CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Concentration of Zinc in Soil, pH and their Synergetic Effect in Dieback Disease of *Dalbergia sissoo* (Shisham)

by

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Dedicated to my family and all important people, whose support made this work possible.

CERTIFICATE OF APPROVAL

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Abstract

Dalbergia sissoo is an economical and multipurpose tree plant. Its population is declined by dieback disease. Different biotic and abiotic factors were observed in this disease. The actual cause of dieback disease in *Dalbergia sissoo* is still unknown. The objective of current study was to investigate available Zinc concentration in soil, pH and their synergetic effect in dieback disease of *Dalbergia sissoo*. Total ninty soil samples of thirty plants were taken from three tehsils of district Rawalpindi. 50% plants were healthy and 50% were diseased plants.Three samples were taken from three depths i.e. 0-20, 20-40 and 40-60 cm for each plant. Samples were analysed and the available Zinc and pH of soil samples were determined by AB-DTPA and pH meter method, respectively. Concentration of Zinc were compared between healthy and diseased plants soil samples. Similarly pH were compared. T test was used to know statistical difference of available Zinc and pH between healthy and diseased groups. T test comparison were done depth wise as well as overall. Significanct difference were not observed in any group. There were not statistical difference of zinc between healthy and diseased plants soil samples. Similarly pH were also showed no statistical difference between healthy and diseased plants soil samples. Correlation of pH and Zinc was measured with pearson's correlation to investigate the synergetic effect of zinc and pH in soil. There was a moderate positive correlation in healthy plants soil and weak negative in diseased plants soil. Therefore, it is concluded from this study that the available Zinc and pH are not directly associated with die back disease of *Dalbergia sissoo*. Synergetic effect of available Zinc and pH in soil is not strong, therefore, it is also not associated with die back disease of *Dalbergia sissoo*.

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Abbreviations

AARI	Ayub Agricultural Research Institute
AAS	Atomic absorption spectrophotometer
AB-DTPA	Ammonium Bicarbonate Diethylene triamine penta acetic acid
DTPA	Diethylene triamine pentaacetic acid
GPS	Global Positioning System
MS	Microsoft
NARC	National Agricultural Research Center
PC 1	Planning commission 1
PFI	Pakistan forest Institute
PFRI	Punjab Forest Research Institute
PPM	Part per million
Zn	Zinc

Chapter 1

Introduction

1.1 Background

Dalbergia sissoo (shisham) is a very precious plant. It is a tree plant and deciduous. It can grow up to 30 m tall and its girth can be more than 80 cm normally. In Pakistan it is mostly called shisham or tali. Its generic name is Dalbergia because of two Swedish brothers Nils and Carls dalberg [1]. There are about 100 species of its genus are in different parts of North America, Australia and tropical Asia. In subcontinent there are about twenty seven species and fifteen of them are native [2]. *Dalbergia sissoo* (shisham) is a very important species of dalbergia genus and is present in tropical and sub-tropicals of Asia. It is also grown in countries like Java, South Africa, Mauritius, Nigeria, Zimbabwe, Srilanka, Kenya and palastine [3]. This plant tree is native of Himalayas, mostly growing along roadsides, railway lines, water channels of agricultural fields and bank canals. Different soil types are ideal for its growth [4].

1.2 Importance of Shisham

Shisham is very economical plant that is why it is very important plant. It is multipurpose species with quick growth [5]. Its wood is famous for furnitures

because of its high quality and textured wood. There many other uses of shisham wood. It is very important ecologically and medicinally.

1.2.1 Ecological Importance

Dalbergia sissoo is multipurpose plant and shows its services to environment. It provides shade to animals. It is a wind break because it slow down the fast moving air and resists against the storms. Its roots are hard, tough and has suckers, it is commonly used against soil-erosion. It is helpful to stop the soil erosion process. This process is nicely controlled by shisham [6]. It is the habitat of many birds and insects because of its bulky size. It can fix nitrogen so it has nitrogen fixation ability, this ability makes this plant very important forest species. It makes nitrogen rich soil by fixing the nitrogen which is considerd as a good contribution for ecosystem [7].

1.2.2 Medicinal Importance

Shisham is also important because of its medicinal properties. Different diseases are cured by this medicinal plant [8]. It is anti-diarrhoeal and is used in diarrhea [10]. Its leaf extract contains alcoholic compounds due to these compounds, shows anesthetic properties [9]. It has shown anti-inflammatory effect in human digestive tract, as there is no any kind of side effect on digestive tract [11]. For the prevention from chronic infection of bacteria, sissoo is used as an antiseptic along with cow urine and Datura stramoium [12].

Its boiled leaves extract is used for hair growth, length and also for the treatment of hair dandruff [13]. Bark and wood is antihelmintic and can be used against helminthic worms. Its bark is useful in the treatment of inflammatory problems because it contains different antioxidants [1]. The bark and wood of shisham are used in treatment of ulcer, dysentery, skin diseases, leucoderma and dyspepsia [12]. It is a very good and cheap source of differents medicines of different diseases [13]. A variety of biochemical processes have been proposed.

1.3 Dieback Disease of Shisham and its Symptoms

Dieback is a worst disease in shisham tree. Slow, gradual and full destruction and damage of crown region of tree is known as dieback. Symptoms appears in three to five years and the plant go to death. Symptoms starts to appear from crown part of the tree and then progressively reach to downward. Thinning of crown part and drying of leaves and brances are the most visible symptoms. When the crown region gets dry up completely it looks like the stag headedness in more severe condition [14]. Necrosis, wilting and cholorosis are symptoms observed in the diseased plants [15]. It kills the plant gradually. Firstly, the plant get slightly infected, then severely infected and at last the plant die. Figure 1.1 is showing the healthy sissoo tree (a and b) and the diseased sissoo tree (c, d and e). Symptoms of shisham dieback are closely resembling with mango dieback disease in Pakistan's southern regions[16].

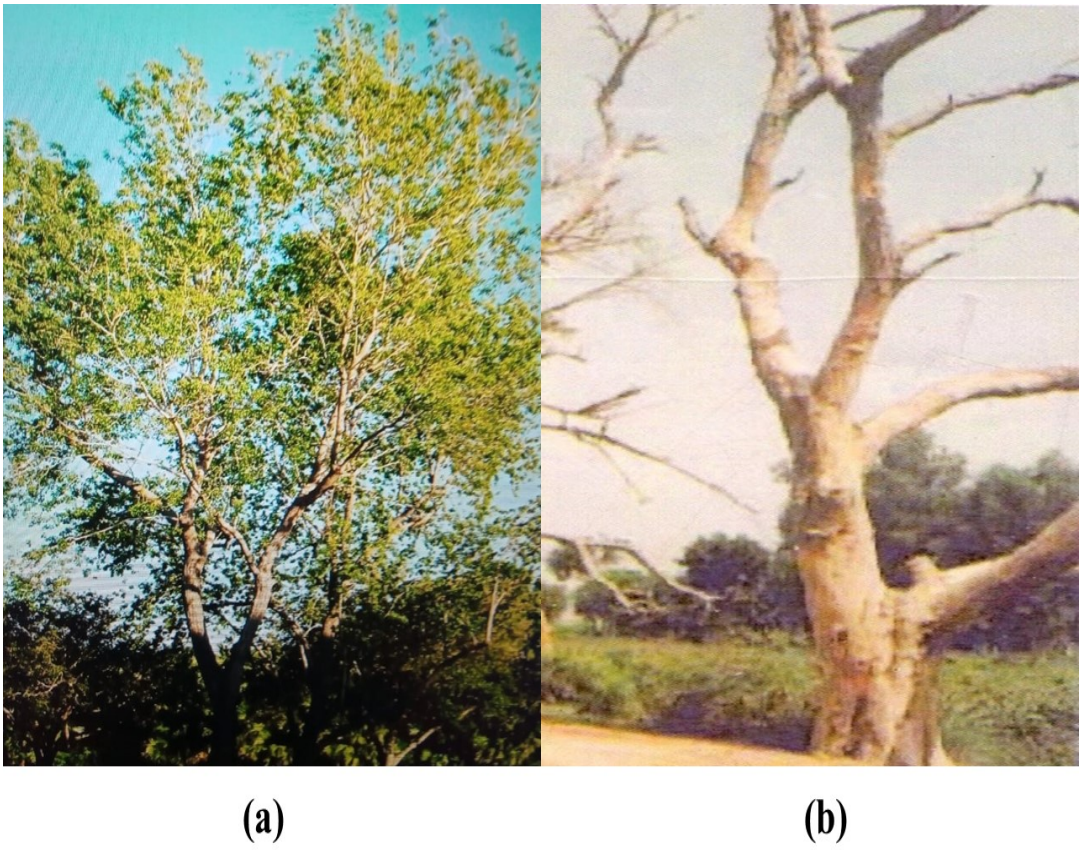

FIGURE 1.1: (a) Healthy and (b) diseased *Dalbergia sissoo* (shisham) tree.

1.4 Cause of Dieback Disease in *Dalbergia sissoo*

It was observed that both biotic and abiotic components of ecosystem were participating in the disease of dieback in shisham, in some tree [17, 18]. Some biotic and abiotic factors were assumed responsible for disease by blocking normal physiological activities in different parts of tree, were heat, pathogens, insects, waterlogging, salanity, drought, etc [1]. *Ganoderma lucidum* fungus was assumed responsible for disease and a disease causing factor. Borers are secondary agents causing disease after infection of fungus in some plants [19]. Its actual reason is still unknown and controversial. soil condition also playing very important role in diseases[14]. Moisture and deficiency of any nutrient may cause disease directly or indirectly. Nutrient imbalance is also an important factor which can cause disease. A little zinc deficiency has been observed in dieback diseased plants [20].

Available Zinc concentration and other factors like pH, organic matter, etc can cause of the disease. Available Zinc is controlled by pH in soil, these two can work synergetically in soil. High pH can cause available Zinc deficiency which can be a possible cause of dieback disease in *Dalbergia sissoo*.

1.5 Soil pH and Bioavailability of Micronutrients

Soil acidity or alkalinity are depend on soil pH. As, pH is a negative log of Hydrogen ions concentration. Soils become acidic when the value of pH is lower than 7 and there nature become alkaline when pH becomes higher than 7. In acidic soils the hydrogen ions are more as compared to the hydroxyl ion, while in basic soils the hydroxyl ions are more than hydrogen ions. Soils with pH of 5 are acidic and lower than that are more acidic. On the other hand, soils with 7.5 are alkaline and above that are very alkaline. The availability many plant nutrients are extensively depend upon the soil pH. Mostly, the plants nutrients are more available form 6.5 to 7.5 but this is not optimal for all types of soils. This range can vary by soil to

soil by their nature. Availability of Nitrogen, phosphorus, potassium and micro nutrients in soil are affected by the pH of soil.
Most of the micronutrients except molybdenum are more available at low pH. They are more available to plants and plants gain these nutrients through soil by their roots. Micronutrients show minimum bioavailability to plants in higher pH. Zn, Cu, Mn, Mo, etc are the micronutrients[21].

1.6 Concentration of Zinc in Soil, its Bioavailability and pH

Soil pH is very important because it has certain possible impacts on biogeochemical and soil processes and reactions. pH is controlling many chemical activities and reactions in soil. These reactions in soil are for different purposes like nutrient recycling and availiblity for crops. These can be for translocation and removal of undesired or toxic nutrients from soil [22].

Zn is one the important micronutrients and its bioavailability is regulated by pH of soil. Soil pH can control solubility of trace elements, it also controls the mobility and bioavailability of these elements. these all are controlled by soil pH. Translocation of trace elements in plants depends upon these. Elements in soil are partitioned in two phases i.e, solid and liquid soil phases. pH is regulating elements concentration into two phases through precipitation-dissolution reactions. When pH is low, the trace elements are more soluble because of low adsorption and maximum desorpton. In this situation the elements are more availible to plants due to maximum concentration of elements in liquid soil phase. While when pH is high, the elements are less soluble for maximum adsorption and minimum desorption. very low concentration of elements are available to plants roots because of low concentration of elements in soil solution or liquid soil phase. Any change in pH of soil shows impacts on element solubility, normally the bioavailible zinc in soil is about 0.5 mg/kg [23].
According to Forster decrease of one unit in pH of soil leads to increase of metal

solubility up to ten-folds [24]. At 7 pH, the zinc solubility was 1mgZn/L of total 1200mg/kg in soil solution. At 6 pH, soluble concentration became 100 and at 5 it was 40mgZn/L. precipitation is also regulated by pH and effect the trace element concentration. Lime used in soil has effect on pH which lowers the concentration of bioaccessible and available Cu[25]. As pH increases the availability of Zinc becomes low. High pH can creates Zinc deficiency in soil which in turn can cause the dieback disease in *Dalbergia sissoo*.

1.7 Synergetic Effect of Zinc and Soil pH

Zinc and soil pH are associated with one another and they work together. As, Zn is a micronutrient and their availability are strongly associated with pH. Mostly, the availability of these micronutrients are decreased with increase in pH. When pH becomes lower their availability in soil becomes more. Zinc becomes less available and becomes deficient in some cases when pH of soil gets higher. If pH becomes lower than the availability of zinc in soil becomes high [26].

When Zinc availability becomes low in soil, it creates the Zinc deficiency. In zinc deficient soil there is possibility that the total zinc in soil is relatively high. Sometimes it happens despite of high total zinc the available zinc is deficicent, this is because of pH. Usually, it happens the pH forces the available zinc to make the zinc complexes those are springly not soluble in available fraction. Plants can only utilize the available zinc from plant available fractions [27]. Main reason of low available zinc soils is pH but there are some other factors which are also contributing in low availability of zinc. The other factors are; clayey texture, sandy texture, ill drained soils, wetlands, high or low organic matter, etc [28].

1.8 Problem Statement

Dalbergia sissoo (shisham) is an economical tree. Dieback disease is damaging its population badly. Its actual cause is not clear. The study was conducted to know

the concentration of Zinc in soil, pH and their synergetic effect investigated in *Dalbergia sissoo*.

1.9 Objectives

- To know concentration of available zinc in soil of dieback diseased and healthy *Dalbergia sissoo* (shisham) tree for comparison.
- To know difference of pH in soil of dieback diseased and healthy *Dalbergia sissoo* (shisham) tree.
- To investigate the synergetic effect of available Zinc and pH in soil of dieback diseased *Dalbergia sissoo* (shisham) tree.

1.10 Current Research Work and Research Gap

All the previous researchers only focused on the dieback diseased plants and did the plants soil analysis to know available zinc concentration and pH of soil. They did not analyse the healthy plant soil to compare the healthy and diseased plants soil. Current study performed analysis on diseased as well as healthy plants soil to compare available Zinc and pH.
Similarly, peer studies only did an overall analysis but current research did depth to depth as well as an overall analysis for a deep comparison and for more batter outcomes.

1.11 Research Questions

- Does the dieback disease of *Dalbergia sissoo* is due to deficiency of available zinc concentration?
- Is there any difference of pH between healthy plants soil and diseased plants soil?

- Is there any synergetic effect of available zinc and pH in soil of dieback diseased plant and causing disease?

1.12 Significance of the Research

The shiaham plant is an economical valued tree. Its dieback disease damaged many tree in Pakistan as well as in other countries. The correct reason of shisham diebck is not clear, it is very important to know it. This research study helped to know the concentration of Zinc in soil and pH and their relative effect on dieback disease of this tree.

The research study will be helpful for further studies on synergetic effect of other nutrients and pH, in shisham's dieback disease.This research study will not only helpful for understanding shisham plant dieback disease but also for many other plants dieback disease and synergetic effects of different nutrients and other factors like pH, organic matter, etc.

Chapter 2

Literature Review

2.1 Introduction and Description of *Dalbergia sissoo*

Shisham is medicinally and economically important tree with many uses. people use it widely for different purposes [29]. Its wood is of good strength that is why used in manufacturing of furniture [30]. It has potential of nitrogen fixation. It is cultivated in countries like, Afghanistan, Pakistan, Bangladesh, Nepal, India and tropical and sub-tropical of Africa [31]. Its domain, kingdom, division and class are eukaryote, plantae, magnoliophyta and magnoliopsida respectively. It belongs to order Fabales,family Fabaceae, genus Dalbergia and species *Dalbergia sissoo*. It is medium to large in size. Its trunk is grey yellow with longitudinal cracks, as shown in Figure 2.1.

FIGURE 2.1: Trunk of *Dalbergia sissoo* obtained from [5]

Its leaves are compound and petiole. Its flowers are sessile and whitish to pink in colour.Its pods are flat, light brown and thin, as shown in fig. Its seeds are flat bean shaped, as shown in figure 2.2. Its flowering time is from March to May [5].

FIGURE 2.2: Leaves and fruits of Dalbergia obtained from [5]

For last 30 years, this Dalbergia sissoo has been most widely cultivated. Now it has been started to die from last few years. diffrent diseases vicitmised this tree [32]. Its symptoms includes yellowing of leaves, cholorosis, wilting, destruction of terminal parts of branches, etc [33]. This disease of shisham is the dieback disease. The whole tree shed its leaves and becomes leafless and branches die. Thinning of branches and dying the crown part of branches leads to death of plant [14].

