SOIL FAUNA OF RANIBARI COMMUNITY FOREST, KATHMANDU, NEPAL

Pratistha Shrestha
T.U. Registration No: 5-2-22-159-2013
T.U. Examination Roll No: 580/074
Batch: 2018

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Entomology

Submitted to

Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kirtipur, Kathmandu
Nepal
July, 2021

DECLARATION

I hereby declare that the work presented in this report has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Date: 9th March 2021

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Pratistha Shrestha

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.
०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology T.U., Kirtipur

RECOMMENDATIONS

This is to recommend that the thesis entitled "**Soil Fauna of Ranibari Community Forest, Kathmandu, Nepal**" has been carried out by Miss Pratistha Shrestha for the partial fulfillment of Master's Degree Science in Zoology with special paper Entomology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: 9th March 2021

Dr. Prem Bahadur Budha
(Supervisor)
Associate Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

Date: 9TH MARCH 2021

Mohan Bikram Shrestha
(Co-supervisor)
Research Officer
Bird Conservation Nepal
Uttardhoka, Kathmandu, Nepal

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
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T.U., Kirtipur

LETTER OF APPROVAL

On the recommendation of supervisor "Dr. Prem Bahadur Budha" this thesis submitted by Miss Pratistha Shrestha entitled "**Soil Fauna of Ranibari Community Forest, Kathmandu, Nepal**" is approved for the examination in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Entomology.

Date: 9th March 2021

....

Dr. Tej Bahadur Thapa
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

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Central Department of Zoology
T.U., Kirtipur

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Miss Pratistha Shrestha entitled "**Soil Fauna of Ranibari Community Forest, Kathmandu, Nepal**" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Entomology.

EVALUATION COMMITEE

………………………	………………………
Supervisor	Head of Department
Dr. Prem Bahadur Budha	Dr. Tej Bahadur Thapa
Associate Professor	Professor
Central Department of Zoology	Central Department of Zoology
TU, Kirtipur, Nepal	TU, Kirtipur, Nepal
………………………	………………………
External examiner	Internal examiner

Date of examination: 2nd July 2021

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Pratistha Shrestha

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
CDZMTU	Central Department Zoology Museum of Tribhuvan University
PCA	Principal Component Analysis
QBS	Soil Biological Quality
RCF	Ranibari Community Forest
TU	Tribhuvan University

ABSTRACT

Ranibari Community Forest (RCF) is the important forest patch present within the highly urbanized Kathmandu valley with information gaps on soil invertebrate fauna. This study aimed to explore soil meso and macro fauna of the forest. Fourteen random quadrats (1m × 1m) were laid, within the seven blocks. Leaf litter samples and soil cores were collected, screened, sieved on a white sheet to extract fauna. Sampling was done once a fortnight from May to November, 2019. The result showed that the diversity, abundance and species richness of soil fauna were seen highest in summer season (H' = 2.897, Abundance = 1973, S = 84) dominated by Collembola. In addition to that, the QBS-ar value was seen highest in summer as well (QBS-ar = 417) which successively decreased in succeeding seasons. In case of the habitat type, soil fauna was seen to be more diverse and evenly distributed in soil layers throughout the seasons but the abundance was greater in leaf litters, particularly in summer. Fauna like Chilopoda, Diplopoda, Haplotaxida and Isopoda were seen to be affected negatively by soil temperature in case of summer and autumn seasons. Soil moisture content was found to be positively correlated with immature insects, earthworms and millipedes in rainy and autumn seasons. Besides, the pH of soil was seen to affect Diplura only in autumn season. The relation of fauna with the physico-chemical parameters (temperature, moisture and pH) and also with other taxa shows their ecological roles and adaptation to specific microclimate.

1. INTRODUCTION

1.1. Background

Soil fauna is the biological community inhabiting permanently or at least in one of their developmental stages on soil or leaf litter (Pereira et al. 2017, Zagatto et al. 2017). It comprises arthropods (insects, arachnids, crustaceans and myriapods), mollusks (snails, slugs) and annelids (earthworms, leeches) along with other small animal *viz*. protozoans, nematodes, turbellarians and rotifers. Approximately, one fourth of global biodiversity is estimated to be soil fauna (Decaëns et al. 2006) including the micro-invertebrates (Protozoa, Nematoda, Turbelllaria, Rotifera, Tardigrada, Crustacea, part of Oligocheata), the meso-invertebrates (smaller than 2 mm e.g. part of Oligocheata, Collembola, Protura, Diplura, Pauropoda, Symphylla, Acari, Pseudoscorpionida and Palpigradi) and the macro-invertebrates (invertebrates larger than 2 mm) (Lavelle 1996, 1997, Lavelle et al. 2006). Representative arthropod taxa found in soil are Cheliceromorpha (scorpions, pseudoscorpions, spiders, harvestmen and mites, and other rarer groups), Crustacea (amphipods and woodlice), Myriapoda (centipedes, millipedes, and rarer groups), and Hexapoda (insects and close allies).

Soil accommodates rich number of organisms due to the resources it provides and the environment it creates of micro-divided aerial and aquatic phases (Lavelle 1996). Besides, the mutual interactions between micro and macro-organisms of soil create differential ecological niche space thus, make them abundant (Lavelle 1996, 1997, Van Straalen 2004) as well as sensitive to the local environmental conditions (Gerlach et al. 2013). Whatsoever, their community composition is dependent upon the specific vegetation type and the specificity in the environmental conditions (Koehler and Born 1989, Wu and Wang 2019, Kooch and Noghre 2020).

Soil invertebrates have been recognized as an important component of biodiversity (Kremen et al. 1993, Pimentel et al. 1997) for their diverse species coverage and various functional roles in the nature as soil engineers, organic decomposers (Szlavecz et al. 2018, Liu et al. 2019, Lubbers et al. 2020), predators and parasites (Daily et al. 1997).

Also, soil fauna are believed to enhance functioning of soil by increasing nutrient turnover (Reichle 1977), water holding capacity and detoxification (Lavelle et al. 2006).

Since past few decades, soil invertebrates, especially meso and macro-fauna have been used as biological parameters for portraying structure of forests, grasslands, croplands and local communities (Szlavecz et al. 2018) as their rarity and diversity provide data for conservation approaches (Oliver and Beattie 1996, Pik et al. 1999). The Soil Biological Quality Index including soil arthropods (QBS-ar) has been recently in use to determine the heath of an ecosystem which focuses on the assumption that higher the quality of soil, higher the number of arthropods adapted to that habitat (Parisi et al. 2003, 2005, Tabaglio et al. 2009, Madej et al. 2011, Menta 2012).

Furthermore, monitoring and recording standard data about distribution pattern, diversity and abundance helps in prioritizing the worth of an ecosystem (Ward and Larivière 2004, Gerlach et al. 2013). But still, the knowledge about assemblage pattern of invertebrates is lagging behind and is rarely considered in the conservation policies (Ward and Larivière 2004, Szlavecz et al. 2018). Here comes the necessity to set the pace for the conservation approach (considering soil invertebrates) from a confined area towards broader range.

The Ranibari Community Forest (RCF) is one of the important remaining forest patches surrounded by dense urban settlement within Kathmandu Valley. Birds of Ranibari Community Forest are well studied by Bird Conservation Nepal (BCN 2019) but the soil fauna are poorly known in the area. So this study aimed to explore the faunal diversity of soil in RCF and their relation with the physical parameters.

1.2. Objectives

1.2.1. Main objective

The main objective of this study is to document the diversity of soil meso and macro fauna in Ranibari Community Forest (RCF), Kathmandu, Nepal.

1.2.2. Specific objectives

The specific objectives are as follows:

i. To explore diversity of soil meso and macro fauna in three seasons (summer, rainy and autumn).
ii. To assess the relation of soil fauna with soil parameters (temperature, pH and moisture) in Ranibari.
iii. To calculate Soil Biological Quality Index including arthropods (QBS-ar Index) of Ranibari Community Forest.

1.3. Rationale of the study

As RCF is an important forest for recreational values within the highly populated Kathmandu valley, assessing the biodiversity within the forest areas may help to know the present condition and the worth of it. Moreover, invertebrate fauna has its importance in indicating environmental condition of a particular area by appropriately responding to the changes. Their diversity, distribution pattern and assemblage provide information about the ecosystem which in turn could help in initiating conservation approach of the forest in upcoming days.

1.4. Limitations

The soil fauna were identified up to genus, and in case of immature forms only up to family level as accessible literatures were limited and type materials were not available.

2. LITERATURE REVIEW

2.1. Diversity of Soil Fauna

Occurrence and assemblage of the soil fauna vary in forest areas, shrub lands and grasslands as woody plants provide greater coverage, high input of surface litter, finest nutrient cycling and dense root system offering suitable and stable microhabitat (Scherber et al. 2010, Bayranvand et al. 2017, Pereira et al. 2017, Kerdraon et al. 2019, Kooch and Noghre 2020, Song et al. 2020), shrubs offer qualitatively and quantitatively different litters than trees whereas in grassland, soil nutrient is completely dependent on rooting system (Čuchta 2020). But also, forest areas may have modified biodiversity seasonally due to habitat diversification and change in microclimate depending upon tree phenology (Martin-Chave et al. 2018).

Higher litter deposition and great litter diversity assure higher diversity of the fauna but not necessarily abundance (Paul et al. 2011, Zagatto et al. 2019a) for soil fauna has specialized preference towards litters as a food source depending upon its quality and chemistry (Warren and Zou 2002). For instance, fresh deciduous leaf litter is favorable for earthworms (Paoletti 1999) whereas coniferous forest with flourishing fungi is favorable for mites, springtails and Enchytraeid worms (Čuchta 2020).

As experimented in mixed plantation with the variety of leaf litters, it is concluded that the most palatable litter is decomposed at higher rate by diverse groups whereas litter with low nutritional values are preferred only when other resources are scarce (Guille et al. 2019, Tresch et al. 2019).

Wu and Wang (2019) has stated that litter mass affect meso fauna solely but in case of macro fauna, multiple factors like plant coverage and soil organic carbon has to be considered along with the litter mass. However, high litter mass is estimated to favor colonization of the fauna (Yang et al. 2020) which if removed cause restriction in food resource and the fauna form different community patterns ultimately affecting the abundance (Tresch et al. 2019, Kooch and Noghre 2020).

