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Characterization of rhizospheric soil of selected Medicinal plants

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The institute wishes the candidate success in her future endeavours.

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TOWHOMITMAYCONCERN

This is to certify that **Miss Saima Iqbal,** a student of M.Sc. Biotechnology (IVsemester), Integral University has completed her four months dissertation work entitled "**Characterization of rhizospheric soil of selected Medicinal Plants"** successfully. She has completed this work from 15 Feb, 2022 -15 June,2022 at the CSIR-Central Institute of Medicinal and Aromatics Plants (CSIR-CIMAP), under the guidance of ***Dr. B. Shivanna (Scientist)***

The dissertation was a compulsory part of his M.Sc. degree. I wish her good luck and a bright future.

(**Dr. Snober S. Mir**)
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DECLARATION

I hereby declare that the embodied in this dissertation entitled **"Characterization of rhizospheric soil of selected Medicinal plants"** has been carried out by me during the time period of February- July 2022, under the guidance of Dr. B. Shivanna , Scientist, Agronomy and Soil Science Department at CSIR-Central Institute of Medicinal and Aromatics Plants (CSIR-CIMAP), Lucknow, Uttar Pradesh, India. This thesis is being submitted to Integral University, Lucknow, towards the partial fulfillment of the requirement for the award of Master of Science in Biotechnology.

I further declare that this project has not been submitted to any other University or Institute for the award of any other degree or diploma.

Saima Iqbal

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Saima Iqbal

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ABBREVIATION

ABBREVIATION	DESCRIPTION
%	Percentage
µ	Mu
°C	Degree Celcius
m	Meter
mm	Millimeter
1	Liter
ml	Milliliter
MLD	Million liters per day
dS/m	Decisiemens per meter
ppm	Parts per million
g	Gram
mg	Milligram
kg	Kilogram
ha	Hectare
mt	Metric tone
Vmax	Maximum rate of reaction
KM	Michaelis constant
EC	Electrical conductivity
OC	Organic carbon
OM	Organic matter
N	Nitrogen
P	Phosphorus
K	Potassium
B	Boron

Al	Aluminium
A	Ashwagandha
S	Salvia
Ba	Barium
Ca	Calcium
Cr	Chromium
Co	Cobalt
Cu	Copper
Fe	Iron
Hg	Mercury
Pb	Lead
Mn	Manganese
Mg	Magnesium
Ni	Nickel
Zn	Zinc
DTPA	Diethylenetriaminepentaacetic acid
ICP-OES	Inductively coupled plasma- optical emission spectrometry

CHAPTER-1

INTRODUCTION

Achievement and maintenance of high nutrient use efficiency together with high crop productivity has become a major challenge in both developed and developing countries with an increasing global demand for food for a growing population, depletion of natural resources, and deteriorating environmental conditions (Cassman, 1999; Matson et al., 1997; Tilman et al., 2002). For example, crop yield in intensive Chinese farming systems has failed to increase in proportion to increasing inputs of chemical fertilizers over the last 20 years, leading to low nutrient use efficiency and increasing environmental problems. The main explanation is that too much effort has been made to increase fertilizer inputs while ignoring the potential benefits of biological processes in crop exploitation of nutrient resources in soil. Plant roots take up nutrients from soils via the rhizosphere , which is the critical zone of interactions among plants, soils, and microorganisms. In addition to adaptation to the soil environment by regulating root plasticity in morphological traits, plant roots can also significantly modify the rhizosphere environment through their physiological activities, particularly the exudation of organic compounds such as mucilage, organic acids, phosphatases, and some specife specific signaling substances which provide a key driving force for various rhizosphere processes . The chemical and biological processes occurring in the rhizosphere not only determine mobilization and acquisition of soilnutrients as well as microbial dynamics, but also control nutrient use efficiency by crops, and thus profoundly influence crop productivity and sustainability (Zhang et al., 2002, 2004.

The concept of rhizosphere was first introduced by Hiltner in 1904 to describe the narrow zone of soil surrounding the roots where microbial

populations are stimulated by root activities, and this has now been extended to include the soil surrounding a root in which physical, chemical, and biological properties have been changed by root growth and activity both in a radial and longitudinal direction along an individual root (Brimecombe et al., 2007; Marschner, 1995). In the plant–soil system, the rhizosphere represents not only an interface zone between roots and soils for an individual plant, but also the central area of interactions among plants, soils, and microorganisms. It is therefore an extremely important and active area in regulating nutrient bioavailability, plant communities, adaptation processes, and the growth environment (Marschner, 1995; Shen and Zhang, 1999; Zhang and Shen, 1999a,b; Zhang et al., 2002).

