SOIL CARBON CONTENT AND MICROBIAL BIOMASS ACTIVITY OF DIFFERENT FOREST TYPES UNDER SEASONAL VARIATIONS

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Dedication

This research is dedicated to my loving, caring, and industrious parents, whose efforts and sacrifices have made my dream of having this degree a reality. Words cannot adequately express my deep gratitude to them.

"O My Sustainer, bestow on my parents your mercy even as they cherished me in my childhood."

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List of Abbreviations

MBS	Microbial biomass
SMB	Soil microbial biomass
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MBP	Microbial biomass phosphorous
TN	Total nitrogen
TP	Total phosphorous
TOC	Total organic carbon
SOC	Soil organic carbon
EC	Electrical conductivity
FAS	Ferrous ammonium sulphate
FE	Fumigation extraction
NT	NUST
MG	Margala
MR	Murree
SP	Shakar parian
MC	Moisture content
DR	Dial reading
USDA	United states department of agriculture
WHC	Water holding capacity
MHC	Moisture holding capacity

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ABSTRACT

Soil carbon is sequestered in soil by plants through photosynthesis and may be stored as soil organic carbon. The increased levels of carbon sequestration may be observed with plants grown on fertilized land. Rainfall and temperature have by far the strongest influence on soil organic matter levels. Increases in temperature have been demonstrated in empirical studies to increase soil heterotrophic respiration and decrease the amount of carbon the soil may uptake. Soil organic matter content is usually higher where rainfall is higher and temperatures are cooler. To analyze the effects of seasonal variations on physico-chemical and biological properties of different forest soil types, a comparative study was conducted to assess the relationship among soil parameters in Murree, Islamabad-Pakistan. Samples were collected from the different forests in the vicinity of Islamabad in all four seasons. Physical, chemical, and biological properties of the soil were analyzed. Results of the study shows that Soil Microbial Biomass (MBS) is highest during summers season while lowest during the winter season for Murree samples. In winter season 380 mg/kg Phosphorus concentration was observed in NUST samples which is highest while it decreases in summer season. Total Nitrogen (TN) of 0.421% was observed at Margalla site in autumn season which is the highest, while during the summer season, TN was reduced to 0.211%. Highest Total Organic Carbon (TOC) was observed in Margalla during winter season whereas the lowest TOC was observed in NUST samples during summer season. Statistical analyses reveals that the TOC has a positive co-relation with MBS, WHC and TN, while MBS had a positive correlation with TOC and Temperature. Negative Correlation was found between soil moisture content and temperature.

CHAPTER 1

Introduction

1.1 Carbon sequestration

An increase in the levels of greenhouse gases (GHGs), mainly carbon dioxide (CO_2), may initiate changes in the climate patterns throughout the world which definitely affects the natural moisture present in the soil, plant cultivation, and also the agricultural patterns (Prayogo, 2013). Pakistan lost 94% of the tree cover from 2001 to 2007. The country's average forest area is 2.5%, of which Azad Kashmir contributes the most significant percentage, which is12% (Global Forest watch, 2018).

Pakistan has an arid and semi-arid region, which receives 50- 250 mm precipitation per year. It has the potential to sequester Carbon with higher woody plants that have the capacity to survive in low moisture levels and in high soil saline area. Research has shown that these lands are able to store 1billion tons of Carbon in soil (Sadeghi and Raeini, 2016).

Carbon sequestration is generally defined as the capturing of Carbon in the soil (and other sinks) from the atmosphere for a more extended period of time. The storage of Carbon in the sinks is effective in mitigating the effects of global warming and climate change effects (Ni *et al*., 2016).

1.2 Soil carbon sequestration

Soil plays two leading roles in the environment, which helps to promote the sustainable activities of development and land degradation neutrality, i.e., land degradation process and rehabilitation and restoration activities of soil (Keesstra *et al*., 2018). In the environment, the soil is the primary sink for the storage of Carbon which helps to manage the difficulties related to agriculture (Novara *et al*., 2016). Moreover, expanding the natural storage for CO_2 is one of the suitable applications for mitigating atmospheric Carbon. Shrub-land, agricultural areas, range land, and forest area are the natural sinks in the semi-arid and arid regions; by increasing these areas, CO_2 uptake and storage will also increase (Trabucco *et al*., 2008).

Some other factors along with carbon sequestration for the reduction of climate change impacts include enhancing the energy efficiency, use of renewable energy sources, less use of fuels that contain carbon, and also increasing the natural pools for the storage of carbon to mitigate the GHGs emissions. The studies have revealed that organic farming has a positive effect on carbon emission and preservation in soil (Di Prima *et al*., 2018; Novara *et al*., 2019). One of the biggest carbons pools in the environment is stored within the soil with the possibility of reduction in the impacts of climate change. (Matovic, 2011).

Different land systems have different potential to sequester carbon which depends upon the factors like soil topography, type of soil, the climate of the region, and different management practices. These factors need to be adequately understood as the carbon accumulation depends on them. Researchers conducted that the level of carbon dioxide present in the environment can be reduced by actively promoting environment-friendly strategies and plans (Hammad *et al*., 2020).

1.3 Carbon sequestration in forests

Forests can sequester a large amount of carbon. According to a study, the world's total biomass can provide 1 Giga ton of carbon per year (1 Gt C yr1) (Ni *et al*., 2016). Collection of soil from forests is a pretty challenging process as the forest soil is composed of a coarse root system and rugged rocks, has high spatial variability, and is not approachable easily, while the above-ground biomass is easy to sample. The methods present these days for the analysis of soil and available underground carbon are relatively long, slow, and exhausting as they require further steps like the collection of soil samples, the removal of moisture, grinding, weighing, and then they undergo for the laboratory analysis. Moreover, these laboratory methods also have some limitations as they require heterogeneous samples to precisely distinguish the soil properties, large sample volumes might be needed, and these methods are also non-affordable sometimes (Nurthup *et al*., 1977).

Types of forests can affect the microbial community and their activities and also help in the determination of the quality and quantity of organic matter present in the soil. Some other factors like; Temperature, precipitation, the composition of organic matter, seasonal variations, and litterfall have a considerable impact on microbial biomass present in soil (Chang *et al*., 2016).

Forests might have to face different natural problems. These difficulties may be divided into two groups, i.e., biotic and abiotic disturbances. Abiotic disturbances include fire, harvesting, storm, leaching, etc., while biotic factors include insects or pathogens attacking and infection in trees due to microbial community (Goetz *et al*., 2012). These forest disturbances can actually occur occasionally or repeatedly as indicators of climate change and global warming. The soil-plant nutrient cycle is linked to the soil by the microbial community, which is accountable for the decaying and mineralization of residues (plant and animal) in the soil (Duffkova and Macurova, 2011).

1.4 Factors effecting soil carbon

Capturing of carbon in soil depends on some factors which can enhance the ability of soil to sequester carbon more; some of these factors are the presence of nutrients in soil, water holding capacity, soil structure, enhanced plantation and soil productivity, less weathered soil, etc. (Guimaraes *et al*., 2013). The difference in elevation is the leading environmental factor that can alter environmental conditions like climate and soil by changing their physical, biological, and chemical cycles (Hutchins *et al*., 1976). The difference in the altitude can cause a change in the climate and soil properties, i.e., Carbon and nutrients, which can further affect the biomass, respiration, and enzymes of the microbial community along the height (He *et al*., 2016).

As the height increases, the temperature of the soil decreases, which limits microbial growth and activity. Hence the retention time of Carbon extends in soil, and the nitrogen level also increases due to the limited mineralization (Prichard *et al*., 2000).

It is hard to understand the complex interaction between this soil condition and the plant community along with the changing elevation, which helps to specify precisely the enhancement needed for microbial processes (Siles and Margesin, 2016). It may now be fueled by our emerging understanding that elevation gradients can be really helpful for understanding the biodiversity in the earth's environment, and it may also foretell the upcoming loss of biodiversity, which can result in the change in climatic conditions (Mayor *et al*., 2017).

1.5 Microorganisms in soil

Microorganisms that are present in soil are an integral part of the forest ecosystem. These soil microbes may influence the biogeochemical cycles in the terrestrial ecosystem. Considerable changes in nutrient conversion in the plant-soil system may be influenced by minute shifts in soil microbes within the active environments (Deng *et al*., 2016). In dry tropical forest soils, its microbial biomass depends on different climatic factors, potential hydrogen of soil, Temperature, and availability of various nutrients, etc. (Singh and Kashyap, 2006).

Carbon acts as an energy provider for microorganisms present in soil which ultimately helps microbes to develop when they come across carbon sources available in rich amounts. The size of microbial biomass depends upon some significant factors, which are enlisted below:

- Carbon and Nitrogen ration (C/N)
- Carbon sources and their accessibility
- Nitrogen availability
- Quality and quantity of organic matter

Various types of microorganisms are present in various soil types, as well as their function and size also vary like the soil in which organic matter is of high content is usually linked with high microbial community and diversity. Soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) act as the nutrient source in soil functioning while they also indicate the size of the microbial community and fertility of the soil (Fu *et al*., 2015).

Soil microbial biomass (SMB) may help in foretelling the natural storage of carbon content in the soil with the proper management practices. SMB (soil microbial biomass) may also sense short-term changes and indicates the number of microbes present in soil (Nautiyal *et al*., 2010). The fertility of the soil and the functioning of the ecosystem can be predicted by the qualities of microbes present in the soil. Moreover, we may say that there are two main essential parameters that represent the soil microorganisms, i.e., SMB (soil microbial

biomass) and fundamental diversity. These two factors are the most sensitive markers of impacts caused by management on the biological characteristics of the soil (Fu *et al*., 2015).

Microbial biomass is composed of main bacteria, fungi, and actinomycetes, and it is the most active and living soil organic matter (Roscoe *et al*., 2006). A large part of Agroecosystems directly depends on the activities of microorganisms; it also requires a sufficient amount of soil microbial biomass (SMB), which is also necessary for its management and production (Mendes *et al*., 2011). Soil microbial biomass carbon present in the soil is also necessary to evaluate because it helps to identify the most functional and dynamic pool of soil organic matter (Mendes *et al*., 2011).