2.2 Dieback Disease Cases and Studies

2.2.1 Initial Cases

First report on dieback in Nepal in year 1933, but officially dieback disease of shisham appeared in 1996-97 and first research study was done on sissoo dieback [34]. high death rate was noticed of sissoo in Bangladesh and the infected plants age range were different, plants were with different ages [35].

2.2.2 Dieback in Pakistan

In May 1998 the shisham diebck was observed as an epidemic in the central irrigated tract of the Punjab and in different parts of the country. Punjab Forest Research Institute (PFRI) started a multidisciplinary study but unluckily the chemical and biological control measures used at PFRI did not successed. Later on PFRI forwarded a PC I project proposal to finance the Punjab Government approved further study. Meanwhile a step was taken by the Chief Executive of Pakistan constituted a Working Group for monitoring and coordination of the research efforts for control of dieback. A developmental project "Survey, research and control of shisham dieback in the Punjab (2001-02 to 2005-06)" was approved with a collaborative research initiative of the Government of the Punjab involved PFRI, Ayub Agricultural Research Institute (AARI) and University of Agriculture Faisalabad at tatal cost of Rs. 18.595 million [36].

The first two, first and second National Seminars on Shisham Dieback disease had been organised on October 27, 2001 and June 29, 2003 to join hands against this issue. These seminars provided very useful infrastructure for sharing the informations and details of ongoing research studies and streamlining future research strategy. A Third National Seminar on Shisham Dieback was arranged in PFRI, Faisalabad on May 11, 2006. Research study on shisham decline in Plant Pathology and Mycology, University of the Punjab, Lahore was started in 2003 after a new Department in 2002 established. The scientists got great success in very short time because of special interest of Vice Chancellor of the University General (R) Arshad Mahmood. Different areas of the province Punjab were surveyed and investigated for various abiotic and biotic factors those were responsible for sufferings of the valuable and important shisham plants. Different diseases were responsible for shisham decline were clearly observed, recognized and studies for their chemical and biological control were undertaken. Most important aspect of the study was to identify 18 shisham varieties including resistant and very vigorous varieties that were recommended for the future cultivation and plantation to save this important plant species from being extinction [37].

Researchers in Pakistan have observed only one disease which is causing shisham

decline and that is dieback. However, after survey of Punjab's different regions, two types of diseases were observed those were wilting and dieback causing shisham decline in the country [37]. In the wilt disease effects on trees were more or less similar as those produced due to drought.

2.2.3 Dieback in India

The first time the disease was noticed by a research in natural forests as well as plants in UP, India. In disease the whole tree shows symptoms. In the initial stage, the affected plant shows drop down of leaves and also branches, due to lack of turgidity. Then the leaflets become yellow, dried up and eventually drop down renders the branches fully bare. All parts of tree gets thin as compared to the healthy dense lush green tree. Death of the diseased plant is more quick and occurs within 4–6 months after the appearing of the symptoms of wilt in crown. However, apart from that similar diseases are where the plant die very quickly i.e. within few weeks plant turns leafless. This quick wilt mostly attack the shisham plants after the end of rainy and through out the autumn season. A similar disease but with little different symptoms has also been noticed where eventually a part or entire plant becomes dry but dry leaves remain fixed with the plant and the entire dried portion turns brown in colour. Further research studies in this regards are under way. In literature dieback disease has been reported as early as 1900 but that has not understand as an threatening or alarming. It was the year 1998 when dieback was observed as an epidemic in central irrigated area of Punjab province [14].

The dieback disease shows more specialized symptoms as compare to the wilt. The symptoms include, thinning of leaves and crown part, dry up of end branches, yellowing of entire plant in some cases, stag-headedness and table topped conditions in severely diseased cases. Small size dry twigs and branches fall continuously and the plant seems like a blunt stub having thick branches. The dieback disease in a plant takes place in successive stages and shows characteristics like, gradually death of crown part, shoots and roots starting at the tip [13]. It was the year 1998

when dieback was observed as an epidemic in central irrigated area of Punjab province.

2.2.4 Pathogenic Fungi on Shisham

Mycologists have observed some 62 pathogenic fungi on shisham. Mostly, the fungi have been studied by many of the mycological aspect and a little has been mentioned on pathological point of view. However, after this epidemic of shisham decline diseases pathologists conducted research studies to investigate the cause of this disease [13]. The study reported that Fusarium solani is the cause of shisham wilt. research also isolated F. solani from roots of diseased plants and assumed this organism causing shisham wilt [38]. Similar observations from other countries have also been reported, countries like Bengladesh, Nepal and India [16].

The disease causing organism of dieback is still controversial and unknown. There are more chances that more than one pathogenic organisms, either singly or in combination, may be contributing for shisham dieback. It is also chance that in different areas with environmental conditions and variable edaphic, different pathogenic organisms may be causing this disease. However, the most probable reason could be the lack of more knowledge and misunderstanding of researchers on the shisham decline diseases. They usually considered different declined diseases as dieback and that is why they isolated different pathogenis organisms from the diseased shisham plants.

A research study reported that F. solani and Ganoderma lucidum are causing root rot in shisham and responsible for large scale destruction [5]. Zakaulla (2006) undertook an intensive and detailed survey of naturally growing regions and irrigated plantations of Peshawar district, roadside plantations and CDR of Attock district under "Forest Sector Research and Development Project" of PFI Peshawar. They isolated six pathogenic species of fungi from affected plants Botryodiplodia thebromoae, Helminthosporium dalbergiaea, Ganoderma lucidum, Xylaria sp., Fusarium solani and Poria ambigua. *B. theobromae* was the most common and pathogenic species. Pathogenicity of *B. theobromae* was tested on the *D. sisso* and re-isolation

of the pathogenic species confirmed the results. From various districts of the Punjab for isolation of disease causing pathogenic organism of shisham diseased samples of shisham were collected [39]. B. theobromae was found the most frequently found after P. cinnamomi and F. solani, respectively. Insects do not play any visible role in causing the dieback [29]. However, termites can attack the plant once its parts become dead and dry. A field survey was conducted by Idree et of 20 districts of the Punjab and isolated 18 different microorganismic species from different infected parts of shisham. Botryodiplodia theoromae was the most frequent fungus pathogenic species isolated from all the plant parts.

This fungus was considered as a causing agent of dieback during the study in 2003-2004. In one of the earlier studies, Fusarium oxysporum was found in frequently in the roots of dying back Shisham plants samples collected from drought affected regoins of Quaid-e-Azam Campus, University of the Punjab, Lahore [31]. However, later on Phytophthora cinnamomi was observed and isolated from samples collected from the roadside of canal. A few researchers also reported that dieback is caused by P. cinnamomi. Like biotic factors and many abiotic factors are also considered to be cause of the initiation and severity of shisham decline diseases [40].

Study recorded the maximum death percentage of 75–80% along the canal banks. It shows that dieback incidence and severity is linked to soil moisture contents. Soil becomes water logged because of water seepage from the canal into the nearby areas. Some other workers and researchers have also observed that high soil moisture contents increase the severity of the disease [41].

2.2.5 Survey of Researches of Punjab University

During the past few years, In Punjab University a considerable number of shisham plants were died either by dieback or wilt diseases during the past few years.

The relative ratio of wilt to dieback incidence in this region was higher as compared to other surveyed regions. It was also observed that disease severity and incidence

was higher on dry soil land patches as compared to irrigated and well-maintained regions. It shows that like water logging, drought stress also makes favourable condition for disease attack and severity. Study conducted surveys of all the shisham growing districts of Sindh those are Nawabshah, Sukhur, Khairpur, Naushahro Feroze, Ghotki, Sanghar and Hyderabad [13]. reported that water logging and drought were the primary causes of drying/dying if shisham trees which created stress conditions and made the trees vulnerable to dieback disease. According to study global warming and eratic rain falls could be the possible reasons of recent shisham decline in the country [5]. Dalbergia sissoo is infected by other disease like, leaf blight, wilt, leaf rust, powdery mildew and collar rot [1].

2.3 Abiotic and Biotic Factors in Dieack Disease

Both abiotic and biotic factors have been reported in dieback disease. These factors contributed in disease differently. Abiotic factors include nonliving factors like, water stress, nutrients, high water table level, etc. Biotic factors includes living factors like different fungi, insects, etc.

2.3.1 Reported Abiotic Factors

The physiological functions of shisham tree are usually modified because of fluctuation in various climatic factors which is worst situation for the tree [42]. Wilting observed in UP, India and symptoms appeared throughout the shisham plantation. When climatic condition are changed, bioavailability of different nutrients are changed and these nutrients usually become less available or unavailable for plant. pH is the most important abiotic factor may affect the plants by nutrient imbalance.

Pathological agents specially different types of fungi attack the shisham plant and become the death cause [1]. When fungus find ideal condition, starts infection. At first stage the crown part of tree start drying and then eventually die. Continuous

presence of high water concentration in root zone area of plants is also a factor which makes the plants more susceptible against the fungal infection. Oxygen is also become low in this condition which also promote more infection. When water table rise up this problem become more severe. It is observed that sissoo can thrive on loosely textured soils but it suffers badly from root diseases in hard stiff clayey soils.

In loosely textured soil the aeration and drainage is proper which promot the healthy root growth. The texture of soil is also a very important factor in disease. It is thought that soil texture or nutrients in soil are the real culprit of the disease and these are the primary cause factors. Secondary agents attack the plants because of these soil factors. Plants with poorly drained soil are more attackable than sandy soils. Pathogens are thought as secondary cause of the disease; the primary cause is something that is undiscovered and still controversial [42].

2.3.2 Wilt and pH

Wilt is observed by the unknown toxin present in cultural filterate and symptoms appeared. Soil texture, pH and incidence of wilt disease are associated in some cases. These factors studied to understand their with nature. Disease was not found in properly drainage soil no disease appeared while soil with more silt and less sand particles showed disease.

The pH range of soil in diseased plants was observed 7.5 – 9.7. The high death rate of shisham was observed in Uttar Pradesh, Haryana and Bihar. The following factors may have a role and playing their role in making shisham more susceptible to the pathogens like Fusarium and other fungi [1].

2.3.3 Texture of Soil

There all sissoo tree became pale yellow and eventually died. Similar symptoms were also observed in Lachiwala Rang. This was not seen in soil of light texture but in clayey soil it has been observed [1].

2.3.4 Water Availability

For irrigation they used shallow channels system for and then superficial root system. After that year, irrigation was blocked in 9 out of 11 coups. After three years disease mortality was spread in all 9 coups. While no disease was seen in the other two coups because of the availability of water. the area with insufficient water, roots of plants are unable to draw water from deep water level, so water availabiliy is also a reason of the high disease death rate of shisham decline [1].

2.3.5 Underground Water Level

An Indian report showed that when the roots of plant approaches the water table, the disease chances has increased fungi mostly the plants specially the attack of Fusarium solani which can cause infection and then the tree die. The plants of 10-12 years age shows death rate at 2-3m deep water table zone while with 2 m water table zone, the death rate was appeared at 5-6 years age and with less water table death started at age of 12-14 years [1].

2.3.6 Biotic Factors

The fungi live in root zone area Ganoderma lucidum is assumsed to be the primary agent of shisham dieback as the fungi infect the root of plants. The root contact and spreads lateraly [43]. It is reported that Fusarium solani was the causal agent of this disease [44]. The fungus has been observed in roots, its hyphae and jelly like material restricted in vessels and cause the wilting in plants. According to a research study Fusarium oxysporum[42]. Different species of insects and fungi were reported in causing sissoo desease in tropical and sub-tropical [35]. In subcontinent the fungaus dieback in association with wilt and canker has also been seen. In north India and Pakistan, F. solani fungus has been reported to cause wilt in sissoo. The larvae of insects like pinhole and beetles were also observed to cause serious damage on shisham plantation. An insect, the pinhole beetle gains

nutrition. It is noticed as the cause of disease in plains area of Punjab. It is observed in the northern region of sub-continent India, Perissus dalbergiae and Agrillus dalbergiae as the causal agent as they gild the stem of plant in favorable conditions. The galleries and the cracks may be surrounded with hyphae which attacking wood of diseased trees in association with some other possible causes. Poria ambigua another fungus associated with root and butt rot are extensively identified associated with beetles. The tunnels are usually with fungus growth. Rhizoctonia, fungus whose habitat is soil, has also been observeded as causal agent to the root system of shisham at high soil moisture level.

A research was conducted by a multidisciplinary experts panel. They reported pinhole bark beetle insect as a primary disease-causing agent, and the secondary disease causing agent was long horn beetle in association with other saprophytic fungi in galleries. *Batocera* and *Dorysthenes spp* are root and bark feeding species of unhealthy and old barks of tree [43]. These species observed decaying barks in the plains of Pakistan and India. These species lay Eggs under bark and wounds on stem, shoot part and twig. Emergence is usually from May to July. *Batocera rubus* beetle insect mostly appears in March to April. Life cycle for *Batocera spp* ranges from 1 to 2 years and life cycle of *Dorysthenes spp* is 3 -4 years or even sometimes it is more. The leaves of infected trees become yellow and the tree start to die slowly after 2 to 3 years. *Agrillus dalbergiae* and *Perissus dalbergiae* have also been observeded as causing girdling of the stem in the northern part of the Sub-continent[44].

2.4 Loss in Subcontinent

Subcontinent is suffering from massive loss because of shisham dieback disease. Since last few decades this plant is struggling for its survival. Many plants has died.

The forest departments was in huge loss because they faced the loss of million dollar because of the high death rate [17]. In subcontinent, the improper planning, use

of susceptible varities and mismanagement of shisham distribution are the three main causes of shisham decline. Millions of shisham tree are cut or smuggled illeagly due to its good wood quality. Timber mafia and forest offernders cut this plant on large scale [1].

2.4.1 Loss in Pakistan

Shisham decline was not thought as an alarming situation but after that in year 1998, it was reported as an epidemic disease and future threat in central Punjab [45]. Shisham decline was thought as a destructive and drastic disease in Sindh, Pakistan[46].

2.4.2 Loss in Bangladesh

The maximum disease death rate was observed in Bangladesh that was 55% [17]. In another report, it was observed that 40% plants along the road and highways and 80% with the bank canals are affected by sissoo decline [45].

2.4.3 Loss in India

In Uttar Pradesh, high death rate of shisham was observed since 1900. A survey was conducted in irrigated sissoo population in Bhagat, Punjab. In Bhagat, Punjab a survey was conducted in irrigated shisham. In another study sissoo plants were affected because of the soil tightness and stiffness [3].

2.5 Dieback and Nutrients Availability

Availability of nutrients is very essential. Low availability of nutrients can cause the dieback disease, as the dieback disease was observed in coffee plants due to insufficient nutrients [47]. A study, however, did not find any relationship of

dieback with soil physiological properties and plant nutrient status [48]. An other research observed available zinc deficiency in sheesham dieback [20].

2.6 Zinc Distribution in Soil

Zinc is one of the transition metals with atomic number 30 and it is the 23rd most abundant element on our earth crust. Its mass number is 65.39 and its oxidation state is +2 [49]. Zinc is the second most abundant element in living organisms after iron, and it is the only metal exist in all six enzyme classes (Enzyme Commission number, EC. Names of enzymes are; oxidoreductases, lyases, transferases, hydrolases, ligases, isomerases [50].

The primary input source of Zn to soils is by the physical and chemical and weathering of old rocks. The lithosphere layer of atmosphere consists 70 to 80 ug Zn g 1, while sedimentary rocks consists of 10 to 120 ug Zn g 1. Soil Zn presents in its three primary fractions named;

1. Water-soluble Zn (which includes zinc ions and soluble organic fractions)
2. Adsorbed and exchangeable Zn in fraction which is colloidal fraction (cocernrd with humic compounds, clay particles, and Aluminium and iorn hydroxides)
3. The third is insoluble Zn complexes and minerals. The distribution and circulation of Zinc between these three soil fractions can be determined by soil complexations, soil-specific precipitation, adsorption reactions [51].

2.7 Influence of Soil pH on Nutrients

Nutrients availability is influenced by different factors. There are different interactions which influence the nutrients availability like microbe-plant-micronutrient

interactions [52]. Micronutrients include, Zn, Cu, Mn, Fe, etc. show distinct interaction with pH, but pH is also dependant on the plant species and genotype of species [26]. Availability of nutrients is controlled by soil properties, microorganisms, surrounding soils, etc. Different nutrients show particular interaction with pH of soil [53].

2.8 Zinc and pH Relationship

The most dominant and crucial factor which determine soil Zn distribution is pH; Zn is more efficiently, readily and quickly adsorbed on cation exchange sites at high pH value and adsorbed Zinc is more readily and efficiently exchanged by CaCl2 at lower pH. Thus, at low pH the soluble Zn and the ratio of Zn ions to organic Zn-ligand complexes, especially for those soils which have low soluble content of organic matter. Zn distribution in soil affected by soil type, soil texture, pH, soil clay, moisture, mineral contents, diffusion, mass flow rates, weathering rates, soil organic matter, soil flora, plant uptake, soil fauna and content of organic matter will also affect Zn distribution. Zn which insoluble consists of >90% of soil Zn and is unavailable for the plant uptake through roots. The range of exchangeable Zn is about from 0.1 to 2 ug Zn g 1 [54]. Zinc is the most important and vital micronutrient of crops and plants. Mostly zinc deficiency appears in crops, particularly for those soils which have high value pH [39]. More recently, substantial plants of crop responses against Zn fertilization have been observed and reported. in India, Australia and Central Anatolia a city of Turkey, the substantial responces of crops to zinc fertilization has been observed where the yields of wheat crop have raised about 600% from the mid-1990s, with a massive annual economic profit of US$100 million [55]. Visible and clear symptoms of Zn deficiency appears only when the relatively severe deficiency exists. When the deficiency is marginal its symptoms not appear but crop and plants quality and yield is affected. Deficiency is there but this hidden and it affect growth and yield. When there is a deficiency of Zinc in plants, it adversely affect the growth of plants because many physiological fuction are disturbed. Disturbance of physiological functions creates many problems

for plants. The Zn is necessary for the normal growth of plants and it has been reported scientifically for about 70 years [56].

