and evenness in a particular location. Shannon Weiner Index and Simpson diversity index were used to calculate the species diversity of macrofungi in each plot.

**Shannon Wiener Index** was calculated by using the following formula (Magurran, 2004):

Shannon Weiner Index (H) = $-\sum Pi \times \text{In } Pi$

Where, Pi = Proportion of individuals found in the $i^{th}$ species for a sampling community. It was calculated as $Pi = ni / N$

Where, ni = Average coverage of each individual species, N = total average coverage of all species

**Simpson's diversity index** was calculated using the following equation (Magurran, 2004):

Simpson's Diversity Index (D) = $N (N -1) \sum ni (ni-1)$

Where, 1-D = Simpson's diversity index, Ni = Total coverage of individual in $i^{th}$ species, N = Total average coverage of all species

$$\text{Ni}: \frac{\text{Average coverage of each individual species}}{\text{Total average coverage of all species}} \times 100$$

**Soil analysis**

**Soil pH and Soil moisture**

Soil pH was determined by using a pH meter (model-HM-1003) in a 1:2 ratio of the soil-water mixture. During the measurement, the pH meter was calibrated using a buffer solution of known pH (pH 4 and pH 7). During the process, 50 mL of distilled water was poured into 25 g of soil sample. The mixture was stirred for at least 30 minutes using a magnetic stirrer and then allowed to settle down for 5 minutes. The electrode was dipped into the mixture and a reading of pH was noted. Triplicate readings were taken from each soil sample.

Moisture content was determined by using the formula by Zobel *et al* (1987). For the calculation of moisture content in the soil, clean and dry crucibles were taken. From each sample, 10 g fresh soil sample was kept inside a hot air oven and heated at 105°C for 48 hrs. Then the crucible was cooled thoroughly and weighed again.

$$\text{Moisture content: } \frac{\text{Weight of fresh soil} - \text{Weight of oven dried soil}}{\text{Weight of oven-dried soil}} \times 100\%$$

**RESULTS**

A total of 66 macro-fungal taxa were documented, of which 54 were identified up to the species level, 7 up to the generic level and 5 were unidentified. Out of 66 species, 61 species belong to 30 genera, 21 families, and 8 orders. Among 21 families, the highest number of species belonged to the family Russulaceae (16) followed by Amanitaceae (9), Boletaceae, and Agaricaceae while the least number of species belonged to the family Xylaraceae, Schizophyllaceae, and Trichomatecae (Fig. 4; Table 1). Agaricales (26) was found as the largest order followed by Russulales (16), and Boletales (9). Among 61 macro-fungal species identified, 60 species belonged to class Basidiomycetes class and only 1 belonged to the Ascomycetes class.

Habitat-wise, a higher number of mushroom species were found on soil (53) followed by wood (11) and leaves (2) (Fig. 5).

According to ethnomycological and morphological character, most of the species were edible (33) followed by inedible (19), poisonous (6) and the least number of species were medicinal (Fig. 6). Based on ecological preferences, 65% of the species were mycorrhizal, 33% were saprophytic, and only 2% were parasitic (Fig. 7).

The diversity index of species was analyzed. Shannon's diversity index (H') and Simpson's diversity index (1-D) were found to be 3.5952 and 0.93, indicating a higher value of the diversity index.