2.2. Relationship of Soil Fauna and Environmental Variables

One of the major factors which determines the community assemblage of soil invertebrates is environmental conditions (Zagatto et al. 2017, Yang et al. 2020, Uhey et al. 2020). Mainly, temperature (of both soil and air) and moisture are found to affect their distribution and density under different land covers (Zagatto et al. 2019b, Kooch and Noghre 2020). Studies on the correlation of soil fauna with physical parameters have shown that lower temperature or high moisture decreases their abundance in soil but not necessarily in litter, in fact, high moisture facilitates these fauna so, humid areas with high temperature promote diversity and density as compared to dryer areas (Laiho et al. 2001, Gonzalez and Seastedt 2001, Zagatto et al. 2019a, 2020). Not only that, this correlation also explains pronounced seasonal variation where higher abundance is seen in autumn and spring and least in winter (Zagatto et al. 2017, Yin et al. 2018, 2019).

Additionally, soil characteristics like lower porosity, bulk density and higher salinity affect abundance of meso fauna inversely (Machado et al. 2019, Zagatto et al. 2019b, Kooch and Noghre 2020) as majority of fauna live in topsoil (Lee and Foster 1991, Yin et al. 2018, 2019). In contrast to this, response of macrofauna was not found significant, except for Chilopoda, for they have relatively higher tolerance and diversity in adaptation (Yin et al. 2019,Yang et al. 2020).

Several studies revealed that there exists significant difference in the community composition in natural systems (including conserved area) and systems involving anthropogenic activities (Koehler 1992, Baretta et al. 2007, Pereira et al. 2017, Santos et al. 2018, Zagatto et al. 2019a, 2020). Specifically, meso fauna such as mites and springtails are found to be greatly affected in different land use systems (Yin et al. 2019, Van Langevelde et al. 2020) but some macro faunal taxa like Chilopoda, Diplopoda, Coleoptera, Hemiptera, Formicidae, Isopoda and Aranea have been reported to be affected as well (Pereira et al. 2017, Yin et al. 2019).

2.3. Soil Fauna as Bioindicators

In case of Collembola, their communities require remarkable time to recover from disturbances (Rusin and Gospodarek 2016, Van Langevelde et al. 2020) and temporarily increased species richness with altered pattern eventually fade away (Čuchta et al. 2019, Snyder and Callaham 2019). In the other hand, land use systems can be differentiated with the morphological characters and abundance of soil fauna, especially of meso fauna (De Souza et al. 2016, Santos et al. 2018, Machado et al. 2019, Zagatto et al. 2019a) as macrofauna has greater dispersal rate in recovery period and are comparatively less sensitive (Van Langevelde et al. 2020).

Environmental toxicology assessment merely with classical approach has some limitations which is why biomarkers are concerned in recent days (Scott-Fordsmand and Weeks 2000) that also with a group of minimum of three invertebrates having different ecological roles (Greenslade 2007).

Taking example of Collembola, laboratory experiment has shown that its species *Folsomia candida* Willem, 1902 has ability to accumulate heavy metals, mainly cadmium (Cd), in tissues up to 10 folds and its survivorship and reproductive responses get affected in the soil containing high concentrations of heavy metals (Fountain and Hopkin 2004a, Fountain and Hopkin 2004b, Buch et al. 2016).

Similarly, it has been proved that Isopoda prefer alkaline soil and its species *Porcellio scaber* Latreille, 1804 has ability to accumulate heavy metals like cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb) and mercury (Hg) with indication of different level of contamination (Dallinger et al. 1992, Hopkin et al. 1993, Heikens et al. 2001, Komarnicki 2005, Pedrini-Martha et al. 2012,Yorkina et al. 2019).

2.3.1 Soil Biological Quality Index (QBS Index)

The use of soil fauna in re-establishment and conservation of forest had been practiced successfully and it was believed that these fauna reduce root mass of dominant species and provide advantages to subdominant species thus, increase local diversity of plant species (De Deyn et al. 2000). But, quantitative and behavioral information including whole community was still in need to create appropriate bioindicators with predictive powers (Stork and Eggleton 1992, Van Straalen 1998, Cortet et al. 1999, Andersen et al. 2004). Gonzalez and Seastedt (2001) had also mentioned the importance of composition and abundance of soil organisms along with analytical tools to predict ecosystem processes.

In recent years it has become practically possible to assess soil condition with the help of Soil Biological Quality Index (QBS-index) which is based on the fact that high quality of the soil houses higher number of micro-arthropod taxa (Parisi et al. 2003, 2005). The EMI used in the QBS Index is the assigned score ranging from 1-20 for each of the micro-arthropod taxa including Myriapoda, Crustacea, Arachnida and Insecta based on their adaptation to the soil (epiedaphic and euedaphic) and life form approach (Parisi et al. 2003, Madej et al. 2011, Ghiglieno et al. 2019). The QBS-ar value of more than 200 is the indicator of stable forest ecosystem considering that the value changes with season and succession (Blasi et al. 2013, Galli et al. 2014, Venanzi et al. 2016). However, the threshold value of QBS is precisely measured to be 93.7 which mean any value more than that would affirm good quality of the soil (Menta et al. 2018). Studies on various land use systems has affirmed that the QBS-ar is a better and reliable tool for biomonitoring both the natural and anthropic soil (especially in the recovering areas) than tools which assess merely diversity and density of those arthropods (Parisi et al. 2003, Madej et al. 2011, Galli et al. 2014, Menta et al. 2018).

3. MATERIALS AND METHODS

3.1. Study Area

Ranibari community forest is one of the few natural forests located in the north western part of the Kathmandu metropolitan city (27.7286° N - 27.73325° N; 85.3200° E - 85.3219° E) with the areas of 69,500 m^2 (Fig. 1). Kathmandu has monsoon type of climate with rainy summer and dry winter with the average annual temperature of 16.1 ˚C and annual rainfall of 2,812 mm (Climate-Data 2021). RCF is situated at the altitude of 1,311 m asl. It is floristically rich and has mixed type of vegetation with large component of bamboo and tree species like *Castanopsis indica*, *Ficus lacor*, *Pinus roxburghii*, *Melia azedarach*, *Quercus glauca*, *Ziziphus incurva*, *Engelhardia spicata* and *Schima walichii*. It is surrounded by the urban settings without any open areas nearby.

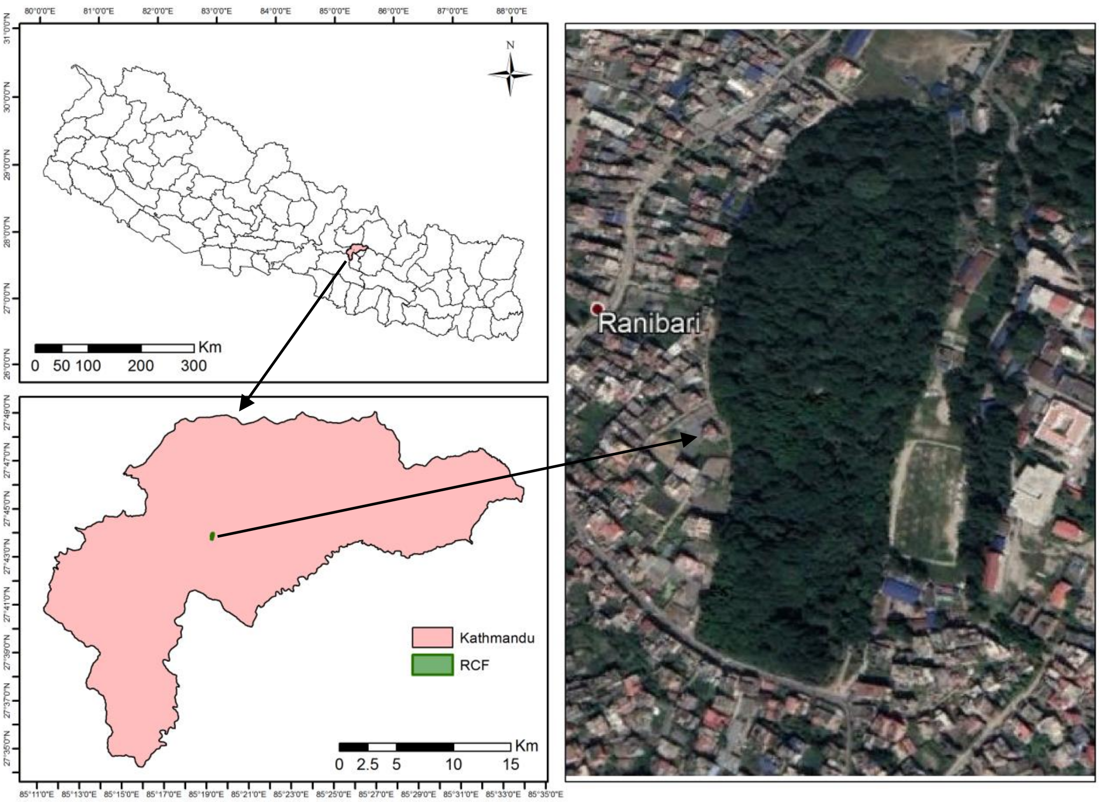

Figure 1: Map of the study site

3.2. Methods

The present study is primarily focused on soil meso and macro fauna.

3.2.1 Sampling methods

The entire community forest was divided into seven blocks each of one hector. From each sample block two leaf litter samples and two soil samples were collected randomly by quadrat method (1x1 m^2). Samples were collected in three seasons from May to November 2019 (Mid-May to Mid-July Summer season; Mid-July to Mid-September Rainy season; Mid-September to Mid-November Autumn season) once a forthnight from 9AM to 5PM.