Worldwide the deficiency of zinc has been observed. It has been more deeply in last a few years. Zn deficiency is one of the most threatening and alarming for world because it affects the growth and food production of plants [57]. It is necessary to understand and study the Zinc deficiency that would certainly help for proper management of this problem. Biovailability of Zinc to plants and crops can be affected by other factors like soil pH, organic matter, soil temperature, moisture content, total soil Zn content, root nature and rhizosphere effects. Zn adsoption to the surface of soil components such as clay minerals, metal oxides, etc is increased by increasing soil pH. This surely concequences in decreases in the solubility. Bioavailability of Zn is become less to plants. When pH is high the desorption of Zn become lower, this makes the Zn less available for plants. Zn precipitates and in the process of precipitation of Zn forms of $ZnCO_3$, $Zn(OH)_2$, and Zn_2SiO_4 when pH is high. The Zn concentration in the soil solution is regulated by pH and it forces the Zn to become either less or more available to plants.

In acidic nature soils where pH is lower the Zn bioavalability depends upon pH value of soil. In sandy soil, the bioavailability Zn become two times when the pH of soil is lowered from 7 to 5 pH value by applying ammonium sulfate. In alkaline or basic soils usually Zn is deficient. It creates many problems for plants. This Zn deficiency can be minimize by Zn application. But the growth and yield of plants can only slightly improved. It is observed that growth of plants on the alkaline soil is more responsive to alkalinity of soil as compared to Zn deficiency. Soil alkalinity is more important for the growth of plants [58].

2.9 AB-DTPA Method and Micronutrients

AB-DTPA method and pH meter method are used to measure the available zinc concentration in soil and pH, respectively [59]. AB-DTPA is an extracting solution. It is made up of ammonium bicarbonate and diethylene triamine pentaacetic acid.

AB-DTPA stands for ammonium bicarbonate diethylene triamine pentaacetic acid and is a very important multi-element test for soil. It was developed by two scientists named, Soltanpur and Schwab in 1977.
This method measure the quantity of elements in soil. It is the most suitable method for measure of the micronutrients in soil like, Zn, Cu, Mn and Fe [60].

2.10 Zinc Analysis by AB-DTPA

Zinc is analysed by AB-DTPA method with atomic absorption spectrophotometer. It determines the quantity of available Zinc ions in soil. Zinc is one of the micronutrient and its available quantity is determined by AB-DTPA method. Air dry soil sample is being mixed with DTPA extraction solution in specific ratio that is 1:2 respectively [61]. The suspension is filtered and available Zinc concentration is measured with the help of standard solutions by atomic absorption spectrophotometer. Zinc is shown by atomic absorption spectrophotometer is the concentration of Zinc in extract, the available Zinc in soil is calculated [59][62].

2.11 Analysis of pH and pH Meter Method

PH meter method is used to measure the pH of soil. Soil is mixed and homogenised with distil water in a fixed ratio that is 1:1 and pH is measured by pH meter [59]. pH meter is caliberated with buffers and the electrode of pH meter is rinsed with distilled water and dipped into the suspension of soil and distilled water and pH is measured [63].

Chapter 3

Material and Method

3.1 Study Area

Samples were collected from three tehsils of district Rawalpindi named Kahuta, Kallar Syedan and Gujjar Khan. District Rawalpindi is located in Pothwar region. It is consists of seven total tehsils. It is semi arid area. It has plains as well as mountains. Its temperature ranges 0-48 celcius and the annual rainfall is about 1000 mm [64].

3.2 Sample Collection

Firstly, the *Dalbergia sissoo* plant was identified. After identification of plant, its different characters like GPS coordinates, girth and health status were noted, as mentioned in table 4.1. After that, three soil samples were taken with the help of auger. Soil samples were taken from 0-20, 20-40 and 40-60, one by one. Then, placed the samples separate and removed roots, stones, leaves, etc. from them. After that, put the samples into zipper bags and labaled the bags with serial number and sample ID. Repeated the same procedure for all the selected plants to take their samples. Total 90 soil samples were taken by 30 sissoo tree, there were

15 healthy tree and 15 tree were suffering from dieback disease. Three sample from each tree with three different depths were taken. After that, the samples were being carried to the Land Resourses Research Institute, NARC Islamabad for analysis of concentration of zinc and pH of samples.

3.3 Soil Sample Processing

The soil samples were kept air-dried at room temperature for 24 hours. After that, the soil samples were crushed into fine powder form, then sieved to <2 mm with the help of sieve and again stored in zipper bags.

3.4 Analysis of Zinc Concentration

The analysis of zinc concentration were performed by an instrument named Atomic absorption spectrophotometer. Atomic absorption spectrometery were being used. This technique is used to determine the concentration of different elements. The AB-DTPA method were used for determining the available Zn concentration in soil.

3.4.1 AB-DTPA Method

Ammonium Bicarbonate-Diethylene triamine pentaacetic acid method is also called AB-DTPA method. It is a soil test. Different elements concentration can be determined in soil by using this method [61]. By this method analysis of micronutrients can be done.

3.4.2 Apparatus

Different apparatus and instruments were used in AB-DTPA method to determine available Zinc concentration. Atomic absorption spectrophotometer were used for

Zinc analysis and the shake machine to homogenise soil with AB-DTPA solution and distilled water. Hotplate were used to prepare the AB-DTPAsolution.

3.4.3 Preparation of AB-DTPA Solution and Standard Solutions

For preparation of AB-DTPA solution, 1.97 g Diethylene triamine pentaacetic acid were weighted. Added into a distilled water washed 1000 ml beaker. Distilled water were added in it to dissolve to DTPA. Then 79.06 g of ammonium bicarbonate were added, then solution were stirred on hotplate. Then pH of the solution were adjusted to 7.6 by using HCl and ammonium hydroxide. Then solution were used for extraction [61].

3.4.4 Preparation of Standard Solutions

Standard solutions were prepared in a series for Zinc in DTPA extraction solution, i.e. 0, 0.2, 0.4, 0.6, 0.8, 1.0 ppm with the help of stock solution. Zn (II) (1 mg/mL) is a stock solution. It was prepared by adding 4.398 g of $ZnSO_4.7H_2O$ in distilled water.

Then, added a few drops of conc. H_2SO_4 and standardized with the help of 8-hydroxyquinoline. The buffer solutions was prepared by dissolving sodium acetate (pH 1–3), HCl, acetic acid and sodium acetate and acetic acid (pH 3.2–7.0), and ammonium chloride and ammonium hydroxide (pH 8.0–12.0) [50].

3.4.5 Procedure of Extraction

The 10 g of air dried soil was added into125 ml flask, then added 20 ml of extraction solution that was AB-DTPA solution, then the suspension was shaked on shake machine for 25 to 30 minutes. After that,the suspension was filtered by Whatman filter paper. Extract was taken in distilled washed bottles and bottles were being

labeled with samples serial and ID as, shown in figure 3.1. Same procedure was repeated for all the samples in different batches.

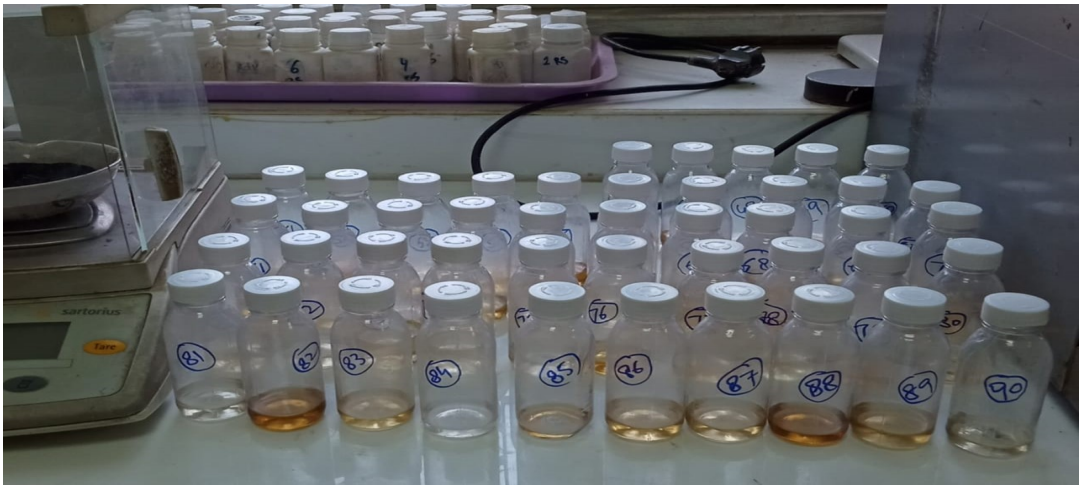

FIGURE 3.1: Filtration for extracts at NARC, Islamabad

3.4.6 Running of Samples Extract on Atomic Absorption Spectrophotometer

The filterate was run on Atomic absorption spectrophotometer step by step. First of all the lamp of Zinc was adjusted in atomic absorption spectrophotometer, then started the spectrophotometer. Standard solutions i.e. 0, 0.2, 0.4, 0.6, 0.8, and 1 ppm were run on atomic absorption spectrometer, by putting the capillary tube of into the standard solutions one by one in sequence. Then run blank. After that, the samples extracts were run on atomic absorption spectrometer on by one. Noted the readings for Zinc concentration in extract solutions on spectrometer.

FIGURE 3.2: Zinc analysis with the help of Atomic absorption spectrophotometer

Blank is the extraction solution, whereas dilution factor is obtained by dividing the amount of extraction solution (AB-DTPA solution) in ml with the weight of soil samples in mg. summary of methodology for zinc analysis is given in figure 3.3.

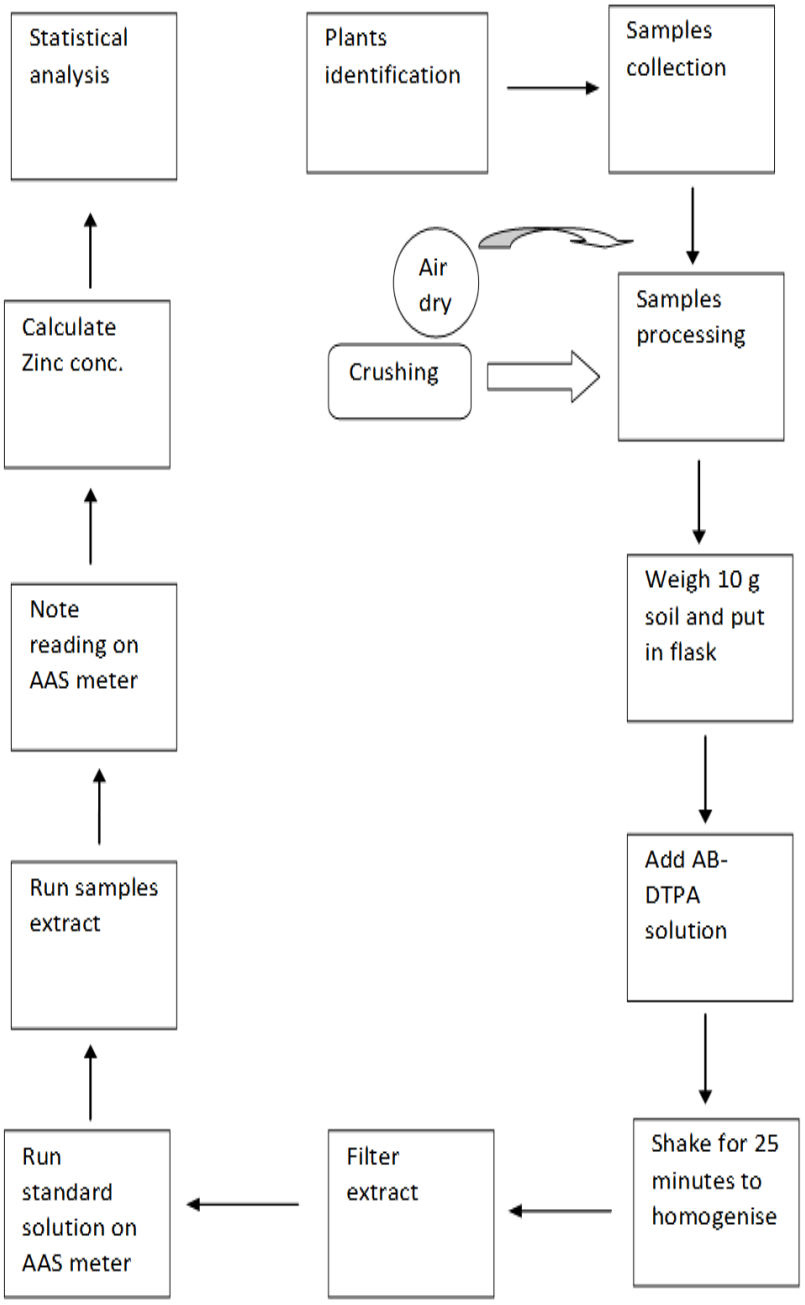

FIGURE 3.3: Flow chart of methodology for Zinc analysis

Then, calculated the available Zinc concentration in mg/kg by subtracting blank values from the readings of spectrometer and then multiplying with the dilution

factor. Dilution factor is obtained by dividing the volume of AB-DTPA solution by weight of soil sample. AB-DTPA solution and soil are taken 2:1 respectively for each sample. Equation were used to calculate the available Zinc concentration in soil;

$$\textbf{Zinc (mg/Kg) = (Spectrometer Reading – Blank) x Dilution Factor} \tag{3.1}$$

3.5 Analysis of pH

pH was measured by the pH meter. Samples were analyzed immediately after the soil sample was suspended in water. Laboratory analyses were typically performed at room temperature (15 to 25 degree celsius).

3.5.1 Equipment and Apparatus for pH Measurement

The following materials and equipments were used for the determination of soil pH: pH meter, Small griffin beaker and electric shake machine. The pH meter were used to measure pH values of samples. Beaker was used to contain the soil and distil water suspension. Electric shake machine was used to homogenized the soil with distil water.

3.5.2 Procedure for Measurement of pH

PH was measured in different steps. Firstly, 20 mg soil was weighted on balance for each sample, then put it into a plastic flasks. Added 20 ml of distilled water in flasks. Then electric shake machine were used to shake and homogenise the soil and water in flasks for 30 minutes.

Different steps were followed to measure the pH of soil samples. First of all, pH meter was powered on by pressing on button. Then, pH meter caliberated by

pressing CAL button. Then placed the electrode of pH meter in buffer solution with pH 4.0. Again caliberated by pressing CAL key then P1 was displayed on screen, accepted by pressing YES. Then, screen displayed ready pressed YES and P2 appeared on screen. Pressed YES and then second buffer was used whose pH was 7.0. READY appeared on screen pressed YES and then MEASURED appeared on screen. Electrode of pH meter was rinsed with distilled water. Electrode of pH meter was dipped into flasks one by one and pH of samples were noted.

3.6 Statistical Analysis

Statistical analysis was done on data obtained from soil samples. T test was used to know the difference of Zinc concentration and pH, between healthy and diseased plants soil samples. Minitab 21 was used for T test. Correlation test was also used to know the strength and he direction of correlation between pH and available Zinc concentration, with the help of minitab 21. MS exel 2007 used for general statistics.

