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POPULATION DENSITY OF FREE-LIVING NEMATODES AND THEIR RELATIONSHIPS WITH SOME SOIL PHYSICOCHEMICAL PROPERTIES OF ALFALFA

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ABSTRACT

The present study was conducted to evaluate the soil's suitability for the alfalfa crop in Nomal Valley, Gilgit Baltistan (GB), using a variety of physicochemical parameters and free-living soil nematodes as indicators. Ten soil samples from different sites (A1-A10) of the valley were collected in zip-lock plastic bags to test for free-living soil nematodes and physicochemical characteristics. Each sample's free-living nematodes were extracted using the modified Baermann funnel method and counted using a compound microscope (at 40 and 100X). About 100 nematodes were identified from each sample to the family level. Based on feeding behavior, nematodes were divided into groups. Nematode population densities ranged from 102 to 507. The most prevalent feeding group members were bacterivores, while predators were the least common. Cephalobidae was the most frequently occurring family, which indicates soil's fertility. Various physiochemical parameters, including soil temperature, pH, electrical conductivity (EC), moisture, bulk density, and texture, were also measured. The ranges for the selected soil parameters were as follows: pH 7.20-7.80, EC 68-252 mS/m, bulk density 0.91-1.37g/cm^2, and soil temperature 22-30 °C. All sites had slit loam soil texture except A-5, which had sandy loam.

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INTRODUCTION

The most important component of sustainable agriculture is soil quality. Various physical, chemical, and biological markers are used to assess soil quality (Ahmad et al., 2015; Ara, 2015; Arshad et al., 1996; Aziz, 2018). With agricultural techniques, the physical and chemical properties of soil significantly change (Begum et al., 2011). Biological markers are significant and are gradually gaining prominence (Begum, 2019). Numerous living things susceptible to various agricultural management techniques are found in soil. This makes using these markers to link crop production to soil quality useful (Begum et al., 2011). A stable soil that can withstand stress and continue nutrient cycling typically has a high level of biological diversity (Bourget and Carson, 1962; Briar, 2007; Briar et al., 2007; Caswell-Chen et al., 1998). One of the major types of microorganisms in soil is nematodes. In general, nematodes are minute, with just a few species large enough to be seen with the unaided eye. They belong to the phylum Nematoda (Chaudhari, 2013; Duan et al., 2019; DuPont et al., 2009; Ensinas et al., 2016; Zhang et al., 2020). They are known as bio-indicators because of their many characteristics and significant roles in soil processes (Fouri, 2018; Franco-Navarro and Godinez-Vidal, 2007). The soil ecosystem's ability to recover from disruptions more quickly and easily depends on diverse soil (Freckman and Caswell, 1985; Gee and Bauder, 1986; Goodell and Ferris, 1980; Hakl et al., 2007). Knowing how nematodes live can provide you with vivid details on soil disturbances. Most significantly, because of their numerous interactions with other soil species and crucial roles in soil nutrient cycling, nematodes offer an understanding of the conditions of the soil food web (Ingham, 2011; Jennings and Nelson, 1998; Kavut and Avcilglu, 2015; Kelling and Schmitt, 2011; Khan and Chandra, 2017; Kitagami et al., 2016; Li et al., 2016). Nematodes are the most prevalent and diversified group of small (0.2–12 mm) vermiform invertebrates in soil (Mcsorley, 2011; Moser and Frankenbach, 2009; Mumtaz, 2013; Neher, 2001). In general, an environment rich in the organic matter has lower concentrations of plant-parasitic nematodes (Mcsorley, 2011). Nematodes in the crop rhizosphere are abundant and diverse, offering a unique perspective on soil biological activities and being regarded as a good bioindicator for determining the sustainability of a production system's soil (Khan and Chandra, 2017). Nematodes can survive in harsh environments and are widespread due to their great tolerance (Kitagami et al., 2016). Since they are more dynamic and frequently more sensitive than physical or chemical soil qualities, changes in soil biological traits may be a sensitive indicator of soil quality (Neher, 2001). Additionally, nematode populations can alter due to agricultural activities such as crop cultivation, fertilizer, herbicides, insecticides, and other chemicals in the soil, as well as monoculture practices. As a result, many statistical methods and indices have been developed to assess the disruption caused by nematodes in the soil environment (Li et al., 2016). The biological characteristics of nematodes link them to ecological processes in soil, such as nutrient recycling, mineralization, and decomposition (Franco-Navarro and Godinez-Vidal, 2007). Nematode physical characteristics, particularly the mouth region, best reflect the feeding behaviors of nematodes and are categorized into trophic stages in accordance with these characteristics. To recycle nutrients and mineralize nitrogen and phosphorus, bacterivores and fungivores feed on bacteria and fungi (Kitagami et al., 2016). Numerous soil species serve as food sources for soil worms in that habitat. In every stage of life, nematodes have a worm-like structure. The body length is between 0.3 and 0.5 mm. The mouth area, which is so developed that it connects to a feeding habitat, demonstrates the variation in form (Orloff, 2007; Pérez-Hernández and Giesler, 2014; Ritz and Trudgill, 1999; Sun et al., 2018). The most prevalent and recently recognized eating groups include omnivores, plant feeders, fungus feeders, and bacterial feeders (Valsecchi et al., 1995; Wang and Hooks, 2011; Williamson and Husssey, 1996; Yeates, 1979; Yeates et al., 1993). For agricultural chemists and farmers, the physicochemical characteristics are essential for plant growth and land management. These offer knowledge to address issues with applying fertilizers and other nutrients to boost crop output (Chaudhari, 2013). Due to its high output, superior feed quality, and adaptability to many climatic situations, alfalfa is the most important forage crop in the world. Alfalfa holds the top spot compared to all other fodder crops, including meadows. Farmers refer to alfalfa as the "Queen of fodder herbs" because of its high digestibility, extensive growing area, and lack of crop rivalry (Kavut and Avcilglu, 2015). Alfalfa is an extremely resilient crop that produces for at least four years. Although it can be productive for up to 10 years, it is frequently rotated with a grain crop due to production reduction. Alfalfa is not significantly reseeded following the immediate conclusion of the same crop as before. It happens because the same prior plant produced autotoxicity, which prevents the new seeds from establishing themselves properly (Yeates et al., 1993). The objectives of this study are as follows 1) to examine the free-living nematodes connected to the alfalfa crop in Nomal Valley, 2) to determine the physiochemical characteristics of soil used for alfalfa cultivation, and 3) to check the soil quality based on the presence of bioindicators (various feeding groups of nematodes).