For agricultural sustainability, understanding the distribution and characteristics of soil is important (Louis, 2010). The availability of nutrients to the plant is very high in rhizospheric soil and soil pH is low than non-rhizospheric soil (Curl and Truelove, 1986; Marschner, 1995; Mishra et al., 2015). Soils with high natural fertility can produce more crop yields without adding any fertilizers and farmers achieve higher yields with additional supply of critical nutrients (Louis, 2010). In rhizospheric soil, living plant roots interact with surrounding mineral, organic and microbial components of the soil (Curl and Truelove, 1986). These interactions play significant role in determining plant nutrition and growthRobertandBerthelin,1980).

Use of soil tests can help to determine the status of plant available nutrients to develop fertilizer recommendations to achieve optimum crop production and manage the disease infections caused by various pathogens. The control of disease infection and increase in yield of the crop determine the profit potential for farmers. Soil organic carbon (SOC) is important parameter of soil fertility (Brady and Weil, 2008).

SOC improves soil physical, chemical and biological properties and thus soil health. However the studies on physico chemical properties inrhizospheric soils of medicinal plants is very meager. Hence the present study has been taken up with the following objectives:

1. To assess the physico-chemical properties of the rhizosphere soils of selected aromatic plants.
2. To assess available nutrient status in Rhizospheric soils.
3. To assess the enzymes activities of rhizospheric soil.

CHAPTER-2

REVIEW OF LITERATURE

Rizosphere

Roots are among the most important organisms in the soil ecosystem and the soil environment surrounding the roots is designated as rhizosphere. The word rhizosphere is from the Greek meaning "the influence of a root on its surrounding" (Pinton and Varanini, 2001). It was first introduced by German scientist Lorenz Hiltner in 1904 to describe the interaction between microorganisms and legume plant roots. Now the term includes all plants and is a topic ofA better understanding of rhizospheric soil processes may provide an effective approach for improving nutrient use efficiency and crop productivity simultaneously through exploitation of biological potential for efficient acquisition and utilization of nutrients by crops, and reducing the overreliance on increased soluble nutrients from application of chemical fertilizers.

PHYSICO-CHEMICAL PROPERTIES OF SOIL

pH

Soil pH is widely accepted as a dominant factor that regulates soil nutrient bioavailability, vegetation community structure, plant primary productivity, and a range of soil processes including soil microbial community structure and activity (Robson 1989). All the soil properties and the value of the soil pH can widely differ in reliance on soil type,

topography, climate, vegetation, and anthropogenic activity, because all these factors influence the spatial variability of the observed soil types (Shi et al. 2009). The value of soil pH is directly influenced by all five soil-forming factors (parent rock, climatic conditions, organisms, topography, and time) and further the value of soil pH is dependent on the season influence, way of management, tested soil horizon, soil water contents, and time limit of sampling for analysis (Troeh & Thompson 2005).The combined application of manure and mineral fertilizer has major effects on soil physical, chemical, and biological properties and it increases crop yields (Hou et al. 2012). Application of fertilizers is one of the causes of soil acidification (Hoyt & Hennig1982). The acidification of soil by N fertilizer is caused by transformation of nitrogen in soil. The uptake of N as ammonium in the crop also contributes to soil acidification (Malhi et al. 1998).

Electrical Conductivity(EC)

EC measures the salinity and electrically charged nutrient ions in a solution (Bluelab, 2015). Even if you cannot see sediment or particles in a solution this does not mean there are no electrically charged nutrient ions. Pure water does not conduct electricity, whereas, irrigation water may be full of impurities which may conduct electricity (Bluelab, 2015). EC does not specifically measure certain ions or salt compounds, but the correlation to concentrations of nitrates, potassium, sodium, chloride, sulfate, and ammonium (Natural Resource Conservation Service). In soils, properties that contribute to EC are clay content and mineralogy, soluble salts, soil water content, bulk density, organic matter, and soil temperature (Corwin &Leh) soil that may contribute to the final EC measurement. Plant roots impacted by salinity can be expressed as

reduced growth rate, changes in leaf color, leaf necrosis, changes in root to shoot ratio and affect how the plant ages (Shannon & Grieve, 1998). There are different factors that influence the salinity in the soil. Some factors are temperature, wind, humidity, light, and air pollution (Shannon & Grieve, 1998). Different plants have different thresholds of salinity in which that they can thrive in. With the right level of EC, favorable effects on yield, quality, and disease resistance are possible outcomes (Shannon & Grieve, 1998).