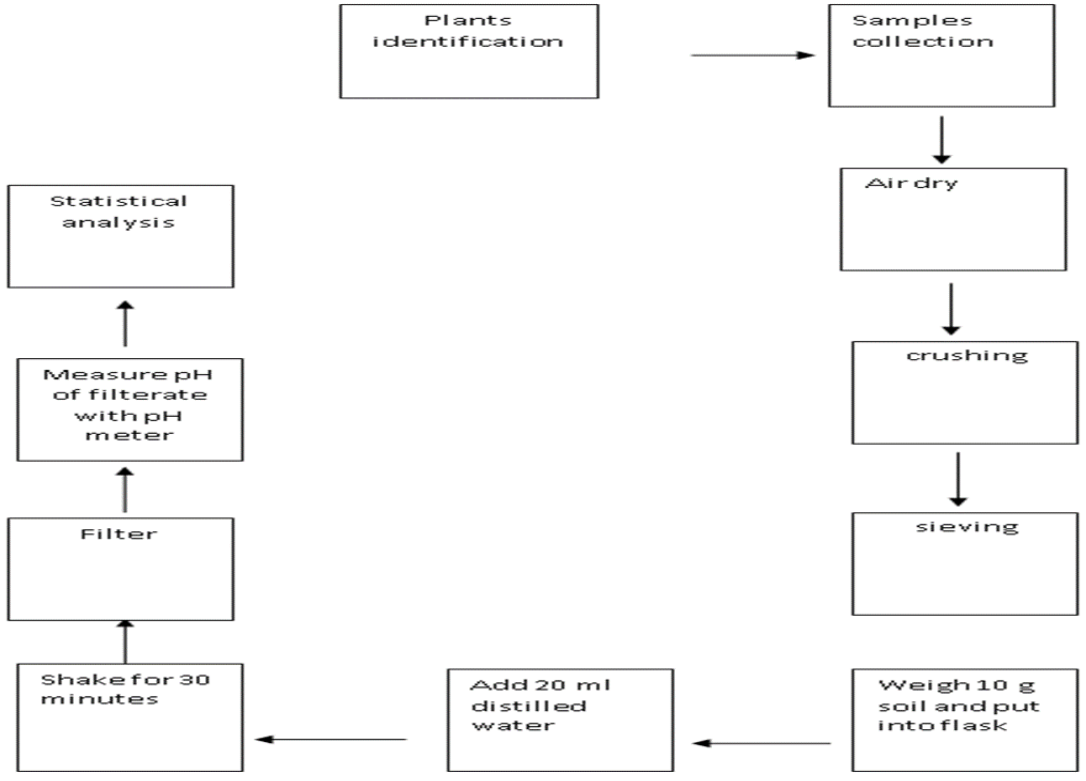

FIGURE 3.4: Flow chart of methodology for pH analysis

Chapter 4

Results and Discussion

4.1 Characters of Thirty Selected Plants

90 soil samples were taken from thirty plants for analysis of available Zinc and pH. Different characters of the selected plants were noted. These characters include, health status of plants, location of plants and girth of plants. Health status noted either the selected plants were healthy or diseased. Location of plants were noted with the help of GPS. Girth of plants in centimeters were also noted with the help of inch tape. All the noted characters are mentioned in table 4.1.

Table 4.1: Informations about thirty selected plants

Sr. No	**Sample I.D**	**Tree no.**	**Status**	**Location (Latitude. Longitude.)**	**Girth (cm)**
1	H1A	1	Healthy	33.5760585, 73.3091258	11
2	H1B	1	Healthy	33.5760585, 73.3091258	11
3	H1C	1	Healthy	33.5760585, 73.3091258	11

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
4	H2A	2	Healthy	33.5764433, 733103412	70
5	H2B	2	Healthy	33.5764433, 733103412	70
6	H2C	2	Healthy	33.5764433, 733103412	70
7	H3A	3	Healthy	33.5767959, 73.3102818	78
8	H3B	3	Healthy	33.5767959, 73.3102818	78
9	H3C	3	Healthy	33.5767959, 73.3102818	78
10	H4A	4	Healthy	33.5779159, 73.3103762	69
11	H4B	4	Healthy	33.5779159, 73.3103762	69
12	H4C	4	Healthy	33.5779159, 73.3103762	69
13	H5A	5	Healthy	33.5765617, 73.3096312	71
14	H5B	5	Healthy	33.5765617, 73.3096312	71
15	H5C	5	Healthy	33.5765617, 73.3096312	71
16	H6A	6	Healthy	33.5813533, 73.3122467	84

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
17	H6B	6	Healthy	33.5813533, 73.3122467	84
18	H6C	6	Healthy	33.5813533, 73.3122467	84
19	H7A	7	Healthy	33.5818717, 73.3126483	183
20	H7B	7	Healthy	33.5818717, 73.3126483	183
21	H7C	7	Healthy	33.5818717, 73.3126483	183
22	H8A	8	Healthy	33.5765548, 73.3078498	33
23	H8B	8	Healthy	33.5765548, 73.3078498	33
24	H8C	8	Healthy	33.5765548, 73.3078498	33
25	H9A	9	Healthy	33.5765017, 73.3097817	29
26	H9B	9	Healthy	33.5765017, 73.3097817	29
27	H9C	9	Healthy	33.5765017, 73.3097817	29
28	H10A	10	Healthy	33.4940245, 73.3859856	133
29	H10B	10	Healthy	33.4940245, 73.3859856	133

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
30	H10C	10	Healthy	33.4940245, 73.3859856	133
31	H11A	11	Healthy	33.4942357, 73.3861981	60
32	H11B	11	Healthy	33.4942357, 73.3861981	60
33	H11C	11	Healthy	33.4942357, 73.3861981	60
34	H12A	12	Healthy	33.3161999, 73.2252292	81
35	H12B	12	Healthy	33.3161999, 73.2252292	81
36	H12C	12	Healthy	33.3161999, 73.2252292	81
37	H13A	13	Healthy	33.3165585, 73.2252685	97
38	H13B	13	Healthy	33.3165585, 73.2252685	97
39	H13C	13	Healthy	33.3165585, 73.2252685	97
40	H14A	14	Healthy	33.3165359, 73.2252043	65
41	H14B	14	Healthy	33.3165359, 73.2252043	65
42	H14C	14	Healthy	33.3165359, 73.2252043	65

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
43	H15A	15	Healthy	33.5743043, 73.3099852	75
44	H15B	15	Healthy	33.5743043, 73.3099852	75
45	H15C	15	Healthy	33.5743043, 73.3099852	75
46	D16A	16	Diseased	33.5765617, 73.3072812	54
47	D16B	16	Diseased	33.5765617, 73.3072812	54
48	D16C	16	Diseased	33.5765617, 73.3072812	54
49	D17A	17	Diseased	33.5814217, 73.3125303	121
50	D17B	17	Diseased	33.5814217, 73.3125303	121
51	D17C	17	Diseased	33.5814217, 73.3125303	121
52	D18A	18	Diseased	33.5814136, 73.3122522	117
53	D18B	18	Diseased	33.5814136, 73.3122522	117
54	D18C	18	Diseased	33.5814136, 73.3122522	117
55	D19A	19	Diseased	33.5841369, 73.3138562	33

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
56	D19B	19	Diseased	33.5841369, 73.3138562	33
57	D19C	19	Diseased	33.5841369, 73.3138562	33
58	D20A	20	Diseased	33.5839552, 73.3138624	23
59	D20B	20	Diseased	33.5839552, 73.3138624	23
60	D20C	20	Diseased	33.5839552, 73.3138624	23
61	D21A	21	Diseased	33.5843138, 73.3141777	44
62	D21B	21	Diseased	33.5843138, 73.3141777	44
63	D21C	21	Diseased	33.5843138, 73.3141777	44
64	D22A	22	Diseased	33.4943255, 73.3856909	133
65	D22B	22	Diseased	33.4943255, 73.3856909	133
66	D22C	22	Diseased	33.4943255, 73.3856909	133
67	D23A	23	Diseased	33.4941642, 73.5788245	120
68	D23B	23	Diseased	33.4941642, 73.5788245	120

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
69	D23C	23	Diseased	33.4941642, 73.5788245	120
70	D24A	24	Diseased	33.3169533, 73.2262289	82
71	D24B	24	Diseased	33.3169533, 73.2262289	82
72	D24C	24	Diseased	33.3169533, 73.2262289	82
73	D25A	25	Diseased	33.3182604, 73.2266173	74
74	D25B	25	Diseased	33.3182604, 73.2266173	74
75	D25C	25	Diseased	33.3182604, 73.2266173	74
76	D26A	26	Diseased	33.3188448, 73.2265509	140
77	D26B	26	Diseased	33.3188448, 73.2265509	140
78	D26C	26	Diseased	33.3188448, 73.2265509	140
79	D27A	27	Diseased	33.5914569, 73.3292536	9
80	D27B	27	Diseased	33.5914569, 73.3292536	9
81	D27C	27	Diseased	33.5914569, 73.3292536	9

Table 4.1 continued from previous page

Sr. No	Sample I.D	Tree no.	Status	Location (Latitude Longitude.)	Girth (cm)
82	D28A	28	Diseased	33.5942518, 73.3384542	25
83	D28B	28	Diseased	33.5942518, 73.3384542	25
84	D28C	28	Diseased	33.5942518, 73.3384542	25
85	D29A	29	Diseased	33.5743998, 73.3101852	27
86	D29B	29	Diseased	33.5743998, 73.3101852	27
87	D29C	29	Diseased	33.5743998, 73.3101852	27
88	D30A	30	Diseased	33.5740034, 73.3101242	42
89	D30B	30	Diseased	33.5740034, 73.3101242	42
90	D30C	30	Diseased	33.5740034, 73.3101242	42

Table 4.1 is showing the serial numbers of plants soil samples, their IDs numbers, tree number, status of tree and location of tree. Serial numbers of samples were 1 to 90. ID number of first samples is H1A. Here, H stands for healthy and1 is representing tree number. Tree numbers were 1 to 30, as total plants were 30.

A is representing the depth 1. Similarly, B is representing depth 2 and C is representing the depth 3. Status of plant showed 50% plants were healthy and 50% were diseased. Table is also showing the coordinates in form of latitudes

and longitudes of all the tree those were selected from three tehsils. The girth of selected plants were ranging from 11 to 183 cm.

4.2 Percentage of Samples Taken from 3 Tehsils

All the 90 soil samples were taken randomly from three tehsils of district Rawalpindi, named Kahuta, Kallar and Gujjar Khan. Heathy as well as diseased plants soil samples were taken from three tehsils. Figure 4.1 is showing the percentage of samples tehsil-wise.

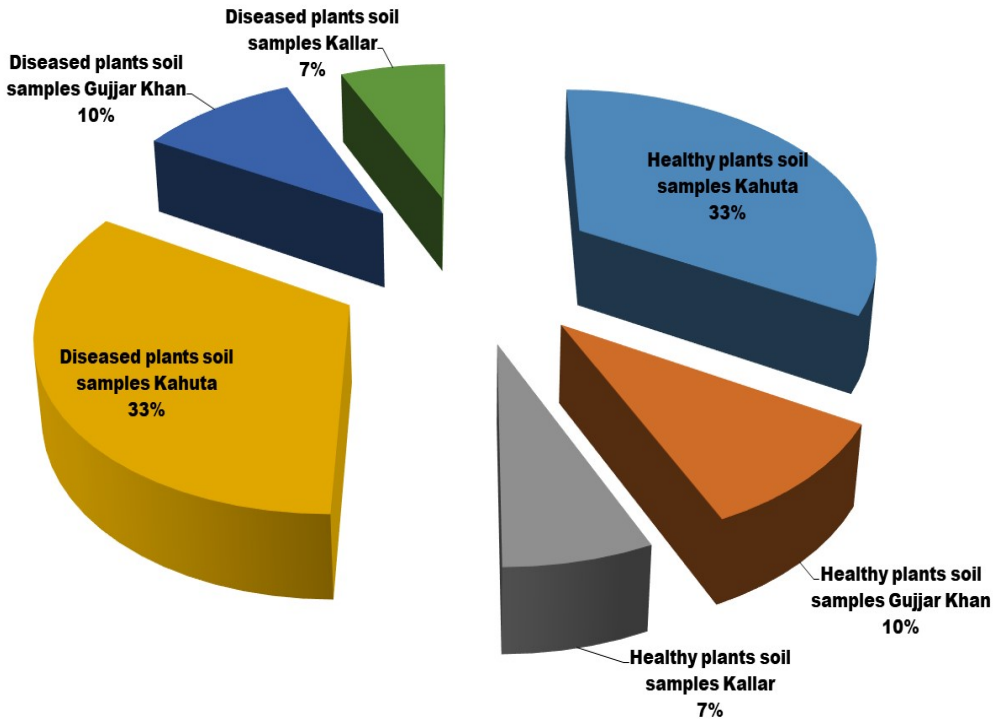

FIGURE 4.1: Percentage of healthy and diseased plants soil samples collection from three tehsils.

Figure 4.1 is showing the percentage of samples, collected from three tehsils i.e. Kahuta, Gujjar khan and Kallar. 33% healthy and 33% diseased plants soil samples were taken from Kahuta. 10% healthy and 10% diseased plants soil samples were collected from Gujjar Khan. 7% healthy and 7% diseased plants soil samples were taken from Kallar. Equal healthy and diseased plants soil samples were taken from a tehsil.

4.3 Age of Selected Plants

Girths of selected plants were noted which help to determine the approximate ages of plants. Selected plants were belonging to different ages. Younger as well as older plants were affected by the dieback disease. Ages of selected plants is mentioned in Figure 4.2.

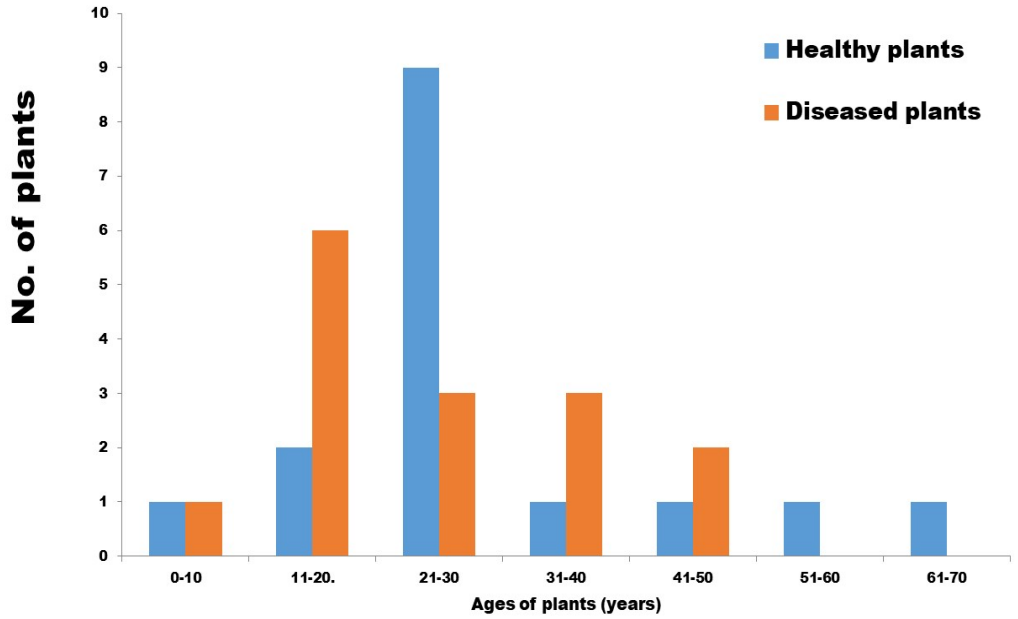

FIGURE 4.2: Ages of the studied plants.

Figure 4.2 is showing the approximate age of the studied plants those were determined by the help of their girths. The left blue coloured columns are showing the healthy plants while right red coloured columns are representing the diseased plants. X axis representing the age groups in years those are; 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70. Y axis representing the number of plants. There was only single healthy and diseased plant, of age 0-10 yreas. Two healthy and six diseased plants, were 11-20 years old. 9 plants were healthy belonging to 21-30 age group and three were diseased plants. In 31-40 years age group, there were 1 and 3 healthy and diseased plants respectively. 1 healthy and 2 diseased plants, were 41-50 years old. In 51-60 and 61-70 years age groups, both groups showed no healthy and 1, 1 diseased plants. In the studied plants, Healthy and diseased plants were belonging to different age groups.

4.4 Comparison of Healthy and Diseased Plants Soil Samples for Available Zinc Concentration

T test was used to compare statistical difference between healthy and diseased plants soil samples. Concentration of available Zinc was compared in depths as well as overall. Mean, standard deviation, range, N, t value and p value of healthy and diseased plants soil samples were analysed depth-wise as well as overall, as mentioned in table 4.2.

TABLE 4.2: Statistical comparison of Zinc concentration in soil samples

		Mean	**Standard deviation**	**Standard Error**	**Range**	**N**	**T-Value**	**P-Value**
Depth 1	Healthy	1.16	0.49	0.05	1.57	15	1.36	0.18
	Diseased	0.95	0.33	0.05	1.21	15		
Depth 2	Healthy	0.69	0.35	0.06	1.51	15	1.54	0.14
	Diseased	0.53	0.17	0.05	0.68	15		
Depth 3	Healthy	0.42	0.30	0.04	1.21	15	0.06	0.95
	Diseased	0.41	0.24	0.04	0.76	15		
Overall	Healthy	0.75	0.26	0.05	1.04	15	1.38	0.17
	Diseased	0.63	0.21	0.04	0.79	15		

Table 4.2 is showing statistical comparison of Zinc concentration between healthy plants soil samples and diseased plants soil samples in three depths, i.e. depth 1, depth 2 and depth 3 as well as overall. Mean, standard deviation, t value and p value were compared between healthy and diseased plants soil samples in all the the three depths as well as overall. N is showing the number of samples. T values showing the difference of available Zinc between healthy and diseased plants soil samples groups. P values showing the significant difference and the level of

significane was 0.05.

Mean of healthy plants soil samples are slightly higher than the diseased plants soil samples but not statistically significant difference observed, in all the three depths. Similar trend appeared in overall comparison between healthy and diseased plants soil samples. There is not statistical significant differene between healthy and diseased plants soil samples, in any of all the three depths as well as overall.

4.4.1 Comparison of Available Zinc Concentration in Depth 1

Available Zinc concentration compared between healthy and diseased group in depth 1 by using t test. First depth of both groups were compared. P and t value showed the statistical difference between the two groups. Means and standard deviations were also compared between two groups as mentioned in figure 4.3.