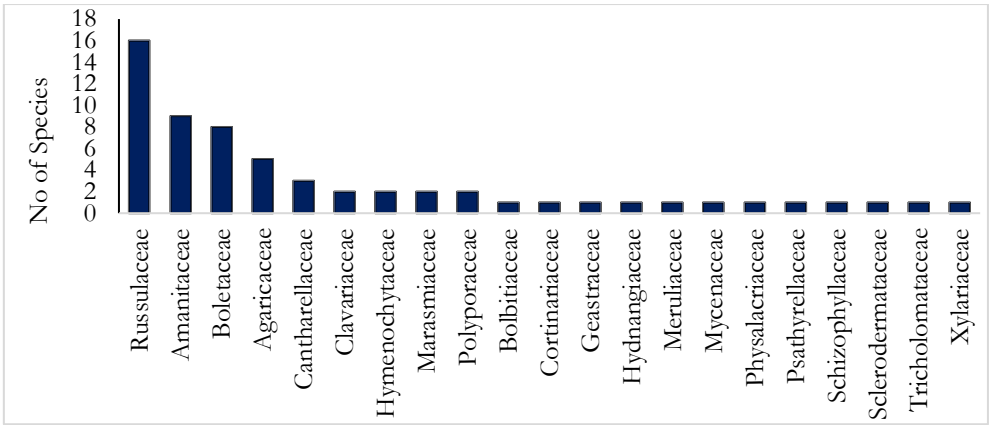


**Figure 4. Number of mushroom species belonging to different families**.

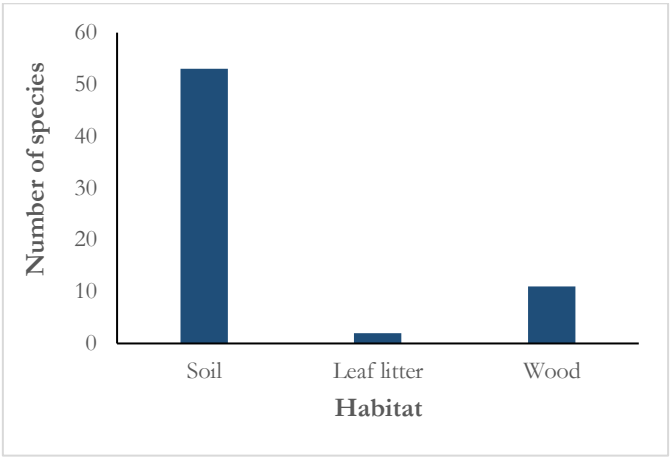


**Figure 5. Number species found in different habitats**

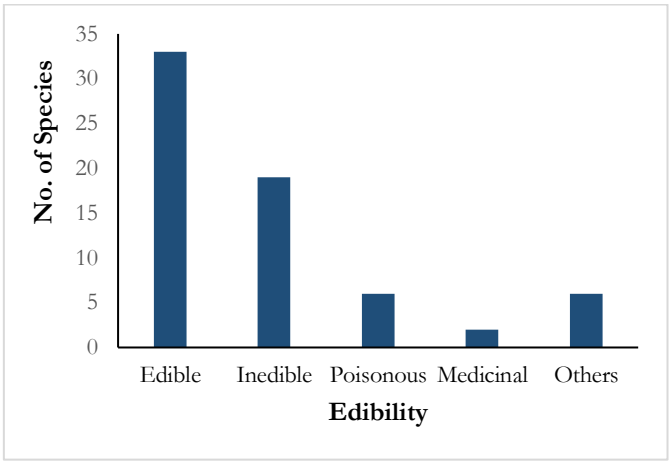


**Figure 6. Number of species with edibility functions**

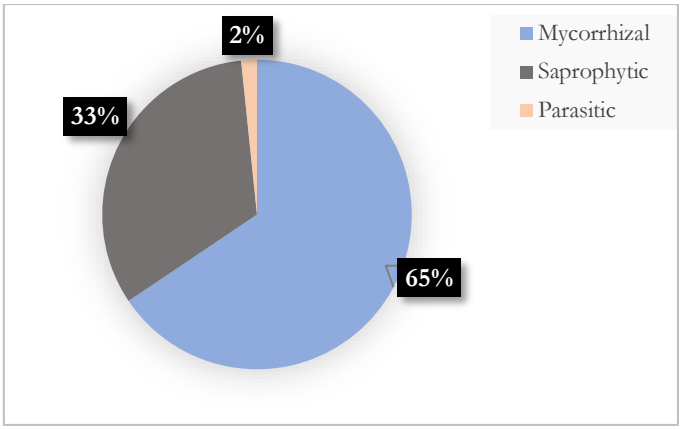


**Figure 7. Pie chart showing ecology (%) of macrofungi**

**Table 1. List of collected mushrooms along with Families, Frequency, Relative Frequency, Density, and Relative Density**