Organic Carbon(OC)

Soil carbon (SOC) affects the chemical and physical properties of the soil, such as water infiltration ability, moisture holding capacity, nutrient availability, and the biological activity of microorganisms. Soil organic C is a heterogeneous material that can be separated into a light and a heavy fraction (Gregorich and Ellert, 1993; Janzen et al., 1992). The light fraction mainly consists of botanical relics and is more responsible for cropping practices than is the heavy fraction (Biederbeck et al., 1994; Gregorich et al., 1994). SOC sequestration cannot occur in the absence of N. The C to N ratio of mineral-associated SOC ranges from 8 to 12. There is a direct relationship between SOC and N (Fig. 3.6). Therefore, increasing SOC requires a concomitant increase in soil N. The SOC sequestration rate promoted in the 4PT initiative would require 100 Tg N y^{-1}, assuming a soil C to N ratio of 12 (van Groenigen et al., 2017). The authors estimated cropland residue N globally to be about 30 Tg N, well below that needed to form stable SOC. This suggests that additional N is required beyond crop demand to meet the goal of 4PT. The need for N would further be exacerbated if crop residues were removed, for example, for biofuel production (Blanco-Canqui and Lal,

2009). Removing crop residues to produce ethanol would remove N, but also C inputs, reducing SOC sequestration potential or leading to its loss.

Nitrogen (N)

Symbiotic organisms such as rhizobia, and to lesser extent nonsymbiotic bacteria such as Azospirillum, can augment plant uptake of N via N fixation (Pepper and Bezdicek, 1990). Nonsymbiotic N fixation has also been well documented (Marschner, 1995; Mengel et al., 2001). Symbiotic N2-fixing bacteria can fix substantial amounts of N, but free-living organisms must compete with all other rhizosphere organisms for substrate, and hence are unlikely to fix large amounts of N. The quantity of symbiotic N fixation by legumes varies from species to species, and depends on environmental conditions. However, contribution of symbiotic N2 fixation by legumes of up to 300 kg ha−1 has been reported (Bezdicek et al., 1978). Free-living organisms can fix about 15–30 kg N ha−1 yr−1 (Pepper and Bezdicek, 1990). A detailed discussion of N fixation by microorganisms is provided under the heading Biological Changes later in this paper. In addition to supplying plants with fixed N, rhizosphere organisms greatly influence the N cycle in the soil-plant system via mineralization, immobilization, nitrification, and denitrification.

Mengel et al. (1990) showed the ability of plants to promote the release of fixed, nonexchangeable ammonium in their rhizosphere. Scherer and Ahrens Downloaded by [UniversitatPolitècnica de València] at 02:30 14 November 2014 1338 N. K. Fageria and L. F. Stone (1996) reported that the depletion of the NH+ 4 ion at the root surface contributes to the

release of non-exchangeable NH+ 4 in the rhizosphere of ryegrass (Lolium multiforum L.) and red clover (Trifolium pretense L.). Furthermore, the mobilization of non-exchangeable NH+ 4 -N in the soil-root interface may be increased by nitrifying and hetrotrophic microorganisms with a higher activity in the rhizosphere, influencing the equilibrium between non-exchangeable NH+ 4 and NH+ 4 in the soil solution, thus favoring the release of NH+ 4 from interlayer of the clay minerals (Nommik and Vathras, 1982; Bottner et al., 1988). According to Grinster et al. (1982) rape (Brassica napus L.) decreased the pH in the vicinity of the roots from 6.2 to 4.5. The relatively high replacing power of H+ is presumably an important factor in mobilizing non-exchangeable NH+ 4 , because H+ leaves the crystal lattice in an expanded state and thus renders the non-exchangeable NH+ 4 more accessible to replacing cations

Phosphorus (P)

Physical, chemical, and biological changes in the rhizosphere are associated with improved concentration of this element in the root vicinity and, consequently, its uptake. Root exudates released in the rhizosphere contain phosphate radicals that will be subject to microbial modification. Furthermore, several microbial processes including mineralization, immobilization, and solubilization of inorganic phosphates also influence P availability to plants. Several studies have reported modification in concentration and uptake of P in the rhizosphere of crop plants. Jianguo and Shuman (1991) reported that in rice, uptake of P increased under low-P conditions, and that this increase in P uptake was associated with a decrease in the rhizosphere pH. Furthermore, these authors reported that H+ secretion by rice roots under low soil-P conditions can be considered a beneficial adaptation. Evidence for the availability of unavailable soil P in some crop species is