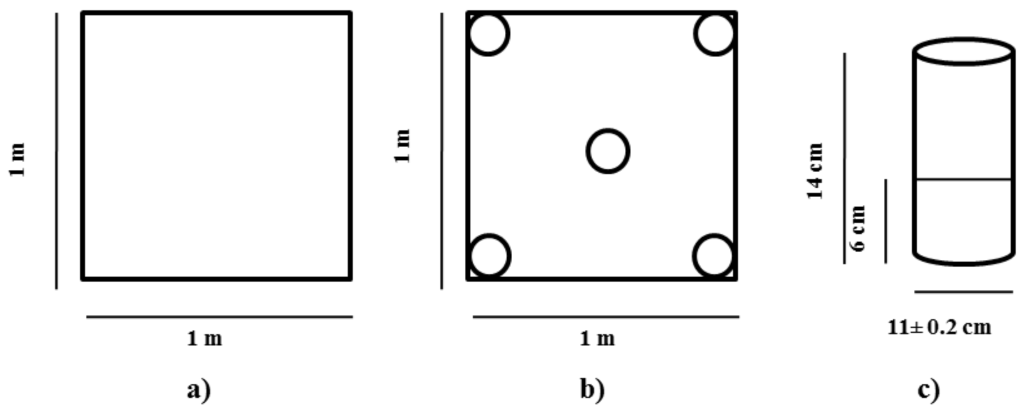

Figure 2: Sampling methods of soil fauna in RCF. a) Quadrat for one leaf litter sample; b) Quadrat with five replicates for one soil core sample; c) PVC pipe ring

For collecting the soil fauna two methods were applied. In the first method, leaf litters from a quadrat was collected in a plastic bag along with surface soil and sieved on white sheet to extract fauna. In second method, for every soil samples, soil core with five replicates (four corners and a center) of diameter 11±0.2 cm and 6 cm depth were collected with the help of metal PVC pipe ring (Fig. 2). Thus collected soil was then screened under a white sheet similar to the first method. Aspirator was used for collecting meso-fauna whereas, forceps were used for macro-fauna. These fauna are then put in vials containing 70% alcohol separately for each sample. Then, the vials were labeled with sample block no., sample plot no. and date.

The disturbance level around the sampling points was measured and field checklist was made based on the presence of walking trails, broken glasses and garbage disposal sites. In addition to that, vegetation type, weather condition and soil temperature of the sampling point were noted as well. Moreover, 200 gm soil from each soil sample (total of 14 in one visit) were collected in a zip-lock bag and labeled so that further analysis of soil could be done in the laboratory.

3.2.2 Soil analysis

a) Moisture

The moisture content of the soil was calculated with gravimetric method (Reynolds 1970). For that, 100 ± 0.5 gm of soil was taken in aluminum tin and kept in an oven at 105 °C dried for 24 hours. Once the soil was completely dried, it was weighed again. Then, the moisture content was calculated by using following formula:

$$\text{Moisture content } (\theta) = \frac{\text{weight of wet soil} - \text{weight of dry soil}}{\text{weight of dry soil}}$$

b) pH

For determination pH of soil, 20 gm of soil was taken in a 100 ml beaker and 50 ml of distilled water was added to it. It was then shaken well with the help of glass rod and let stand for half an hour. Then, the pH meter (already calibrated in buffer solutions of pH 7 and 4) was immersed in the upper portion of the suspension and waited for the stable reading (Brietbart 1988). The reading was then noted in a data sheet.

3.2.3 Identification and preservation

Collected fauna were brought to the laboratory, sorted, identified and photographed by Sony Cyber-shot DSC-W710 under the stereo-microscope. The fauna were identified with the help of relevant keys (Gupta 1985, Tikader 1987, Julka 1988, Mitra et al. 2004, Johnson and Triplehorn 2005, other journal articles and monographs).

Fauna were kept in a separate vial containing 70 % ethyl alcohol and labeled properly with the mention of taxonomic information of species, date of collection, place of collection and name of collector. These specimens were deposited in the CDZMTU, Kirtipur, Nepal.

3.2.4 Data analysis

All the data were managed in MS excel 2010. Data were analyzed by using Shannon-Weiner Diversity Index (H'), Evenness (J), Richness (S), Jaccard's Similarity Index (J') and Soil Biological Quality Index (QBS-ar) in MS Excel 2010. In addition to that, the correlation of the taxa with physical parameters was analyzed with the help of Principal Component Analysis (PCA) in R-studio (version 4.0.3) with prcomp and ggbiplot packages.

4. RESULTS

4.1. Diversity of Soil Fauna in RCF

Approximately, 90 % of the fauna of RCF was recorded to be occupied by arthropods in which 60 % fauna were insects. The earthworms occupied only 11.84 % of the overall fauna whereas snails were recorded the least (0.32 %) (Fig. 3).

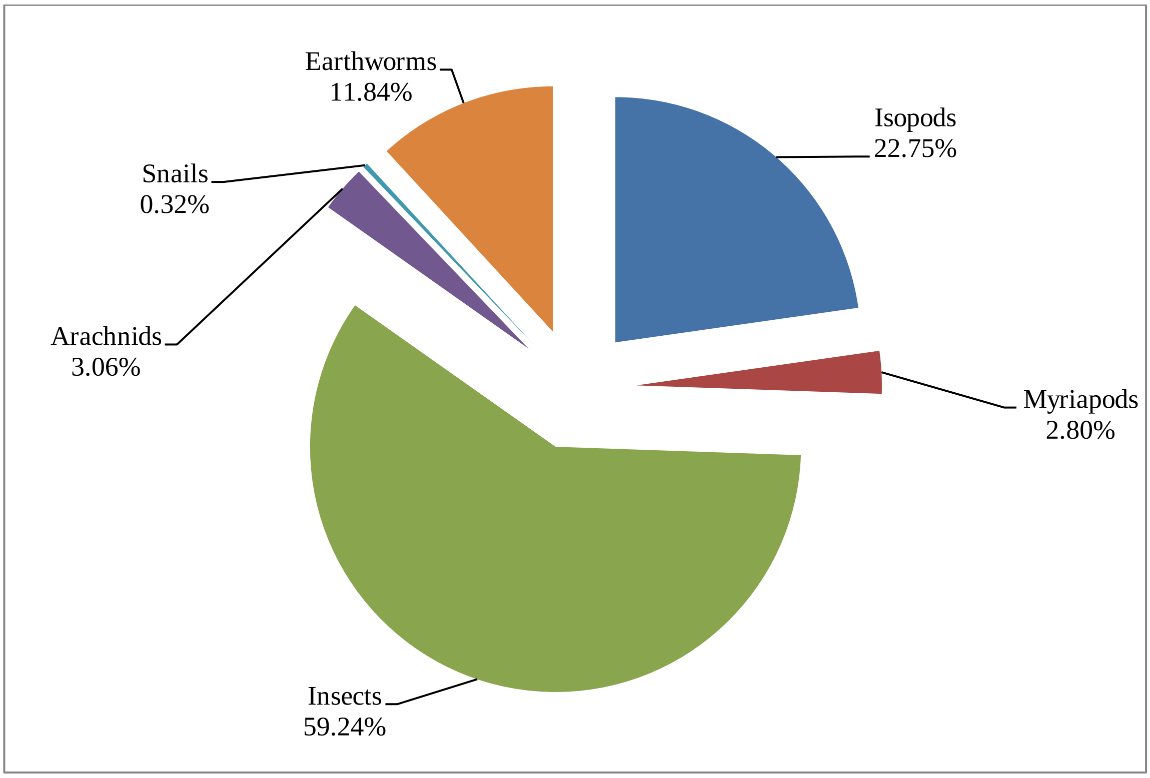

Figure 3: Soil fauna of RCF (including all taxa)

Within the insect groups, the order Collembola was seen the highest (63.54 %), followed by Hymenoptera, specifically ants (18.84 %). Some orders such as Orthoptera, Lepidoptera, Dermaptera and Diptera were seen least in overall study period (collectively occupied only 0.80 % of the total fauna) (Fig. 4).

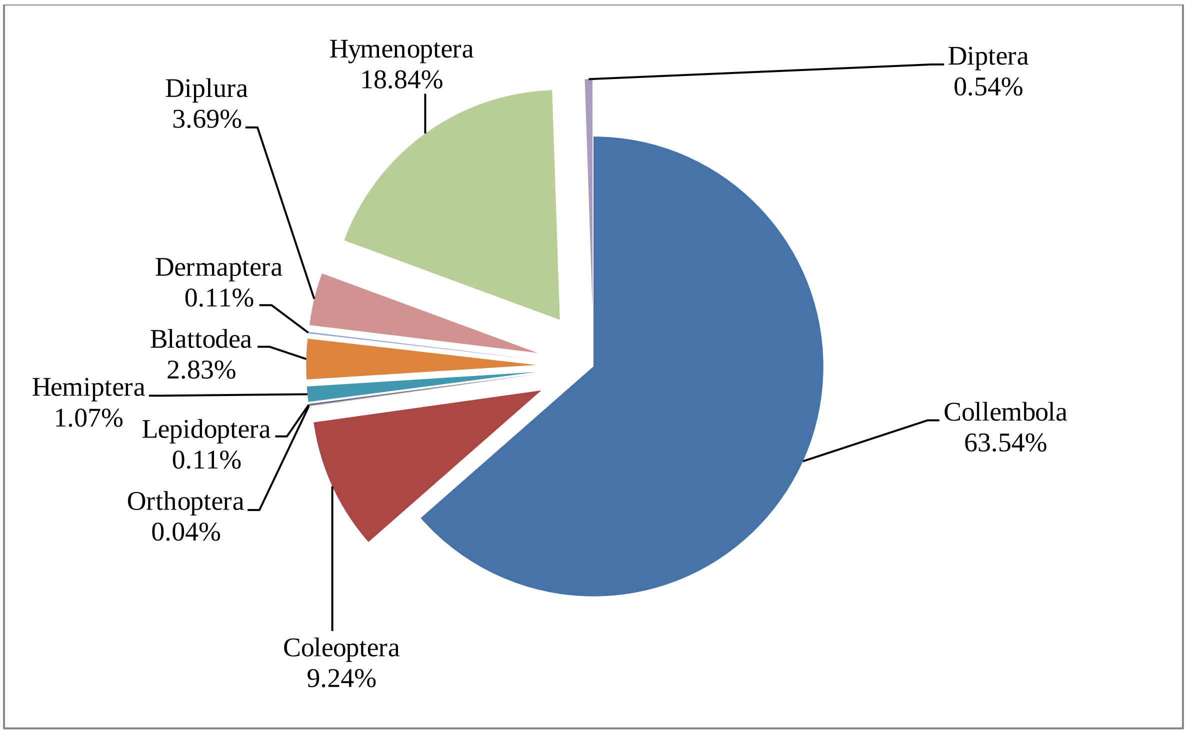

Figure 4: Soil insect fauna of RCF

Among the 4,693 specimens belonging to 90 species collected throughout the sampling period, 86 species were of phylum Arthropoda (6 species of Myriapoda, 2 species of Crustacea, 68 species of Insecta and 10 species of Arachnida) and two species were of Phyla Annelida and Gastropoda each. In case of the class Insecta, the fauna were of two forms; mature and immature (larvae or nymph). Amongst 68 species of insects 15 immature species were recorded (Table 1).