| S.N | Name of Species | Family | Frequency (%) | Relative frequency (%) | Density | Relative Density (%) |
|---|---|---|---|---|---|---|
| 1 | *Agaricus placomyces* | Agaricaceae | 10.00 | 1.03 | 0.13 | 1.280 |
| 2 | *Agaricus sp 1* | Agaricaceae | 3.33 | 0.34 | 0.11 | 1.083 |
| 3 | *Agaricus sp 2* | Agaricaceae | 20.00 | 2.05 | 0.14 | 1.378 |
| 4 | *Amanita battarae* | Amanitaceae | 6.67 | 0.68 | 0.03 | 0.295 |
| 5 | *Amanita caesarea* | Amanitaceae | 43.33 | 4.45 | 0.19 | 1.870 |
| 6 | *Amanita chempangiana* | Amanitaceae | 10.00 | 1.03 | 0.08 | 0.787 |
| 7 | *Amanita fulva* | Amanitaceae | 6.67 | 0.68 | 0.1 | 0.984 |
| 8 | *Amanita longistrata* | Amanitaceae | 3.33 | 0.34 | 0.07 | 0.689 |
| 9 | *Amanita mutisquamosa* | Amanitaceae | 36.67 | 4.79 | 0.79 | 7.776 |
| 10 | *Amanita pantherina* | Amanitaceae | 26.67 | 2.74 | 0.12 | 1.181 |
| 11 | *Amanita sp* | Amanitaceae | 6.67 | 0.68 | 0.04 | 0.394 |
| 12 | *Amanita vaginata* | Amanitaceae | 3.33 | 0.34 | 0.04 | 0.394 |
| 13 | *Armillaria mellea* | Physalacriaceae | 3.33 | 0.34 | 0.01 | 0.098 |
| 14 | *Boletellus emoedensis* | Boletaceae | 6.67 | 0.68 | 0.04 | 0.394 |
| 15 | *Boletus sp 1* | Boletaceae | 46.67 | 3.77 | 1.25 | 12.303 |
| 16 | *Boletus sp 2* | Boletaceae | 3.33 | 0.34 | 0.06 | 0.591 |
| 17 | *Boletus strobilaceus* | Boletaceae | 26.67 | 2.74 | 0.12 | 1.181 |
| 18 | *Borofutus dhakanus* | Boletaceae | 33.33 | 3.42 | 0.26 | 2.559 |
| 19 | *Cantharellus cibarius* | Cantharellaceae | 3.33 | 0.34 | 0.02 | 0.197 |
| 20 | *Coltricia cinnamommea* | Hymenochaetaceae | 3.33 | 0.34 | 0.05 | 0.492 |
| 21 | *Coltricia perennis* | Hymenochaetaceae | 3.33 | 0.34 | 0.05 | 0.492 |
| 22 | *Cortinarius varius* | Cortinariaceae | 13.33 | 1.37 | 0.14 | 1.378 |
| 23 | *Craterellus cornucopiodes* | Cantharellaceae | 3.33 | 0.34 | 0.05 | 0.492 |
| 24 | *Craterellus lutescens* | Cantharellaceae | 3.33 | 0.34 | 0.33 | 3.248 |
| 25 | *Cystoagaricus trisulphuratus* | Agaricaeae | 3.33 | 0.34 | 0.03 | 0.295 |
| 26 | *Filoboletus manipularis* | Mycenaceae | 3.33 | 0.34 | 0.03 | 0.295 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 27 | *Geastrum triplex* | Geastraceae | 10.00 | 1.03 | 0.26 | 2.559 |
| 28 | *Laccaria laccata* | Hydnangiaceae | 43.33 | 4.45 | 0.76 | 7.480 |
| 29 | *Lactarius volemus* | Russulaceae | 56.67 | 5.82 | 0.54 | 5.315 |
| 30 | *Lepista sordida* | Tricholomataceae | 3.33 | 0.34 | 0.04 | 0.394 |
| 31 | *Leucocoprinus fragillissimus* | Agaricaceae | 16.67 | 1.71 | 0.11 | 1.083 |