now overwhelming (Jones, 1998). Many plant-induced changes in the rhizosphere, produce this phenomenon. Those can include the manipulation of root morphology (hair length/density), the provision of extra C for mycorrhizal exploitation of non-rhizosphere soil, the release of phosphatases to release organically bound soil P, and the release of organic acids and H+ to solubilize inorganic P (Jones, 1998). Enhanced secretion of acid phosphatases (APase) and phytases by plant roots and also by rhizosphere microorganisms under Pdeficient conditions may contribute to P acquisition by hydrolysis of organic P esters in the rhizosphere (Neumann and Romheld, 2001). Some dicotyledonous plant roots, and especially non-mycorrihizal plants such as Lupinas albus and Brassica napus, are capable of releasing large amounts of organic acids into the rhizosphere in response to P deficiency (Gerke, 1994; Jones, 1998). Mallic, citric and tartaric appear to be the primary organic acids released by roots under P deficiency (Jones, 1998; Wang et al., 2000). Downloaded by [UniversitatPolitècnica de València] at 02:30 14 November 2014 Changes in Rhizosphere and Nutrient Availability 1339 The depletion of soil solution P ions in the rhizosphere occurs as a consequence of uptake of P by roots (Hinsinger and Gilkes, 1996). This depletion prompts a replenishment of P from the solid phase (Morel and Hinsinger, 1999). However, the replenishment of depleted P depends on time and physicochemical conditions of the soil (Nye, 1981; Darrah, 1996; Hinsinger and Gilkes, 1996; Hinsinger, 1998).

Potassium (K)

Potassium (K) uptake is higher in crop plants and its availability to plants in sufficient amounts is fundamental for higher productivity. Unlike N, it does not form any gases in the soil that could be lost to the atmosphere. Its fixation in the soil-plant system is also limited. It does not cause major environmental problems as N and do P do. The original

source of K is from primary minerals such as micas (biotite and muscovite), feldspar (orthoclase and microcline), and their weathering products. Availability of non-exchangeable K to plants has been reported to increase due to an exchange reaction and mineral dissolution in the rhizosphere (Moritsuka et al., 2004). Increased exudation of sugars, organic acids, and amino acids has been detected in maize as a response to K limitation (Neumann and Romheld, 2001). Hinsinger et al. (1993) reported that dissolution of phlogopite structure occurred in the rhizosphere of brassica (Brassica napus L.), probably due to proton excretion by roots. The release of nonexchangeable K from feldspar has also been attributed to exudation of acids such as like citric and oxalic (Song and Huang, 1988; Drever and Stillings, 1997; Wang et al., 2000)

. The release of non-exchangeable K from soil minerals requires a very low concentration of K in the soil solution (Sparks, 1987; Fannings et al., 1989). The root-induced release of nonexchangeable K contributes up to 80% of the uptake of the plants in soils, whereas the release of nonexchangeable K would have been expected to be negligible when considering the concentration of K in the bulksoil solution (Niebes et al., 1993; Hinsinger, 1998). Exchangeable Ca^{2+}, Mg^{2+}, and Na^{+} are supplied to plant roots mainly by mass flow. Furthermore, due to their lower demand for plant growth than the supply by mass flow from the non-rhizosphere, these cations are often accumulated in the rhizosphere and adsorbed on the exchange sites of the solid phase (Yanai et al., 1996; Moritsuka et al., 2000; Moritsuka et al., 2004). The adsorption of these cations on the exchange site in the rhizosphere may be an important process in the release of interlayer K (Mengel, 1985; Sparks, 1987). Moritsuka et al. (2004) reported that release of nonexchangeable K in the rhizosphere by the adsorption of cations Ca^{2+}, Mg^{2+}, or Na^{+} plays an important role in upland soils whose rhizosphere

[UniversitatPolitècnica de València] at 02:30 14 November 2014 1340 N. K. Fageria and L. F. Stone pH is usually above the range of intensive mineral dissolution as a result of the supply of N to plants, mainly in the form of NO_3^- .

DIETHYLENETRIAMINEPENTAACETIC ACID(DTPA EXTRACTABLE)MICRONUTRIENTS

A DTPA soil test was developed to identify near-neutral and O Studies on the reactions of metal chelates in soils calcareous soils with insufficient available Zn, Fe, Mn, or Cu for (Lmdsay et al., 1967; Norvell and Lmdsay, 1969, 1972; maximum yields of crops. The extractant consists of 0.005M DTPA Lindsay and Norvell, 1969; Halvorson and Lindsay, 1972, (diethylenetriaminepentaacetic acid), 0.1M tricthanolamine, and 1977; Norvell, 1972; Lindsay, 1974, 1979). As these 0.0lMCaCl, with a pH of 7.3.. The soil test consists of shaking 10 g of air-dry soil with 20 ml of extractant for 2 hours. The leachate is filtered, and Zn, Fe, Mn, and Cu are measured in the filtrate by atomic absorption spectrophotometry. Development of the soil test was based, in part, on theoretical considerations. The extractant is buffered at pH 7.30 and contains $CaCl_2$ so that equilibrium with $CaCO_3$ is established at a CO_2 level about 10 times that of the atmosphere. Thus, the extractant precludes dissolution of $CaCO_3$ and the release of occluded nutrients which are normally not available to plants. DTPA was selected as the chelating agent because it can effectively extract all four micronutrient metals. Factors such as pH, concentration of chelating agent, time of shaking, and temperature of extraction affect the amount of micronutrients extracted and were adjusted for maximum overall effectiveness.