MBC (microbial biomass carbon) and MBN (microbial biomass nitrogen) are known as the active nutrient reservoir in the soil, as they indicate the level of soil fertility and the size of microbial communities. The high nitrogen content in the soil does have a negative impact on the microbial communities present in the soil, as it limits their functional diversity by modifying the characteristics and source of organic matter (Nair and Ngouajio, 2012). Any alteration in the ecosystem may be detected as a quick response by microbial biomass as it plays the role of a vital ecological indicator (Powlson and Jenkinson, 1981). The total percentage of microbial biomass carbon present in the soil is probably 1 to 3% of the total organic carbon of soil (Heijboer *et al*., 2016).

The potential hydrogen (pH) is an utmost important factor of the soil which helps in sculpturing the structure of the microbial community present in the soil; nevertheless, studies have shown the negative relationship of pH with the biomass of soil (Ai *et al*., 2015).

It has been proven by the study that physicochemical qualities represent that the productivity of the soil is related to the soil texture, and it can also define the capacity of the particular soil type (Patnaik *et al*., 2013).

Nutrient availability and the presence of moisture also have an impact on the texture of soil; hence the ecosystem of the microbes and their activity are also gets affected (Naveed *et al*., 2016).

One of the most critical elements of the soil is the organic Carbon present in the soil. SOC (soil organic carbon) has an encouraging effect on the aeration, permeability, and structure of soil while sustaining the warmth and moisture of soil, hence providing for the efficiency of the ecosystem (Prescott *et al*., 2000).

Another essential function of the SOC (soil organic carbon) is to retain the fertility of the soil. The nutritional stress can arise if the organic Carbon of soil drops from 1%, while on the other hand, if the soil is rich in organic Carbon, it will also be rich in microbial biomass (Kallenbach *et al*., 2011).

Furthermore, the variation in season also impacts the properties of soil and causes instabilities, i.e., the Temperature of the soil, moisture content, quantity of organic matter, activity of the root, quantity, and quality of the microbial community, etc. (Chen *et al*., 2003)

Different microbial populations have different minimum and maximum thresholds levels in which their growth and activity vary, so seasonal variations in the Temperature and humidity of the environment will ultimately alter the size and activity rate of the microbial biomass population (Barbhuiya *et al*., 2004)

Moreover, it is not possible to scrutinize the mutual impacts of these factors on soil microbial biomass (SMB) because of their long- and short-term influence; however, the studies with restricted data of field with the division of time and space can be executed out in various land types (Moazzam *et al*., 2016).

1.6 Objectives

The objectives of the study were:

a. To assess and relate carbon and microbial assessment in different soil types under seasonal variations.
b. To determine the relationship between pH, temperature, rainfall on MBS, TOC, TN, and phosphorous content present in soil.

CHAPTER 2

Literature review

In 2013, researchers conducted a study claiming that other than temperature, the availability of moisture and nutrients also fluctuates the composition of soil microbial biomass (Serna-Chavez *et al*., 2013).

Shen and his co-workers performed a study and generated a theory that a rise in altitude may have some different effects on the make-up and activity of microbial biomass in various ecosystems. Their study proved the significant rise in the functional genes of microbes along the rise in elevation gradient (Shen *et al*., 2016).

A study revealed the variation in the microbial community along with the altitude gradients (especially in large altitudinal scales), which is proved by Bryant and his colleagues in 2008 that the bacterial community decreases drastically from the lower altitude to the higher, i.e., from 2460m to 3380m in Rocky Mountains of Colorado (Bryant *et al*., 2008).

A relevant study showed that the widest variety of bacteria present in the soil at the altitude of 820m, which is considered a medium altitude in the mountainous subtropical forests of China revealed that fungal diversity decreased monotonically with increasing altitudes (Meng *et al*., 2013).

The research in the Hyrcanian forests of northern Iran provides the results, revealing that the variation in the fungal community decreases as the altitude increases (Bahram *et al*., 2012).

In 2020 Hammad and his co-workers proved that forest lands have a great potential for C sequestration than crop areas (Hammad *et al*., 2020). They conducted a study by comparing C sequestration stocks in different land regions, i.e., forest land cropland, agro forest land, and orchids in the arid regions of Pakistan. Soil samples were collected and analyzed for different physio-chemical properties like pH, EC, NPK, SOM, and soil organic carbon. Plant biomass and C content were also determined. Their results revealed that the high C sequestration value was 64.55 Mg ha^{-1} in the forest land, while the lowest C sequestration potential was found to be in cropland, which is 33.50 Mg ha^{-1}. It was also concluded that forests sequester more C content amount, so reforestation can help in lowering the impacts of climate change.

Another study was conducted by Omar and his researchers in south New Mexico, US. They analyzed almost 21 soil properties from the fall of 2015 to the summer of 2016. Three sampling sites were chosen, which include alfalfa, Upland cotton, and beacon. Of 21 measurement soil properties, some are soil organic matter (SOM), pH, soil electrical conductivity, nitrate-nitrogen (NO_3-N), extractable phosphorous (P), extractable potassium (K), and some micronutrients. They concluded that soil managers need to account for seasonal variability in order to assess the changes in soil quality. Their results deduced that soil organic matter (SOM) was significantly higher in the winter season while values of soil quality indicators like (NO_3-N), K, and P were lower in the spring season (Omar *et al*., 2016).

In 2016, Smith assessed the potential for harmful emissions from soil carbon sequestration and biochar addition to land and also the potential global impacts on land use, water, nutrients, albedo, energy, and cost. Results indicate that soil carbon sequestration has practical negative emission potential (every 0.7 GtCeq. Yr^{-1}) and that they potentially have a lower impact on land, water use, nutrients, albedo, energy requirement, and cost, so they have fewer disadvantages than many negative emissions technologies NETs. Limitations of soil carbon sequestration as a NET center around issues of sink saturation and reversibility (Smith, 2016).

Zifcakova and his team conducted a study in 2016 on Picea abies-dominated coniferous forest soil in two contrasting seasons, i.e., summer and winter. The microbial community was characterized by the high activity of fungus by recording the difference in abundance between the summer and winter seasons. Moreover, different parameters of soils were analyzed, some of which are dry mass, organic matter (OM), pH, Carbon, Nitrogen, and Phosphorous. The amount of C, N, and P recorded in summer soil was 30.0%, 1.5%, and 72.0 (μg g^{-1}), while in winter soil, the values recorded were 29.5%, 1.5%, and 68.0 (μg g^{-1}), respectively (Zifcakova *et al*., 2016).

To investigate the spatial and seasonal variation, another study was conducted in 2014 in China by Zhao and his team. Two main attributes, i.e., soil organic carbon (SOC) and total nitrogen (TN) were analyzed and then correlated with the different soil properties like soil moisture, salinity, carbon-nitrogen (C/N), and carbon phosphorus (C/P) ratio. The soil was collected from five different sampling sites during three seasons. Their study proved that

carbon-nitrogen ratios were higher in the summer season and lowered in the spring season. The soil organic carbon density end total nitrogen density was ranked higher in the summer season while they are recorded as lower in the autumn season (Zhao *et al*., 2016).

In another study conducted in Spain by Hueso-González and the research members under Mediterranean climatic conditions, parameters like SOC, pH, and EC were examined over the time period of two years. The readings were noted under present natural conditions first, and then the soil was amended with different types of biochar like straw mulching, sheep manure, etc. then, and after some specific time interval, the change in the readings was observed. The data of experimental sites were recorded in Mediterranean climatic conditions having an annual mean temperature of 18 ℃. Under natural conditions at experimental site the values of TC, TN, pH, EC were recorded as 12.5, 0.2, 8.0 and 501.0 $\mu S\ cm^{-1}$ respectively (Hueso-González *et al*., 2014).

A study was conducted in 2009 by Almagro and his research team in dry Mediterranean areas of southern Spain. Three different sites were selected, including a forest area, an abandoned agriculture field, and a rainfed olive grove, for the analysis of soil temperature, soil respiration, and soil water content along with the soil carbon dioxide efflux rate. The experiment was performed over a time period of two years (Jan 2006-Dec 2007) in which Co_2 efflux was measured monthly. As we are interested in forest areas, the lowest Temperature was recorded in the month of December 2006, which was 5.5 ℃, and the highest Temperature for the forest area was recorded as 29 ℃. The values for the soil water content of forest areas, the lowest and highest values, were recoded as 3.5% and 22%, respectively (Almagro *et al*., 2009).

A field experiment was conducted in deciduous forests of Ghana to examine the microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP). This field experiment consists of three amendment sites, one control, and a cropping system. Samples were taken within each cropping season after three weeks intervals time period. This study revealed that microbial biomass carbon has a positive correlation with soil organic carbon. However, microbial biomass nitrogen indicated more temporal fluctuations than biomass carbon, while microbial biomass phosphorus also revealed negative values at 42 - 63 days after amendment (logah *et al*., 2010).

To analyze the effects of altitude and seasons on the soil microbial biomass carbon (SMC), soil microbial biomass phosphorus (SMP), and soil microbial biomass nitrogen (SMN), a comparative study was carried out between two forests named the Tarai Sal Forest (TSF) and hill Sal Forest (HSF) in Nepal. The fumigation extraction (FE) method was adopted to analyze the soil samples in the summer, rainy, and winter seasons. Non-fumigated samples were taken as a control. The results revealed that microbial biomass carbon was 66% higher in HSF than in TSF. Distinct seasonality was also observed in soil microbial biomass. In summer, microbial biomass carbon was maximum, while in the rainy season, it was minimal in both forests. The microbial biomass Carbon was reduced to 46 from 67% in HSF and by 32 to 80% in TSF (Bhattarai *et al*., 2020).

The spatial variability of soil microbial biomass of three sites, i.e., agricultural land, grazing land, and natural scrub forests in Pakistan, was investigated by a group of researchers. A hundred samples from each site were collected in the form of soil monolith. The analysis for soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) was carried out in laboratories for all of these sites, and the results were compared. The outcomes of this study revealed that MBC, MBN, and MBP were comparatively maximum in the forest samples. Soil microbial biomass has a negative correlation with pH and electrical conductivity, while SMB has a positive relation with total organic carbon (TOC), NO_3-N, and available phosphorus (P) (Moazzam *et al*., 2016).

Arunachalam and Arunachalam presented that the effects of season have been observed to have the same effects on the humid subtropical north Indian forests of India (Arunachalam and Arunachalam, 2000).

Sudden changes in the environmental conditions like drying of soil, temperature fluctuations in soil may be the reason of the low amount of MB-C, MB-N, MB-P because of the death of microbial biomass. The mineralization done by microbes is the start of the plant growing season was also revealed by the study (Singh *et al*., 2010).