Table 1: Soil fauna of RCF in three different seasons

Taxa	Summer	Rainy	Autumn
Haplotaxida			
Pheretima sp.1 (Plate-D, Fig. 32)	335	136	57
Pheretima sp.2	23	0	0
Chilopoda			
Ethmostigmus sp. (Plate-D, Fig. 29)	3	3	4
Lithobius sp.1	43	18	0
Lithobius sp.2 (Plate-D, Fig. 28)	4	3	0

Taxa	Summer	Rainy	Autumn
Mecistocephalus sp.	4	2	0
Diplopoda			
Anauliciulus sp. (Plate-D, Fig. 31)	19	9	8
Orthomorpha sp. (Plate-D, Fig. 30)	12	0	0
Crustacea			
Burmoniscus sp. (Plate-D, Fig. 27)	168	378	525
Platorchestia sp.	1	0	0
Collembola			
Dicranocentrus sp.1 (Plate-A, Fig. 2)	146	46	5
Dicranocentrus sp.2	79	123	145
Willowsia sp.1	483	191	254
Willowsia sp.2 (Plate-A, Fig. 3)	122	46	59
Willowsia sp.3	58	16	1
Coleoptera			
Aleochara sp.1 (Plate-B, Fig. 16)	1	0	0
Aleochara sp.2	16	1	0
Aleochara sp.3	1	0	0
Aleochara sp.4	1	0	0
Aleochara sp.5	3	0	0
Aleochara sp.6	2	0	0
Anthicus sp.	6	0	0
Astenus sp.1	3	0	0
Astenus sp.2	1	0	0
Atholus sp. (Plate-B, Fig. 13)	0	1	0
Axonya sp. (Plate-B, Fig. 11)	9	4	0
Carabidae larva 1 (Plate-C, Fig. 17)	7	6	0
Carabidae larva 2	1	0	0
Clivina sp.	1	1	7
Elateridae larva (Plate-C, Fig. 18)	9	2	1

Taxa	Summer	Rainy	Autumn
Eobruscus sp.1 (Plate-B, Fig. 9)	6	3	1
Eobruscus sp.2 (Plate-B, Fig. 10)	4	2	0
Eobruscus sp.3	2	0	0
Gonocephalum sp. (Plate-B, Fig. 12)	13	2	0
Lampyridae larva (Plate-C, Fig. 20)	17	26	0
Mesomorphus sp.	2	3	0
Othius sp.1	23	3	0
Othius sp.2	3	0	1
Othius sp.3	2	0	0
Philonthus sp. (Plate-B, Fig. 15)	1	0	0
Rugilus sp. (Plate-B, Fig. 14)	3	0	0
Scarabaeidae larva (Plate-C, Fig. 19)	20	8	1
Tenebrionidae larva	1	0	0
Orthoptera			
Acrididae nymph	1	0	0
Lepidoptera			
Noctuidae larva 1	2	0	0
Noctuidae larva 2	1	0	0
Hemiptera			
Aethus sp.1 (Plate-A, Fig. 8)	1	0	0
Aethus sp.2	1	0	0
Cydnidae nymph (Plate-C, Fig. 23)	1	0	0
Lygaeidae nymph (Plate-C, Fig. 24)	1	4	0
Blattodea			
Blattidae nymph (Plate-C, Fig. 22)	1	31	43
Dermaptera			
Labia sp.	0	1	2
Diplura			
Lepidocampa sp. (Plate-A, Fig. 1)	18	26	59

Taxa	Summer	Rainy	Autumn
Hymenoptra			
Aenictus sp. (Plate-A, Fig. 5)	7	33	0
Aphaenogaster sp.	11	4	0
Brachyponera sp.1	80	118	12
Brachyponera sp.2 (Plate-A, Fig. 6,7)	2	3	0
Cerapachys sp.	2	0	0
Crematogaster sp.	0	17	84
Dorylus sp.1 (Plate-A, Fig. 4)	9	5	0
Dorylus sp.2	8	6	0
Formica sp.1	29	1	0
Formica sp.2	1	0	0
Lasius sp.1	0	3	0
Lasius sp.2	2	0	0
Lasius sp.3	1	0	0
Meranoplus sp.	2	2	0
Monomorium sp.	1	2	0
Ooceraea sp.	7	0	0
Paratrechina sp.	2	0	0
Phidologeton sp.	5	0	0
Tetramorium sp.1	0	58	3
Tetramorium sp.2	3	0	0
Tetramorium sp.3	5	21	0
Tetraponera sp.	1	0	0
Diptera			
Bibionidae larva	3	0	2
Fanniidae larva (Plate-C, Fig. 21)	4	0	0
Tipulidae larva	3	3	1
Aranea			
Unidentified sp.1	16	0	0

Taxa	Summer	Rainy	Autumn
Unidentified sp.2	19	3	2
Unidentified sp.3	7	8	18
Unidentified sp.4	10	3	0
Unidentified sp.5	5	1	0
Unidentified sp.6	4	9	2
Acari			
Parasitinae sp.1 (Plate-D, Fig. 25)	10	1	2
Parasitinae sp.2	2	0	0
Pergamasinae sp.	18	8	0
Trombiculidae sp. (Plate-D, Fig. 26)	8	0	0
Pulmonata			
Nanina sp.	1	3	11
Pyramidula sp.	0	3	0
Total	**1973**	**1410**	**1310**

Among the fauna collected throughout the sampling period, 42.04% of the specimens were reported solely from the summer season. The soil fauna were found to be diverse, abundant and rich in the summer season (H' =2.897, abundance= 1973, S = 83) followed by rainy season (Table 2). However, the fauna were evenly distributed in the rainy season (J = 0.68) as compared to summer and autumn.

Table 2: Diversity of soil fauna in three different seasons

Indices	Summer	Rainy	Autumn
Abundance	1973	1410	1310
Richness (S)	84	52	27
H_{max}	4.431	3.951	3.296
Shannon Diversity Index (H')	2.897	2.671	1.968
Evenness (J)	0.65	0.68	0.60

The soil fauna were recorded abundant from leaf litters in all the seasons than from the soil layers. However, the fauna were seen diverse and evenly distributed in the soil layers as compared to the leaf litters. The fauna were rich in leaf litters in summer and rainy seasons but in autumn the fauna were seen rich in soil layers (Table 3).

Table 3: Diversity of soil fauna in soil layers and leaf litters

Indices	Soil layers			Leaf litters		
	Summer	Rainy	Autumn	Summer	Rainy	Autumn
Abundance	464	330	290	1509	1080	1016
Richness (S)	59	30	23	67	48	18
H_{max}	4.078	3.401	3.135	4.205	3.871	2.890
Shannon Diversity Index (H')	3.019	2.400	2.319	2.630	2.523	1.723
Evenness (J)	0.74	0.71	0.74	0.63	0.65	0.60

The similarity between the occurrences of fauna was moderately higher in case of the summer and rainy seasons as compared to other seasons ($J'_{(S,R)}$ = 0.55) (Table 4).

Table 4: Jaccard's Similarity Index in three different seasons

	Summer	Rainy	Autumn
Summer	1		
Rainy	0.55	1	
Autumn	0.26	0.46	1

4.2. Correlation of Soil Fauna with Physico-chemical Parameters

In the Principle Component analysis (PCA), of all the variables of RCF explained only 29.40%, 30.30% and 34.40% of data variability in summer, rainy and autumn seasons respectively. It showed the correlation of each taxon with the physico-chemical parameters (temperature, moisture and pH) as well as other taxa. The PCA of all three seasons are presented below;

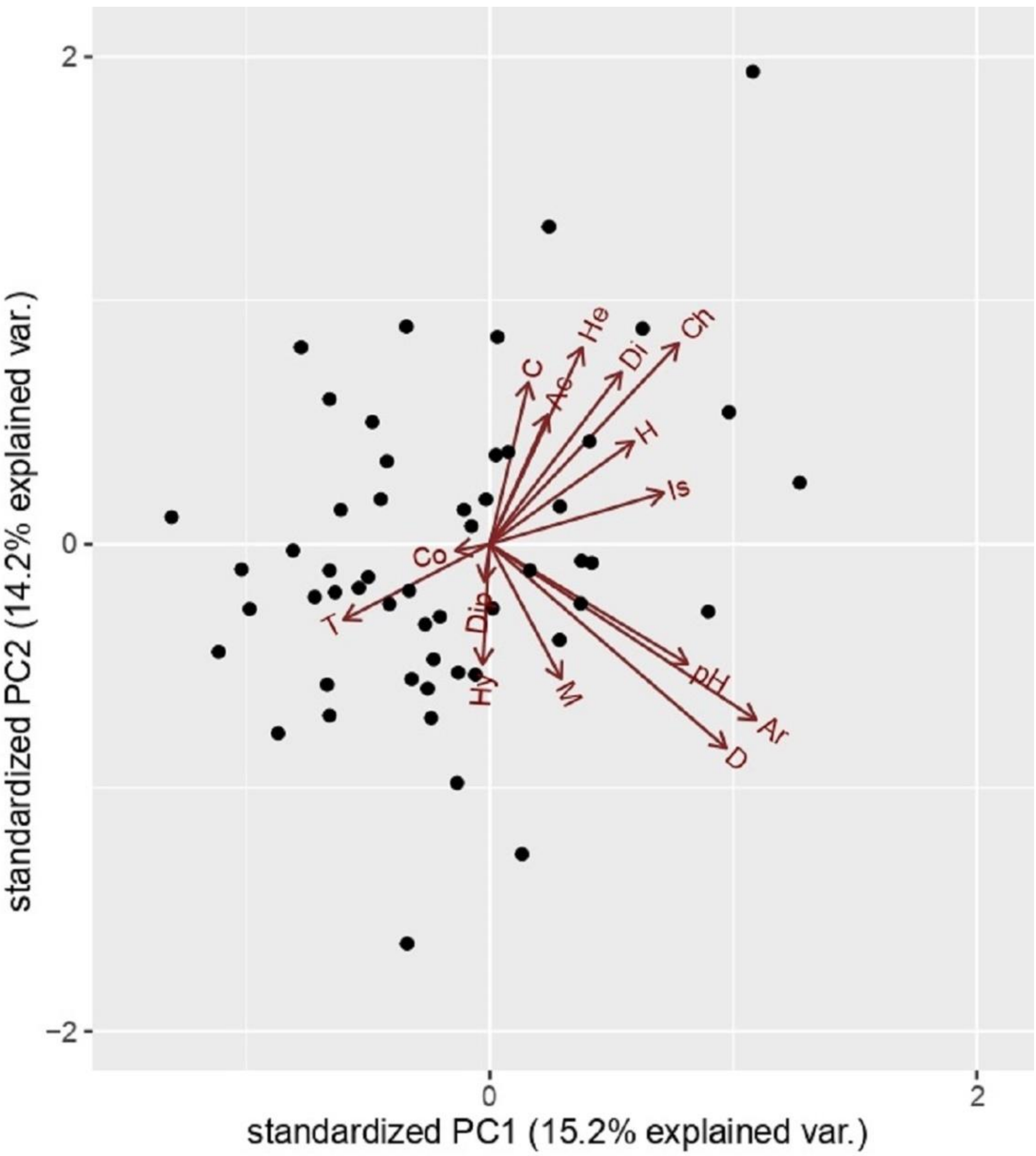

Figure 5: PCA of soil fauna in summer season

[M= Moisture; T= Temperature; Ha= Haplotaxida; Ch= Chilopoda; Di= Diplopoda; Is= Isopoda; Co= Collembola; C= Coleoptera; He= Hemiptera; D= Diplura; Hy= Hymenoptera; Dip= Diptera; Ar= Aranea; Ac= Acari]

