Chapter 3

Materials and methods

In compliance with the proposed objectives, the following materials and methods were followed. The details of the study area, materials used, methodology applied, and the measures which were taken have been discussed in this chapter.

3.1 Study area

Pavagadh Hill is situated in Panchmahal district of Gujarat at a distance of 50 km north-east of The M. S. University of Baroda, Vadodara. At the base of the hill, the historic city of Champaner is located. With Champaner and Pavagadh Hill, the Champaner-Pavagadh Archaeological Park is formed. It is one of the 38 World Heritage Sites of India which has been inscribed by UNESCO on the World Heritage List as Tangible Cultural Property of Universal value in the UNESCO's 28th session held at Beijing in China on 7th July 2004. Inscription on this confirms the exceptional and universal value of a cultural site that requires protection for the benefit of all humanity. The architecture of Champaner is one of the superb examples of the pre-Mughal township. The elegant monuments present in the area stand testimony to the harmonious synthesis of the local tradition of ornamentation and Islamic building traditions.

Champaner-Pavagadh Archaeological Park is a 16th century Medieval Sultanate capital city which was buried beneath a thick forest cover and is a highly complex heritage site whose landscape characterized by mounds, plateaus, and streams (Modi, 2008). An area of Core Zone of approx. 14 sq. km and Buffer Zone of 30 sq. km is covered by it. The famous temple of Kalimatha is situated at the top of Pavagadh hill, which is a well-known religious destination visited by lakhs of pilgrims throughout the year. It is also a place of historic importance having palaces, temples, monuments, mosques, arches, step-wells, and agricultural fields. The magnificent view of the Pavagadh hill invites an innumerable number of tourists from various places. The main Pavagadh hill is the highest point in the district, rising to a height of 823 m surrounded by several small hillocks of height ranging from 200m-300m (Figure 7). From the hill, it can be viewed Baroda on one side and Godhra on the other side. Naulakhi, Mauliya, Bhadrakali, Machi, and Atak are the five successive plateaus. The district is drained by seven major rivers and amongst them, the Mahi is the longest river. The river Vishwamitri originates from Pavagadh hills and flows through Halol Taluka before entering the Vadodara district.