CHAPTER 3

Methodology

3.1 Study area

The study was conducted in four different sites of Islamabad and its surroundings. These sites are Murree, Margalla, Shakar Parian and NUST. The soil was collected from undisturbed forestland. About twelve representative samples were collected, three from each site. Each sample was the mixture of three sub-samples. Different names along with the different seasons was assigned to each sample. Some physical parameters were performed on-site, and readings were calculated. These samples were than stored in airtight bags and refrigerated at 4℃ in IESE/SCEE NUST laboratories. Furthermore, different analysis was performed by using these samples in specific laboratories. The samples were air-dried for 24 hours and passed through a 2 mm sieve. Their physicochemical properties were determined and were stored for later use.

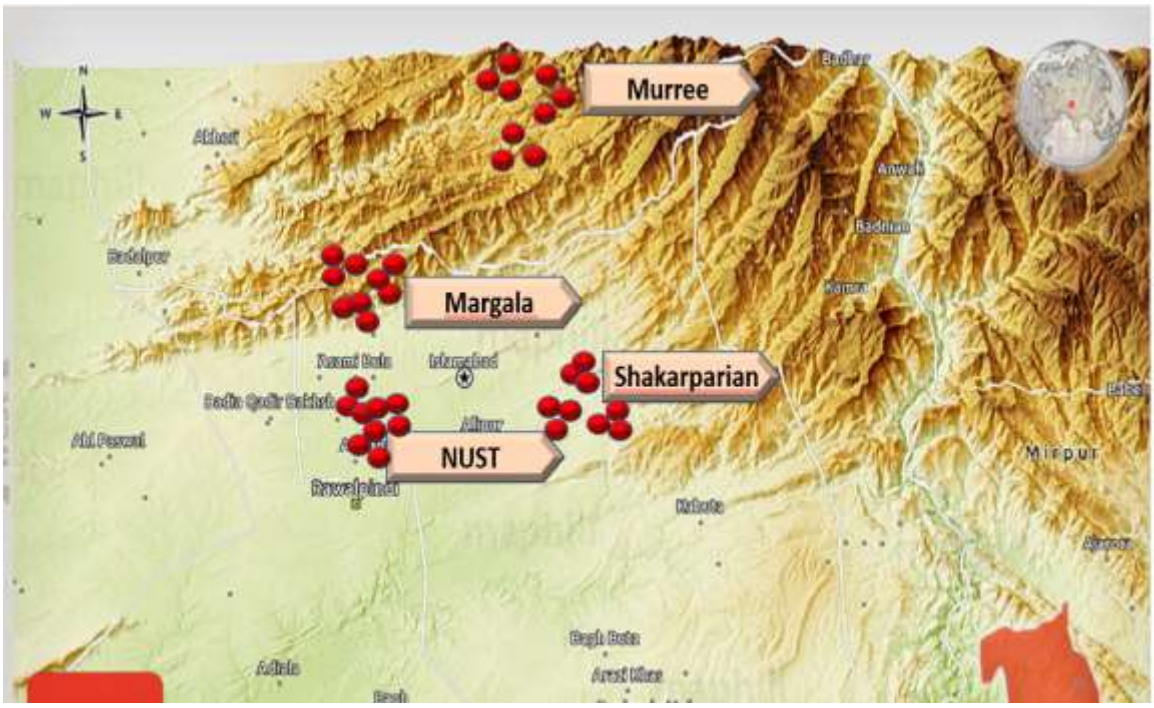

Figure 3.1 Satellite map of the study area

Table 3.1 Coordinates and elevations of sites

Sr.no	Site name	Elevations	Coordinates	
		(m)	(N)	(E)
01	Murree	1374.79	33.85	73.38
02	Margalla	1140.12	33.76	73.07
03	Shakar Parian	570.41	33.69	73.07
04	NUST	72.995	33.64	72.99

Table 3.2 Coding of samples and sites along with seasonal variation

Sample names	Spring	Summer	Autumn	Winter
	Coding	Coding	Coding	Coding
NUST 1	Nt 1-sp	Nt 1-su	Nt 1-au	Nt 1-wn
NUST 2	Nt 2 - sp	Nt 2-su	Nt 2-au	Nt 2-wn
NUST 3	Nt 3-sp	Nt 3-su	Nt 3-au	Nt 3-wn
Shakarparian 1	Sp 1-sp	Sp 1-su	Sp 1-au	Sp 1-wn
Shakarparian 2	Sp 2-sp	Sp 2-su	Sp 2-au	Sp 2-wn
Shakarparian 3	Sp 3-sp	Sp 3-su	Sp 3-au	Sp 3-wn
Murree1	Mr 1-sp	Mr 1-su	Mr 1-au	Mr 1-wn
Murree 2	Mr 2-sp	Mr 2-su	Mr 2-au	Mr 2-wn
Murree 3	Mr 3-sp	Mr 3-su	Mr 3-au	Mr 3-wn
Margala 1	Mg 1-sp	Mg 1-su	Mg 1-au	Mg 1-wn
Margala 2	Mg 2-sp	Mg 2-su	Mg 2-au	Mg 2-wn
Margala 3	Mg 3-sp	Mg 3-su	Mg 3-au	Mg 3-wn

3.2 Prepration of the soil

After the collection of soil samples from various sites, packed soil samples were collected in small quantities, which then air dried for specific parameters from time-to-time. The dried samples were then crushed with the help of mortar and pestle. The flattened soil was then sieved with the help of 2 mm sieve mesh and then used for furthur analysis.

3.3 Physical Parameters

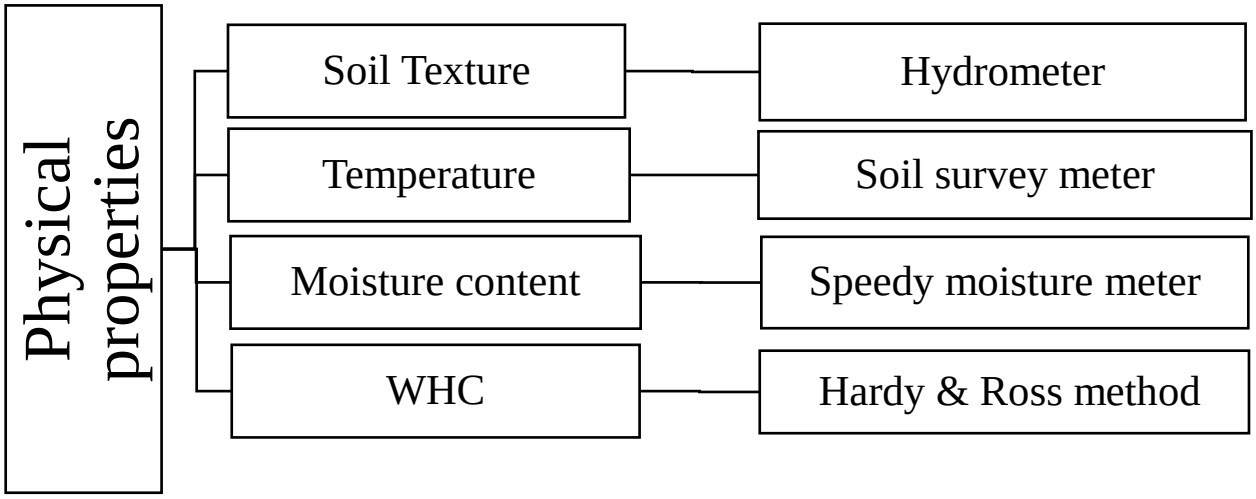

Figure 3.2 Flow diagram for physical properties

3.3.1 pH

The potential of hydrogen present in different forms is represented as pH. The soil was air dried for the analysis and then subjected to further process. The pH meter used for this process is known as the Lutron, Taiwan multimeter (WA 201). For analysis, the soil was dissolved in distilled water with a ratio of 1:10 or 1:20. Take 1g of soil and dissolve it in 100ml of distilled water to make a solution. The mixture was gradually swirled from time to time and let sit for 20 or 30 minutes before analyzing. After some time, the solution was tested by pH meter by placing the probe of the meter into the solution directly until the reading on the LCD settled down and produced the beep sound. Note down the reading, reccord three readings of each sample, and repeat the same procedure for other samples.

3.3.2 Temperature

The temperature of soil was measured on sampling site by using the soil survey on-site instrument meter. This instrument can measure the Temperature of the soil in Fahrenheit and Celsius degree scale. Before using the device, it is calibrated to zero. The results of soil temperature (℃) are then noted directly form the LCD present on the device.

3.3.3 Water Holding Capacity

The water holding capacity of soil was determined as per methods described by Harding and Ross (1964). A funnel containing filter paper was placed on a measuring cylinder. On the filter paper, 25 g soil was placed, and 25 mL water was poured over it gently. The

excess water was allowed to filter through for 30 minutes until the water stopped dripping. The final volume was noted to determine the maximum water holding capacity of soil.

3.3.4 Soil Moisture

Soil moisture is an important parameter which plays part in the characterization of soil. The speedy tester is a portable system comprising a vessel with an integral pressure gauge, a weighing scale, and a carry case. The water content of the soil samples taken from the field was also found with the Speedy Moister Tester. This device consists of a low-pressure vessel equipped with a pressure gauge and an analogue scale and test accessories and is used to measure practically moisture content of various materials such as soil, aggregate, dust, and powders. Moisture measurements are made by mixing the soil sample of a certain weight with a calcium carbide reagent in a closed pressure vessel. Reagent reacts chemically with water in the soil sample producing acetylene gas that in turn increases the pressure within the vessel. The pressure increase in the chamber is proportional to the amount of water in the sample and the dial reading is read directly from the pressure gauge of the device. The accuracy of the device is 0.5% and the test speeds range from 45 seconds to 3 min.

To obtain the water content with the Speedy Moister Tester, 20 g soil sample was poured into the chamber of Speedy vessel. After those pulverizing balls were placed into the chamber and was added two full scoops of reagent to the Speedy cap cavity. The sample was mixed with the reagent holding the Speedy horizontally and shaking it in an orbital motion to make the balls spin around inside the Speedy vessel for 20 seconds. After resting 20 seconds, repeat this process twice or thrice. Water content was read directly from the pressure gauge, keeping the Speedy horizontal and at eye level. Free moisture within the sample reacts with the reagent to produce a gas and pressure rise within the vessel that is proportional to the amount of moisture. The dial reading was noted, and the moisture content value was obtained by using the following equation (Kurtuluş *et al.*, 2019).

$$\boldsymbol{Mc\ (\%) = \frac{DR \times 100}{100 - Dr}}$$

Where, Mc is moisture content of soil in percentage while DR represents the dial reading of the speedy moisture meter.