MATERIALS AND METHODS

Study area and soil parameters

The soil samples for the research were collected from Nomal valley, GB. The valley is 25 km away from Gilgit city. Ten alfalfa sites (A1-A10) were selected and samples were taken from each site covering the whole valley. The sampling plots of alfalfa were chosen randomly. The soil samples were only collected in the first week of July 2019. Two parameters (biological and physiochemical) were selected to test the quality of soil associated with alfalfa. These parameters determine the quality of soil and the quantity of alfalfa production. Free-living nematodes were investigated as a biological parameter; soil temperature, bulk density, moisture, texture, pH, electrical conductivity (EC), and moisture contents were considered under physiochemical characteristics (Ahmad et al., 2015; Aziz, 2018).

Soil sampling

Three sub-samples were collected from each site in zip-locked plastic bags i.e. for bulk density, and two composites (4 to 5) samples for free-living nematodes and physicochemical parameters. The soil temperature was recorded in the fields along with a GPS reading. A short survey was also conducted to get field history (e.g. type of fertilizer used, irrigation time, previous crop, etc.). A corer was used for bulk density. The samples for nematodes were collected from the rhizosphere of alfalfa plants. All the samples were labelled and transported immediately to the laboratory for further processing. The composite samples for nematode were kept at low temperatures in the refrigerator to avoid any fluctuation before extraction. The samples for soil physicochemical parameters were dried at room temperature (in the shade). Soil samples were sieved via a 2 mm mesh size sieve after drying at room temperature (Ahmad et al., 2015; Aziz, 2018).

Extraction of free-living nematodes

The collected soil samples were subjected to nematode extraction. The modified Baermann funnel method was used to extract the free-living nematode community. About 50 g soil was taken for the extraction process which was kept for three days and extracts from three consecutive days were collected in the centrifuge tubes. This method guaranteed almost 90% extraction of nematode present within the soil sample in 24 h (Goodell and Ferris, 1980; Hakl et al., 2007).

Preservation of nematodes

The extraction of nematodes was followed by preservation. For this purpose, hot and cold formalin (5%) shocks were given to each sample, along with a few drops of glycerin.

Counting of nematodes

Under the compound microscope, all the free-living nematodes from each preserved sample were counted at 40× magnification. A special counting dish with grids was used for this purpose.

Identification of nematodes

Each sample identified a hundred nematodes up to the family level. Identification was carried out using a compound microscope under a 40× and 100× lens, using an appropriate identification key proposed by De Nematoden Van Nederland.

Physicochemical parameters

The soil samples were investigated for the most vital physicochemical parameters concerning their suitability for alfalfa production. The following physicochemical parameters were tested.

Soil temperature

The soil temperature was observed at the sampling spot. A soil temperature probe measured the temperature at three points at each sampling field and the mean temperature was calculated.

Bulk density

Bulk density is the measurement of dry soil weight per unit volume. The sampling for bulk density was done separately by using the corer method. Wet soil weight was firstly calculated and then the soil was dried overnight in an oven at 105°C. After measuring the dry weight of each sample, the Bulk density was computed using the formula (Wang and Hooks, 2011).