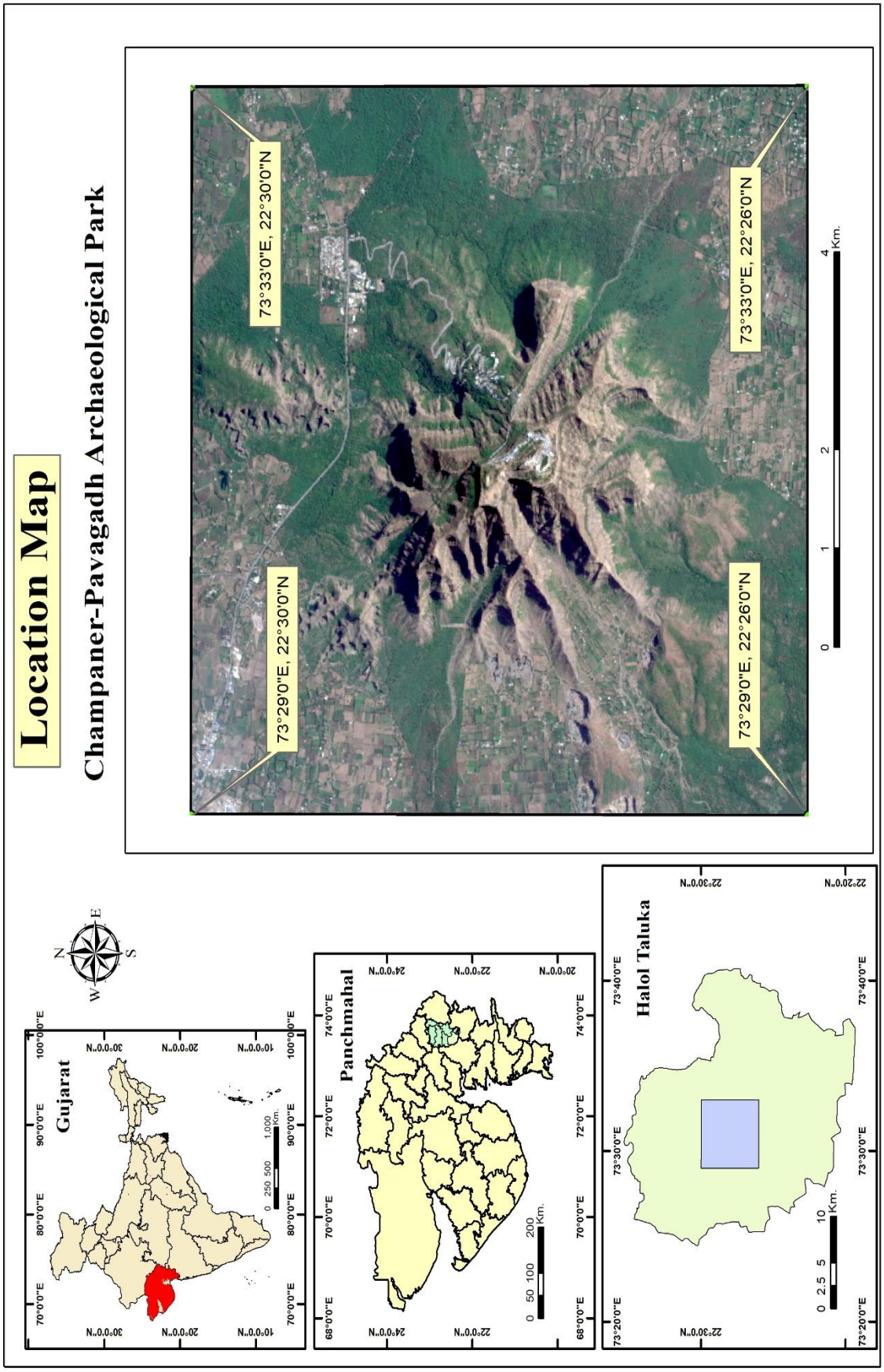

Figure 7: Image depicting the location of the study area

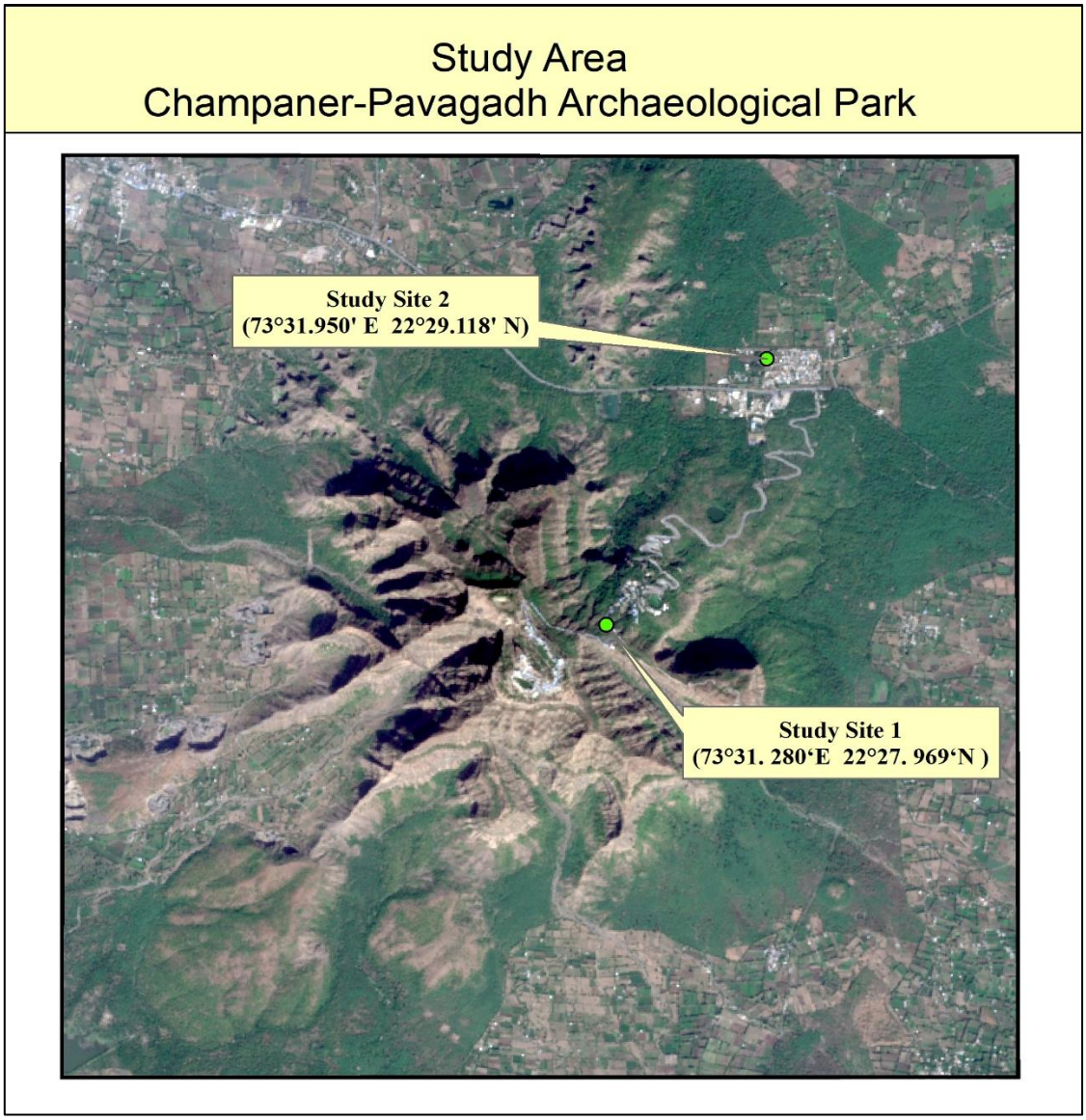

Figure 8: Image showing the study sites used in the study where study site represents the foothills of Pavagadh hill and site 2 shows the Champaner area

Climate: The climate of the study area is hot and dry except in the monsoon season. The year may be divided into four seasons: - summer season, monsoon season, post-monsoon, and winter. Essentially, the rainfall in the area is by the south-west monsoon during monsoon months. Rainfall is very irregular and erratic. Total rainfall received varies widely from year to year and from place to place. In the months of January to May humidity reaches around 30-40 %. Climatic conditions affect the diversity and ecology of butterflies. Ecological studies of butterflies help to know the environmental changes and if any disturbances occur in the area. Rainfall of 700 mm to 1024 mm range occurs here annually. The relative humidity is high

during the monsoon season and in months like July and August it reached up to 50% to 80%. It became around 30- 40 % in the months of January to May.

Soil: The composition and constitution of the soil vary in the study area. It was chiefly sandy loam and black in nature. The Pavagadh Hill area has generally very shallow and poor soils and was totally devoid of soils at many places (Revdandekar, 2014).

3.2 Study Period

The study was carried out for a period of 3 consecutive years i.e., January 2017 to December 2019 in all four seasons of the year. Butterflies were mostly found in all habitats like forest areas, garden areas, agricultural fields, near the monuments, and hilly areas. Butterflies are diurnal in nature except for a few species like Common Evening Brown which are usually active at dusk. Thus, for the collection of the butterfly's day time was preferred. The sampling was done in the morning hours from 8:00 am to 12:00 pm under suitable weather conditions