3.3.5 Soil texture

Different texture of the soils was determined by hydrometer (Bouyoucos, 1962). The silt and clay measuring hydrometer method depends on the impact of particle size on the dissimilar settling velocities in the water column. Percentages of sand, silt and clay were determined by hydrometer method for each soil. After measuring the percentages, USDA textural triangle was used to assign the soil a textural class. The relative proportions of the soil give various soil textures within textural triangle. By using the hydrometer method and following the USDA textural triangle our three different soil textures were classified as sandy loam, silt loam and silty clay loam.

3.4 Chemical parameters

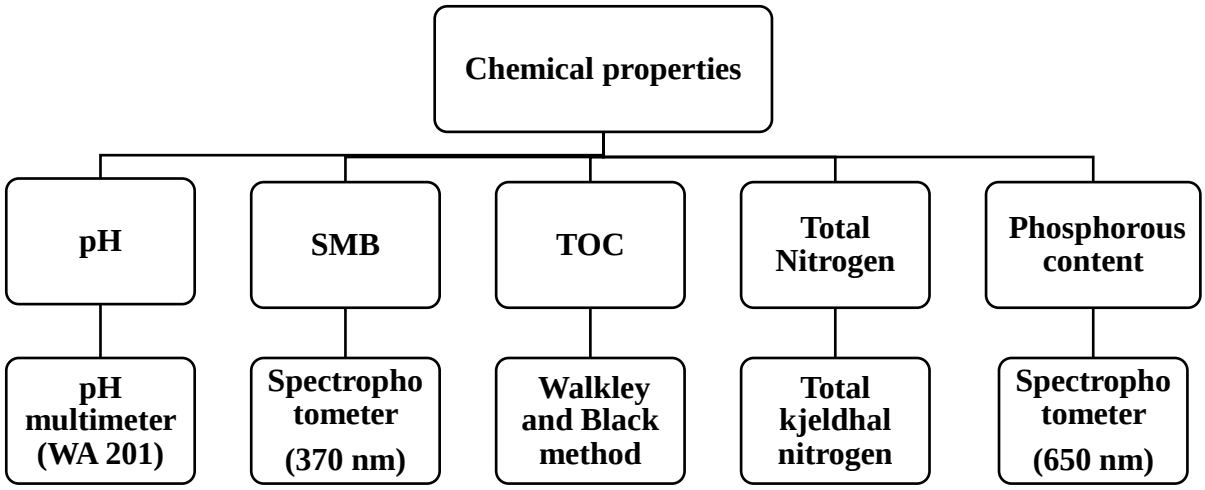

Figure 3.3 Flow diagram of a methodology for chemical properties

3.4.1 Phosphorous

The main role of phosphorous in the soil is to accumulate and transfer the energy resulting from photosynthesis, used by plants for their reproductive and growth activities. The phosphorous in the soil is present in two forms, i.e., organic and inorganic. Organic phosphorous is not available for plants and is in immobilized

form, while organic phosphorous can be readily available for plants through mineralization (Bhantana *et al*., 2021).

Sample Preparation

Molybdate blue method was used for the determination of phosphorous concentration in the soil. Take the soil sample and place it in the oven for the night at 50°C to remove the moisture and let it cool down completely. Carefully cover it with foil paper so that it drys the soil to avoid the breathing of dust coming off from the dry soil (Doolitle, 2014).

Take a 250 mL volumetric flask and add 50 ml distilled water to the flask. Add 0.75 g of ammonium sulfate to this solution, let it dissolve, and then slowly add 5 mL of concentrated sulphuric acid to the solution. The ammonium sulfate solution will get hot. Let it cool, and then dilute the solution up to the mark with distilled water. Take 10g of dry soil, add 200 mL of ammonium sulfate mixture, and seldomly shake over half an hour. Now filter the sample through fine filter paper and keep it a side. The color of the sample will be slightly brown or maybe clear.

Preparation of standard

Standars of phosphate solution should be prepare with different quantities of KH_2PO_4 so that variety of range may be obtained.

First of all prepare standard phostphate solution. For preparing the standards of phosphates,take out 0.220 g of solid potassium di-hydrogen phosphate (KH_2PO_4) in 500 ml volumematric flask now, pippet out 10 ml of this phosphate standards solution into 200, 250, 500, 1L of volumetric flask and dilute it upto the mark, these will considered as the standards of 15, 12, 6, 3 ppm solution respectively. For 4.5 ppm solution, prepare 15 ml of standard solution in 1L of volumertric flask and dilute it with distilled water upto the mark. Label each solution their respective concentrations and store it in a safe place.

Preparation of Complex

Dissolve 5 g of ammonium molybdate into 100 mL of water. Transfer this to a 500 mL volumetric flask. To this add very slowly 160 mL of concentrated sulfuric acid. If the flask becomes very hot, stop, and wait for it to cool over 15 minutes. Once all the acid has been added, dilute the solution to 500 mL with water – add the water slowly with stirring. Collect 10 mL of sample in a 150 mL conical flask and add 20 mL of water, 2 mL of molybdate

solution and a spatula of ascorbic acid crystals. Heat this slowly to boiling (a deep blue/green color should develop) and then allow it to cool. Repeat this for all the standards.

To 500 ml volumetric flask, add 5 g of ammonium molybdate and add 100ml of distilled water.

To this solution, add 160 ml of concentrated sulphuric acid very slowly. The solution will become realy hot, stop the procedure and wait for it to cool.

When the solution accomplished the tempreature of room, then add distilled water to dilute the upto 500 ml and swerl it gently.This solution will be named as molybdate solution.

In 150 ml conical flask, add 10 ml of filtered sample, 20 ml of distilled water 2 ml of molybdate soltuion and a spatula of a ascorbic acid crystals.

Heat this solution slowly untill it starts to boil (greenish or bluish colour will develop) and then allow this misture to cool. Repeat this procedure for all the standards

Spectrophotometric Analysis

1. For the blank, fill spectrophotometric tube with water (this will be considered as a blank while working with spectrophotometer) and place it into the spectrophotometer. Set the absorbance to 650 nm (this is reddish light) takes an absorbance reading. If there is a 'zero' adjust, or a 'blank' function on the spectrophotometer, use this water sample to zero the spectrophotometer.

2. Place the solution of lowest concentration (3 ppm from above) in the cuvette and take a reading. After recording the absorbance, wash the tube and repeat the measurement on the next most concentrated standard, until all the standards have been measured.

3. Place the sample into the spectrophotometer tube. Take an absorbance reading, and record.

Result Calculations

1. Draw a standard curve, by plotting on a graph the absorbance of your standard solutions (y-axis) versus the concentration of the standards (x-axis). The straight line will be achieved. Keep taking the readings until the curve is achieved, take the concentration which resembles to absorbance. This is the concentration of phosphate in your liquid sample.

2. These readings can be used furthure for dilution accuracy.

3.4.2 Microbial biomass determination

Standard Preparation

Standard solution containing 137.5 mg L^{-1} glucose in a volumetric flask was diluted to prepare the final concentrations of 10, 20, 30, 40 and 50 mg L^{-1}. These values were used as a standard to calculate soil microbial biomass. Using distilled water as blank, absorbance was measured at 350 nm. The calibration curve developed for soil microbial biomass is presented in Figure 3.4.

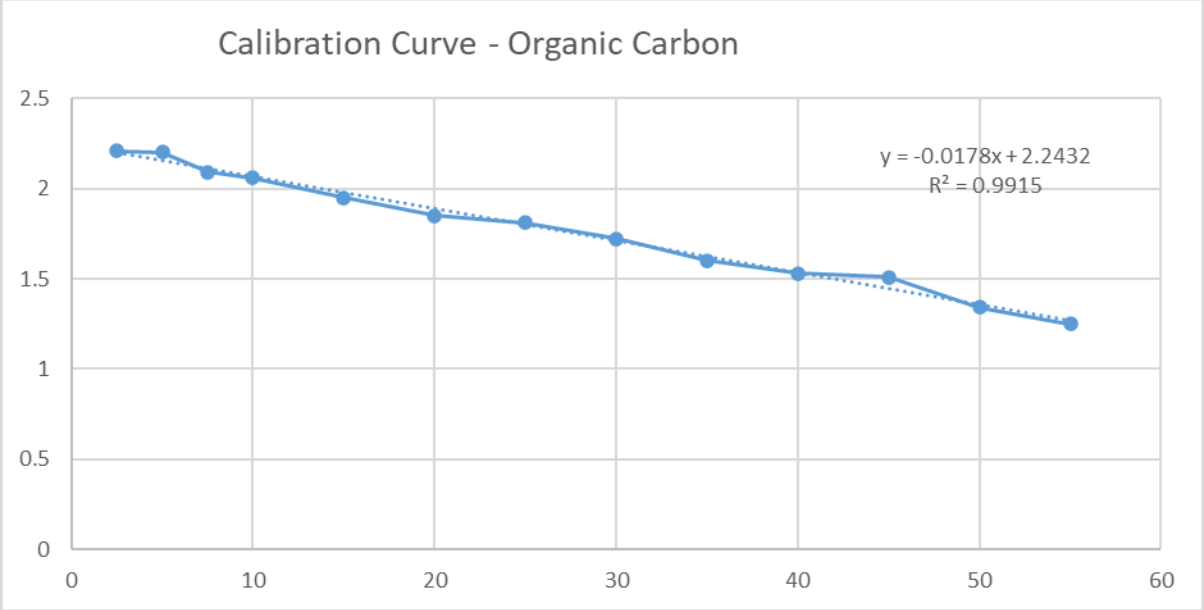

Figure 3.4 Standard curve of total organic carbon

Oxidant Solution

In 40 mL distilled water, 0.128 g of potassium dichromate ($K_2Cr_2O_7$) was added and mixed well. To this, 200 mL concentrated sulfuric acid was added.

Microbial biomass determination

A rapid chloroform fumigation extraction method (Witt *et al.*, 2000) was used for the estimation of microbial carbon under different antibiotic concentrations over a period of 15 days. The sieved soil samples were adjusted to 40% maximum water holding capacity (MWHC).