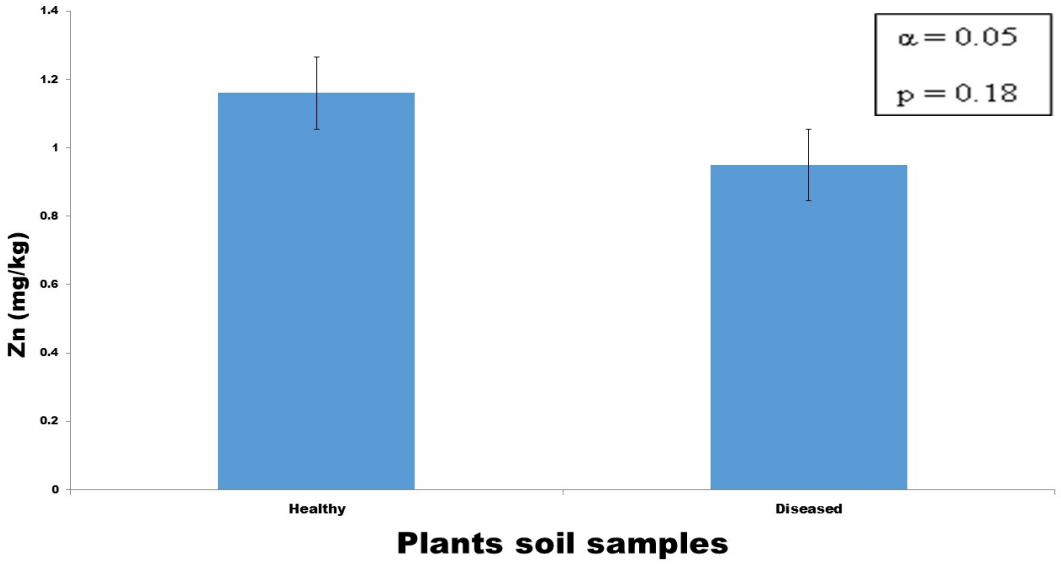

FIGURE 4.3: Comparison of the available Zinc concentration between healthy and diseased plants soil samples in depth 1.

Figure 4.3 is showing comparison of available Zinc concentration in first depth, between healthy and diseased soil samples. X-axis has two categories healthy and diseased plants soil samples groups while, Y-axis is showing the available Zinc concentration in mg/kg. Columns are representing the mean of each category

while bars showing the standard deviations. Standard deviations are showing the variations of samples in population from means. Mean of healthy group is slightly greater than the mean of diseased group. But the bars are overlapping each other which is clearly showing there is no significant difference between healthy and diseased plants soil samples, in depth 1.

4.4.2 Comparison of Available Zinc Concentration in Depth 2

Available Zinc concentration was compared between healthy and diseased group in depth 2 by using t test. Second depth of both groups were compared. P and t value showed the statistical difference between the two groups. Means and standard deviations were also compared between two groups as mentioned in figure 4.4.

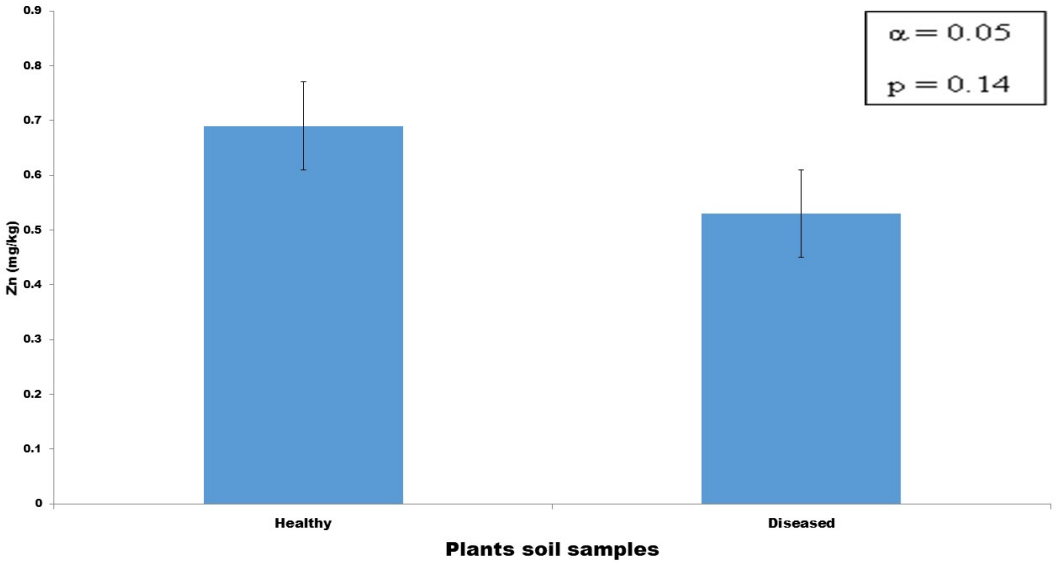

FIGURE 4.4: Comparison of the available Zinc concentration between healthy and diseased plants soil samples in depth 2.

Figure 4.4 is showing the comparison between healthy and diseased plants soil samples in second depth. X-axis contains two categories i.e. healthy and diseased plants soil samples groups. Y-axis is showing available concentration of Zinc in mg/kg. The columns are representing the means of healthy and diseased groups

respectively. The bars are showing the standard deviations of each group. Standard deviations are showing the variation of samples in population. The mean of healthy group is slightly higer than the mean of the diseased group but the bars are not overlapping each other. Therefore there is no significane difference between healthy and diseased plants soil samples, in depth 2.

4.4.3 Comparison of Available Zinc Concentration in Depth 3

Available Zinc concentration was compared between healthy and diseased group in depth 3 by using t test. Third depth of both groups were compared. P and t value showed the statistical difference between the two groups. Means and standard deviations were also compared between two groups as showned in figure 4.5.

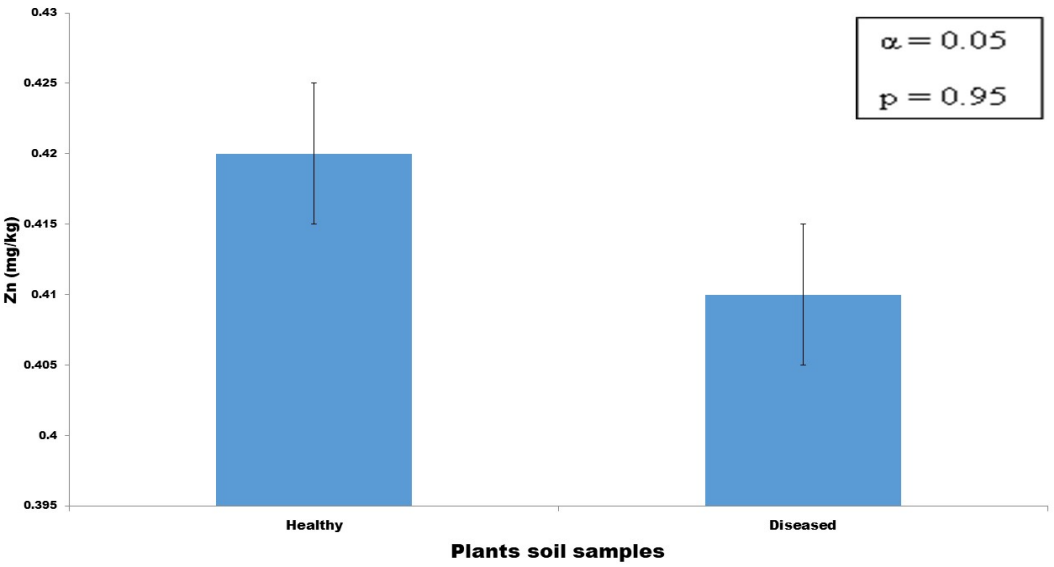

FIGURE 4.5: Comparison of the available Zinc concentration between healthy and diseased plants soil samples in depth 3.

Figure 4.5 is showing a comparison of available Zinc concentration between healthy and diseased plants soil samples categories . X-axis showing healthy and diseased plants soil samples categories while Y-axis showing available concentration of Zinc in mg/kg. columns are showing the means of healthy and diseased plants soil samples. Bars are showing the standard deviations. Standard deviations are

showing the variation of samples from means in population. Mean of healthy group is slightly greater than mean of diseased group but there is overlapping of bars which means there is no significant difference of available Zinc concentration between two groups in depth 3.

4.4.4 Overall Comparison of Available Zinc Concentration

Available Zinc concentration was compared between healthy and diseased group overall by using t test. Average of all the three depths was calculated for all the plants and then compared the healthy and diseased plants soil samples. Overall, both groups were compared. P and t value showed the statistical difference between the two groups. Means and standard deviations were also compared between two groups as showned in figure 4.6.

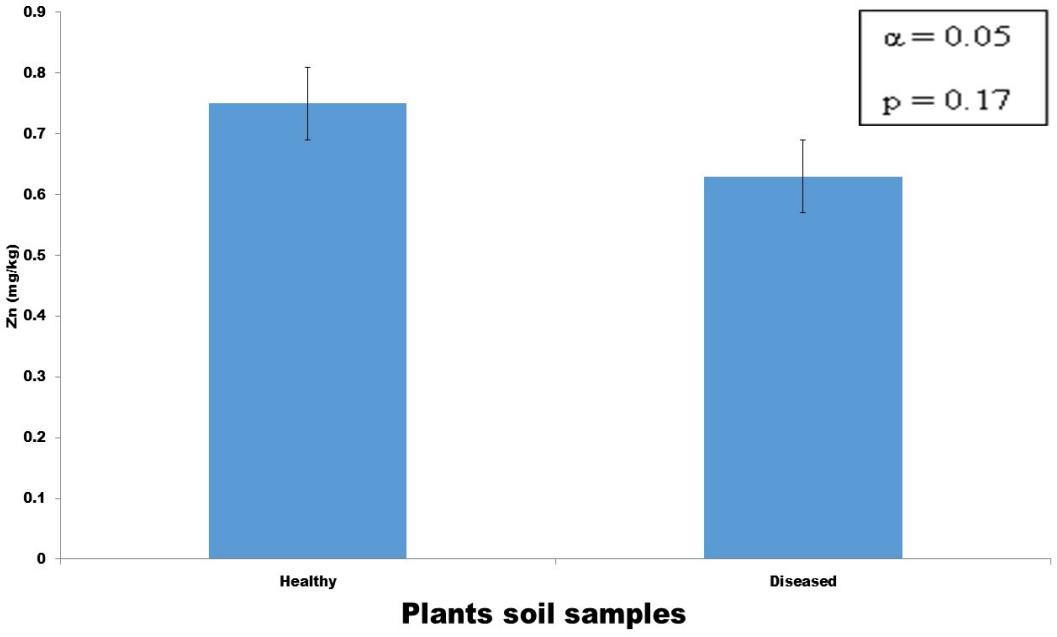

FIGURE 4.6: Comparison of the available Zinc concentration between healthy and diseased plants soil samples for average of all the three depths.

Figure 4.6 is showing overall comparison between healthy and diseased plants soil samples. X- axis showing healthy and diseased plants soil samples and Y-axis showing the available Zinc concentration in mg/kg. Columns are representing means of healthy and diseased groups. Bars are showing the standard deviations.

Standard deviations are representing the variation of samples from means in population. Healthy group is showing slightly more mean than the diseased group, but there is no gap between bars so the healthy group showed slightly more zinc concentration as compared to the diseased group, but did not show significant difference between the two groups. Overall, there is no significant difference between healthy and diseased plants soil samples.

4.4.5 Percentage of Zinc Deficiency in Depth 1

Zinc concentration was analysed and Zinc deficiency appeared in plants soil samples. Numbers of Zinc deficient soil samples counted and their percentage was calculated in healthy samples as well in diseased samples of depth 1. Similarly Zinc sufficient samples were also counted and their percentage calculated. Percentages are shown in figure 4.7.

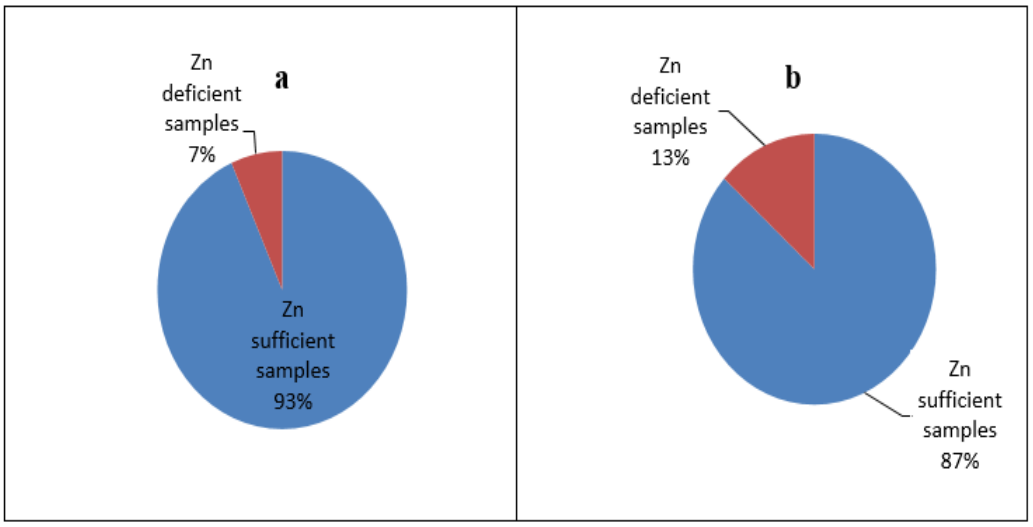

FIGURE 4.7: The comparison of the percentage Zinc deficient soil samples between healthy and diseased groups of depth 1.

Figure 4.7 is showing the percentage of Zinc sufficient soil samples and percentage Zinc deficient soil samples of healthy and diseased plants soil samples in depth 1. (a) is representing the Healthy plants soil samples and (a) is showing that there are 93% samples having sufficient available zinc concentration and 7% samples with

Zinc deficiency. (b) is representing the diseased plants soil samples, (b) is showing that there are 87% soil samples having sufficient amount of Zinc concentration and 13% showing Zinc deficiency. In depth 1, the healthy plants soil samples showed 7% Zinc deficiency while in diseased plants soil samples deficiency is 13%.

4.4.6 Percentage of Zinc Deficiency in Depth 2

In depth 2 Zinc concentration was analysed. There were Zinc deficiency in plants soil samples. Numbers of Zinc deficient soil samples counted and their percentage was calculated in healthy samples as well in diseased samples of depth 2. Similarly Zinc sufficient samples were also counted and their percentage calculated. Zinc deficient and sufficient Percentages are shown in figure 4.8.

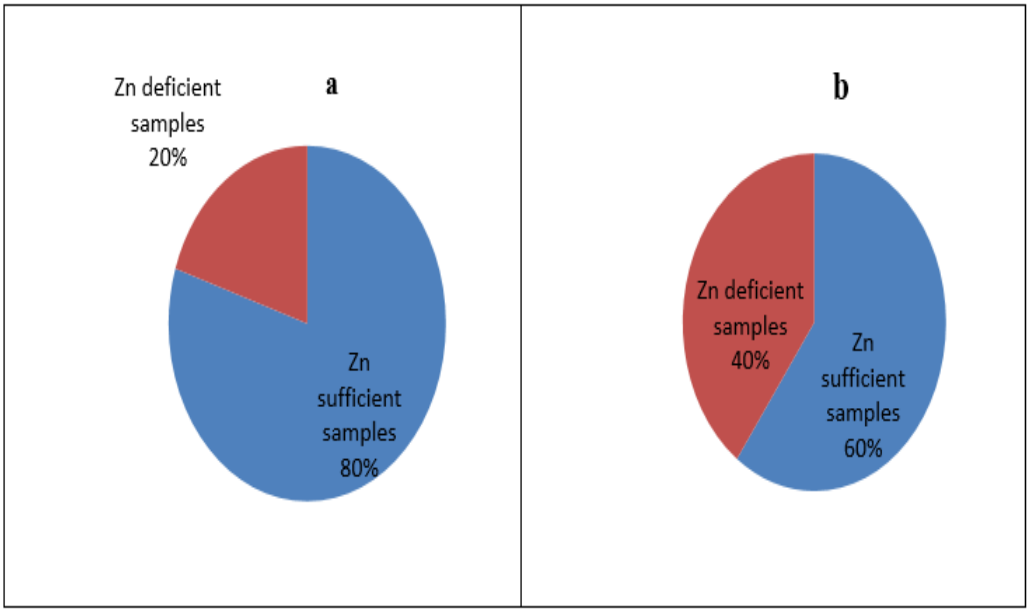

FIGURE 4.8: The comparison of the percentage Zinc deficient soil samples between healthy and diseased groups of depth 2.

Figure 4.8 is showing the percentage of Zinc sufficient soil samples and Zinc deficient soil samples of healthy and diseased plants soil samples, in depth 2. (a) is showing healthy plants soil samples and these are 80% Zinc sufficient and 20% are Zinc deficient. (b) is showing the diseased plants soil samples and these are showing that 60% samples are Zinc sufficient and 40% samples are Zinc deficient,

in depth 2. 20% Zinc deficiency observed in healthy plants and 40% in diseased plants.