In summer, certain taxa *viz.* Diplopoda, Chilopoda, Isopoda and Haplotaxida, were seen to have moderate negative correlation with the soil temperature. And, the soil moisture content was seen to affect Coleptera negatively. In addition to that, the taxa Aranea and Diplura were positively correlated (Fig. 5).

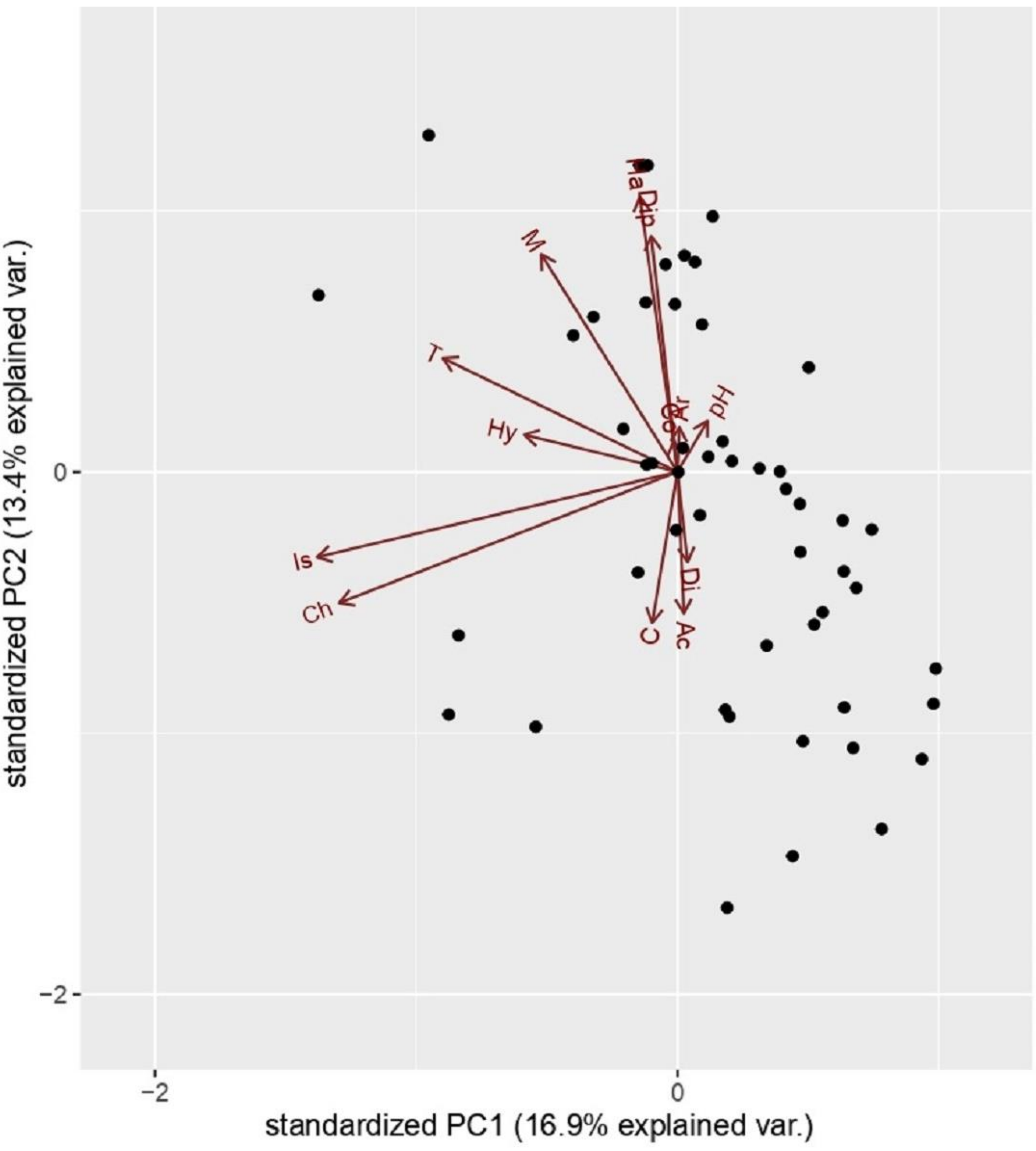

Figure 6: PCA of soil fauna of rainy season

[M= Moisture; T= Temperature; Ha= Haplotaxida; Ch= Chilopoda; Di= Diplopoda; Is= Isopoda; Co= Collembola; C= Coleoptera; Hy= Hymenoptera; Dip= Diptera; Ar= Aranea; Ac= Acari]

In case of the rainy season, the pH of soil, abundance of Aranea and Collembola were poorly represented. And, the temperature was not seen to affect any of the taxa significantly. However, the earthworms and the dipteran larvae were seen to be affected positively by the soil moisture content. And the taxa Isopoda and Chilopoda were positively correlated (Fig. 6).

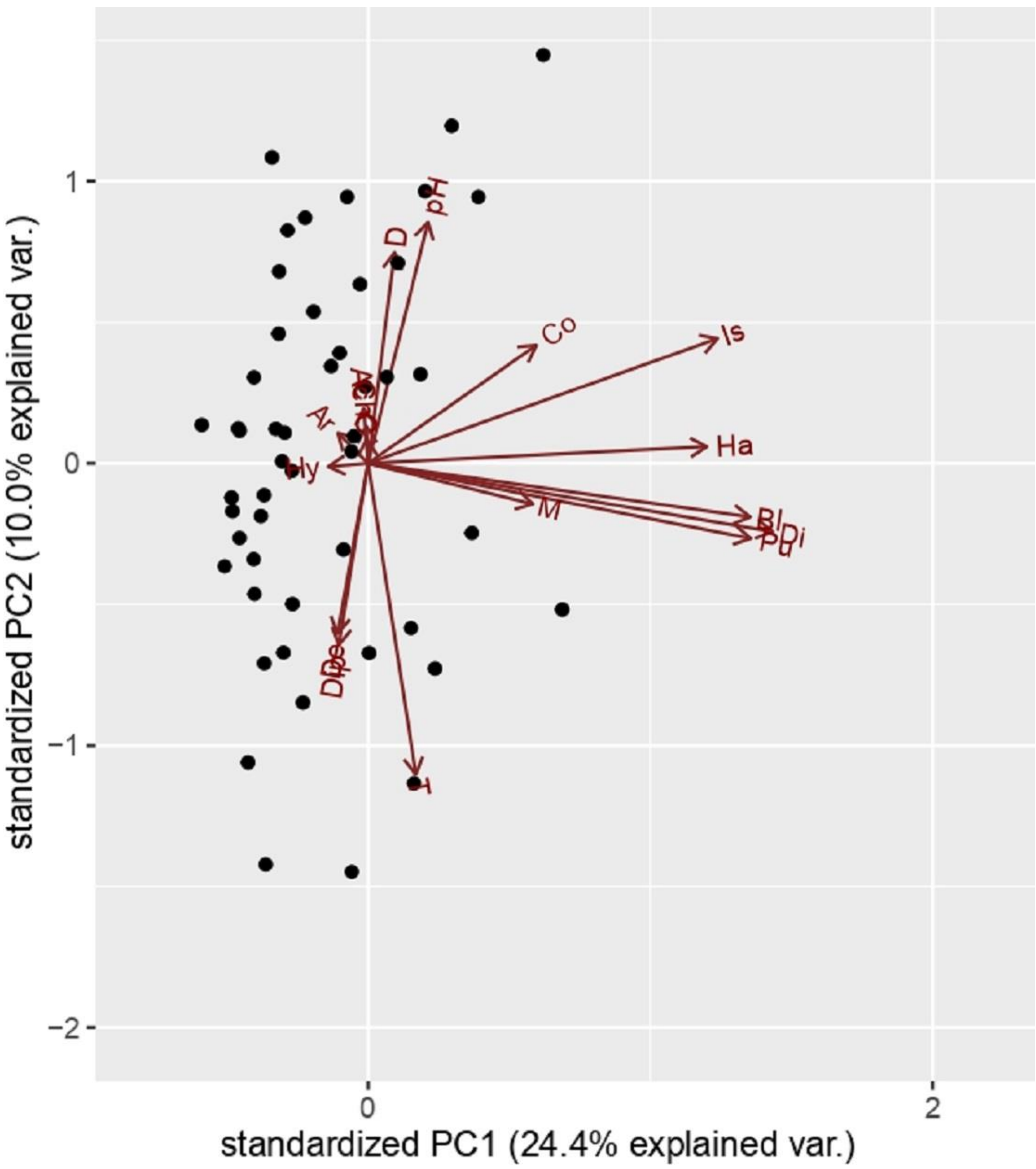

Figure 7: PCA of soil fauna of autumn season

[M= Moisture; T= Temperature; Ha= Haplotaxida; Ch= Chilopoda; Di= Diplopoda; Is= Isopoda; Co= Collembola; C= Coleoptera; Bl= Blattidae; De= Dermaptera; Di= Diplura; Hy= Hymenoptera; Dip= Diptera; Ar= Aranea; Ac= Acari; Pu= Pulmonata]

In case of the autumn, Diplura was affected positively by the pH of the soil whereas, negatively by the soil temperature. Besides, pH was also seen to negatively affect Dermaptera. The taxa Blattodea and Diplopda were positively correlated with each other and with the soil moisture content (Fig. 7).