| 32 | *Lyoperdon perlatum* | Agaricaceae | 3.33 | 0.34 | 0.01 | 0.098 |
| 33 | *Marasmius maximus* | Marasmiaceae | 6.67 | 0.68 | 0.02 | 0.197 |
| 34 | *Marasmius sullivanti* | Marasmiaceae | 10.00 | 1.03 | 0.07 | 0.689 |
| 35 | *Microporus xanthopus* | Polyporaceae | 6.67 | 0.68 | 0.02 | 0.197 |
| 36 | *Pholiotina sp* | Bolbitiaceae | 43.33 | 4.45 | 0.55 | 5.413 |
| 37 | *Podoschypha petalodes* | Meruliaceae | 26.67 | 2.74 | 0.27 | 2.657 |
| 38 | *Pulveroboletus revenelli* | Boletaceae | 20.00 | 2.05 | 0.12 | 1.181 |
| 39 | *Ramariopsis kunzei* | Clavariaceae | 3.33 | 0.34 | 0.02 | 0.197 |
| 40 | *Ramariopsis spp* | Clavariaceae | 16.67 | 1.71 | 0.14 | 1.378 |
| 41 | *Russula alboareolata* | Russulaceae | 13.33 | 1.37 | 0.06 | 0.591 |
| 42 | *Russula cyanoxantha* | Russulaceae | 20.00 | 2.05 | 0.11 | 1.083 |
| 43 | *Russula delica* | Russulaceae | 6.67 | 0.68 | 0.02 | 0.197 |
| 44 | *Russula densifolia* | Russulaceae | 30.00 | 3.08 | 0.11 | 1.083 |
| 45 | *Russula emetica* | Russulaceae | 40.00 | 4.11 | 0.24 | 2.362 |
| 46 | *Russula flavida* | Russulaceae | 6.67 | 0.68 | 0.02 | 0.197 |
| 47 | *Russula luteotacta* | Russulaceae | 23.33 | 2.40 | 0.14 | 1.378 |
| 48 | *Russula nigricans* | Russulaceae | 43.33 | 4.45 | 0.21 | 2.067 |
| 49 | *Russula nitida* | Russulaceae | 3.33 | 0.34 | 0.35 | 3.445 |
| 50 | *Russula pectinata* | Russulaceae | 3.33 | 0.34 | 0.01 | 0.098 |
| 51 | *Russula poichilochroa* | Russulaceae | 33.33 | 3.42 | 0.26 | 2.559 |
| 52 | *Russula rosea* | Russulaceae | 16.67 | 1.71 | 0.08 | 0.787 |
| 53 | *Russula sanguinea* | Russulaceae | 13.33 | 1.37 | 0.06 | 0.591 |
| 54 | *Russula vesca* | Russulaceae | 10.00 | 1.03 | 0.05 | 0.492 |
| 55 | *Russula virescens* | Russulaceae | 3.33 | 0.34 | 0.02 | 0.197 |
| 56 | *Schyzophyllum commune* | Schizophyllaceae | 3.33 | 0.34 | 0.07 | 0.689 |
| 57 | *Scleroderma cepa* | Sclerodermataceae | 3.33 | 0.34 | 0.02 | 0.197 |
| 58 | Sp 38 | Unknown | 16.67 | 1.71 | 0.11 | 1.083 |
| 59 | Sp 48 | Unknown | 3.33 | 0.34 | 0.03 | 0.295 |
| 60 | Sp 53 | Unknown | 3.33 | 0.34 | 0.01 | 0.098 |
| 61 | Sp 66 | Unknown | 3.33 | 0.34 | 0.03 | 0.295 |
| 62 | Sp 8 | Unknown | 6.67 | 0.68 | 0.27 | 2.657 |
| 63 | *Tramates hirsuta* | Polyporaceae | 6.67 | 0.68 | 0.19 | 1.870 |
| 64 | *Tylopilus plumbeoviolaceus* | Boletaceae | 16.67 | 1.71 | 0.13 | 1.280 |
| 65 | *Tylopilus virense* | Boletaceae | 26.67 | 2.74 | 0.28 | 2.756 |
| 66 | *Xylaria polymorpha* | Xylariaceae | 20.00 | 2.05 | 0.07 | 0.689 |