Dehydrogenase enzyme activity

Soil dehydrogenase activity is a function of soil management system as it is directly or indirectly influenced by the orchard ground-floor management systems (Chu et al., 2007). Generally the enzyme activities in the soil were closely related to the organic matter content. Application of balanced amounts of nutrients and manures improved the organic matter and microbial biomass carbon status of soils, which corresponded with higher enzyme activity (Mandal et al, 2007). Dehydrogenase activity with application of organic sources might be linked to more substrate availability in the soil. This reflects the greater biological activity in the soil and the stabilization of extracellular enzymes through complexation with humic substances (Basak et al., 2013). It has been reported that the increase in dehydrogenase activity and microbial biomass were proportional to the addition of number and amount of nutrients. (Manjaiah and Singh, 2001). In the present study, a similar trend was observed. According to Pramanik et al. (2010) dehydrogenase activity is influenced more by the quality than by the quantity of organic matter incorporated into soil. Thus, the stronger effects of vermicompost or microbial inoculants on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms.

Phosphotase Enzyme Activity

The term phosphatase has been used to describe a broad group of enzymes that hydrolyse organic phosphorus (P) compounds, pyrophosphates, metaphosphates, and inorganic polyphosphates which occur in soils. It is generally accepted that plants utilize only inorganic P and since a large proportion of soil P is organically bound, the mineralization of this organic fraction can be an important factor in

plant nutrition. Phosphatases in soils hydrolyze C–O–P ester bonds in organic P compounds and release inorganic P. Phosphatase activity is, therefore, an important factor in maintaining and controlling the rate of P cycling through soils, particularly for soils with deficient P because most P in soils of this study is organically bound (Chen et al., 1996). Dick and Tabatabai, (1993); reported that phosphatase activity can be a good indicator of the organic phosphorus mineralization potential and biological activity of soils. Phosphatase activity is related to soil organic matter, organic P, inorganic P, and N availability in soil. Juma and Tabatabai, (1978); Chen et al., (2000), found that phosphatase activity in soils are mostly associated with upper surface soils and decreased with depth of soils.

CHAPTER-3

MATERIALS AND METHODS

Thepresent investigation entitled '' **Characterization of rhizosphere soil of selected medicinal plants** '' at CIMAP farm ,Lucknow was conducted to determine the availability of nutrients present in soil.This work was carried out in the Department of Agronomy and Soil Science at CSIR-Central Intitute of Medicinal and Aromatic plants(CSIR-CIMAP),Lucknow,Uttar Pradesh,India.The study includes the collection of Soil Samples from four medicinal plants Ocimum,Cammomile,Ashwagandha and Salvia for their soil analysis.The details of Method and Materials followed in this investigation are described in this chapter.

3.1 Location of Study Area

CSIR- Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, Uttar Pradesh. Located at 26°5' N latitude80°5'E longitude with an elevation of about 120 meter above mean sea level under the sub-tropical plains of the North-India. (Nilofer et al; 2018). Therhizospheric soil samples were collected from four different medicinalplants of CSIR-CIMAP.

Figure- 3.1: Satellite map location of CSIR-CIMAP, Lucknow, Uttar Pradesh.

CSIR-Central Institute of Medicinal and Aromatic Plants, popularly known as CIMAP, is a frontier plant research laboratory of Council of Scientific and Industrial Research (CSIR). Established originally as Central Indian Medicinal Plants Organisation (CIMPO) in 1959, CIMAP is steering multidisciplinary high quality research in biological and chemical sciences and extending technologies and services to the farmers and entrepreneurs of medicinal and aromatic plants (MAPs) with its research headquarter at Lucknow and Research Centres at Bangalore, Hyderabad, Pantnagar and Purara. CIMAP Research

Centresare aptly situated in different agro-climatic zones of the country to facilitate multi-location field trials and research.

Figure 3.2 CSIR-CIMAP

3.2 Collection of soil samples and soil sampling

Medicinal plants associated soil samples were collected from experimental farmlands from 0-2mm distance from roots during the rabi season of 2022. Clean plastic pails were used to collect and store samples.The soil samples were air-dried, crushed and then mixed thoroughly to obtain a homogenous mixture for each sample separately. Further, collected soil samples were analyzed for NPK, electrical conductivity, SOC and pH.The soil nitrogen was analyzed by Kjeldal method, phosphorous by Spectrophotometric method and potassium by flame photometer (ELICO). Soil pH and EC were determined using digital electronic pH meter and electrical conductivity meter, respectively. The SOC was estimated by Walkley-Blacktitration method.