4.3. QBS-ar Index of RCF

The Eco-Morphological Index (EMI) value assigned for each micro-arthropod species present in the soil of RCF was aggregated according to their corresponding orders and life forms then to overall phylum (Annex 1). The summed EMI value *i.e.* QBS-ar of all the arthropod taxa was recorded highest in the summer season (QBS-ar = 417) as compared to rainy and autumn seasons. The QBS-ar value successively decreased in succeeding seasons (Table 5).

Table 5: Soil Biological Quality Index (QBS-ar)

Order	EMI-summer	EMI-rainy	EMI-autumn
Chilopoda	40	40	10
Diplopoda	20	10	10
Isopoda	10	10	10
Collembola	5	5	5
Coleoptera	21	9	3
Orthoptera	1	-	-
Hemiptera	4	1	-
Blattodea	5	5	5
Dermaptera	-	1	1
Diplura	20	20	20
Hymenoptera	95	65	15
Larvae	110	50	40
Aranea	6	5	3
Acari	80	40	20
QBS-ar	**417**	**261**	**142**

5. DISCUSSION

5.1. Diversity of Soil Fauna in RCF

Majority of the soil fauna were arthropods (especially insects) in this study with temporal variations during sampling periods. Soil fauna were highly abundant, rich and diverse in the summer season primarily driven by Collembola and Haplotaxida whereas fauna were evenly distributed in rainy season. In addition to that, similar fauna were recorded in summer and rainy seasons. Some studies (Zhu et al. 2010, Yin et al. 2018, Zagatto et al. 2019b, Kooch and Noghre 2020) have shown that the faunal diversity varies seasonally due to change in local environmental conditions such as precipitation, temperature (of both soil and air) and soil organic matter. Yin et al. (2018) reported that the abundance, diversity and evenness of the soil fauna was dependent upon the rainfall pattern. In contrast, Sylvain and Wall (2011) claimed that the lower precipitation rate directly correlate with less abundance and richness of the fauna. Studies on the correlation of soil fauna with physical parameters have shown that the combination of high temperature and rainfall increases the number of individuals of soil fauna as compared to the combination of lower temperature and little rainfall because high moisture facilitates survival of these fauna (Laiho et al. 2001, Gonzalez and Seastedt 2001, Zhu et al. 2010, 2011, Zagatto et al. 2019a, 2020).

Earthworms were the dominant macrofauna in this study. They were seen highest in summer when the temperature was high in the moist soil. As Warren and Zou (2002) explained, in a sandy soil, the moist soil moisture and texture play important role in the distribution and abundance of the earthworms rather than the leaf litter deposition. Similarly, Tondoh (2006) reported that the diversity of earthworm is dependent upon the soil moisture but does not correlate with the rainfall pattern.

Previous studies (Palacios-Vargas et al. 2007, Paul et al. 2011, Zagatto et al. 2019a) showed that the number of individuals of Collembola were highest in the summer and decreased significantly in the autumn when the leaf litter decomposition would be high. The same pattern was observed in this study as well (Table 1).

In case of the Crustacea (specifically the order isopoda), the abundance increased in the succeeding seasons of the sampling period (Table 1). In the same manner, Zagatto et al. (2019a) reported the higher density of isopoda in the areas with wet leaf litter as compared to the dryer areas. It is due to the fact that the soil isopods are terrestrial organisms which respire with gills thus moisture is the essential factor for their survival (Warburg 1987, Vona-Túri et al. 2019).

In this study, the fauna were recorded abundant in leaf litters however the diversity and evenness were found greater in soil layers. Similarly, Gonzalez and Seastedt (2001) showed that the fauna are abundant in the leaf litter than in soil layers. Seitz et al. (2015) concluded that the bare lands or only soil layers are generally less abundant in fauna. It is due to the fact that microclimatic conditions of the particular areas influence the distribution pattern of these fauna (Gonzalez and Seastedt 2001, Zhu et al. 2010, De Souza et al. 2016, Martin-Chave et al. 2018, Yin et al. 2018). Moreover, the litters protect the fauna from the unsuitable microclimate (Seitz et al. 2015). In addition, the soil compaction in this study may have resulted in the less abundance of the fauna in soil as these fauna are more sensitive towards the soil compaction (Beylich et al. 2010).

5.2. Correlation of Soil Fauna with Physico-chemical Parameters

In this study, effect of temperature is seen moderate with the soil fauna. It is seen negative in summer but positive in autumn. It means the soil communities are temperature sensitive and any fluctuation in the temperature affected their abundance. Gonzalez and Seastedt (2001) showed the positive relation of temperature to the fauna and stated that the increase in the temperature is associated with the shortened developmental time and thus increase populations. Furthermore, studies have shown that the correlation of temperature to the abundance of fauna is positive only when the soil is wet (Haimi et al. 2005). Lindberg (2003) claimed that the soil warming in the dry condition affect soil fauna negatively by triggering them to migrate.

Similarly, soil moisture content is seen to correlate slightly positively with earthworms and dipteran larvae than other fauna. As these fauna are sensitive to desiccation (Menta and Remelli 2020). Furthermore, Lindberg (2003) showed positive relation of soil moisture and faunal abundance. It is because moisture content is necessary for the survival of the fauna. In addition to that, the fungal growth is affected by the moisture content thus influence fungivorous communities and eventually earthworms (Gonzalez and Seastedt 2001). Thus, these intertwined relationships make soil communities associate with the moisture content of the soil.

In this study, the pH was fairly positively correlated with the abundance of the fauna. Similarly, Salmon et al. (2008) showed that the lower the pH value, lesser the faunal abundance. In contrary, Wang et al. (2015) showed that the abundance is negatively associated with pH of soil. As species of soil fauna have difference preferences, their association with soil pH might be different (Kautz et al. 2006). Also, fluctuation in the nutrients availability and adsorption quality of soil driven by pH indirectly affect microclimate for soil fauna (Salmon et al. 2008, Mulder and Elser 2009).

As the soil communities are affected by biotic and abiotic components like litter quality, and other physico-chemical parameters of the soil, the abundance of the fauna are dependent upon such components (Frouz et al. 2008, Korboulewsky et al. 2016). Since, soil fauna are either periodic geophiles (eg. grubs) or geobiont (eg. earthworms), effect of soil physico-chemical parameters are species dependent (Menta and Remelli 2020). Whatsoever, the correlation of the parameters here was not seen significantly strong. One of the major reasons for this can be the soil compaction caused by roots of trees, trampling and forest management practices and presence of sandy loam soil. Certain taxa such as Diplura, Colepteran adults, Chilopoda, Diplopoda, Diptera larvae negatively react to soil compaction (Menta 2012). In addition to that, soil compaction is believed to affect the physical and chemical properties of the soil and eventually affecting the biodiversity of the forest (Tan et al. 2005, Tan and Chang 2007, Tan et al. 2007, Beylich et al. 2010, Nawaz et al. 2012).

Likewise, there are certain taxa which correlated with each other than with other parameters. It might be due to the similar response pattern and composition, which can be defined by functional groups with similar habitat demands, to common determinants rather than depending on one another (Su et al. 2004, Wolters et al. 2006, Qian and Ricklefs 2008).

5.3. QBS-ar Index of RCF

The QBS-ar Index of the forest was seen highest in summer which eventually was lessened in succeeding seasons. It might be because of the greater population pressure in later seasons as anthropogenic disturbances affect the fauna (Menta et al. 2018). However, certain studies (Tabaglio et al. 2009, Madej et al. 2011, Galli et al. 2014) have shown that the index is affected by the seasonal variation as well.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

As the result of this study suggests, microclimatic conditions affect the soil invertebrates in their abundance and distribution pattern. Also, the physico-chemical parameters have great influence in these fauna. The QBS-ar Index showed high biological quality of soil of RCF. However, the disturbances induced by area modification by humans should be mitigated so as to maintain the quality of the forest in long-term.

6.2. Recommendations

Some recommendations made by this study are given below;

i. Species-level identification of the fauna should be done.
ii. Garbage and litters should me managed in this area or may be particular dumping site should be made.
iii. High level of anthropogenic disturbances should be minimized in the forest whenever possible.

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ANNEX

Tables related to main text

Annex 1: Eco Morphological Index (EMI) of soil arthropods

Taxa	Summer	Rainy	Autumn
Chilopoda			
Ethmostigmus sp.	10	10	10
Lithobius sp.1	10	10	0
Lithobius sp.2	10	10	0
Mecistocephalus sp.	10	10	0
Diplopoda			
Anauliciulus sp.	10	10	10
Orthomorpha sp.	10	0	0
Crustacea			
Burmoniscus sp.	10	10	10
Collembola			
Dicranocentrus sp.1	1	1	1
Dicranocentrus sp.2	1	1	1
Willowsia sp.1	1	1	1
Willowsia sp.2	1	1	1
Willowsia sp.3	1	1	1
Coleoptera			
Aleochara sp.1	1	0	0
Aleochara sp.2	1	1	0
Aleochara sp.3	1	0	0
Aleochara sp.4	1	0	0
Aleochara sp.5	1	0	0
Aleochara sp.6	1	0	0
Anthicus sp.	1	0	0
Astenus sp.1	1	0	0

Taxa	Summer	Rainy	Autumn
Astenus sp.2	1	0	0
Atholus sp.	0	1	0
Axonya sp.	1	1	0
Carabidae larva 1	10	10	0
Carabidae larva 2	10	0	0
Clivina sp.	1	1	1
Elateridae larva	10	10	10
Eobruscus sp.1	1	1	1
Eobruscus sp.2	1	1	0
Eobruscus sp.3	1	0	0
Gonocephalum sp.	1	1	0
Lampyridae larva	10	10	0
Mesomorphus sp.	1	1	0
Othius sp.1	1	1	0
Othius sp.2	1	0	1
Othius sp.3	1	0	0
Philonthus sp.	1	0	0
Rugilus sp.	1	0	0
Scarabaeidae larva	10	10	10
Tenebrionidae larva	10	0	0
Orthoptera			
Acrididae nymph	1	0	0
Lepidoptera			
Noctuidae larva 1	10	0	0
Noctuidae larva 2	10	0	0
Hemiptera			
Aethus sp.1	1	0	0
Aethus sp.2	1	0	0
Cydnidae nymph	1	0	0
Lygaeidae nymph	1	1	0