$P = M_S \,/\, V_t$

Where;

P= Bulk Density

M_S= Oven-dried Soil Weight

V_t= Corer Volume

Soil moisture

Soil Moisture is the total water content present in the soil. The values of wet soil weight and oven-dried soil weight was utilized for calculating the total soil moisture content. The gravimetric method was used to analyze soil moisture (Gee and Bauder, 1986);

$$\%\ \text{Soil Water} = \frac{\text{weight of wet soil (g)} - \text{the weight of dry soil (g)}}{\text{Weight of dry Soil (g)}} \times 100$$

Soil texture

For the determination of soil texture, the hydrometer method was used. About 51 g of air-dried soil was used for this purpose. The following equations were used to determine the percent clay, sand, and silt (Gee and Bauder, 1986).

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% Clay = 2* hydrometer reading
% Sand = 2*∑ weight of sand retained on sieves
% Silt = 100 − % Clay − % Sand
The values of the soil particles (clay, sand, and silt) were compared with the USDA Soil texture triangle to find the nature of soil texture.

Soil pH

A 1:1 solution of soil and distilled water was prepared for pH measurement. A portable pH meter was dipped, left to stable, and the stable value displayed on the probe was noted (Ahmad et al., 2015).

Electric conductivity

Electric conductivity was measured following a similar process for pH, 1:5 solution of soil and distilled water was prepared. An electric conductivity meter was dipped into the samples, andnoted the electric conductivity value.

RESULTS AND DISCUSSION

The owners of each sampling site/field (A1-10) were interviewed to get a detailed overview of the standard practices related to the Alfalfa crop. A summary of all the sampled sites is mentioned in Table 1.

Mostly cherry trees were found along with the alfalfa crop, a type of agroecosystem. Both organic manure and inorganic fertilizers were used in the fields. Alfalfa is a high-quality, high-yielding and manure-desiring crop.

It requires high phosphorus, potassium, and nitrogen. Manure application to alfalfa has several potential environmental, agroeconomic, and management advantages (Kelling and Schmitt, 2011).

Table 1: Details of each alfalfa site of Nomal Valley, Gilgit Baltistan.

Sites				GPS		
	Existing crops	Fertilizers	Irrigation	North	East	Altitude
A-1	Alfalfa + cherries	Animal manure, white and black	2 days before the sampling	36º05.1	074º17.3	1538 m
A-2	Alfalfa with small cherry trees		1 or 2 days before sampling	36º05.5	074º16.7	1645 m
A-3	Alfalfa + Trifolium repens	Animal manure + inorganic fertilizers	1 day before sampling	36º05.6	074º17.2	1638 m
A-4	Alfalfa	Urea + Animal manure	1 day before sampling	36º05.6	074º17.3	1607 m
A-5	Alfalfa (big andtall) + small cherry trees	Animal Manure + Inorganic fertilizers		36º03.4	074º17.0	1563 m
A-6	Alfalfa	Animal manure + inorganic fertilizers	One side irrigated 2 days before sampling with one side irrigated on the day of sampling	36º05.6	074º17.4	1613 m
A-7	Alfalfa	Animal manure + inorganic fertilizers	1-2 days before sampling	36º05.7	074º17.3	1610 m
A-8	Alfalfa	Animal manure + Nitrophos fertilizers	2-3 days before sampling	36º05.7	074º17.4	1611 m
A-9	Alfalfa + Small cherries	Urea with Animal manure	1-2 before sampling	36º05.2	074º17.2	1603 m
A-10	Alfalfa + Cherries	Animal manure	3-4 days before sampling	36º04.0	074º17.1	1584 m

Analysis of soil free-living nematodes

Soil free-living nematodes are essential members of almost all kinds of soil fauna. They contribute to many soil processes like mineralization and decomposition and are good indicators of soil health (Franco-Navarro and Godinez-Vidal, 2007). The free-living soil nematodes from alfalfa soil (50 g) showed that the population density was highest in A-10 with 507 nematodes and lowest in A-7 with a population density of 102 nematodes (Table 2 and Figure 1). According to Ingham

(2011), agricultural soil can support 100 nematodes in a teaspoon of soil. Grassland can hold up to 500 nematodes, whereas forest soil contains several hundreds of nematodes in a spoonful of soil. The results for the population density in our research resemble that of agricultural soil reported by Ingham (2011). The extracted and identified free-living nematodes of soil were classified into five feeding groups, namely: bacterivores, fungivores, plant parasites, omnivores, and predators (Yeates et al., 1993). The results for feeding groups showed that bacterivores were the most abundant among all the other feeding groups. Fungivores were found to be the second most abundant feeding group (Figure 2). Cephalobidae was the most common bacterivore family found in all soil samples. The soil managed by organic farming practices contains more bacterivores than conventional farming practices (Mumtaz, 2013). Nematodes are present in all kinds of soil. Their presence indicates the quality of soil and its suitability for alfalfa production. Bacterivores and fungivores feed on bacteria and fungi, respectively, and release nutrients into the soil (Begum, 2019). Hence, the abundance of these feeding groups proves the soil is fertile and suitable for the alfalfa crop.