4.4.7 Percentage of Zinc Deficiency in Depth 3

Zinc concentration in depth 3 was analysed. The Zinc deficiency observed in plants soil samples. Numbers of Zinc deficient soil samples were counted and their percentage was calculated in healthy samples as well in diseased samples of depth 3. Similarly Zinc sufficient samples were also counted by numbers. Then percentage of sufficient soil samples was calculated. Zinc deficient and sufficient Percentages are shown in figure 4.9.

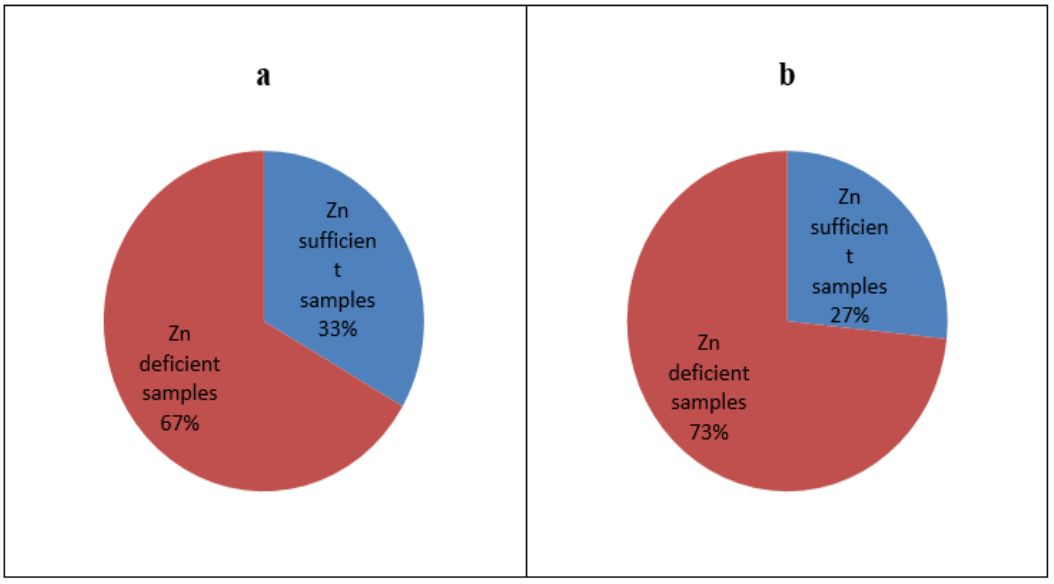

FIGURE 4.9: The comparison of the percentage Zinc deficient soil samples between healthy and diseased groups of depth 3.

Figure 4.9 is showing the percentage of the Zinc sufficient soil samples and Zinc deficient soil samples of healthy and diseased plants soil samples, in depth 3. Healthy plants soil samples are represented by (a) and showing that there are 33% soil samples are Zinc sufficient and 67% samples are Zinc deficient. Diseased plants soil samples are represented by (b) and showing that 27% samples are Zinc sufficient and 73% samples are Zinc deficient, in depth 3. Healthy plants soil showed 67% Zinc deficiency and diseased plants soil showed 73%, in depth 3.

4.4.8 Percentage of Zinc Deficiency in all the Three Depths

In all the three depths Zinc concentration was analysed. Zinc deficiency in plants soil samples observed. Numbers of Zinc deficient soil samples counted and their percentage was calculated in healthy samples as well in diseased samples of all the three depths. Similarly Zinc sufficient samples were also counted and their percentage calculated. Zinc deficient and sufficient Percentages are shown in figure 4.10.

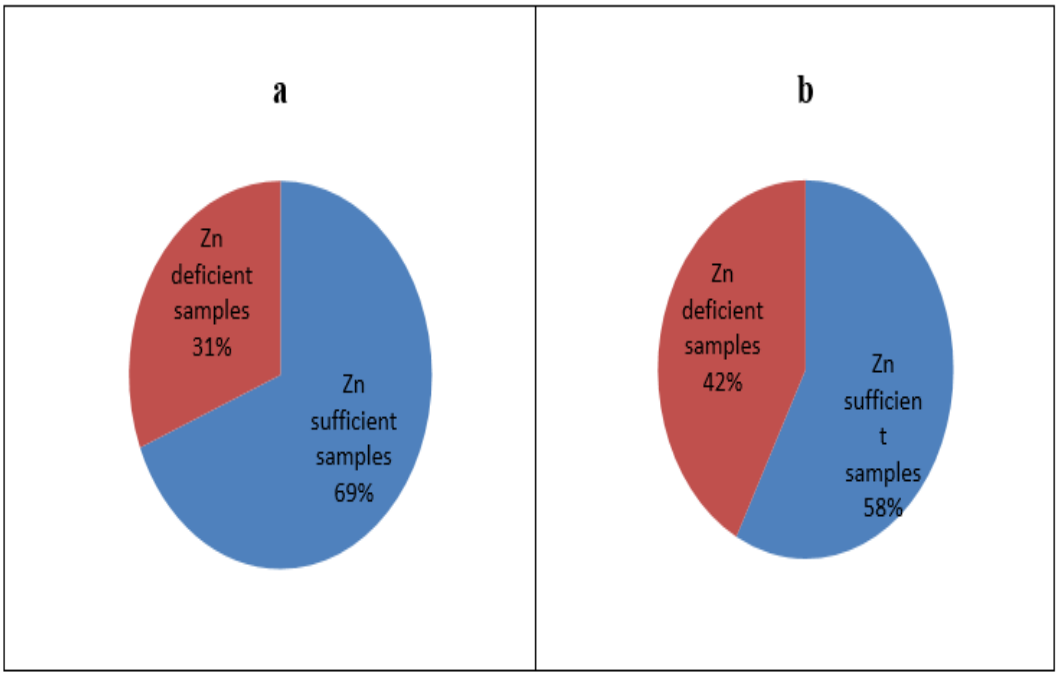

FIGURE 4.10: The comparison of the percentage Zinc deficient soil samples between healthy and diseased groups of all the three depths.

Figure 4.10 is showing the comparison of the percentage of Zinc sufficient soil samples and Zinc deficient soil samples for all the three depths, between healthy (a) and diseased plants soil samples (b). Healthy plants soil samples (a) is showing that there are 69% soil samples with sufficient available Zinc concentration and 31% soil samples with Zinc deficiency. On the other hand, graph of the diseased plants soil samples showed that 42% samples are Zinc deficient and 58% samples are Zinc sufficient. Zinc deficiency in both groups is almost similar, so zinc deficiency is not creating problem for diseased plants soil samples because, healthy plants soil samples group is also showing the Zinc deficiency.

4.5 Comparison of Healthy and Diseased Plants Soil Samples for pH

T test was used for comparison and to compare statistical difference between healthy and diseased plants soil samples. PH was compared between healthy and diseased groups in all the three depths one by one, as well as overall comparison was also done. Mean, standard deviation, t value and p value of healthy and diseased plants soil samples were analysed depth-wise as well as overall. Comparison of depth 1, 2 and 3was done and then overall comparison was done. Results of comparisons are mentioned in table 4.3.

TABLE 4.3: Statistical comparison of pH in soil samples

		Mean	**Standard deviation**	**Standard Error**	**Range**	**N**	**T**	**P**
Depth 1	Healthy	8.01	0.26	0.05	1.17	15	-1.0	0.06
	Diseased	8.19	0.22	0.05	0.68	15		
Depth 2	Healthy	8.10	0.24	0.05	0.66	15	-1.5	0.12
	Diseased	8.24	0.23	0.05	0.69	15		
Depth 3	Healthy	8.22	0.18	0.04	0.65	15	-1.2	0.22
	Diseased	8.30	0.17	0.04	0.57	15		
overall	Healthy	8.11	0.19	0.05	0.80	15	-1.86	0.07
	Diseased	8.24	0.18	0.04	0.56	15		

Table 4.3 showing statistical comparison of pH in healthy plants soil samples and diseased plants soil samples, of all the three depths as well as overall. Mean, standard deviation were compared in between healthy and diseased plants soil samples. N is representing the number of samples that are 15 for each group.

In depth 1, pH of diseased plants soil samples were slightly more than the healthy but no significant difference observed. Similarly, in depth 2 and 3, pH was slightly

higher in diseased plants soil samples as compared to healthy plants soil samples but there was no significance difference between two groups. Similarly, the overall analysis of pH showed that there is no statistiacal difference between healthy and diseased plants soil samples, inspite of slight difference between their means.

4.5.1 Comparison of pH in Depth 1

Comparison of pH was done in first depth between healthy and diseased plants soil samples. T test was used for comparison between healthy and diseased groups. Means and standard deviations were compared, as figure 4.11 is showing the comparison.

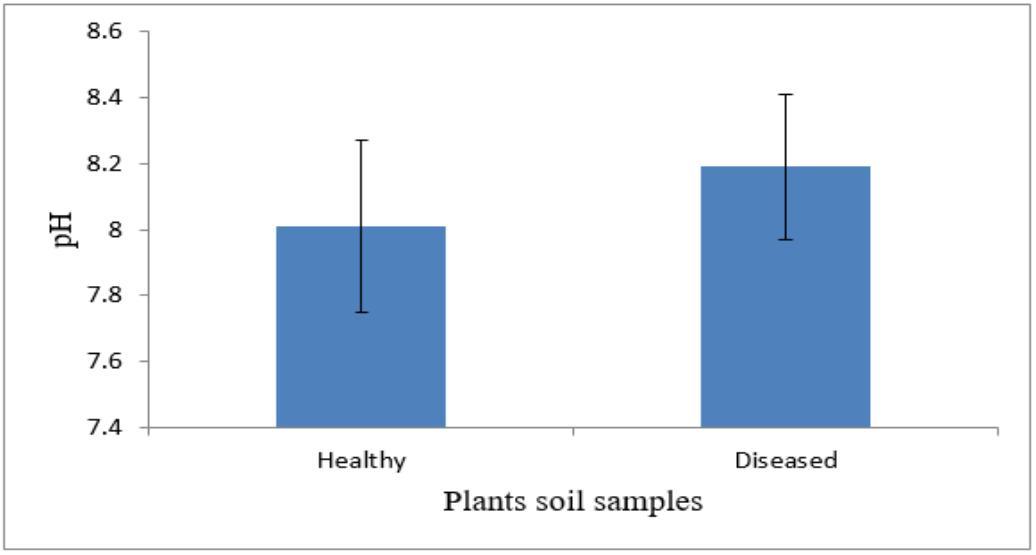

FIGURE 4.11: Comparison of the pH between healthy and diseased plants soil samples in depth 1.

Figure 4.11 is showing a comparison of pH, between healthy and diseased plants soil samples. X-axis contains two categories i.e. healthy plants soil samples and diseased plants soil samples. Y-axis showing pH in depth 1. Columns are representing the means of two groups i.e. healthy and diseased group. The bars are representing the standard deviations, which show the variations of samples in population, it can be predicted how the samples are showing variation from their means. PH of the diseased group is slightly more than healthy group but there is

no gap between bars. The bars are overlapping each other, therefore there is no significant difference between healthy and diseased groups.There was not observed significance difference of pH, between the healthy and diseased plants soil samples in depth 1.

4.5.2 Comparison of pH in Depth 2

Comparison of pH was done in depth 2, between healthy and diseased plants soil samples. T test was used for comparison between healthy and diseased plants soil samples. Means and standard deviations were compared, as figure 4.12 is showing the comparison.

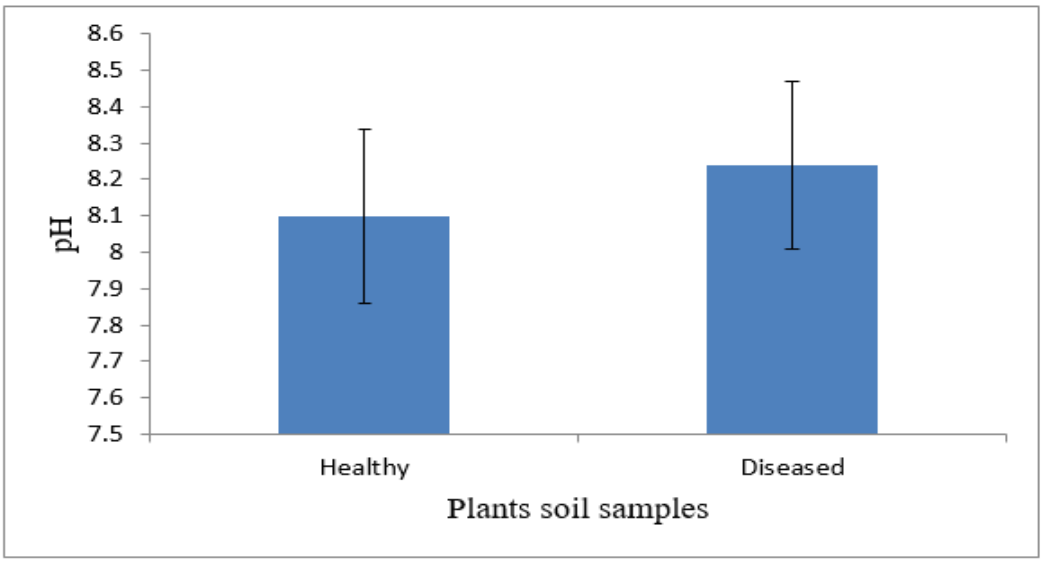

FIGURE 4.12: Comparison of the pH between healthy and diseased plants soil samples in depth 2.

Figure 4.12 is showing a comparison of pH, between healthy and diseased plants soil samples. X-axis contains two groups i.e. healthy plants soil samples and diseased plants soil samples. Y-axis showing pH value in depth 2. Columns are representing the means of two groups i.e. healthy group and diseased group. The bars are showing the standard deviations, which show the variation of samples in population, both healthy and diseased plants soil samples are showing variation in population. Samples of both groups showed a certain deviation in population.

PH of the diseased group is slightly more than healthy group but there is no gap between bars. The bars are overlapping each other, therefore there is no significant difference between healthy and diseased groups. There was not observed significance difference of pH, between the healthy and diseased plants soil samples in depth 2.

4.5.3 Comparison of pH in Depth 3

Comparison of pH was done for third depth between healthy and diseased plants soil samples. T test was used for comparison between healthy and diseased groups. Means and standard deviations were compared, as figure 4.13 is showing the comparison.

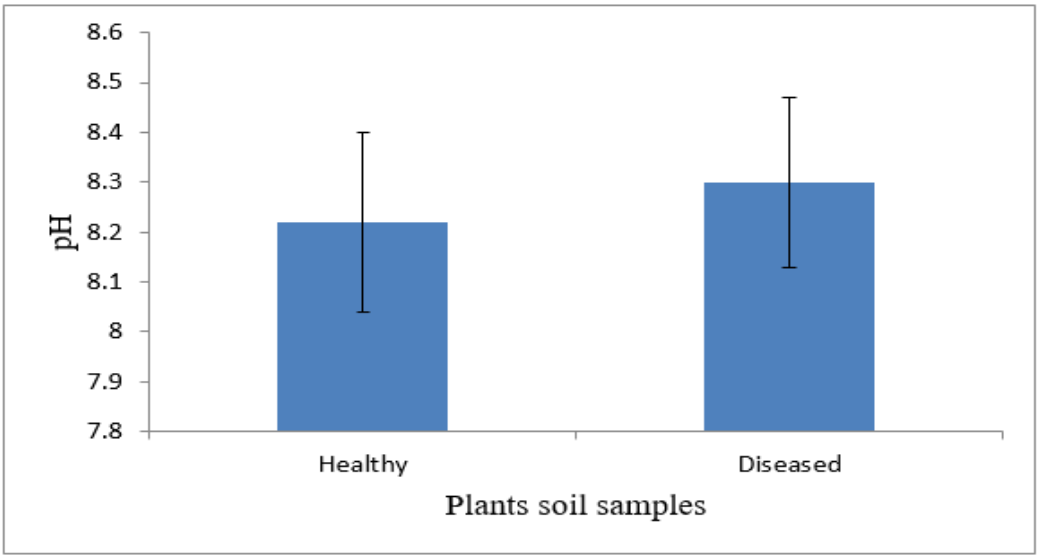

FIGURE 4.13: Comparison of the pH between healthy and diseased plants soil samples in depth 3.

Figure 4.13 is showing a comparison of pH, between healthy and diseased plants soil samples. X-axis is showing two groups i.e. healthy plants soil samples and diseased plants soil samples. Y-axis showing pH in depth 3. Columns are showing the means of two groups and the bars are representing the standard deviation. Standard deviation shows the variation of samples in population. PH of the diseased group is slightly more than healthy group but there is no gap between bars. The bars are

overlapping each other, therefore there is no significant difference between healthy and diseased groups. There was not observed significance difference of pH, between the healthy and diseased plants soil samples in depth 3.

4.5.4 Overall Comparison of pH

Overall comparison was done between healthy and diseased plants soil samples. T test was used for comparison between healthy and diseased groups. Means and standard deviations were compared, as figure 4.14 is showing the comparison.