Taxa	Summer	Rainy	Autumn
Blattodea			
Blattidae nymph	5	5	5
Dermaptera			
Labia sp.	0	1	1
Diplura			
Lepidocampa sp.	20	20	20
Hymenoptra			
Aenictus sp.	5	5	0
Aphaenogaster sp.	5	5	0
Brachyponera sp.1	5	5	5
Brachyponera sp.2	5	5	0
Cerapachys sp.	5	0	0
Crematogaster sp.	0	5	5
Dorylus sp.1	5	5	0
Dorylus sp.2	5	5	0
Formica sp.1	5	5	0
Formica sp.2	5	0	0
Lasius sp.1	0	5	0
Lasius sp.2	5	0	0
Lasius sp.3	5	0	0
Meranoplus sp.	5	5	0
Monomorium sp.	5	5	0
Ooceraea sp.	5	0	0
Paratrechina sp.	5	0	0
Phidologeton sp.	5	0	0
Tetramorium sp.1	0	5	5
Tetramorium sp.2	5	0	0
Tetramorium sp.3	5	5	0
Tetraponera sp.	5	0	0

Taxa	Summer	Rainy	Autumn
Diptera			
Bibionidae larva	10	0	10
Fanniidae larva	10	0	0
Tipulidae larva	10	10	10
Aranea			
Unidentified sp.1	1	0	0
Unidentified sp.2	1	1	1
Unidentified sp.3	1	1	1
Unidentified sp.4	1	1	0
Unidentified sp.5	1	1	0
Unidentified sp.6	1	1	1
Acari			
Parasitinae sp.1	20	20	20
Parasitinae sp.2	20	0	0
Pergamasinae sp.	20	20	0
Trombiculidae sp.	20	0	0
QBS-ar	**417**	**261**	**142**

Annex 2: List of species of RCF deposited in the CDZMTU, Kirtipur, Nepal

Taxa	No. of specimen	Plot no.	Date of collection
Haplotaxida			
Pheretima sp.1	38	P1, P2, P5	2019/05/24 – 2019/07/19
Pheretima sp.2	2	P4, P5	2019/05/24
Chilopoda			
Ethmostigmus sp.	2	P1, P6	2019/05/24 – 2019/07/19
Lithobius sp.1	6	P1, P3, P5	2019/05/24 – 2019/08/16
Lithobius sp.2	3	P1, P5	2019/05/24 – 2019/08/16
Mecistocephalus sp.	3	P2, P6	2019/05/24 – 2019/08/16
Diplopoda			
Anauliciulus sp.	5	P1, P5, P6	2019/05/24 – 2019/08/16
Orthomorpha sp.	7	P5, P7	2019/05/24 – 2019/07/19
Crustacea			
Burmoniscus sp.	11	P1, P2, P6, P7	2019/05/24 – 2019/08/16
Platorchestia sp.	1	P2	2019/07/19
Collembola			
Dicranocentrus sp.1	9	P1, P4, P5, P6	2019/05/24 – 2019/07/19
Dicranocentrus sp.2	7	P1, P2, P3	2019/05/24 – 2019/06/08
Willowsia sp.1	22	P1, P2, P5, P6	2019/05/24 – 2019/07/19
Willowsia sp.2	6	P6, P7	2019/05/24 – 2019/06/08
Willowsia sp.3	13	P2, P3, P7	2019/05/24 – 2019/07/19
Coleoptera			
Aleochara sp.1	14	P2, P3	2019/05/24 – 2019/06/08
Aleochara sp.2	1	P2	2019/05/24
Aleochara sp.3	5	P1, P3	2019/06/29
Aleochara sp.4	1	P2	2019/05/24
Aleochara sp.5	1	P2	2019/06/29
Aleochara sp.6	1	P5	2019/05/24

Taxa	No. of specimen	Plot no.	Date of collection
Anthicus sp.	1	P4	2019/05/24
Astenus sp.1	2	P6	2019/05/24
Astenus sp.2	1	P5	2019/06/08
Atholus sp.	1	P6	2019/08/03
Axonya sp.	8	P4, P6	2019/05/24 – 2019/06/08
Carabidae larva 1	2	P3	2019/06/08
Carabidae larva 2	1	P2	2019/06/08
Clivina sp.	1	P1	2019/05/24
Elateridae larva	2	P1, P3	2019/05/24
Eobruscus sp.1	2	P4, P7	2019/05/24
Eobruscus sp.2	2	P7	2019/06/29 – 2019/07/19
Eobruscus sp.3	6	P1	2019/05/24
Gonocephalum sp.	5	P1, P7	2019/05/24 – 2019/06/08
Lampyridae larva	8	P1, P4	2019/06/08 – 2019/06/29
Mesomorphus sp.	4	P6	2019/05/24 – 2019/06/08
Othius sp.1	4	P4	2019/06/08
Othius sp.2	2	P1	2019/07/19
Othius sp.3	1	P7	2019/06/08
Philonthus sp.	1	P7	2019/06/29
Rugilus sp.	3	P1	2019/05/24
Orthoptera			
Acrididae nymph	1	P7	2019/06/08
Lepidoptera			
Noctuidae larva 1	1	P5	2019/06/29
Noctuidae larva 2	1	P6	2019/05/24
Hemiptera			
Aethus sp.1	2	P6	2019/05/24
Aethus sp.2	1	P1	2019/05/24
Cydnidae nymph	3	P6	2019/05/24 – 2019/06/08
Lygaeidae nymph	4	P3, P4	2019/08/03

Taxa	No. of specimen	Plot no.	Date of collection
Blattodea			
Blattidae nymph	7	P1, P3, P6	2019/08/03 – 2019/08/16
Dermaptera			
Labia sp.	1	P3	2019/08/16
Diplura			
Lepidocampa sp.	6	P2, P3	2019/05/24
Hymenoptra			
Aenictus sp.	18	P4, P5	2019/09/01
Aphaenogaster sp.	2	P7	2019/06/29
Brachyponera sp.1	110	P1, P2, P4, P5	2019/05/24 – 2019/09/01
Brachyponera sp.2	16	P1, P7	2019/05/24 – 2019/08/16
Cerapachys sp.	4	P5	2019/07/19
Crematogaster sp.	2	P3	2019/08/16
Dorylus sp.1	5	P1, P7	2019/05/24
Dorylus sp.2	3	P4, P5	2019/05/24
Formica sp.1	1	P6	2019/07/19
Formica sp.2	1	P7	2019/06/29
Lasius sp.1	2	P4	2019/08/03
Lasius sp.2	1	P1	2019/06/08
Lasius sp.3	1	P1	2019/05/24
Meranoplus sp.	21	P1, P4	2019/07/19 – 2019/08/16
Monomorium sp.	7	P7	2019/07/19
Ooceraea sp.	4	P3, P5	2019/07/19
Paratrechina sp.	1	P4	2019/06/08
Phidologeton sp.	15	P3, P5	2019/05/24 – 2019/06/08
Tetramorium sp.1	12	P2, P4	2019/08/03
Tetramorium sp.2	3	P4	2019/07/19
Tetramorium sp.3	3	P4	2019/08/03
Tetraponera sp.	1	P2	2019/05/24

Taxa	No. of specimen	Plot no.	Date of collection
Diptera			
Fanniidae larva	2	P6	2019/06/08
Aranea			
Unidentified sp.1	2	P1	2019/05/24
Unidentified sp.2	1	P3	2019/05/24
Unidentified sp.3	1	P4	2019/05/24
Unidentified sp.4	1	P4	2019/05/24
Unidentified sp.5	1	P4	2019/05/24
Unidentified sp.6	1	P6	2019/05/24
Acari			
Parasitinae sp.1	11	P2, P4	2019/05/24 – 2019/07/19
Parasitinae sp.2	22	P1, P2	2019/05/24 – 2019/06/08
Pergamasinae sp.	4	P6	2019/07/19
Trombiculidae sp.	3	P3, P7	2019/05/24 – 2019/07/19