Table 1: Population density (PD) of free-living nematodes of soil.

Parameters	Minimum	Maximum	Mean	Std. Deviation
PD	102	507	215.70	119.619

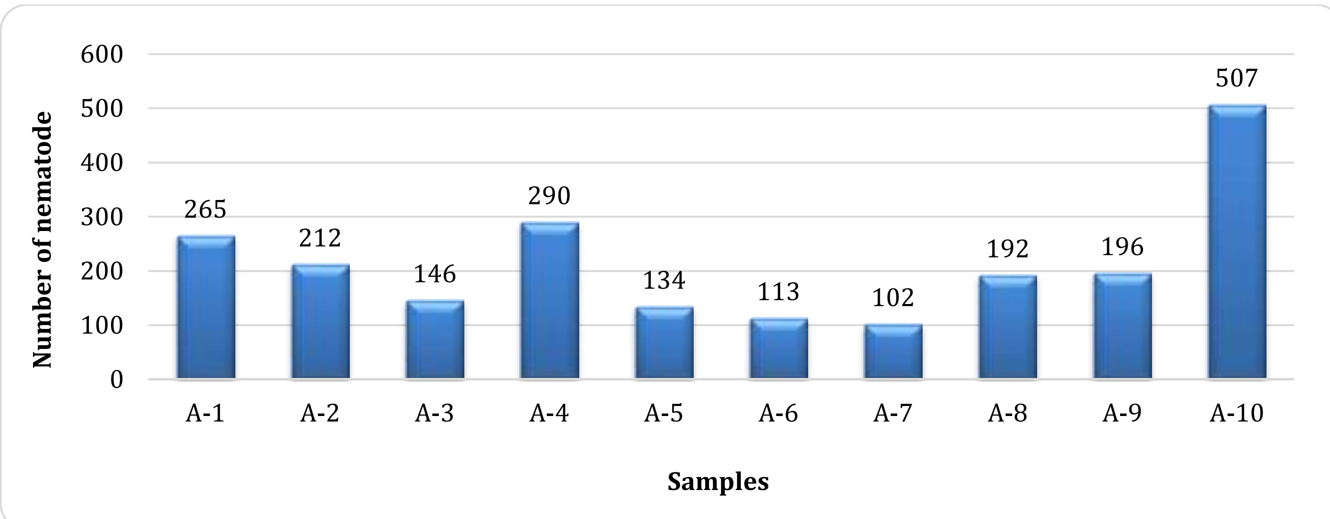

Figure 1: Total number of nematodes in each sample.

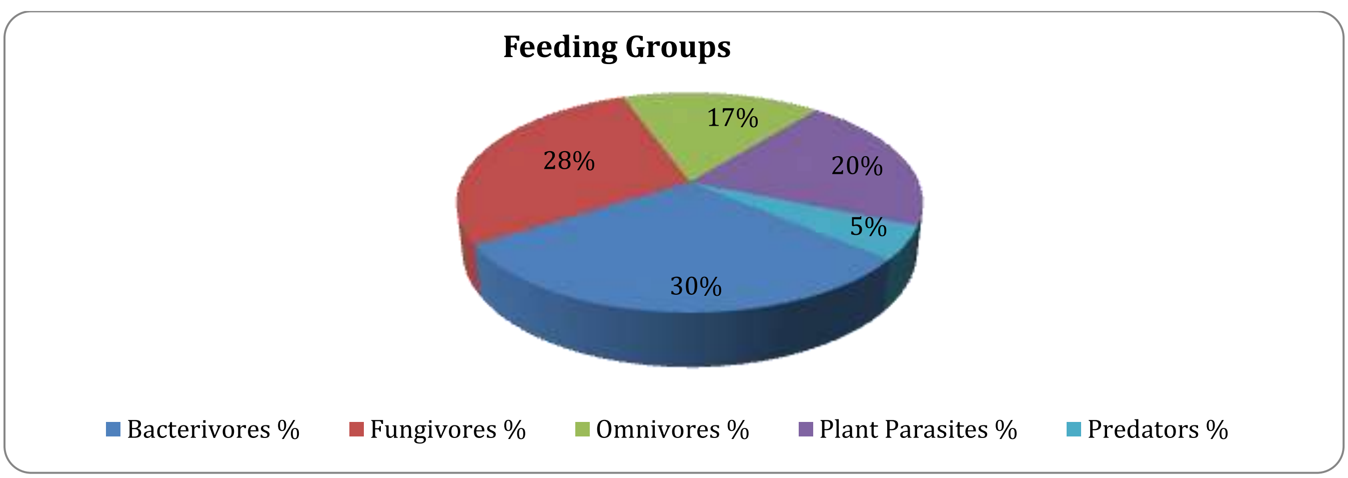

Figure 2: Percentage of all feeding groups of nematodes.