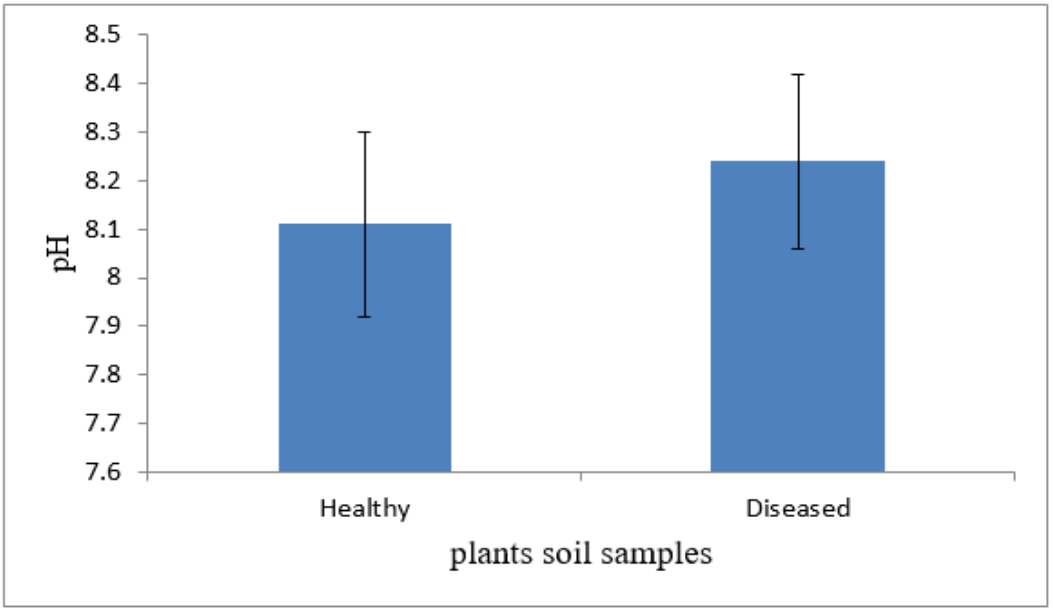

FIGURE 4.14: Comparison of pH between healthy and diseased plants soil samples for average of all the three depths.

Figure 4.14 is showing overall comparison between healthy and diseased plants soil samples. Average of all the three depths was calculated for all the plants and then compared the healthy and diseased plants soil samples. X- axis showing healthy and diseased plants soil samples and Y-axis showing pH. Columns are showing means of two groups and bars are representing the standard deviation. Healthy group showed slightly lower pH as compared to the diseased group, but did not show significant difference between the two groups. Overall, there is no significant difference between healthy and diseased plants soil samples.

4.6 Relative Comparison of P values of Healthy and Diseased Plants Soil Samples in between Zinc and pH.

T test was applied to determine the statistical difference between healthy and diseased groups. The p values revealed the extent of difference between groups. Different healthy and diseased groups were compared with each other to determine statistical difference in form of p value. Then the p values of Zinc concentration and pH were compared of each depth and overall, as shown in table 4.4.

TABLE 4.4: P values of Zinc and pH

	Zinc				**pH**			
	Depth 1	Depth 2	Depth 3	overall	Depth 1	Depth 2	Depth 3	Overall
P-value	0.186	0.140	0.956	0.17	0.066	0.126	0.227	0.07
conclusion	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Note; n.s = non-significant, s = significant and h.s = highly significant.

Table 4.4 is showing the p values of Zinc concentration and pH in three depths and overall. Level of significance was 0.05. Non significant is represented by n.s, significant is represented by s and highly significant is represented by h.s. P values of all pairs are mentioned in table. In depth 1, no significant difference observed between healthy and diseased groups and there was also non significant difference of pH. Both Zinc and pH showed no significant difference. Similar trend was observed in depth 2 and 3. These depths did not Showed significant difference between healthy and diseased groups. Overall comparison of p values between healthy and diseased groups showed no significant difference of available Zinc concentration and pH. Therefore, there was not more fluctuations of Zinc concentration because of lesser pH change between healthy and diseased groups.

4.7 Correlation between pH and Available Zinc Concentration

Available Zinc concentration in soil is controlled by pH. Correlation of pH and zinc concentration was measured to know the synergetic effect of pH and available Zinc concentration in healthy as well as diseased groups. Correlation values and directions were measured for all the three depths as well as overall. Table 4.5 is showing the correlation values and directions for each group. Correlation between pH and Zinc

TABLE 4.5: Correlation between pH and Zinc

	Healthy group		**Diseased group**	
	R value	**Strength**	**R value**	**Strength**
Depth 1	0.45	weak	-0.515	Moderate
Depth 2	0.24	Very weak	0.09	Very weak
Depth 3	0.01	Very weak	-0.34	Weak
Overall	0.58	moderate	-0.32	Weak

Table 4.5 showing the strength of correlation between healthy and diseased group. Depth 1 of healthy plants group showed a weak positive correlation between pH and Zinc concentration, depth 2 and 3 showed very weak correlation and overall in all the three depths there were very weak correlation. For diseased plants soil samples group, depth 1 and 3 showed moderate negative and weak negative correlation respectively, while depth 2 showed very weak positive correlation.

4.7.1 Correlation between pH and Zinc Concentration of Healthy Group in Depth 1

Correlation between pH and available Zinc concentration measured in depth 1. Healthy plants soil samples of depth 1 showed a correlation shown in figure 4.15.

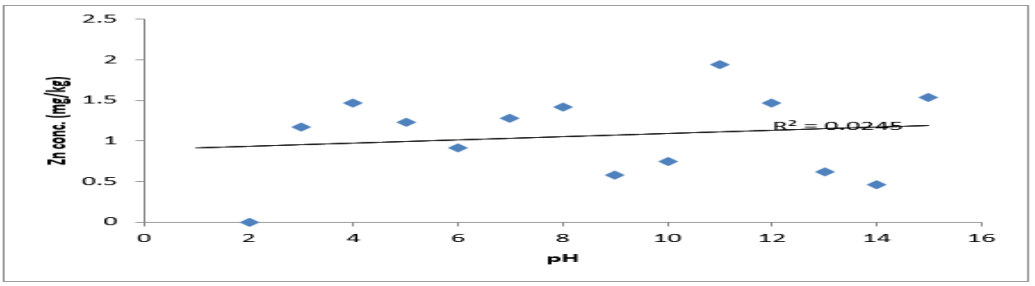

FIGURE 4.15: Correlation between pH and the available Zinc concentration of the healthy plants soil samples in depth 1.

Figure 4.15 is showing the correlation between pH and Zinc concentration of depth 1 for healthy plants soil samples. X-axis showing independent variable i.e. pH, while Y-axis showing dependent variable that is Zinc concentration in mg/kg. A weak positive correlation observed between pH and Zinc concentration , in depth 1 for healthy plants soil samples. It is observed, when pH increased the available Zinc concentration also increased but its strength is weak. Correlation of pH and available Zinc was not so strong and effective in depth 1 of healthy plants.

4.7.2 Correlation between pH and Zinc Concentration of Healthy Group in Depth 2

Correlation between pH and available Zinc concentration observred in depth 2 to determine how these two factors interacting in soil of healthy plants in second depth. After correlation analysis healthy plants soil samples of depth 2 showed a correlation shown in figure 4.16.

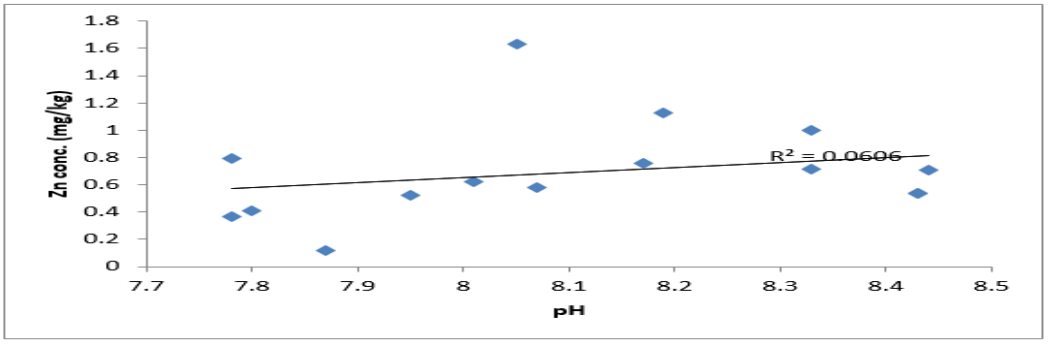

FIGURE 4.16: Correlation between pH and the available Zinc concentration of the healthy plants soil samples in depth 2.

Figure 4.16 is showing correlation between pH and the zinc concentration of healthy plants soil samples in depth 2. X-axis showing pH and Y-axis showing available Zinc concentration in mg/kg. A positive correlation observed. It means, zinc concentration increased with incease in pH. Although, correlation was positive but it was very weak.

4.7.3 Correlation between pH and Zinc Concentration of Healthy Group in Depth 3

Correlation between pH and available Zinc concentration measured in depth 3. Healthy plants soil samples of depth 3 showed a correlation shown in figure 4.17.

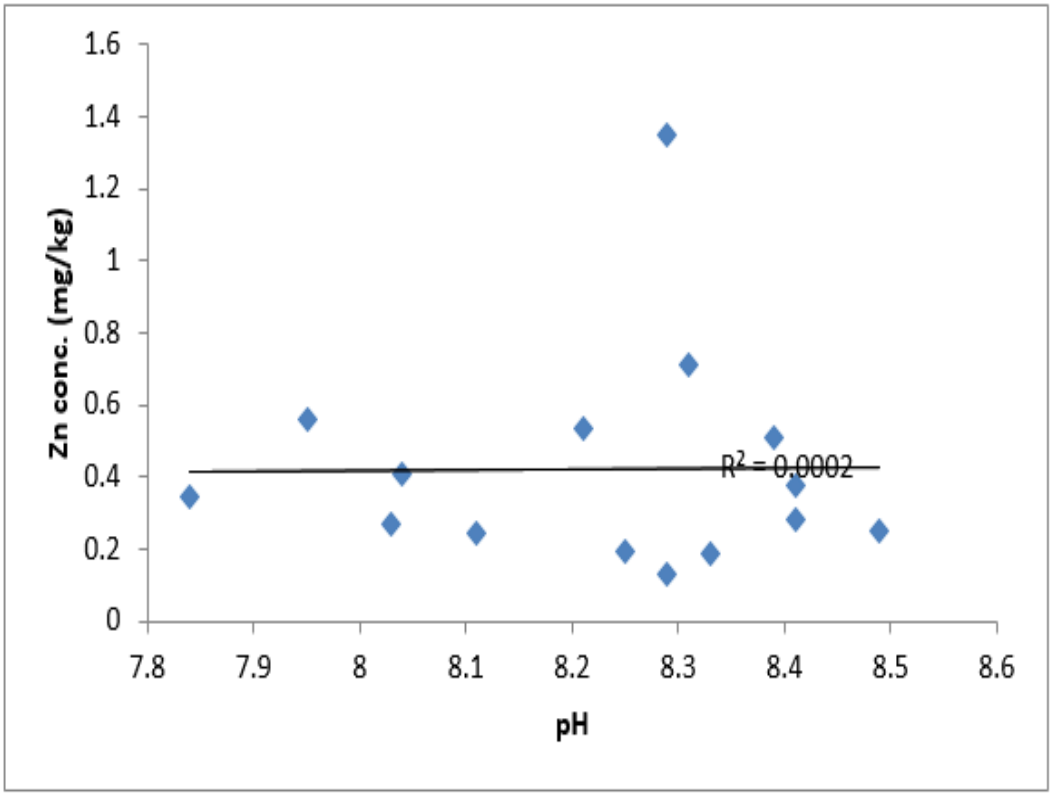

FIGURE 4.17: Correlation between pH and the available Zinc concentration of the healthy plants soil samples in depth 3.

Figure 4.17 is showing the correlation between pH and Zinc concentration of healthy plants soil samples in depth 3. X and Y axis showing pH and Zinc concentration, respectively. Very weak positive correlation was observed in depth 3. By

increasing pH, availability of Zinc increased but in very minute extent. Strength of correlation was very weak almost near to zero.

4.7.4 Overall Correlation between pH and Zinc Concentration of Healthy Group

Overall correlation between pH and available Zinc concentration measured of healthy group. Correlation between pH and Zinc showed how these two factors interacting each other and how these Healthy plants soil samples overall showed a correlation shown in figure 4.18.

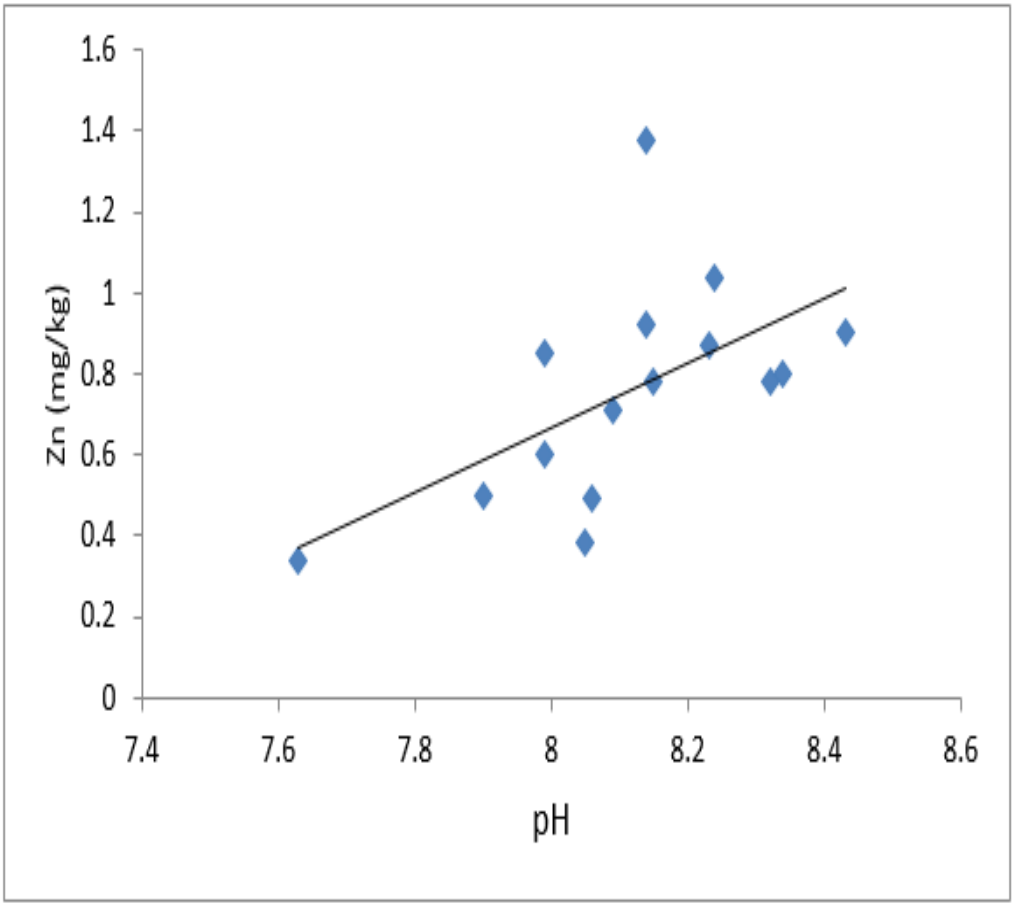

FIGURE 4.18: Correlation between pH and the available Zinc concentration of the healthy plants soil samples for the average of all the three depths.

Figure 4.18 is showing the correlation between pH and available Zinc concentration overall, for the healthy plants soil samples. X-axis is showing the pH and Y-axis

is showing the available Zinc concentration. As, the pH is increasing the available Zinc concentration is also increasing and showing moderate positive correlation. Overall, there was a moderate positive correlation between pH and available Zinc concentration, in healthy group.

4.7.5 Correlation between pH and Zinc Concentration of Diseased Group in Depth 1

Pearson's correlation was used to determine the correlation between pH and available zinc concentration. In first depth of the diseased group also measured and there was a correlation between pH and zinc concentration. Correlation is shown in figure 4.19.

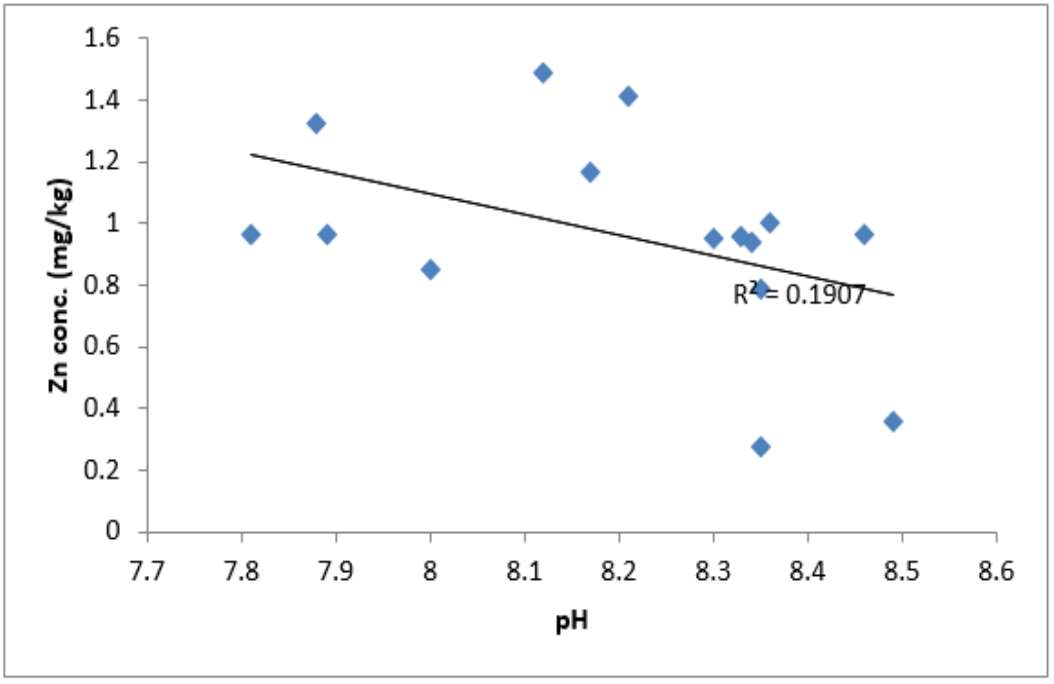

FIGURE 4.19: Correlation between pH and the available Zinc concentration of the diseased plants soil samples in depth 1.