Following medicinal plants were taken for the collection of rhizospheric soil samples :

(a)Ashwagandha (b)Cammomile

(c) Ocimum (d) Salvia

Figure.3.3

The soil for analysis was taken to the research laboratory where the soil samples were air dried then sieved through a 2 mm and 0.2 mm stainless steel mesh. Roots and stones were removed from the sieving process. Each sieved sample was homogenized in the collecting tray and placed in a paper bag (Aston, 1998).

3.3-Determination of Physico-chemical properties of rhizospheric soils.

Soil physico-chemical properties include pH, Electrical conductivity, Organic carbon, available Nitrogen, available Phosphorus and available Potassium. Details of the determination of these parameters are given below.

3.3.1- Determination of ph of soil

A soil water suspension was prepared in the ratio of 1:2:5, 10 gm of the soil sample was taken in a 50 ml. In which 25 ml distilled water has been added and stir well for about 5 minutes, and it has been kept for half an hour. Calibration was done by immersing the electrode in abuffer solution of pH-7.0. The sample was stirred properly again just before immersing the electrode, and reading was observed.(Aboukila and Norton, 2017; Ryti, 1965; Peech, 1965).

3.3.2- Determination of Electrical conductivity of soils

A soil water suspension was prepared in the ratio of 1:2:5, 10g of soil sample was taken in a 100ml beaker. 25ml of distilled water was added, and it was shaken intermittently for 1 hour on a mechanical shaker. After this, the solution was allowed to stand until a clear solution was obtained. The conductivity bridge was calibrated with the help of a standard KCl solution. The supernatant liquid's conductivity was determined by immersing the electrode in the solution, and the reading was observed.(Aboukila and Norton, 2017).

3.3.3- Determination of soil Organic carbon of soil-(Walkley and Black, 1934)

There are many available methods to determinate soil Organic carbon (OC) from which one of the most convenient methods is oxidation with K2Cr2O7 in the presence of sulfuric acid (F2SO4)(Walkley and Black,1934).

0.5 g of soil sample was taken in a conical flask. 10ml of IN $K_2Cr_2O_7$ and 20ml of H_2SO_4 was added to it. Then it was swirted and kept on asbestos sheet for 30 minutes. 200ml of distilled water was added slowly in the solution. Then 10 ml of Orthophosphoric acid was added 1 ml of the Diphenylamine indicator was added to it. 0.5 N Ferrous ammonium sulphate was taken in the 50 ml burette. The content was titrated until the green colour start appearing (Walkey and Black,1934). The OC% was calculated as follows:

$$\text{Organic Carbon(\%) in soil} = \frac{10(\text{blank-sample})/\text{blank} \times 0.003 \times 100}{\text{Wt. of soil}}$$

3.3.4- Determination of Mineralizable Nitrogen of soil (Subbiah and Asija, 1956).

Nitrogen processor involves digestion and distillation. The easily mineralizable N is estimated using alkaline KNMNO4, which oxidizes the organic matter present in the soil and hydrolyzes the liberated ammonia , which is condensed and absorbed in boric acid and titrated against standard acid.

10g of soil sample was taken in 800 ml Kjeldahl flask. The soil was moistened with about 10 ml of distilled water, wash downed the soil adhering to the neck of flask, if any, 100 ml of 0.32% $KMnO_4$ solution

was added. Limited glass beads or fragmented pieces of glass rod were added, 2-3ml paraffin liquid was added, contact with upper part of the flask's neck was avoided. In 250 ml of a conical flask, 20ml of mixed indicator containing 2% boric acid was taken and placed in under the receiver tube 100 ml of 2.5% of NaOH was added an immediately attached to the rubber stopper fitted in the alkali trap. The header was switched on, and distillation was continued until about 100 ml of distillate is collected. The flask containing distillate was titrate against 0.02 N H_2SO_4 in the burette until the pink color appearing (Subbiah and Asija,1956). The N was calculated as follow :-

$$\text{Available N (kg ha}^{-1}\text{)} = \frac{\text{(Sample) x N of } H_2SO_4 \text{ x } 0.014 \text{ x } 2 \text{ x} 10^8}{\text{Wt. of soil}}$$

3.3.5- Determination of available phosphorus of soil

The most common method are used for determining the available P in soils are , Olsen method used for neutral and alkaline soils.

0.5 M $NaHCO_3$ solution in the most suitable for neutral to alkaline soils. It is meant to manage the ionic activity of calcium through the solubility product of $CaCO_3$, thus extracting the foremost reacting forms of P from Al^-, Fe^- and Ca-P (Olsen's et al.,1954).