3.3 Sampling Sites

In order to carry out the diversity studies of butterflies in the Pavagadh Hill, the entire study area was divided into different sub-study sites, depending on the type of vegetation, habitat structure, and convenience of data collection. The co-ordinates and elevation of each site were taken with the help of the Global positioning system (GPS: Garmin Oregon 550 Figure 9). Pavagadh's total area is 6356.98 ha and legally constitutes a reserve forest. Forest area, which is situated at the foot area of the Pavagadh hill of Pavagadh falls under Halol Range of Godhra Division (Figure 7,8, 10). The total forest area of Pavagadh is 2100.35 ha, where 2099.63 ha is considered as a Reserved Forest area and 0.72 ha is coming under Protected or Unclassed Forest (Revdandekar, 2014). The majority of the forest area had a mixed type of vegetation which includes herbs, shrubs, trees, and climbers (Annexure II). A public garden is also present at the base of Pavagadh Hill. This area also constitutes cultivated, ornamental as well as wild plants. Some of the plants were Chrysanthemum, Ixora coccinea, Ocimum sanctum, Jasminum sambac, Vinca rosea, Nerium oleander, etc. It was also surrounded by forest and has monuments and mosques (Figure 11). The garden area was more exposed to human interference because lakhs of tourists visit this area annually. The study of the diversity of butterflies along the slope was carried out in order to see the relationship between species diversity and height. The Pavagadh hill was divided into three altitudes starting from the base

to the top of the hill i.e. lower altitude (230m to 430m), Middle altitude (430 to 630 m), and higher altitude (630 m to 830m). Each altitude was selected for a distance of 200 m in height.

Figure 9: Image showing the co-ordinates and elevation of each site taken by Garmin Oregon 550

Figure 10: Image display the area at the foothills of Pavagadh

Figure 11: The public garden at the base of Pavagadh hill

3.4 Sampling Protocol

3.4.1 Field Survey

A systematic approach was followed to monitor the diversity of butterflies in the study area. The study was conducted from January 2017 to December 2019. The butterflies and plants were observed directly in the field and for documenting the butterflies Pollard Walk method was utilized with modification. The Pollard Walk method is a predominant type of monitoring of butterflies. At a site butterflies are recorded along a fixed-route walk is established on a regular basis under favourable conditions of weather (Pollard, 1997). Each path is walked through for 2-3 hours continuously at a slow and equal pace and kept visibility of 10 meters at both the sides and front side of the recorder. The same method is repeated for all the seasons and visits were made at least twice a month. The selected paths were visited from 08:00 am to 12:00 pm as it was the peak time for the butterfly activities. Repetition of counting butterflies more than once was avoided. The hilly areas were studied with a modification in the method. We explored along with the steps which are already present for the pilgrims to climb and reach the temple and the other sides of the hill were not accessible. So, we followed the same path and studied diversity in three different altitudes for a period of 2-3 hours continuously keeping the visibility of 10m. This sampling method is formed a very crucial step in achieving the objectives of this study.

3.4.2 Collection of Data

Collection of butterflies were restricted to those which could not be identified in the field with the help of field guides. The butterflies were captured with the help of a butterfly net (Figure 12: Insect Trapping Net: A polyester mesh with stainless steel rod. Outer diameter is approximately 12'' net ring for collection with 24'' depth and 2mm net hole for avoiding butterflies flying out) and after taking photographs they were released back into the same habitat. Selected species of butterflies were captured for morphometric analysis. After taking measurements they were released.

A) Photography: Photographs were clicked with the help of a Canon EOS 750D camera. Most of the butterflies were photographed in live conditions from the study sites during their flight or nectaring time for identification.