**Photo plates of some identified species and their spores**


**Photo plate 1: *Agaricus placomyces* and its spores**


**Photo plate 2: *Amanita caesarea* and its spores.**


**Photo plate 3: *Amanita multisquamosa* and its spores.**


**Photo plate 4: *Amanita vaginata* and its spores.**


**Photo plate 5: *Armillaria mellea* and its spores.**


**Photo plate 6: *Boletus strobolaceus* and its spores.**


**Photo plate 7: *Boletellus emoedensis* and its spores.**


**Photo plate 8: *Borofutus dhakanus* and its spores.**

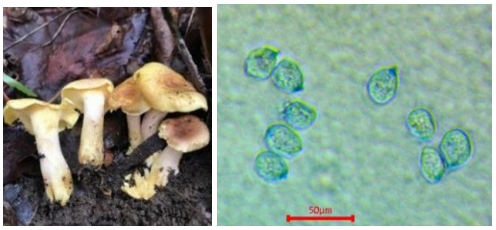


**Photo plate 9: *Cantharellus cibarius* and its spores.**


**Photo plate 10: *Coltricia cinnamomea* and its spores**


**Photo plate 11: *Craterellus lutescens* and its spores**


**Photo plate 12: *Cortinarius varius* and its spores**


**Photo plate 13: *Cystoagaricus trisulphuratus* and its spores**


**Photo plate 14: *Filoboletus manipularis* and its spores**

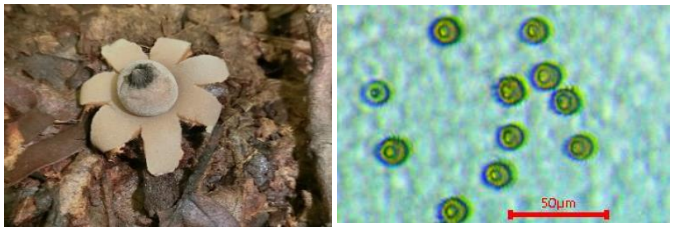


**Photo plate 15: *Geastrum triplex* and its spores**


**Photo plate 16: *Laccaria laccata* and its spores**


**Photo plate 17: *Lactarius volemus* and its spores**

**Photo plate 18: *Lepista sordida* and its spores**

**Photo plate 19: *Leucocoprinus fragilissimus* and its spores**

**Photo plate 20: *Lycoperdon perlatum* and its spores**

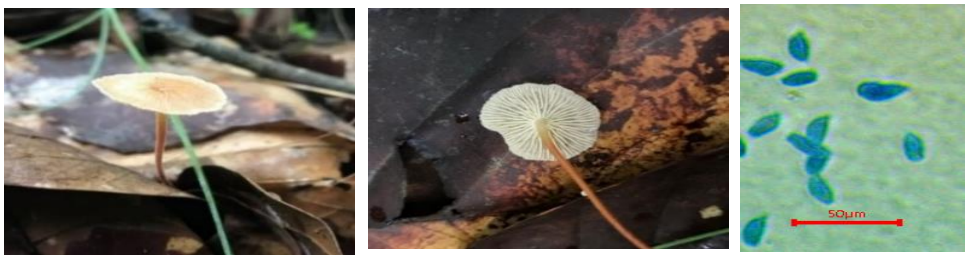

**Photo plate 21: *Marasmius maximus* and its spores**

**Photo plate 22: *Microporous xanthopus* and its spores**

**Photo plate 23: *Pholiotina* sp. and its spores**


**Photo plate 24: *Pulveroboletus revenelli* and its spores**


**Photo plate 25: *Ramariopsis kunzei* and its spores**


**Photo plate 26: *Russula delica* and its spores**


**Photo plate 27: *Russula flavida* and its spores**


**Photo plate 28: *Scleroderma cepa* and its spores**


**Photo plate 29: *Tylopilus virense* and its spores**

**Relation of Macrofungi species richness with environmental variables**

There was a positive trend between species richness of macrofungi and environmental variables, i.e., tree canopy (Fig. 8), soil pH (Fig. 9), soil moisture (Fig. 10), and leaf litter (Fig. 11). They showed a positive relationship that means species increased with increasing these environmental variables.