2.5 g of soil sample was taken in the 100 ml conical flask. 50 ml of Olsen's reagent was added in it with a pinch of activated charcoal. It was shaken for 30 minutes on a mechanical shaker. Using Whatman No. 1 filter paper, the solution was filtered. 5 ml clear and colourless aliquot was taken into the 25 ml volumetrick flasks. 5 ml of ammonium

molybdate solution was added (drop by drop) and shaken a little to dry out the CO_2 evolved and diluted to about 22 ml. 1 mi diluted $SnCl_2$ added and mixed by stirring a little, and volume was made up. A blank solution was run without soil under identical conditions. The blue colour intensity of the solution was measured using spectrophotometer a 660nm wavelength. (Olsen's et al., 1954).

The P was calculated as follows:

$$\text{Available } P_2O_5 = \frac{\text{Graph ppm x Vol. of extractant x Vol. made x 2.24 x2.29}}{\text{Wt. of soil x Aliquot}} \quad (\text{kg h}^{-1})$$

3.3.6- Determination of Available Potassium of soil (Hanway and Heidel, 1952)

5 g of soil sample was taken in a conical flask. 25 ml of neutral 1 N ammonium acetate solution was added and shaken for 5 minutes. Filtration was done through Whatman No. 1 filter paper. The concentration of the sample was measured using a Flame Photometer (Hanway and Heidel, 1952). The K was calculated as follows:

$$\text{Available } K_2O = \frac{\text{Graph ppm x vol. of extractant x 2.24 x 1.20}}{\text{Wt. of soil}} \quad (\text{kg ha}^{-1})$$

3.4- Estimation of plant available micronutrients by diethylenetriaminepentaacetic acid (DTPA-extractable)

Diethylenetriaminepentaacetic acid (DTPA), is a reagent that acts as a chelating agent that combines with free metal ions in solution complexes. The reduction within the ionic activity of the solution results in desorption. When the extractant is added to the soil, additional Ca^{++} and some Mg^{++} enter the solution. This is often due to the protonated triethanolamine (TEA) exchanges ionic concentration of CA^{++} within the solution, which successively helps in suppressing the dissolution of $CaCO_3$. DTPA extractant has the aptitude to chelate Zn, Cu, Fe and Mn in comparison with Ca^{++} and Mg^{++}. (Lindsay and Norvell, 1978).

10 g of soil sample was taken in a 100 ml conical flask. 20 ml of DTPA extractant was added to it. The sample was shaken for 2 hours on a mechanical shaker. The sample was filtered using Whatman No.42 filter paper. Then the filtered was collect and observed under ICP-OES. (Lindsay and Norvell, 1978). The DTPA was calculated as follows:

$$\text{DTPA(Cu/Fe/Mn) (ppm)} = \frac{\text{Graph ppm x Vol. of DTPA extract}}{\textbf{Wt. of soil}}$$

3.5- Soil enzyme activities

Soil enzyme activities include Dehydrogenase, alkaline Phosphatase and acidic phosphatase. Details of the determination of these parameters are given below.

3.5.1- Determination of Dehydrogenase enzyme activity in soil

The most common method areused for determining the Dehydrogenase enzyme activity in soil, Casida method.

1. Thoroughly mix 0.2 g of CaCO3 and 20 g of air-dried soil (<2 mm), and place 6 g of this mixture in each of three test tubes.

2. To each tube add 1 mL of 3% aqueous solution of TTC and 2.5 mL of distilled water. There should be a small amount of free liquid at the surface of the soil after mixing.

3. Mix the contents of each tube with a glass rod, and stopper the tube and incubate it at 37°C. After 24 h, add 10 mL of methanol, stopper the tube, and shake it for 1 min.

4. Unstopper the tube, and filter the suspension through a glass funnel plugged with absorbent cotton, into a 100-mL volumetric flask. Wash the tube with methanol and quantitatively transfer the soil to the funnel, then add additional methanol (in 10-mL portions) to the funnel until the reddish color has disappeared from the cotton plug. Dilute the filtrate to a 100-mL volume with methanol. Measure the intensity of the reddish color by using a spectrophotometer at a wavelength of 485 nm and a 1-cm cuvette with methanol as a blank.

5. Calculate the amount of TPF produced by reference to a calibration graph prepared from TPF standards. To prepare this graph, dilute 10 mLof TPF standard solution to 100 mL with methanol (100 μg of TPF mL-1). Pipette 5-, 10-, 15-, or 20-mL aliquotes of this solution into 100-mL volumetric flasks (500, 1000, 1500, and 2000 μg of TPF 100 mL-respectively), make up the volumes with methanol, and mix thoroughly.