Analysis of physical parameters

Soil temperature

Soil temperature is very important for seed germination and the growth of alfalfa plants. The temperature was determined at the time of sampling. The minimum temperature was 22.06°C at A-1 and the maximum temperature was 30.26°C at A-2 (Figure 3). Alfalfa crop can grow better and puts all its energy into making top growth at the temperature of 25-30°C. At this temperature range, alfalfa grows faster after harvesting (Undersander et al., 1997). The mean temperature for every sample in our study and the literature are similar, thus representing soil suitability for Alfalfa cultivation.

Soil bulk density

Bulk density is a dynamic property which depends on soil texture, organic matter, and relative proportion of sand, silt, and clay. It increases with profile depth due to changes in compaction, porosity, and organic matter content. Hakl et al. (2007) stated that the bulk density of alfalfa is 1.33 g/cm^2 on average in the second year and later. The average bulk density in our study was 1.18 g/cm^2 with the highest value of 1.28 g/cm^2 as shown in Figure 4. The soil of Nomal valley is silt loam. Arshad et al. (1996) found that 1.40 g/cm^2 bulk density is best for root growth in silt loam. The results of our study are slightly lower, indicating that the soil is not ideally suitable for alfalfa to get a huge yield.

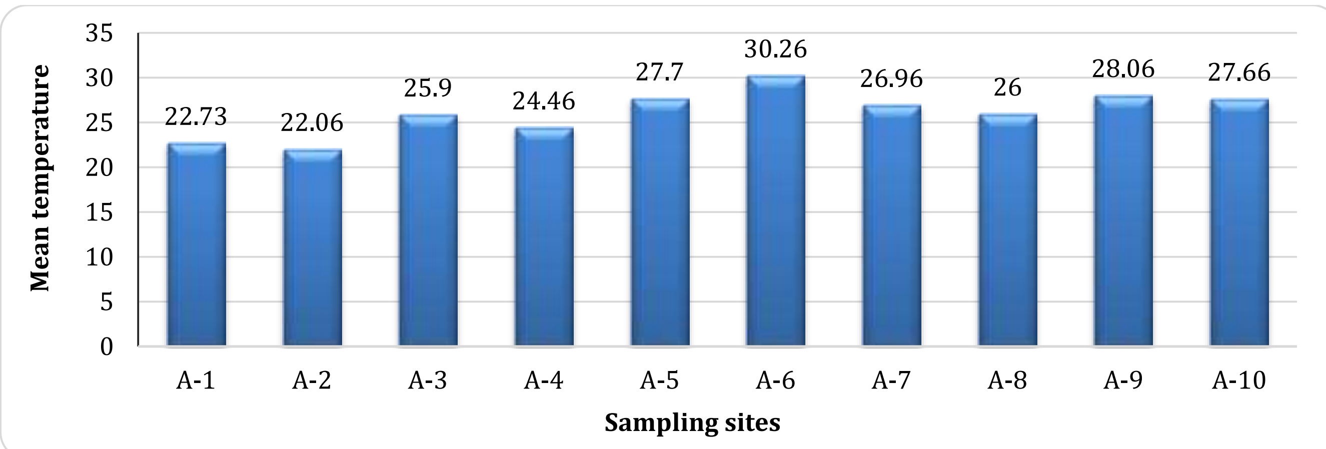

Figure 3: Average temperature of sites of samples.

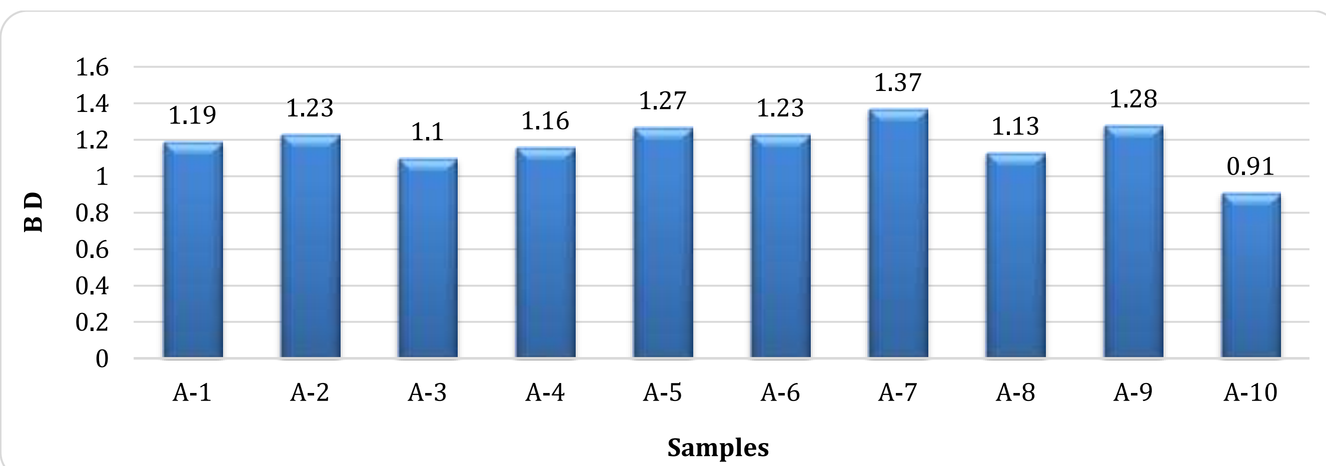

Figure 4: Bulk density at each sampling site.