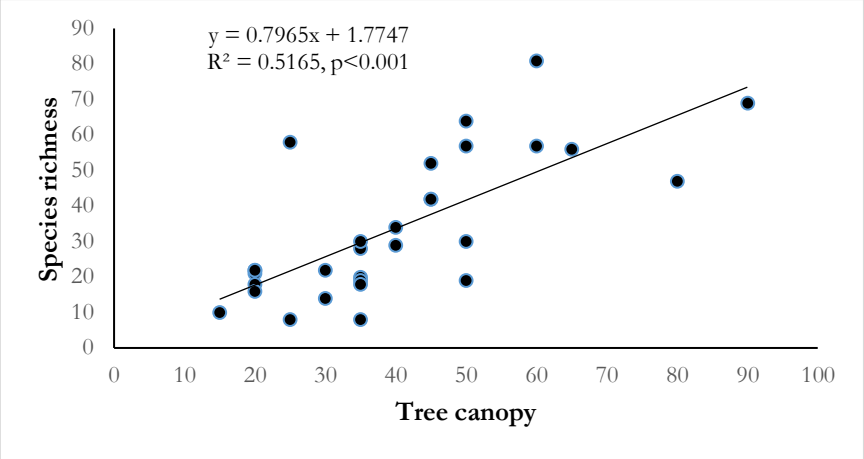


**Figure 8. Relation of species richness with tree canopy cover**

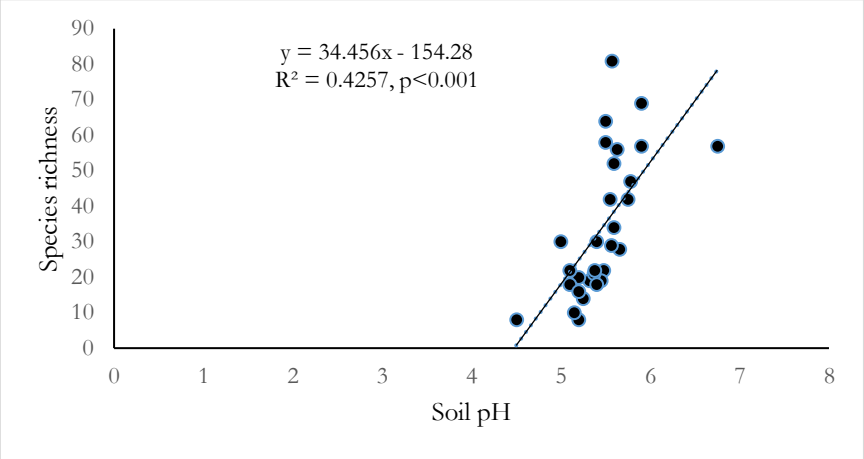


**Figure 9. Relation of species richness of macro-fungi with soil pH**

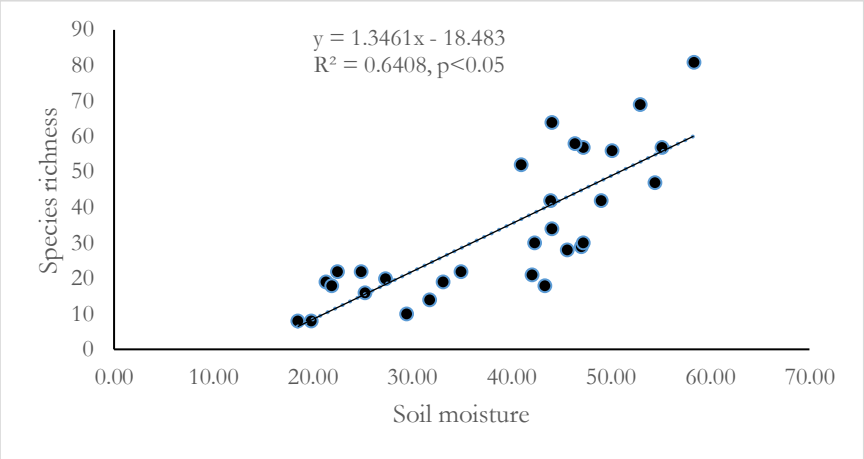


**Figure 10. Relation of macro-fungal species richness with soil moisture**

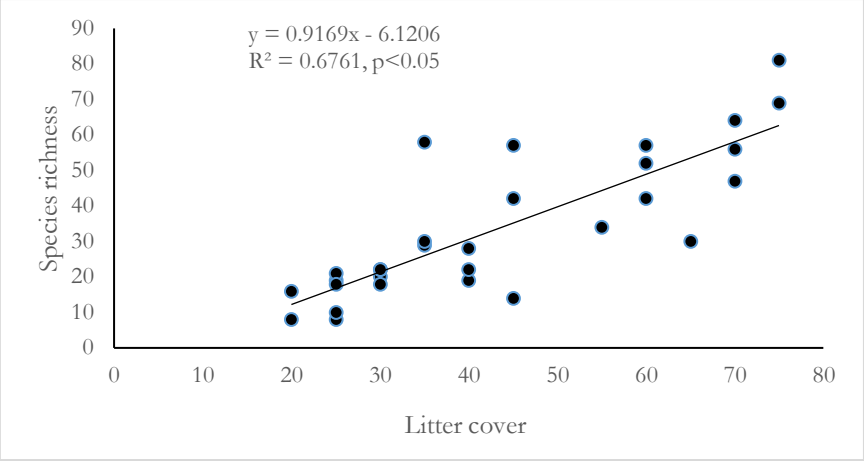


**Figure 11. Relation of macrofungal species richness with litter cover**

### Effect of Environmental variable on species composition

The first and second axis of the plot have respective eigenvalues of 0.4293 and 0.2434, explaining 42% and 24% of the variation. The length of the gradient, i.e., axis length is 3.0920 which is higher than 2.5 s.d. units. The CCA was performed only for those species having high density, i.e., more than 2.5. The effect of tree canopy on species composition, Canonical Correspondence Analysis (CCA) showed that the species composition was significantly variable (p=0.027) with environmental variable. The CCA plot showed that the mushroom species like *Leucocoprinus fragillissimus,* and *Tylopilus virense,* were high towards high canopy cover, and species *Tylopilus virense, Leucocoprinus fragillissimus, Amanita chempangiana* were found in high moisture whereas *Borofutus dhakanus,* and *Amanita chempangiana* were influenced by high pH, Species like *Russula sanguinaria, Ramariopis* sp., *Amanita caesarea* by leaf litter. But *Russula emetica, Laccaria laccata, Amanita pantherina, Cortinarius varius, Pulveroboletus revenelli,* etc. were found everywhere irrespective of any environmental variable (Fig. 12).