For the experiment, soil samples were at each test day split into two portions for fumigation and non-fumigation in screw cap vials. Non-fumigated soil samples taken as control were extracted with 5 mL 0.5 M K_2SO_4 immediately, shaken for 60 minutes at 35 rpm, and

filtered using Whatman No. 42 filter papers. The extracts were frozen until further use. For fumigation, 57 µL chloroform was added to each soil sample, followed by incubation for 24 h in dark at 25°C. After the incubation period, chloroform was allowed to evaporate from the samples by placing them in a fume hood for 30 min. The microbial Carbon was then extracted using potassium sulfate as done for non-fumigated samples.

From the extracts, 1.6 mL was pipetted out in screw cap vials and 2.4 mL oxidant solution was added to it. The vials were then placed in the COD reactor at 150°C for 30 min to achieve biomass C oxidation. Spectrophotometric analysis of samples was then measured at 350 nm using a UV-1700 spectrophotometer (PG-Instruments-T60UV, UK) (Witt *et al.*, 2000). Biomass C was calculated using the following formula:

$$\text{Biomass} = EC/KEC$$

where EC is the difference of extractable C between the fumigated soil samples and the non-fumigated ones. The extractable part of microbial C (KEC) for the proposed method was given as 0.51.

3.4.3 Soil organic carbon

Prepration of soil

Total Organic Carbon was determined through Walkley and Black (1934) method. This method may be used for the the analysis of organic carbon in variety of different samples i.e. residues of plants and animals, soil, coal etc. level of organic Carbon is ususally higher in surface soil. The soil was air dried, grounded and the sieved through 0.50 mm sieve mesh and placed in 500 mL Erlenmeyer flask.

Walkley and Black method is actually a wet oxidation method that determines the organic Carbon in the soil. Oxidation in soil takes place by 1N $K_2Cr_2O_7$ solution. This reaction is assisted by the generation of heat produced upon adding H_2So_4 in the dichromate solution. The remaining dichromate is titrated with Ferrous Ammonium Sulphate (FAS) which is freshly prepared (Jha *et al*., 2014).

Reagents

Pottasium Dichromate (1N)

Dissolve 49.04 g of pottasium dicromate in distilled water and dilute it to 1000 ml.

Ferrous Ammonium Sulphate (FAS) (0.5N)

To prepare 0.5N FAS, dissolve 196 g of FAS in distilled water, add 20 ml H_2So_4 and dilute it to 1000 ml in a volumetric flask.

H_2So_4 (Conc.)

Orthophosphoric acid

Di-phenyl indicator

Take 0.25 g of diphenylamine, add 10 mL of distilled water and 50 mL of H_2So_4 and swirl it softly. Store this indicator in dark bottle (De Vos *et al*., 2007).

Procedure

1. Weigh 0.5 g of air dried soil sample in a conical flask and add 10 ml of already prepared pottasium dicromate solution in it.
2. Pour 20 ml of Conc. H_2So_4 slowly and swirl the mixture as it will get hot due to exothermic reaction. Let this mixture cool down.
3. Add 200 ml of distilled water and 10 ml of orthophosphoric acid in the solution.
4. Add 4,5 drops of di-phenylamine indicator in the soultion, the color of solution starts to turns dark blue.
5. Titrate this solution with prepared 0.5N FAS solution until the colour of solution turns from blue to green. This will be the end point.
6. Calculate the results and deduce the answer.

For Blank: use the same procedure as explained above without soil and calculate the readings. Now these reading will be used as blank's results.

$$\textbf{Organic carbon \%} = \frac{10(B-T)}{B} * 0.003 * \frac{100}{S}$$

3.4.4 Nitrogen determination by TKN method

Kjeldhal nitrogen has been widely used method from the past century to determine the nitrogen content in the organic and in-organic samples. It is an extremely versatile method which can handle a wide range of samples i.e Food (dairy, meat, grains) environment (seeds, soil, water, sludge), beverages, chemical and pharmaceutical indutries (paper, textile, polymere, plastic) etc.

Total kjeldhal nitrogen (TKN) is usually composed of organic nitrogen, ammonia (NH_3) and ammonium (NH_4^+) in the chemical analysis of different compounds. furthermore to calculate the total nitrogen (TN) the concentration of nitrate-N and nitrite-N are calculated and then they are added to the TKN (Roig et al., 2012).

This TKN method is usually composed of three major steps:

1. Digestion
2. Distillation
3. Titration

In the digestion process all the nitrogen bonds breakdown and the sample converts organically bounded nitrogen into the ammonium ions (NH_4^+). The Carbon and hydrogen that are present in their organic forms, combines together and makes carbondioxide (CO_2) After addition of acid the sample changes its color and turns black. After the complete decomposition of the sample, the liquid becomes clear after one to two hours of boiling, which determines the end of chemical reaction.

During the distillation process, sodium hydroxide reagent is added to raise the pH end to convert ammonium to ammonia. The released ammonia is distilled into and indicating boric acid solution. The ammonia (NH_3) is transferred to the receiving vessel by the steam distillation.

Moving on to the third step which is titration, for the titration process the boric acid (as an absorbing solution), The acid-base titration is performed by using this standard solution of sulfuric acid or hydrochloric acid and the mixture of indicators.

Moreover depending on the normalities, the acids are set to 0.01N-0.5N Which depends on the concentration of ammonium ions present. the endpoint will be the shift of color from green to pink and then the amount of ammonia is calculated by using different formulas.

for the blank, prepare the balnk with the same sample amount and distilled water. Add all the requiring reagents. Perform TKN necessary steps (digestion, distillation and titration) and calculate the values.

Process scheme

Add 1 gram of soil sample, 20 ML of distilled water, 20 ML of concentrated H_2So_4, 7 g of K_2SO_4 end 0.2 g of $CuSo_4.5H_2O$ in the digestion flask. Mix the sample homogeneously by gently swirling the digestion tube.

Turn the tap water on and check the level of NaoH and distilled water in the scrubber bottles.

Turn on the digester and let the digestion unit complete the process. After the completion of process let the digester solution cool down at room temperature.

The digestor tube is placed in the Distillation unit, after its Temperature is back to normal.

Distill the contents of kjeldhal flask, Where ammonia (NH_3) is condensed and captured into the 50 ML indicating boric acid solution.

When ammonia reacts with the boric acid solution it turns from red violet color to light green color. This process can take approximately 5 minutes.

For the titration, within the titrate add four to five drops of mix indicator and titrate this solution with 0.02 N of H_2SO_4 until the solution turns pink. This is the end point of titration.

Reagents

1. Digestion reagents
    a. 20ML distilled wated
    b. 20ML Conc. H_2So_4
    c. 7g K_2So_4
    d. 0.2g $CuSo_4.5H_2o$
2. NaoH- $Na_2S_2O_3$ reagent

    Dissolve 500g of NaoH and 25g of $Na_2S_3O.5H_2O$ in distilled water and dilute it to 1000ML.
3. Mix-indicator solution

    Dissolve 0.2 grams of methyl red indicator in 100ml ethyl or isopropyl (95%) alchol. Dissolve 0.1 gram methylene blue in 50ml ethyl or isopropyl (95%) alcohol. combine these two reagents to make mix-indicator solution.
4. Boric acid solution

Take 20g of H_3Bo_3 in distilled water, add 10ml mixed indicator solution and dilute it to 1000ml with distilled water.

Calculations

$$\text{TN in mg/g} = \frac{Vol.of\ H2So4 * Normality * 14}{Mass\ of\ sample}$$

$$\text{N(\%)} = \frac{mg/g}{10}$$

CHAPTER 4

Results and discussions

4.1 Soil physical parameters

Table 4.1 Soil physical parameters influenced by seasonal variations

Seasonal variation	Sampling sites	Soil physical parameters		
		Soil Moisture (%)	Atm. Temperature (ºC)	pH
Spring	Nt	10.62	31	7.00
	Sp	18.20	32	7.33
	Mr	11.61	28	6.83
	Mg	15.47	31	7.17
Summer	Nt	4.23	41	7.02
	Sp	11.36	40	7.36
	Mr	9.17	36	6.92
	Mg	9.65	39	7.19
Autumn	Nt	9.05	17	7.01
	Sp	16.55	17	7.14
	Mr	12.87	18	7.00
	Mg	19.13	16	7.19
Winter	Nt	20.57	15	6.99
	Sp	22.04	14	7.23
	Mr	23.55	13	7.01
	Mg	25.09	15	7.09

Results of soil physical properties are presented in Table 4.1, along with seasonal variations for different soil samples. The highest moisture content is found in margala soil samples during winter, which is 25.09%, while the lower moisture content is observed in NUST soil samples, which is 4.23% during summer.

Table 4.1 also shows that the maximum atmospheric temperature is observed in the NUST soil sampling site during the summer season, which is 41℃, and the lowest temperature in the Murree site, i.e., 13℃.

Moreover, pH is also presented in this table which depicts the highest pH of samples is in the neutral range. For better microbial biomass, optimum moisture, adequate nutrients, and pH are essential (Sadeghi and Raeini, 2016).

Table 4.2 Soil physical parameters uninfluenced by the seasonal variation

Sampling sites	**Physical properties uninfluenced by seasonal variation**	
	WHC (%)	**Soil texture**
NUST	10.0	Sandy loam
Shakar parian	14.67	Loam or silt loam
Murree	18.00	Loam or silt loam
Margala	19.33	Loam or silt loam

Table 4.2 shows the results of some physical properties of soil samples, which are not affected by seasonal variations. Table 4.2 depicts that the soil of the Margala sampling site is loam or silt loam, having the highest water holding capacity (WHC). I.e., 19.3%while lowest WHC is observed in NUST samples which are 10.01%, because of the sandy nature of the soil.