Photo Plates

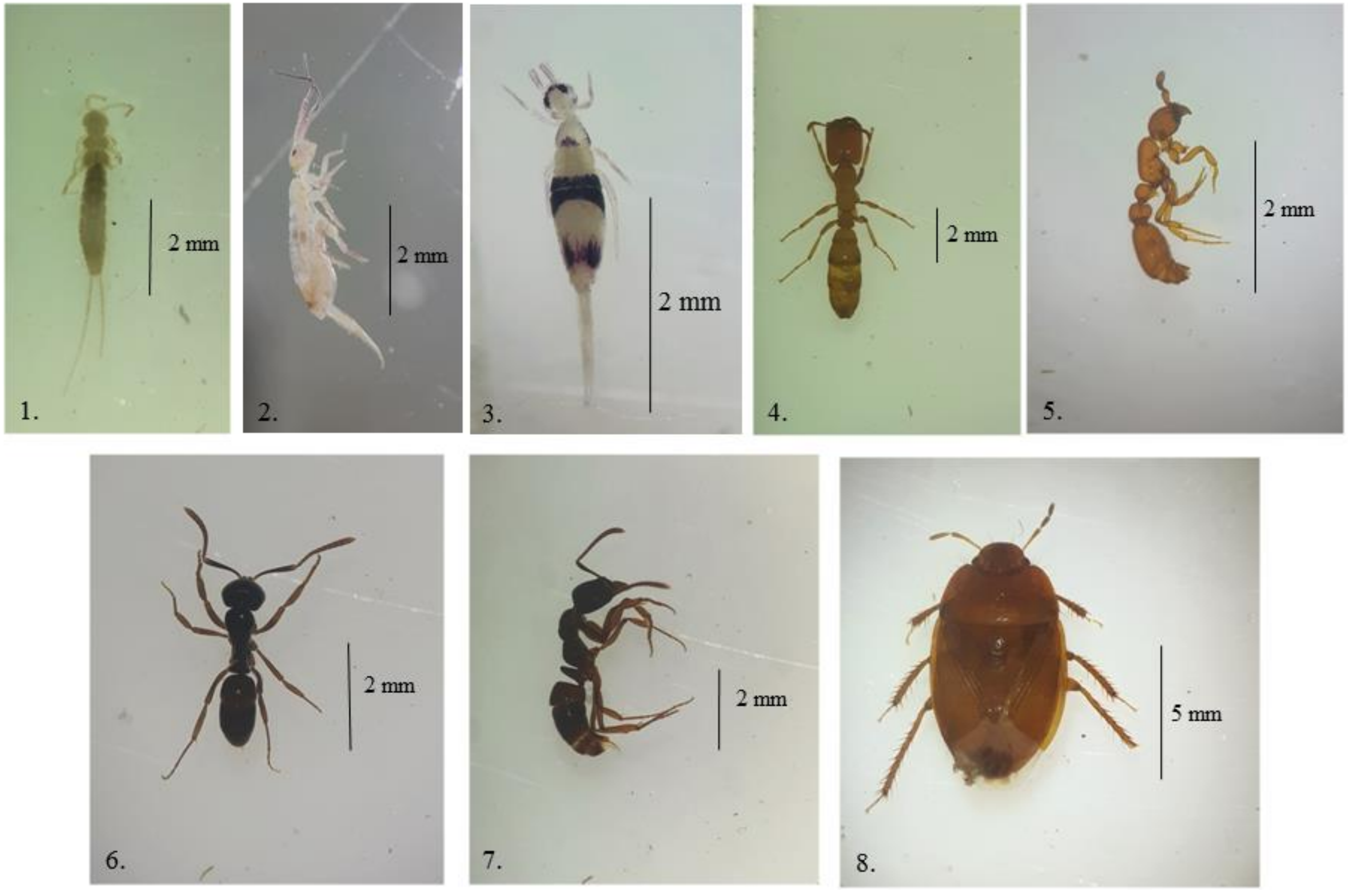

Plate-A: 1. *Lepidocampa* sp.; 2. *Dicranocentrus* sp.1; 3. *Willowsia* sp.2; 4. *Dorylus* sp.1; 5. *Aenictus* sp.; 6. *Brachyponera* sp.2 (dorsal); 7. *Brachyponera* sp.2 (lateral); 8. *Aethus* sp.1

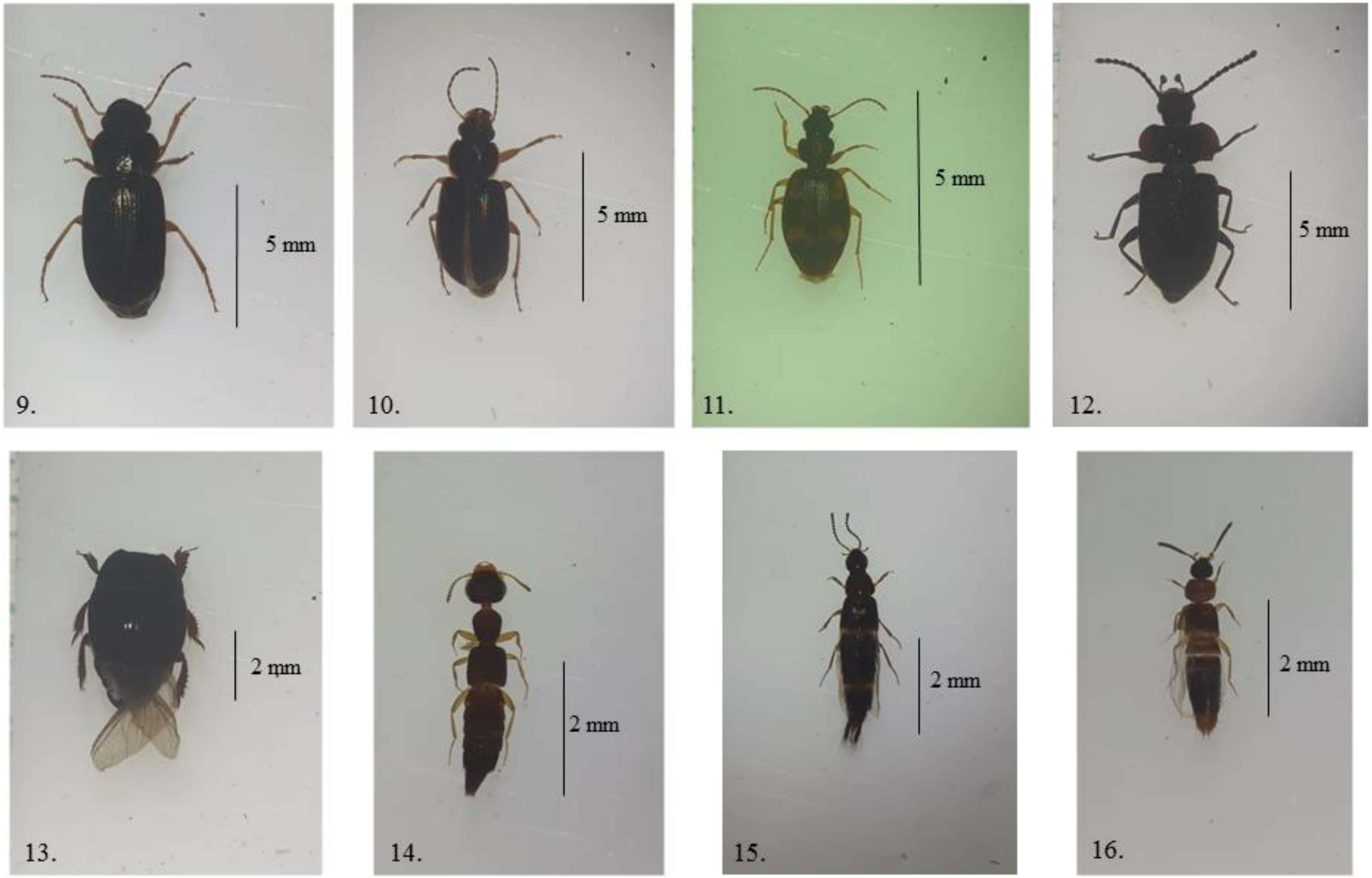

Plate-B: 9-16. Coleoptera; 9. *Eobruscus* sp.1; 10. *Eobroscus* sp.2, 11. *Axonya* sp.;
12. *Gonocephalum* sp.; 13. *Atholus* sp.; 14. *Rugilus* sp.; 15. *Philonthus* sp.; 16. *Aleochara* sp.1

Plate-C: 17-24. Immature forms; 17. Carabidae; 18. Elateridae; 19. Scarabaeidae; 20. Lampyridae; 21. Fanniidae; 22. Blattidae; 23. Cydnidae; 24. Lygeidae

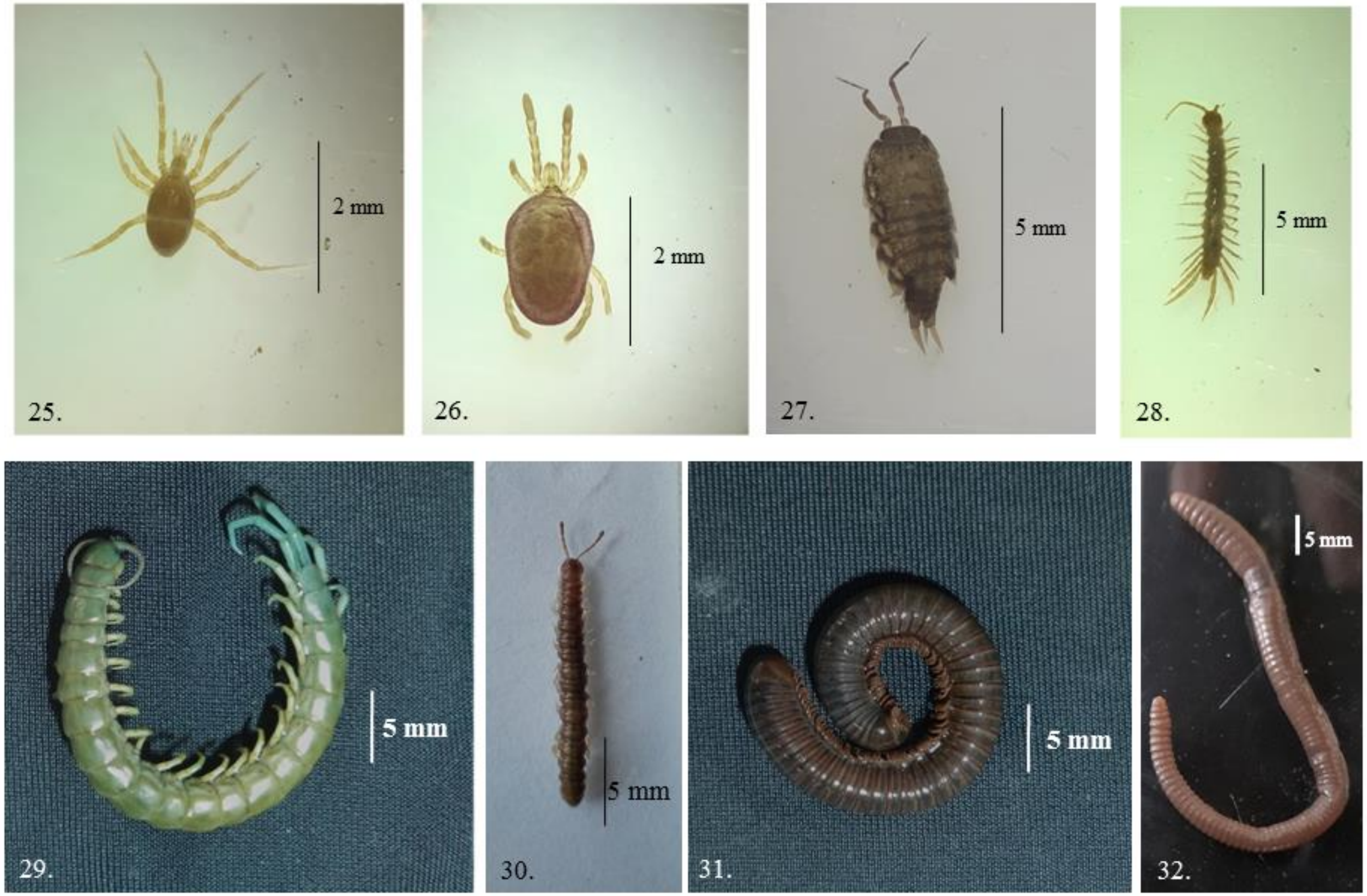

Plate-D: 25. Parasitinae sp.1; 26. Trombiculidae; 27. *Burmoniscus* sp.; 28. *Lithobius* sp.2; 29. *Ethmostigmus* sp.; 30. *Orthomorpha* sp.; 31. *Anauliciulus* sp.; 32. *Pheretima* sp.1