Soil moisture contents

The soil moisture content was found to be lowest at 7.14% at Alfalfa-8, while Alfalfa-3 and Alfalfa-4 had the highest soil moisture content with a slight difference. Both samples have high moisture content due to irrigation. The plots were irrigated just a day before the sampling. All the values are shown in Figure 5. Water is not equally available throughout the range of soil moisture to all plants. The increase in soil moisture causes a consistent decrease in the root yield of alfalfa (Bourget and Carson, 1962). Alfalfa consumes a higher water concentration and causes desiccation of water in lower layers of soil. About 50-90% of soil moisture is required to achieve the optimum alfalfa yield, but the older the alfalfa fields' gets lower the soil moisture (Sun et al., 2018). The alfalfa plot samples in our study are older than four years which was the reason for the low moisture content in the soil samples. Alfalfa-3 and Alfalfa-4 are cultivated a year before, so they had a high moisture content.

Table 3: Summary of all physicochemical parameters of soil.

Parameters	Minimum	Maximum	Mean	Std. Deviation
ST	22.06	30.26	26.1790	2.52182
BD	.91	1.37	1.1870	.12517
SM	7.41	50.53	23.5380	15.29190
Silt	24.02	50.54	38.6920	6.98305
Sand	31.46	55.98	42.2060	6.70683
Clay	18.00	22.00	19.1000	1.37032
pH	7.20	7.80	7.4700	.18886
EC	68.00	252.00	151.6400	56.00048

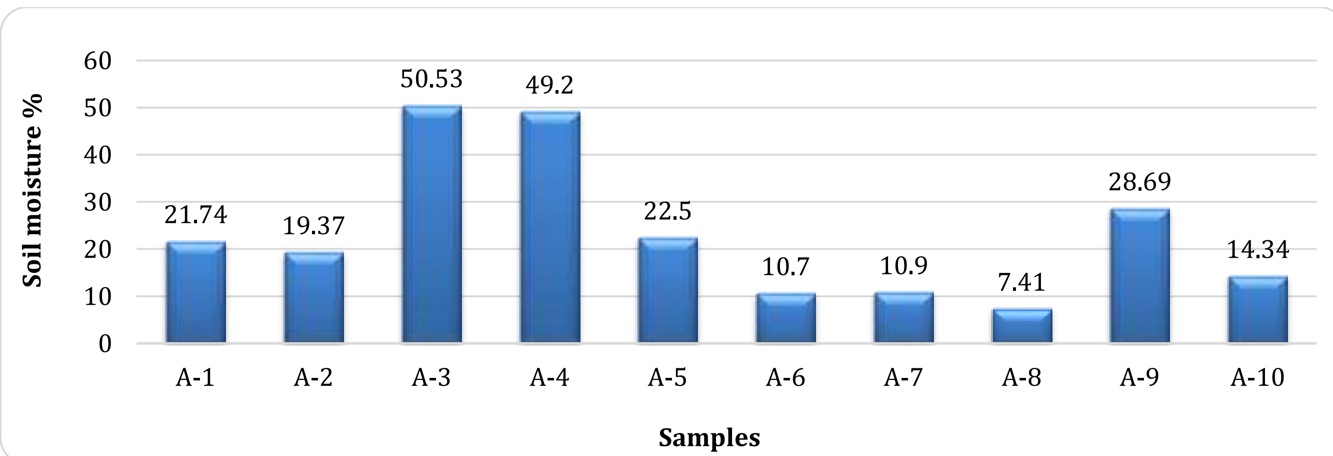

Figure 5: Soil moisture at each sampling site.

Soil texture

Each sample's silt, sand, and clay contents were examined separately to determine the soil texture. Soil texture was found to be silty loam except for Alfalfa-5 which had a texture of sandy loam. Alfalfa crops can grow in a wide range of soil textures but silty loam and sandy loam best suit this crop (Orloff, 2007). The results of both this research and the literature are the same. The overall soil texture of the selected soil samples is given in Figure 6.

Chemical parameters

Soil pH

pH is an important parameter to be determined for soil quality. The results for pH were found in a range of 7.2-7.8. Figure 7 shows the pH values of each sample. According to Orloff (2007), the ideal pH for alfalfa production is 6.3-7.5. The results of our study were slightly higher due to the addition of manure to the cropping fields by the farmers of the region.