4.2 Chemical properties of soil

4.2.1 Soil organic carbon

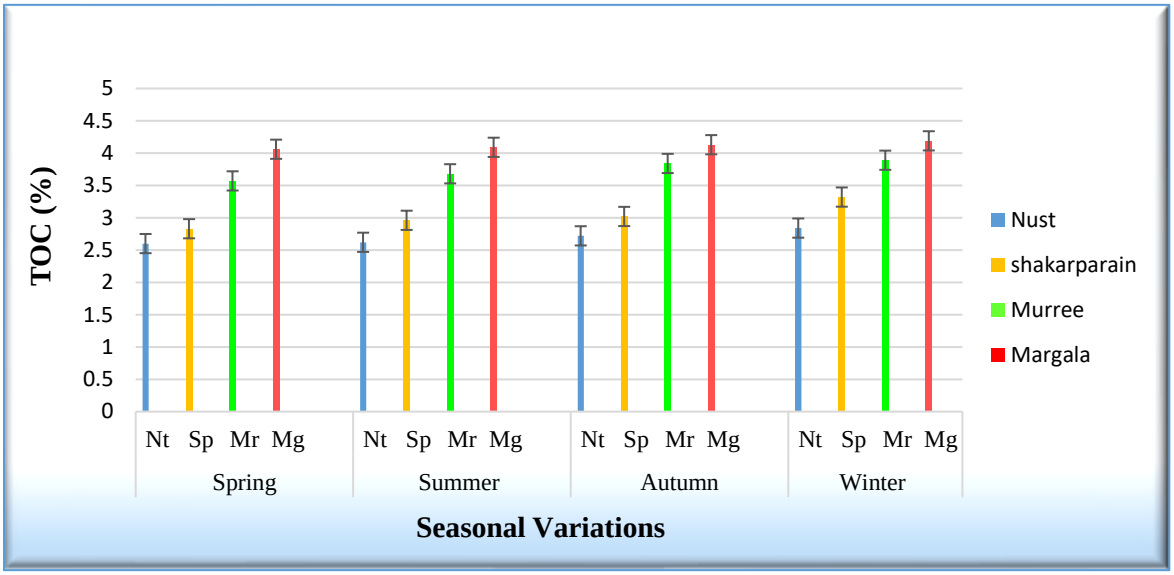

Figure 4.1 TOC in soil samples along with the seasonal variations

Figure 4.1 shows the soil organic carbon (SOC) concentration in different samples during four seasons of the year. Results show that the highest SOC was found in winter season (margala soil) which is 4.19 %, and the lower SOC was found in NUST soil samples during the spring season, i.e., 2.60 %. In terms of seasonal variation, the SOC concentration decreases in the order of winter, autumn, summer, and spring. The higher SOC in the winter is due to the fact that in the spring, summer, and autumn seasons, the plant litter deposits in the soil and mostly become part of the soil in winters (Babur and Dindaroglu, 2020).

In 2018, Babur assessed the forest soils of Karstic areas of the eastern Mediterranean. Soil organic carbon concentrations are analyzed in the study areas. Results showed that in winter, autumn, summer, and spring, the soil organic concentration was 4.62, 4.56, 4.45, and 4.33%, respectively. As in this study, the winter season had higher soil organic carbon content while in spring the soil organic carbon concentration was the lowest. This trend is similar to the current study, with the higher soil organic carbon concentration in winters and the lowest in the spring season (Babur and Dindaroglu, 2020).

Similar organic carbon content in soil is found in the study conducted by Siles and fellow researchers in South Tyrol, Italy. They analyzed the soil organic carbon (SOC) for the spring and autumn seasons. Results of this study depicted that the soil organic carbon in

autumn is higher than that of the spring, which is 29.25 and 18.19%, respectively. So, in the present study, the SOC is also higher in autumn than in summer (Siles *et al*., 2016).

4.2.2 Microbial biomass

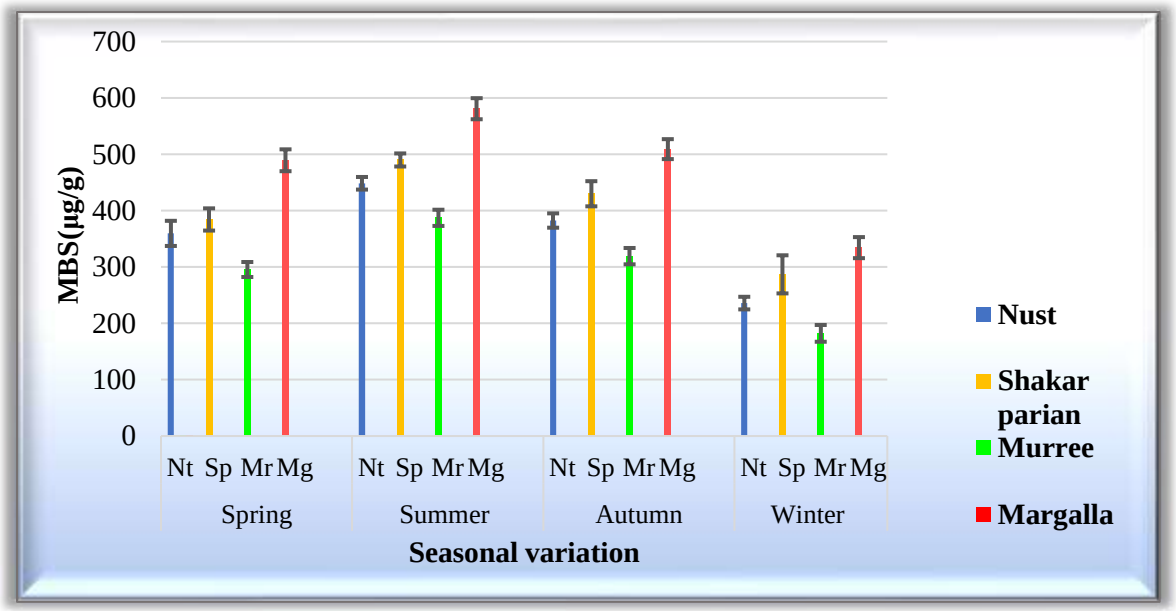

Figure 4.2 Represents microbial biomass content with seasonal variations

Figure 4.2 shows that the highest microbial biomass is found in the margala hill forest in the summer season, which is 580.8 µg/g. At the same time, the lowest microbial biomass is found in Murree at winter, which is 182.2 µg/g. Figure 4.2 also depicts that in terms of seasonal variation in microbial biomass concentration, the overall microbial biomass is higher in summer while it was lowest in winter for all selected samples.

Microorganism needs a set of optimum climatic conditions and nutrient availability for their maximum reproductive activities and growth. Regarding nutrient availability, the important nutrients essential for microbial growth are organic Carbon, available nitrogen, and available phosphorous (Khan *et al*., 2010). On the other hand, certain climatic conditions are also essential for microbial growth, i.e., Temperature (27-30°C) and moisture holding capacity (20%) (Borowik & Wyszkowska, 2016).

Similar results are also found in the study conducted by Bhattarai and Mandal in Tarai Sal and Hill Sal Forest, Nepal. They analyzed the soil microbial biomass carbon in the study area. Results of MB-C in the study depicts that MB-C is highest in summer season which

is 442.7 µg/g while lowest MB-C is found in summer season that is 350 µg/g (Bhattarai & Mandal., 2020).

In 2020, Lepcha & Devi analyzed the soil MB-C in Himalayan soils near north Sikkim, India. Results found out that the MB-C in summer is the highest which is 429.13 µg/g, while it was lowest in winter season i.e., 338.46 µg/g (Lepcha & Devi., 2020).

As, the MB-C in summer is found to be highest because in summer the plant growth and nutrient demand is minimal, so the maximum accumulation of carbon takes place in the soil (Bhattarai & Mandal., 2020).

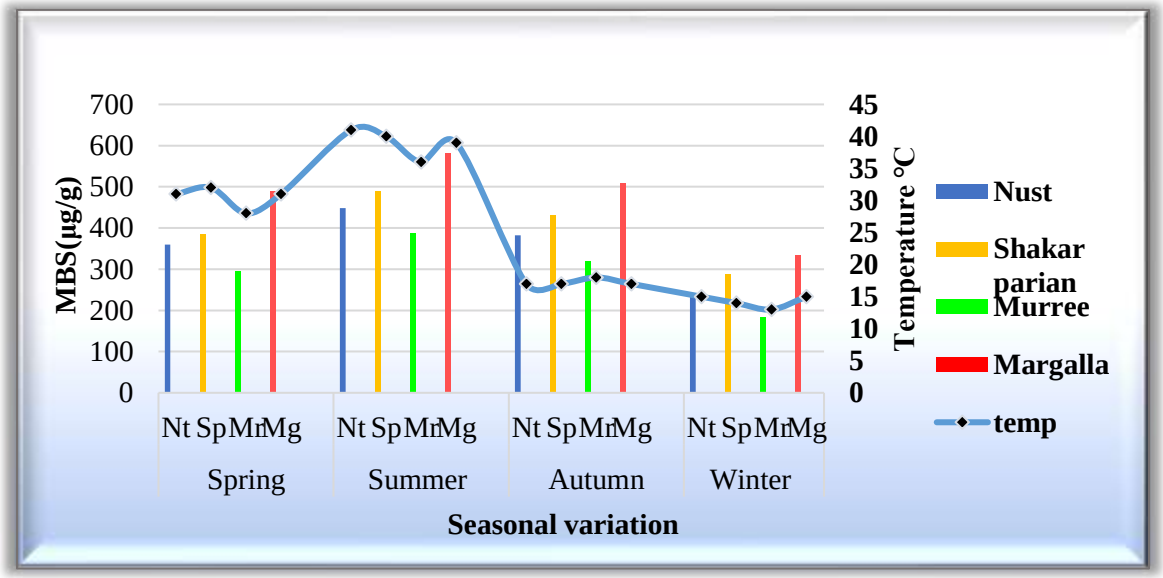

Figure 4.3 Association between microbial biomass and temperature

Figure 4.3 represents the association between temperature and MBS. It represents that MBS and temperature have a direct relationship as they are both highest in summer and the lowest in winter. Temperature has a great influence on microbial activity. As the temperature of soil increases, it speeds up the mineralization of nitrogen rate, which overall increases the microbial activity (Onwuka and Mang, 2018). Statistical analysis has also proved the positive relationship through the Pearson co-relation matrix between MBS and temperature, which is 0.601 at a 0.01 significance level.