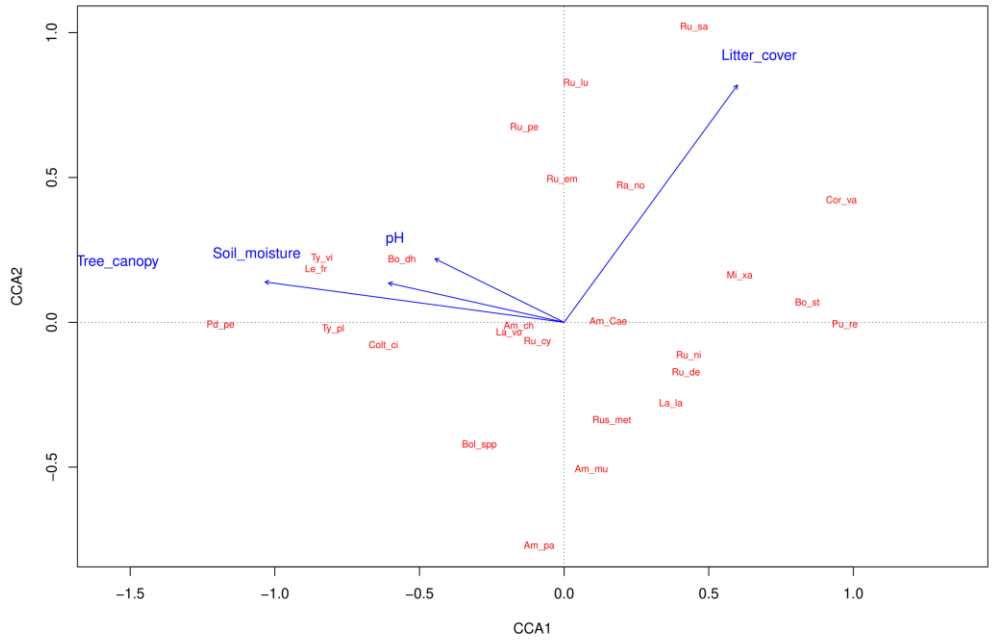


**Figure 12. Relationship between environmental variable and species composition**

## DISCUSSION

### Species richness and diversity

The study explored 66 species of mushrooms in the study area which belong to 30 genera, 21 families, and 8 orders. The study area was rich in macrofungal diversity which might be due to the study area being dominated by the *Shorea robusta,* which accounted for 20-90% of the canopy cover. Compared to leaf litter and wood, the soil was the most important substrate for mushroom growth. Species richness and diversity were found to be

significantly different between the habitats. About 80% of the mushroom species were found on soil followed by wood and leaf litter which is similar to Ghate and Sridhar (2016). Their study showed that the highest macrofungi were found on soil followed by wood and leaf litter. According to the edibility of the mushroom species, a maximum number of species were edible which was followed by inedible and poisonous. A total of 34 species of mushrooms were found edible followed by inedible, poisonous, and medicinal. This result coincides with Ullah *et al.* (2021).

The ecological preferences of the species in the study area showed that the maximum numbers of species were mycorrhiza (40 species) and then followed by saprophytic (20 species), and parasitic (1). The result of the study was similar to Shrestha *et al.* (2021). They found that various species of ectomycorrhiza were associated with the different tree species growing in the *Shorea robusta* forest with varied basidiocarps. The rich biodiversity of ectomycorrhiza in the present forest was due to high rainfall during the rainy season. The ectomycorrhizal fungi appear 2.4 times more diverse and 2.1 times more productive than saprobes (Richard *et al.*, 2004). Islam *et al.* (2007) also reported that the Dipterocarpaceae have an association with ectomycorrhizal. In the current finding, most of the mycorrhiza belongs to the family Russulaceae, Amanitaceae, and Boletaceae which are similar to Shrestha *et al.* (2021); Islam *et al.* (2007). As a result of the study, mycorrhiza had higher richness than saprophytic and parasitic. The most frequent mycorrhizal species which are found in the study area are *Amanita caesarea, Amanita multisquamosa, Russula nigricans, Russula cyanoxantha, Laccaria laccata,* and *Tylopilus virense.* Saprophytic species are *Tramates hirsuta, Xylaria polymorpha, microporous xanthopus, Agaricus placomyces,* etc. Only one species was found parasitic i.e., *Armillaria mellea.*