Electrical conductivity of soil

Electrical conductivity is a crucial soil health indicator. The optimum EC levels in soil range from 110-570 mS/m (Fouri, 2018). Our study results showed EC in a range of 68-252 mS/m for the tested soil samples associated with the alfalfa crop. The values for every sample are represented in Figure 8. The samples analyzed in the lab showed the optimum range of EC except for Alfafa-6, 8, and 9.

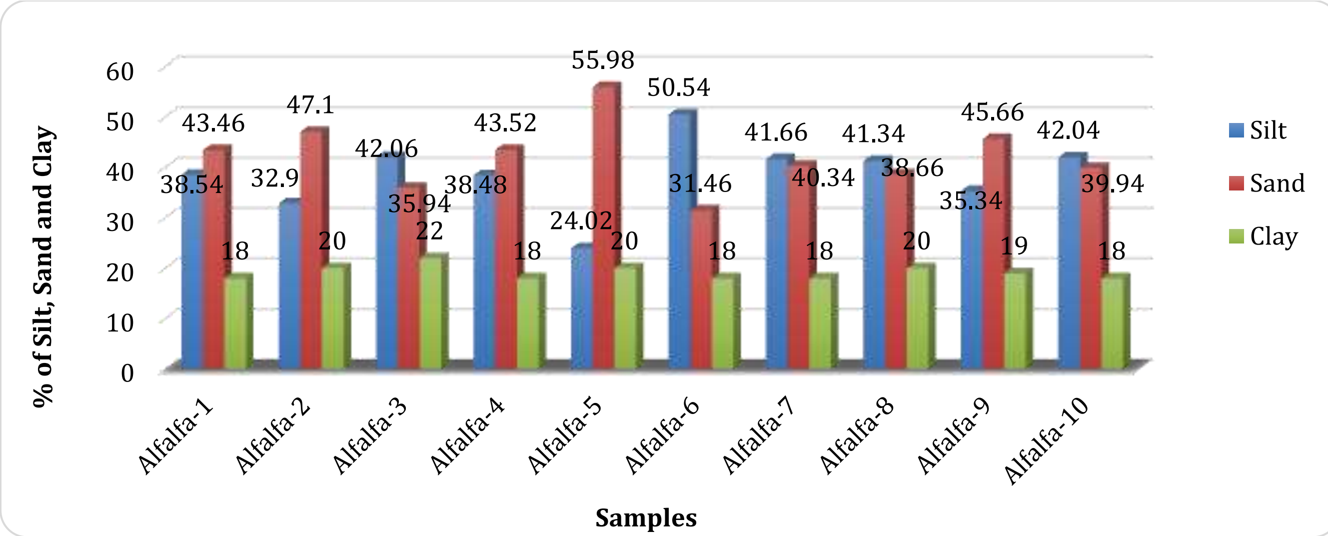

Figure 6: Percentage composition of sand, silt, and clay in each sample.

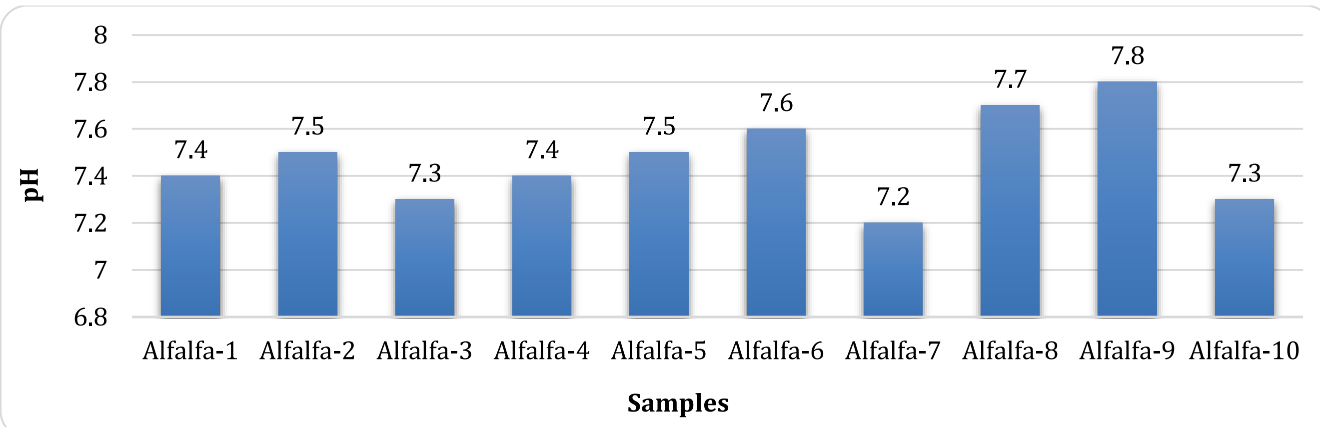

Figure 7: Soil pH of sampling sites.