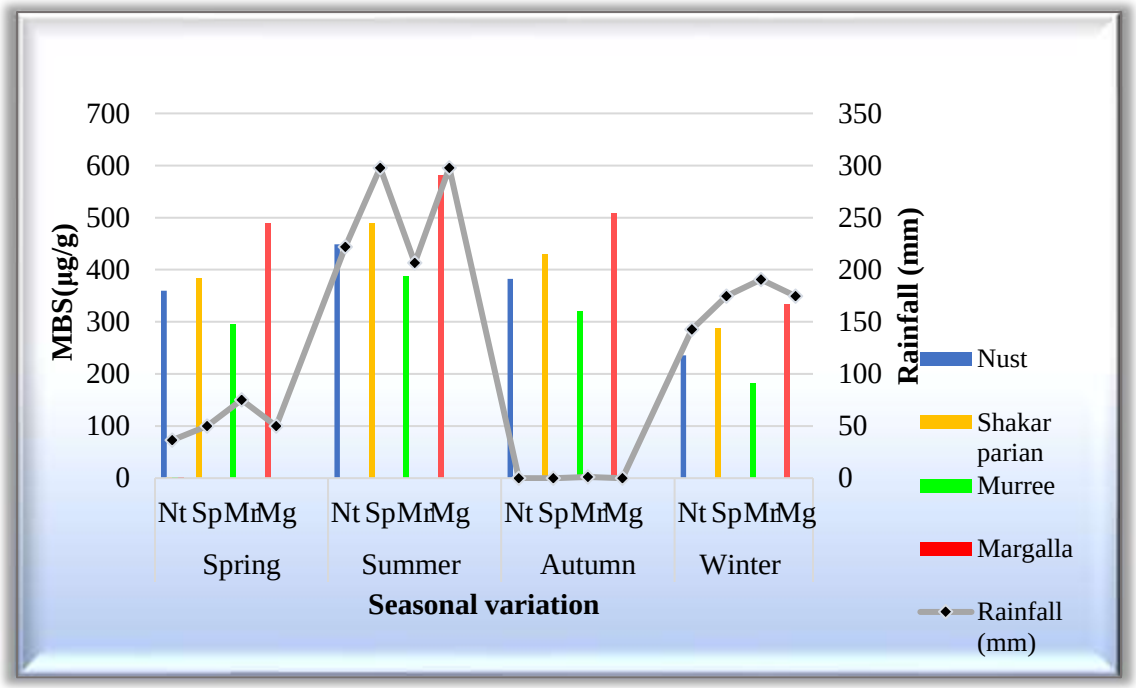

Figure 4.4 Relationship between microbial biomass and ppt

Figure 4.4 shows the association between MBS and precipitation in different samples during four seasons of the year. In figure 4.4, it can be observed that during the summer season, MBS, and precipitation (Ppt) are maximum. Still, in the autumn season, MBS is relatively higher (319.2-509.1µg/g), and the precipitation lowest is 0-0.01mm. So, MBS and precipitation represent anomalies. This is because of the fact that MBS is directly related to moisture content, and precipitation is only one factor in terms of the moisture content of the soil. Other factors that affect soil moisture content are atmospheric Temperature, WHC, soil texture, etc. (Borowik and Wyszkowska, 2016).

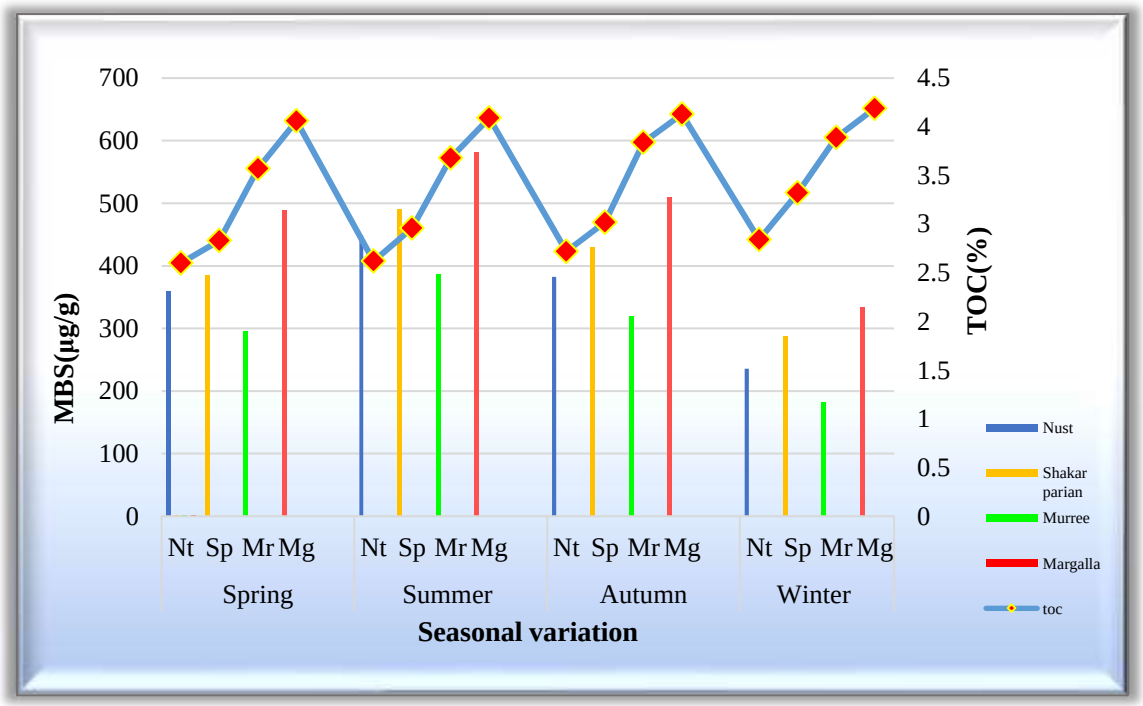

Figure 4.5 Association between microbial biomass and TOC

Figure 4.5 shows the relationship between total organic carbon (TOC) and microbial biomass (MBS) in forest soils of selected sampling sites. Figure 4.5 depicts that TOC has a direct relationship with the SMB. The microbial biomass increases with the total organic content concentration in the soil. Previous studies revealed that SMB concentration in the soil highly depends upon Temperature, soil WHC (moisture), TOC, soil pH, essential nutrient availability, and other climatic factors. Microorganisms depend upon the organic carbon present in the soil for their reproduction, growth, and increase in biomass (Siles *et al*., 2016; Bhattarai & Mandal., 2020).

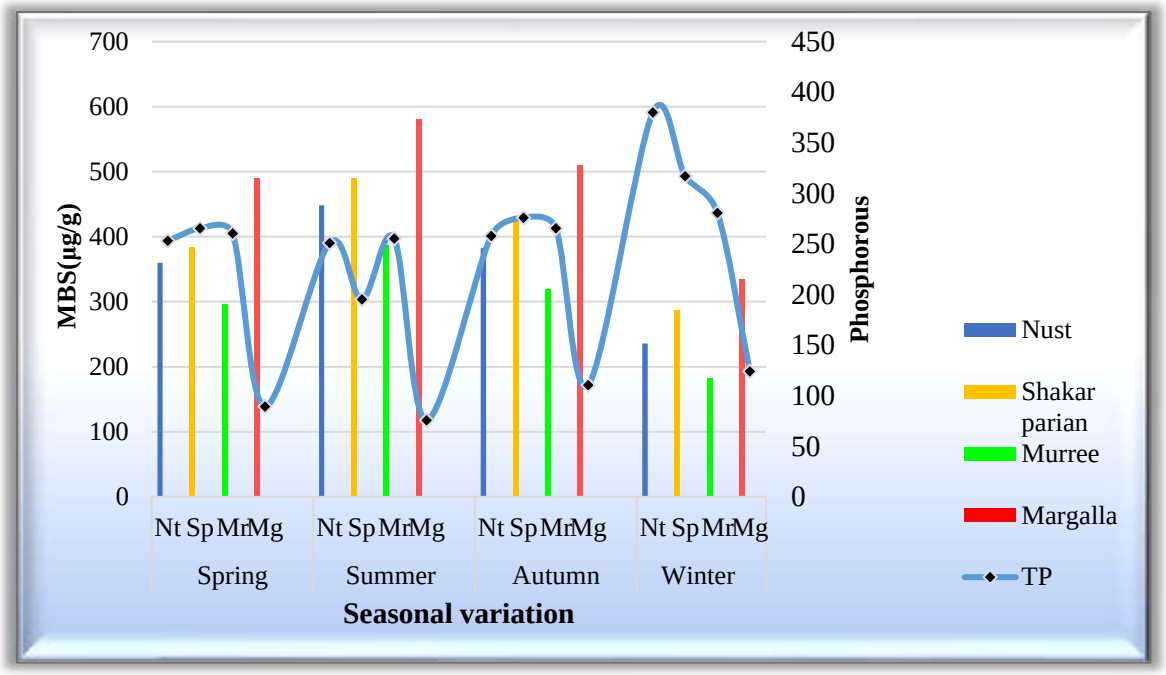

Figure 4.6 Association between MBS and phosphorous

Figure 4.6 shows the relationship between microbial biomass and total phosphorous in different seasons for all samples. In Figure 4.6, it can be observed that MBS and phosphorous content do not have a clear relationship. MBS is highest in summer, while phosphorous concentration is highest in winters. Phosphorous is an essential nutrient for the microbial process for mineralization of organic and inorganic phosphorous and storing it in soil biomass (Tian *et al*., 2021). Some other optimum climatic and environmental conditions are also essential for microbial growth, so it can be concluded that phosphorous has a role in SMB. Still, it is not the only factor, so that's why the clear relationship has not been observed.

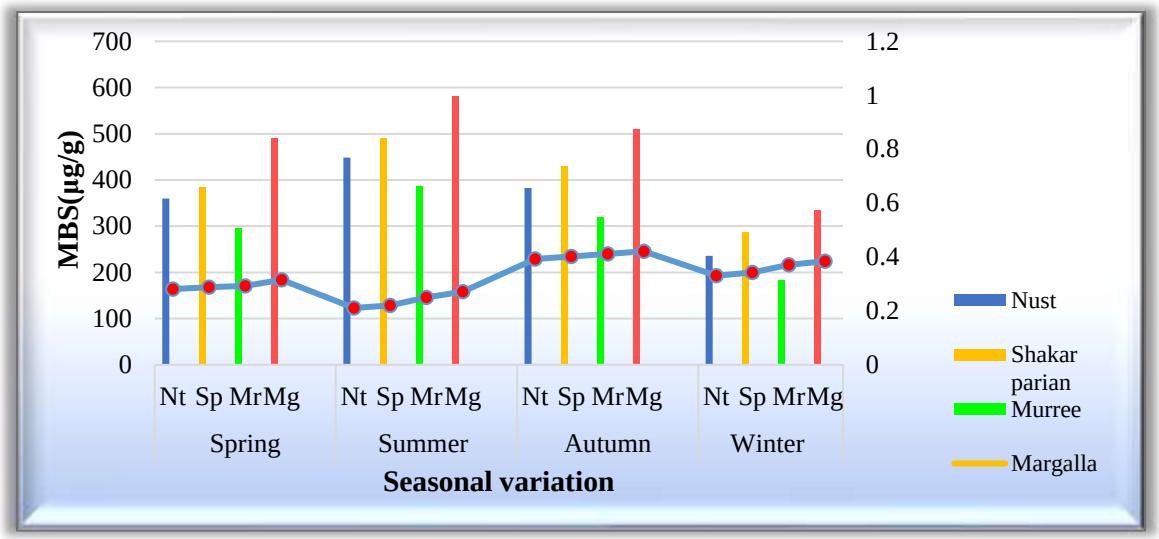

Figure 4.7 Relationship between MBS & TN

Figure 4.7 shows the relationship between MBS and TN at different sampling sites in all four-year seasons. Figure 4.7 shows that MBS and TN show different trend, in all seasons except spring.TN concentration depends directly upon the litter falls from plants, because the source of nitrogen directly comes from plants. As litter is high in autumn season, while it takes times for microbes to convert TN into different form of nitrogen, so that's why the concentration of TN is high in the autumn season. On the other hand, MBS is highly dependent upon optimum temperature and the dependency of TN on temperature is comparatively lesser (Allison *et al.*, 2010).