Figure 4.19 representing the correlation between pH and Zinc of diseased plants soil samples in depth 1. X-axis showing pH and Y-axis representing zinc concentration. A moderate negative correlation was observed in depth 1 for diseased healthy plants soil samples. As pH is increased the zinc concentration decreased.

4.7.6 Correlation between pH and Zinc Concentration of Diseased Group in Depth 2

Pearson's correlation was used to determine the correlation between pH and available zinc concentration. Correlatio showed how the pH and available Zinc interacted each other in depth 2 of diseased group. This showed how one factor affected by change of other one. In second depth of the diseased group measured and there was a correlation between pH and zinc concentration. Correlation is shown in figure 4.20.

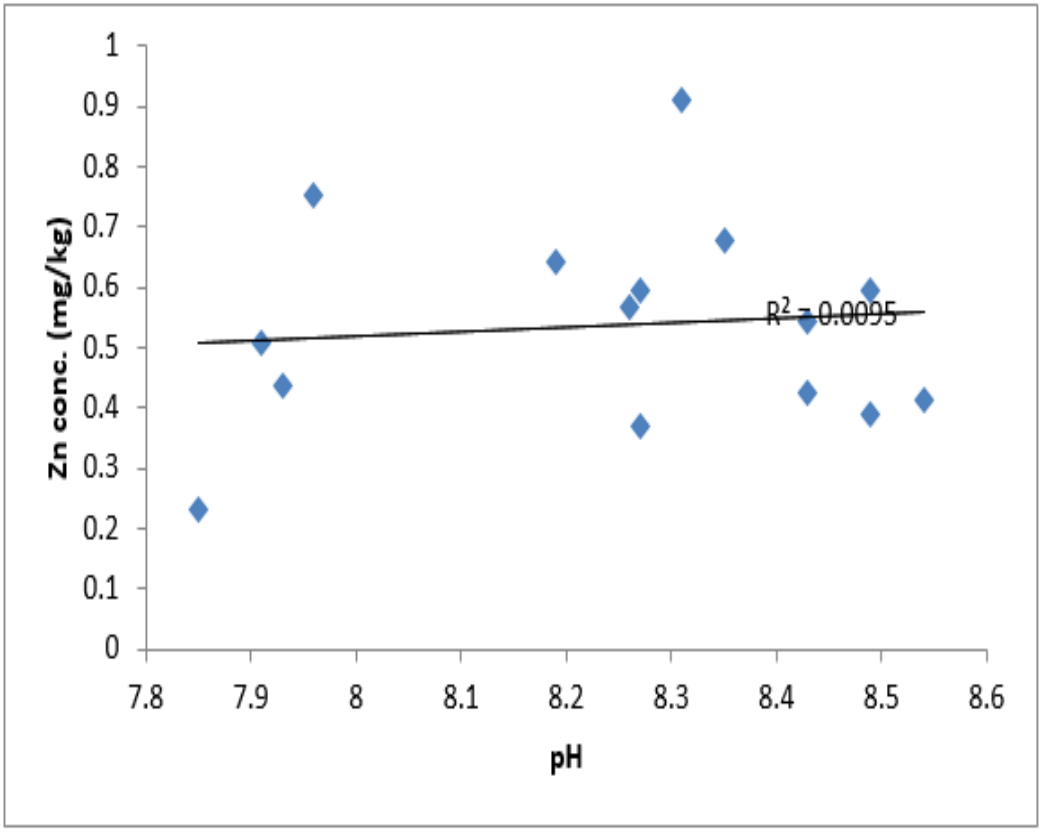

FIGURE 4.20: Correlation between pH and the available Zinc concentration of the diseased plants soil samples in depth 2.

Figure 4.20 showing the correlation between pH and Zinc concentration of diseased plants soil samples in depth 2. X and Y axis showing the pH and Zinc concentration, respectively. A very weak positive correlation observed for diseased plants soil samples in depth 2. Zinc concentration increased with increase in pH, but its strength was very weak.

4.7.7 Correlation between pH and Zinc Concentration of Diseased Group in Depth 3

Pearson's correlation was used to determine the correlation between pH and available zinc concentration. In third depth of the diseased group also measured and there was a correlation between pH and zinc concentration. Correlation is shown in figure 4.21.

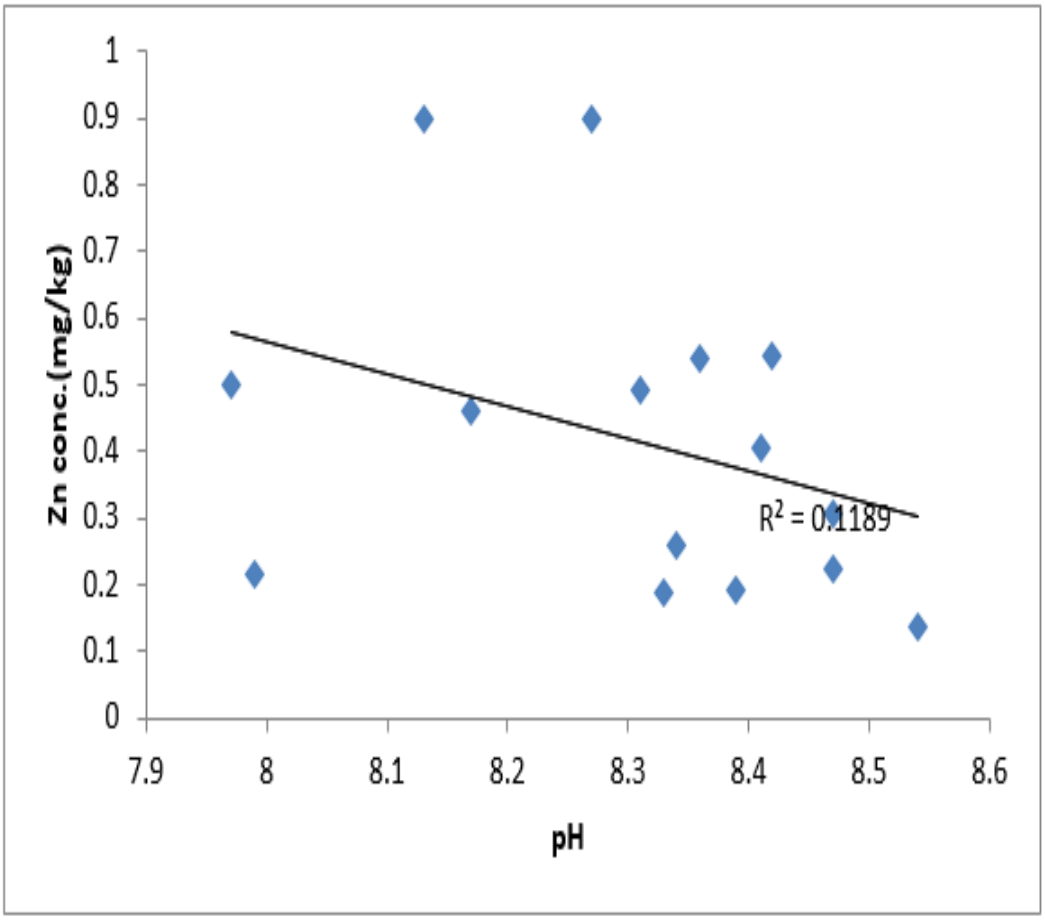

FIGURE 4.21: Correlation between pH and the available Zinc concentration of the diseased plants soil samples in depth 3.

Figure 4.21 showing the correalation between pH and Zinc concentration of diaseased plants soil samples in depth 3. X and Y axis representing pH and Zinc concentration, respectively. The graph is showing a weak negative correlation between pH and Zinc concentration in depth 3. As the pH increased the Zinc concentration decreased, but its strength was weak.

4.7.8 Overall Correlation between pH and Zinc Concentration of Diseased Group

Pearson's correlation was used to determine the correlation between pH and available zinc concentration. Correlation showed how available Zinc and pH interacted and how they affect each other. Overall the diseased group measured and there was a correlation between pH and zinc concentration.
Correlation is shown in figure 4.22.

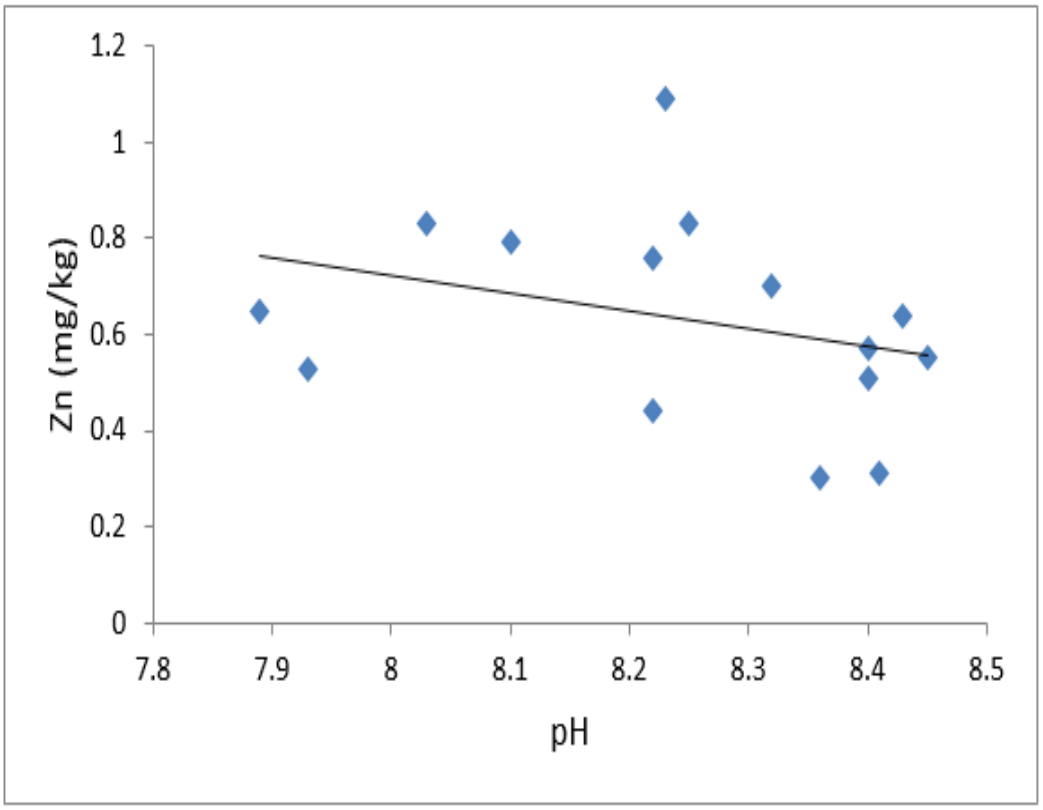

FIGURE 4.22: Correlation between pH and the available Zinc concentration of the diseased plants soil samples for the average of all the three depths.

Figure 4.22 is showing the correlation between pH and available Zinc concentration overall, for the diseased plants soil samples. X-axis is showing the pH and Y-axis is showing the available Zinc concentration. As, the pH is increasing the available Zinc concentration is decreasing and showing weak negative correlation. Overall, there was a weak negative correlation between pH and available Zinc concentration, in diseased group. Overall a weak negative correlation observed in diseased plants.

4.8 Discussion

Dalbergia sissoo is very important multipurpose plant [5]. It is important because of its medicinal and ecological benifits. It is distributed in tropical and sub-tropicals of Asia. It is mostly growing along roadsides, railway lines, water channels of agricultural fields and bank canals [1]. It is a fast growing plant and different soil types are ideal for its growth. Dieback disease is badly damaging this plant [63]. Present study was conducted to know the available zinc concentration and pH of soil and their synergetic effect in dieback disease of Dalbergia sissoo. Present research showed that there were Dalbergia sissoo plants of different ages from 4 to 70 and giths from 11 to 183 as shown in figure 4.2 And table 4.1, respectively. A previous researcher observed that there were available Zinc deficiency in soil samples of dieback diseased plants and the deficiency was observed in one third of total samples [20]. Current study, showed resembling results, as there was also Zinc deficiency in the soil samples of dieback diseased plants and the Zinc deficiency is almost one third of the total samples. The observations of recent study showed contrasting result with a previous study which revealed there was no deficiency of any nutrient in soil of dieback diseased plants [48].

Previous researches only focused on the soil samples of the dieback diseased plants but the current study observed soil samples of healthy as well as diseased plants and compared their results to understand more batterly. Analysis of soil samples of healthy plants revealed that there was also deficiency of Zinc in soil samples of healthy plants. This Zinc deficiency is one third of total samples. Both groups, showed the available Zinc deficiency in soil samples which was about one third of total samples. The previous research stated that soils of Pothwar usually show nutrient deficiency which has also been observed in current study [66]. Zinc deficiency did not influenced Dalbergia plants and there is no relation of available Zinc concentration with dieback disease in this plant. Depthwise, there were not significant difference of available Zinc concentration between healthy and diseased plants soil samples, in any one of the three depths (depth 1, 2 and 3). Another trend of available Zinc concentration was observed that the Zinc concentration

decreased gradually in depths downward. both the healthy as well as in diseased plants the soil samples showed gradual decrease of Zinc concentration from depth one to three. A similar trend was also observed in a previous study, in three depths of the diseased plants [23].

Micronutrients, organic matter and pH are important for availability of nutrients in soil [64]. Nutrients like Zn, P, Mn and Fe are lass available when pH is high, greater than 7.5 [65]. When the pH become lower the availability of Zn and other micronutrients become more, when the pH rages from 5-7. Pothwar (includes district Rawalpindi) soil usually have high pH [63]. Current investigation, observed high pH and low Zinc availability in soil samples of healthy as well as in diseased plants. This study, observed there was no significant difference of pH between soil samples of healthy plants and diseased plants. Therefore, pH is also not contributing for dieback disease of *Dalbergia sissoo*.Cureent study showed pH range from 7.2-8.4 in healthy plants soil and from 7.8-8.5 in diseased plant soil. The current study, observed that there was a moderate positive correlation of pH and the available Zinc concentration in soil samples of healthy plants and a weak negative correlation in soil samples of diseased plants. There is no any stronge correlation of pH and available Zinc concentration in soil samples of any depth of healthy as well as dieback diseased plants.

A very minute synergetic effect of available Zinc and pH has been observed in soil of diseased plants but it did not showed any contribution or influence in dieback disease of Dalbergia sissoo (Shisham).

Chapter 5

Conclusion

Dalbergia sissoo is an important tree plant. Dieback disease is reducing its population.The current research study was conducted to investigate the synergetic effect of available Zinc in soil , pH and their synergetic effect in dieback diseased plants. Zinc concentration in soil and pH were analysed and revealed valuable results. Available Zinc concentration and pH compared between healthy and diseased plants soil. The study showed that there was no significant difference of available Zinc concentration in soil, between healthy plants and dieback diseased plants. Even single depth did not show significance difference of Zinc concentration in soil. Although, there was zinc deficiency in diseased plants soil but the healthy plants soil also showed similar deficiency. Therefore, the dieback disease in *Dalbergia sissoo* (shisham) is not directly connected with the deficiency of available concentration of Zinc in soil.There was no significant difference of pH in any of the three depths, between healthy and diseased plants soil. overall, the pH between healthy and diseased plants soil is also not significantly different from each other and not associated with dieback disease in *Dalbergia sissoo*.

Correlation between pH and available Zinc concentration in soil was moderate positive in healthy plants soil. While, in diseased plants soil, the correlation was weak negative in diseased plants soil. Very samall synergetic effect of concentration of Zinc and pH of soil was observed in soil of the diseased plants. Therefore, the synergetic effect of concentration of zinc and pH of soil is not contributing in

dieback disease of *Dalbergia sissoo*.

Dalbergia dieback disease is very alarming common issue of Asian countries, especially for South Asian countries, Pakistan, Sri lanka, Bangladesh, Mayanmar, Bhutan, India, etc. Dieback disease of this plant has been reported in above mentioned countries but in India, Pakistan and Bangladesh its damage is far more than other country, so Governments and the research institutes of these countries should take steps to do multinational collaborative research. Therefore, data and researches should be exhchanged among different conrened national as well as international research institutes quikly and strongely to solve this issue. On national level, different concened departments should do different collaborative studies with each other. Similarly, multi-disciplinary scientists teams should be made to identify the causal agent of this disease and to recommend the measures and remedies.

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