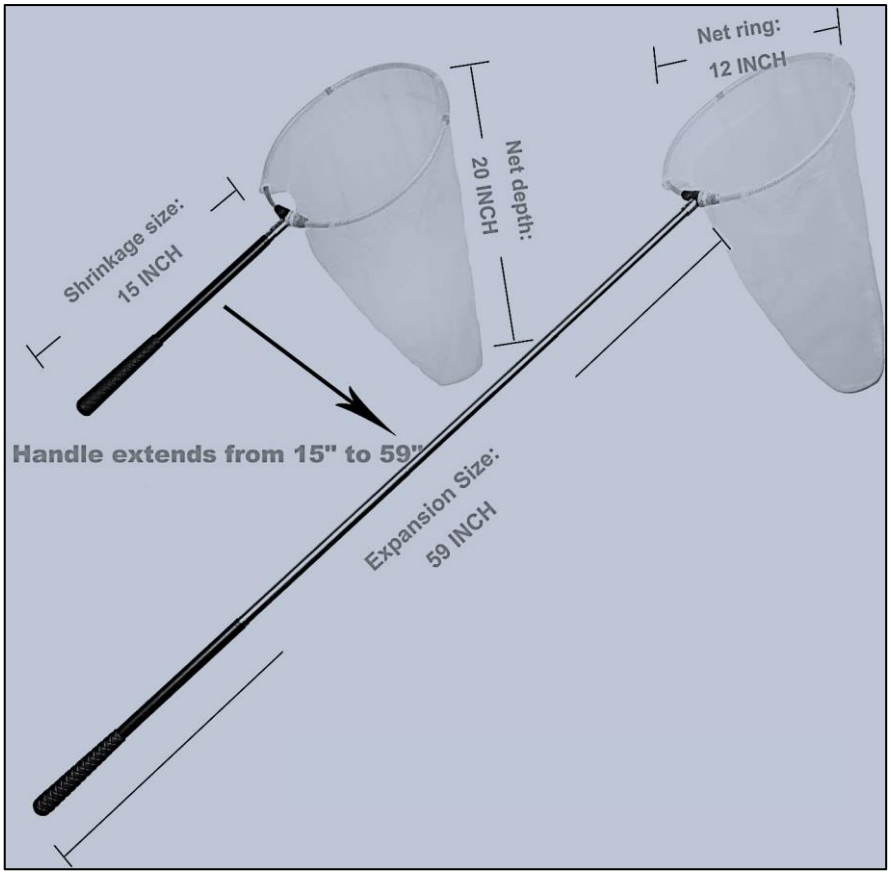

Figure 12: Representative image of Insect trapping net

B) Taxonomic Identification of Butterflies: Taxonomic identification of butterflies was done using multiple pictorial guides and standard books. The pictorial guide of (Kunte, 2000), (Kehimkar, 2008), and (Bhakare & Ogale, 2018) was used for the species-level identification of butterflies.

C) Taxonomic Identification of Plants: Details of the plants visited by butterflies to feed most frequently were observed. The plants are identified with the help of field guides. Flowering season, flower colour, corolla shape, and type of plant were observed. The host plants and nectar plants were identified by Prof. Neeta Pandya of the Department of Botany, Faculty of Science, The M. S. University of Baroda.

D) Seasonal Distribution of Butterflies: To study the effect of different seasons on the abundance and diversity of butterflies, monthly visits were made to the selected study area. The entire year was divided into four seasons depending on availably of rainfall and temperature fluctuation (Table 3). The study was carried out for the seasons of three consecutive years starting from January 2017 to December 2019.

Sr. No.	Season	Months
1	Summer	March, April, May
2	Monsoon	June, July, August
3	Post-monsoon	September, October, November
4	Winter	December, January, February

Table 3: Climatological details considered during the study

E) The abundance of Butterflies: To study the abundance of butterflies, the observed butterflies were categorized into 5 different groups on the basis of their presence or absence of sightings during each visit in the study area. They are VC- Very Common (Above 75 sightings), C-Common (75-50 sightings), UC- Uncommon (50-25 sightings), R-Rare (25-10 sightings), and Very Rare (Below 10 Sightings). This categorization of butterflies was completely dependent on the local availability of them.