4.2.3 Phosphorous concentration

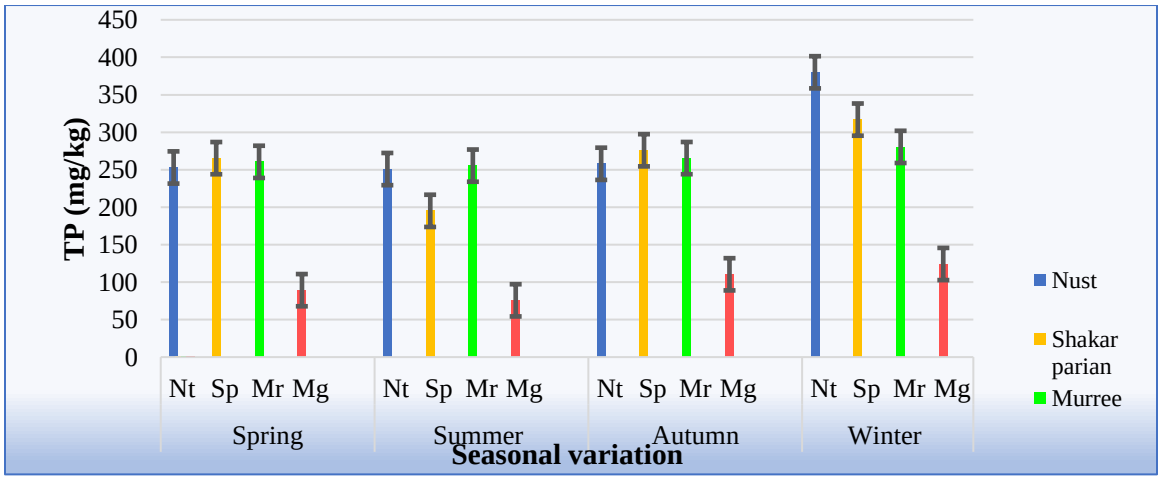

Figure 4.8 Seasonal variation of phosphorous present in the soil

Seasonal variation influences phosphorous concentration present in the soil. Figure 4.8 shows that the highest phosphorous is found in the winter, followed by autumn, spring and summer.

In 2008, Chacón and his co-workers analyzed the soil characteristics of the Mapire river, Venezuela floodplain. The findings of the study depict that the total phosphorous concentration in May (summer) is 270.3 µg/kg, which is lower than the concentration of phosphorous found in November (winters), which is 296.7 µg/kg. So, this trend is similar to the trend shown in the current study (Chacón *et al*., 2008)

Total phosphorus concentration in soil is influenced by climatic factors such as temperature, moisture, aeration, etc. These factors influence the phosphorus mineralization from the decomposition of organic matter. Furthermore, in the current study, the total phosphorus concentration is lower in summer season as compared to the winter season because phosphorus concentration highly depends upon erosion and runoff in soil, which is higher in summer due to high rainfalls in the study area (Luo *et al*., 2021).

4.2.4 Total nitrogen

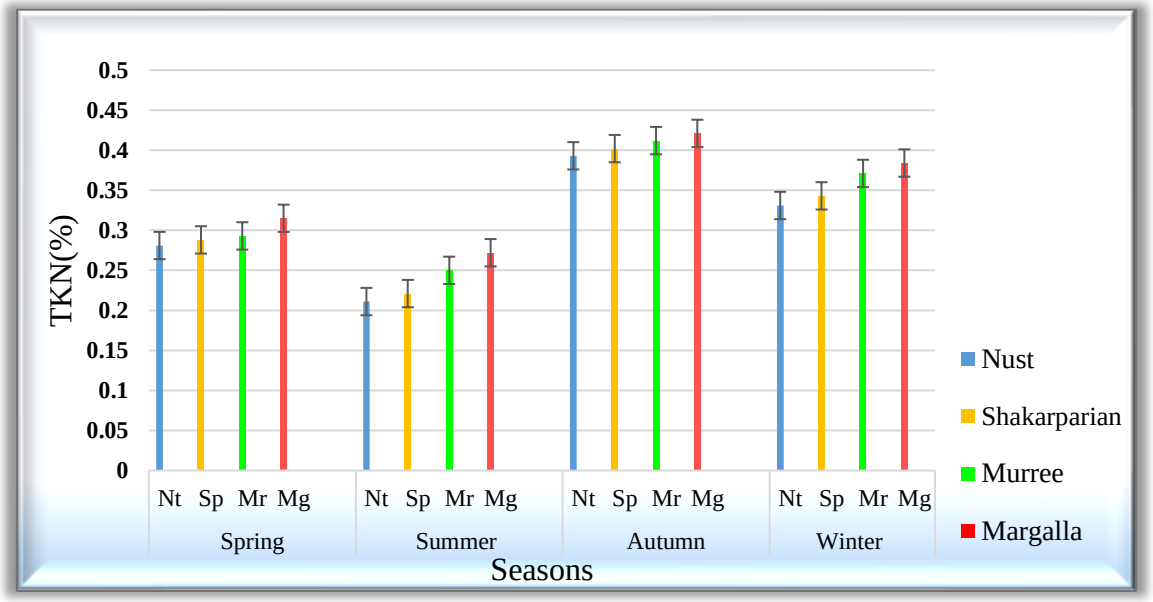

Figure 4.9 Seasonal variation of TN between soil samples

Figure 4.9 shows the total nitrogen (TN) concentration in different samples during four seasons of the year. Figure 4.9 shows that the highest TN was found in margala soil during autumn, which is 0.421%, and the lowest TN was found in NUST soil samples during the

summer season, i.e., 0.21%. In terms of seasonal variation, the TN concentration decreases in the order of autumn, winter, spring, and summer.

Similar trends in total nitrogen (TN) concentration in natural forest soil, in terms of seasonal variations, are found in the study of Salim and co-researchers in 2015 at Jhilmil jheel wetland, Haridwar-Uttrakhand, India. They observed higher concentrations of TN in the autumn season and lower TN concentrations in the summer season (Salim et al., 2015). In another study (conducted by khan and co-researchers in 2010 for characterization of the Quercus forest of Chitral, Pakistan) the TN concentration was found in the range of 0.07 to 0.52% at different soil samples. These values are nearer to the results of TN concentration in the current study (Khan *et al*., 2010).

4.3 Statistical Analysis

The statistical evaluation was carried out on data by using software SPSS-16.0 to determine the association between different physico-chemical parameters in different forest types by applying Pearson correlation matrix analysis. MBS showed a significant positive relation with temperature, season, and sites at the significant level of 0.01 and 0.05. On the other hand, TOC also presented a positive so-relation with soil texture, water holding capacity (WHC), soil moisture content ($P \leq 0.05$), TN ($P \leq 0.01$), and seasonal changes. TN and phosphorous concentration also depicted the positive association between them. Furthermore, WHC showed a negative correlation with temperature and pH. No association was found between SMC and temperature.

Pearson correlation matrix

Soil parameters	Sites	Season	MBS	TOC	P CON.	TN	temp	pH	ST	WHC	SMC
Sites	1										
Season	0.000	1									
MBS	.286*	.470**	1								
TOC	.966**	0.191	0.118	1							
P CON.	-.637**	0.269	-.691**	-.533**	1						
TN	0.247	.593**	-0.278	.354*	0.038	1					
temp	-0.047	-.779**	.601**	-0.205	-.331*	-.874**	1				
pH	0.006	0.000	.469**	-0.133	-0.230	-0.023	0.058	1			
ST	.645**	-0.072	0.262	.566**	-.375**	-0.079	0.198	0.212	1		
WHC	.973**	0.000	0.189	.939**	-.517**	0.234	-0.057	-0.018	.734**	1	
SMC	.304*	.617**	-.486**	.372**	0.123	.557**	-.737**	0.276	0.126	.319*	1

Table 5.1 Pearson correlation matrix between parameters of soil

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

CHAPTER 5

Conclusions and Recommendations

5.1 Conclusions

In the present study, characterization of forest soils was conducted against various seasons in the vicinity of Islamabad-Murree Forest areas of Pakistan. Soil sampling was conducted from four different forest sites in Islamabad during four seasons of the year. Physical parameters of the soil, such as atmospheric temperature, water holding capacity, and moisture content, are analyzed on site. Chemical parameters of soil, which include pH, TOC, TN, Phosphorous concentration, and MBS, are analyzed using standard procedures in the laboratories of IESE and NUST. Statistical data analysis was conducted using Pearson correlation test to evaluate significance of data. Results of study show that overall, for all sampling sites, highest TN and TOC were found during winters, whereas lowest TOC was recorded during spring season. Phosphorous concentration in different sampling sites was highest in winter and lowest in summer season. In comparison, MBS was observed to be higher in all samples during the summer and was lowest in winter. Statistical analysis shows a positive correlation between MBS and TOC which is 0.118 in 0.05% significance level, while Correlation between Phosphorous concentration and WHC was found to be negative.

5.2 Recommendations

1. All the soil parameters should be analyzed for the period of 2-3 years to obtain the more comprehensive soil profile.
2. Furthermore, Soil characterization should also be conducted by adding, additives in soil like synthesize fertilizers, biochar, natural manure against seasonal variation.
3. Studies should be conducted to identify the optimum season for maximum growth of vegetation.

CHAPTER 6

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