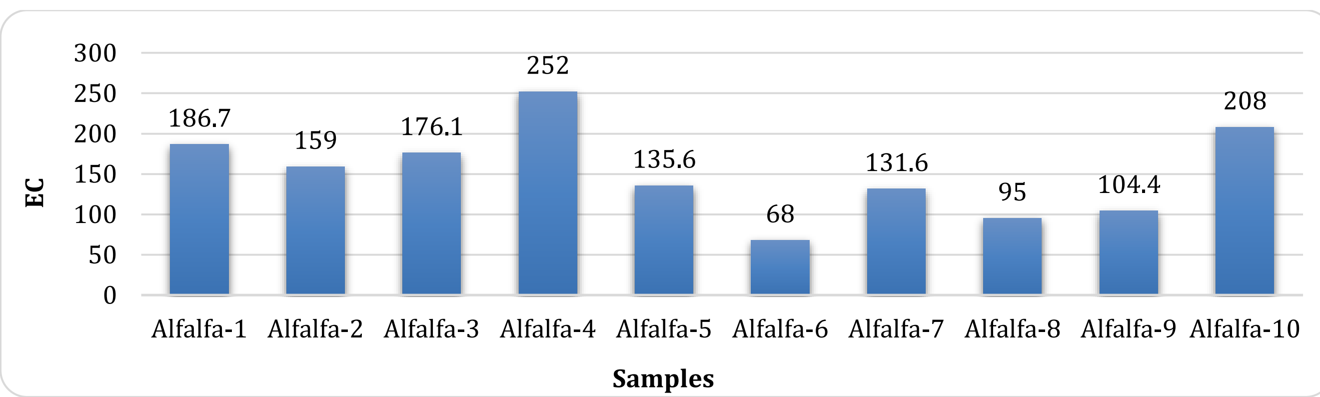

Figure 8: Electrical conductivity of the samples of each site.

Pearson's correlation among various soil parameters

Pearson's correlation was used to observe the correlation among analyzed soil parameters. Pearson's correlation showed a negative correlation between pH and EC while a positive correlation between pH and bulk density and between soil moisture and clay. Silt-bulk density and Silt-sand are negatively correlated (Table 4).

pH is the measure of only the H+ ions, but EC is the measure of the presence of total salts. It never gives information on whether the salts are there due to irrigation or by the addition of fertilizers, and it does not indicate which salt might be present. However, the addition of sulfur fertilizers, a good source of FeS can cause the EC to be negatively correlated with pH (Fang et al., 2018). Soil pH is highly significant with the soil bulk density indicating an increase in pH with an increase in bulk density (Duan et al., 2019). The smaller clay size increases the surface area to hold more water, indicating a positive correlation between clay and soil moisture (Ahmad et al., 2015). Soil texture is mainly linked with population density, but textural classes separately describe this association. Pérez-Hernández and Giesler (2014) found a strong association between sand and population density and found silt and sand negatively correlated.

Table 4: Pearson's correlation among analyzed physical, chemical, and biological parameters.

	pH	EC	BD	SM	PD	Silt	Sand	Clay
pH	1							
EC	-0.605	1						
Bulk density	0.193	-.472	1					
Soil moisture	-0.197	.602	-.129	1				
Population density	-0.214	.636	-.810	.024	1			
Silt	-0.125	-.172	-.266	-.162	.051	1		
Sand	0.111	.202	.293	.092	.017	-.981	1	
Clay	0.099	-.116	-.076	.374	-.350	-.296	.104	1

EC: Electric Conductivity, PD: Population density, BD: Bulk density, SM: Soil moisture

CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, the nematode community was found in the range of 102-507. Bacterivores were found profusely, while predators were found the least. Cephalobidae was the most profoundly existing family of nematodes in the soil samples. Physicochemical parameters were found in normal ranges with slight differences indicating soil suitable for the cultivation of alfalfa. However, this study has some drawbacks, which can be overcome in future studies. We think one-time sampling is not enough to analyze the soil quality and sampling should be repeated. Furthermore, there should be a key for nematode identification that clearly describes the soil nematode community of Nomal Valley, GB. The physical and chemical parameters must be examined regularly to check soil suitability for cultivating the alfalfa crop. Alfalfa is the widely cultivated forage crop in Nomal Valley and in other valleys of GB; therefore, more research is recommended to get information about the cultivation practices, nematode community, physicochemical properties of soil, and effects of phytopathogenic nematodes on alfalfa crops.

AUTHORS' CONTRIBUTIONS

NP performed lab and field experiments and wrote the initial draft; SM assisted in sampling for the research, worked on the initial draft, and assisted in lab work; MS thoroughly read the manuscript, discussed the results and critically reviewed the manuscript; MM checked physicochemical analysis; AA assisted in manuscript formatting; FH helped in data collection and analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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