F) Morphometry of Butterflies: To study the coevolutionary relationship between butterflies and plants, morphometry of proboscis of selected species of butterflies were done with the help of Dial Calliper (Dial Calliper: Mitutoyo Dial Vernier Calliper). Flowers that were frequently visited by the butterflies have identified. Most preferred flowers were only taken into consideration for performing morphometric analysis. To facilitate the morphometry of butterflies, five species each from the families Pieridae, Nymphalidae, Papilionidae, and Lycaenidae were selected based on their occurrence in the habitats. To measure the length of the proboscis of butterflies they were captured with the help of a butterfly net. After that, they were removed from the net, and the length of proboscis was measured by inserting a needle into the centre of the coiled proboscis and straightening them out. The length of the proboscis was measured as the distance between the bases of the labial palps to the tip of the proboscis with the help of Dial Calliper. After measurement butterflies were released into the same habitat without much damage. Measurements were made of body length (in mm), wingspan (in mm), and proboscis length (in mm) and the values presented in the table for the proboscis length are the Mean ± Standard Deviation, n=5 (Table 10).

G) Morphometry Corolla of Flowers: To study the corolla tube length of the preferred nectar flower, morphometry of the corolla tube was done. It was characterized by the measurement of the length of the corolla tube with the help of Dial Calliper of flowers of the preferred nectar plants. The values for corolla length taken as the Mean ± Standard Deviation, n=5 (Table 12)

Figure 13: Dial Calliper used in morphometry studies

3.5 Statistical Analysis of the Data

Alpha diversity parameters were used to analyse the butterfly diversity in the study area using Statistical Software PAST 3.14. To study the Diversity, Species Diversity and Species Evenness were calculated by using Shannon-Wiener Diversity Index and Pielou's Evenness Index.

A) Shannon-Wiener Diversity Index: It is the most commonly used diversity index in ecology. The Shannon-Wiener Index does the quantification of the uncertainty associated, it predicts the identity of new taxa, given a number of taxa and also evenness in abundances of individuals in each taxon. Values of H′ typically range from 1.5 to 3.5, but it can range from 0 to 5. The Shannon-Wiener index assumes that the sample for the site was collected randomly.

$$H' = -\sum (ni/N \times \ln ni/N)$$

Where, ni= is the number of individuals of amount (biomass) of each of the i species

N = is the total number of individuals (or biomass) for the site.

B) Pielou's Evenness Index (J'): The distribution of individuals over species is called evenness. The values of Evenness describe how evenly the individuals are distributed among the different species. It is calculated as:

$$\text{Pielou's Evenness Index } J = H' / \ln S$$

Where S = number of species present in the site

To study the co-evolutionary relationship among butterflies and plants, correlation of the length of proboscis of butterflies, and length of the corolla of the preferred nectar plant. Correlation refers to the strength of the relationship between two variables. It is a method in which one can establish the relationship between two variables. Values of the correlation coefficient always lie between -1 to +1. If the value is positive, then it is positively correlated and the value of X increases with the corresponding Y value. If the value is negative means the value of X decreases with the corresponding value of Y. The value 0 indicates there is no correlation between the two corresponding variables. This analysis was done by using GraphPad Prism version 9.0, GraphPad Software, San Diego California USA.

C) Karl Pearson's Coefficient of Correlation: It is also known as the Product Moment correlation of coefficient. It is used to measure the magnitude of the relationship between two

variables. It measures the level of relation between linear related variables. The letter ‘r’ represents the coefficient of correlation.

D) Regression Analysis: It is a mathematical measure to show the average relationship between the two variables. It indicates the cause-and-effect relationship between the variables and establishes a functional relationship between them

$$Y = a + b\,X + \epsilon$$

Where: Y – Dependent variable

X – Independent (explanatory) variable

a – Intercept

b – Slope

ϵ – Residual (error)

In order to achieve the objectives, the above-mentioned materials and methods were implemented at different stages for the efficient conduct of the research work. The results obtained are discussed in the next chapter in detail.