The species like *Lactarius volemus*, *Amanita multisquamosa*, *Amanita caesarea, Russula nitida, Laccaria laccata*, *Boletus sp*, *Amanita pantherina, Podoschypha petalodes, Tylopilus virense, Leucocoprinus fragillissimus, Borofutus dhakanus* had a high frequency vary from 16.66 to 56.7% and relative frequency ranges from 1.72 to 5.82%. The relative density of these species ranges from 0.098 to 12.30%. Similar results were found by Kumar *et al.* (2013). Their results showed that the species like *Agaricus arvensis, Agaricus langei, Lepiota lilacea, Lepiota magnispora, Auricularia auricula-judae, Boletus aestivalis, Cantharellus cibarius, Hypsizygus tessulatus, Pleurotus pulmonarius, Panus fulvus, Lactarius hygrophoroides, Cookeina sulcipes, Schizophyllum commune,* and *Lepista irina* had a frequency range from 25 to 66.6%. The same study also found by Shrestha *et al.* (2021), the ectomycorrhizal species like *Amanita ovoidea, Amanita vaginata, Cantharellus cibarius, Laccaria laccata, Lactarius hygrophoroides, Russula flavida, Russula brevipes, Scleroderma cepa, etc.* exhibited relative frequency ranges from 1.43 to 15.71%, relative density from 0.58 to 16.53%. As per the diversity indices, the Shannon Weiner index and Simpson diversity index were found to be 3.59 and 0.93 respectively indicating a higher diversity index.

**Relation of species richness with environmental variables**

The species diversity of macrofungi was affected by certain environmental factors. Factors like canopy cover, soil nutrient and pH, leaf litter, light, humidity, and geographical location greatly influence the development of macrofungi**.** Likewise, plant species affect the macrofungal species because plant constitutes the habitat and energy source of macrofungi (Lodge *et al.*, 2004). Canopy cover plays important role in the growth of mushrooms. The present study showed that the maximum number of species were found under a high tree canopy. That means macrofungal species increase with an increase in the tree canopy. This result coincides with Bhandari and Jha, (2017) and Baral *et al.* (2015). The opening of the forest canopy caused the extinction of fungal species. Because the leaf decomposing fungus has superficial mycelia and hyphal stands that are very sensitive to drying (Lodge & Cantrell, 1995).

Soil pH is a very important factor for the mycelial growth of fungi. Mushrooms can grow in a wide range of pH of the medium and produced maximum mycelial growth in slightly acidic to neutral pH (Kalaw *et al.*, 2016). The result of the present study showed that the soil pH has a positive relation with species richness. The pH value was found to be 5-6.5 in most plots. So, the soil-inhabiting species (mycorrhizal) was high than the saprotrophic species. The ectomycorrhizal species grow high at pH value 5-6 but saprotrophic species at pH value 7-8 (Yamanaka, 2003).

Litter is an important factor in the growth of macrofungi and is the main source of organic matter. Litter depth can influence the fungal communities through its impact on moisture content and water holding-capacity (Dowson *et al.*, 1988). Litter removal slowed the decomposition of leaf litter by fungi. Increasing leaf litter is likely to favor the rapid spread of mycelium. Experimentally doubled amount of litter increases sporocarp production (Tyler, 1991). The present study showed that the species increase with the increase in litter cover, these findings are similar to Baral *et al.* (2015). When there is a layer of decomposed leaves, the macrofungi favors by the organic matter. Hedger (1985) found that some species of *Lepiota* grow well on leaf litter that has been decomposed previously by some macrofungi (*Marasmius* sp.).

Soil moisture plays an important role in the growth and composition of macrofungi. Fruiting bodies require a high level of moisture for the formation of the mushroom cap. The present study showed that species diversity increases with an increase in soil moisture. This result coincides with Bhandari and Jha, (2017).

## CONCLUSIONS

The present study came to the conclusion that the macrofungal diversity in the Brahakshetra community

forest, Ghorahi, was high. There were 66 species in the study area, which are divided into 30 genera, 21 families, and 8 orders. The largest order, Agaricales, was discovered, followed by Russulales and Boletales. There was a significant correlation between species richness and environmental factors (canopy cover, soil pH, soil moisture, litter cover), indicating that a variety of environmental factors had an impact on macrofungal diversity. This study has helped mycoflora exploration in some still-unexplored areas. As a result of the mushroom's broad range of functions (for forest ecosystem), proper estimation and documentation are crucial. The fruiting bodies of more macrofungi will be studied molecularly in future research.

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**AUTHORS CONTRIBUTION STATEMENT**

ST: Field work, lab work, data analysis and manuscript writing; SS: field work, lab work, review and editing; SKJ: research design and conceptualization, contribution for supervision, critical revision of the results and manuscript finalization.

**CONFLICT OF INTEREST**

The authors do not have any conflict of interest pertinent to this work.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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