Measure the intensity of the red color of TPF as described for the samples. Plot the absorbance readings against the amount of TPF in the 100-mL standard solutions.

3.5.2- Determination of Phosphotase activity

1.Place 1 g of soil (<2 mm) in a 50-mL Erlenmeyer flask, add 0.2 mL of toluene, 4 mL of MUB (pH 6.5 for assay of acid phosphatase of pH 11 for assay of alkaline phosphatase), 1 mL of PNP solution made in the same buffer, and swirl the flask for a few seconds to mix the contents. Stopper the flask, and place it in an incubator at 37°C.

2. After 1 h, remove the stopper, add 1 mL of 0.5 M CaCl2 and 4 mL of 0.5 M NaOH, swirl the flask for a few seconds, and filter the soil suspension through a Whatman no. 2v folded filter paper.

3. Measure the intensity of the yellow color of the filtrate with a spectrophotometer with wavelength adjusted to 410 nm.

4. Calculate the p-nitrophenol content of the filtrate by reference to a cali-bration graph that plots standards containing 0, 10, 20, 30, 40, and 50 µg of p-nitrophenol. To prepare this graph, dilute 1 mL of the standard p-nitrophenol solution to 100 mL in a volumetric flask and mix the solution thoroughly. Then pipette 0-, 1-, 2-, 3-, 4-, or 5-mL aliquots of this diluted standard solution into a 50-mL Erlenmeyer flask, adjust the volume to 5 mL by addition of water, and proceed as described for p-nitro-phenol analysis of the incubated soil sample (i.e., add 1 mL of 0.5 M CaCl2 and 4 mL of 0.5 M of NaOH mix and filter the resultant suspension). If the color intensity of the filtrate exceeds that of 50 µg of the p-nitrophenol standard, an aliquot of the filtrate should be diluted with water until the colorimeter reading falls within the limits of the calibration graph.

5. To perform controls, follow the procedure described for assay of ß-glucosidase activity, but make the addition of 1 mL of PNP solution after the additions of 0.5 M CaCl2 and 4 mL of 0.5 M NaOH, immediately before filtration of the soil suspensions.

CHAPTER-4

RESULT

The present investigation was undertaken to study **"Charactrization of rhizospheric soil of selected medicinal plants".**The result of the investigation are presented in this chapter.

4.1- Soil physicochemical properties

The rhizosphere condition have a direct impact on crop growth and yield.Thephysico-chemical properties of rhizospheric soils of medicinal plants were estimated through laboratory analysis and are presented below.

4.1.1- pH

The pH analysis showed a little variations in the different rhizospheric soils of medicinal plants ranged to alkaline such as Ashwaganda mean-8.82 (range: 8.49-8.83), Chamomile mean-8.53 (range:8.47- 8.61),salvia mean- 8.14 (range: 8.04 - 8.57), and ocimum mean-8.34 (range:8.25-8.52) in A,Ca,O, and S respectively (Table- 4.1). The results indicate that A,Ca,O, and S was alkaline.

4.1.2- Electric Conductivity (EC)

Analysis of EC, presented variation in the different rhizospheric soils of medicinal plant such as: mean-2.655 mS/cm (range: 2.361-2.945 mS/cm), mean- 3.480 mS/cm (range:3.384-3.550), mean-2.28 mS/cm (range:2.221-2.343), and mean- 2.180 mS/cm (range:2.18-2.29) in Ashwagandha ,Chamomile , Ocimum and Salvia respectively. (Table 4.1). The EC of Ca is higher as compared to the EC value of A and O. And it is probably critical for crop growth.

4.1.3- Organic Carbon (OC)

Analysis of OC, exhibited a dissimilarity in the different rhizospheric soils of medicinal plants such as :mean-0.57% such as (range:0.51-0.66%), mean-0.48% (range:0.45-0.51%), mean:0.24% (range: 0.21-0.27%) mean-0.84% (range: 0.75-0.87%),) in Ashwagandha ,Chamomile , Ocimum and Salvia respectively. (Table-4.1). An excellent build up OC was seen in Salvia as compared to the OC values of A, Ca, and O.

4.1.4- Mineralizable Nitrogen (N)

The determination of N in four different rhizospheric soils of medicinal plants ranged from low to medium such as: mean- 175.92 kg/ha (range -172.48-178.92 kg/ha), Cammomile mean-222.65 kg/ha (range – 221.29-225.79 kg/ha),Ocimum mean-202.48.7 (range-200.7-210.11), Salvia mean- 222.65(range –215.07-225.79) in Ashwagandha ,Chamomile , Ocimum and Salvia respectively (Table- 4.1). Among these Ca, O and S have highest mean then A.

4.1.5- Available Phosphorus (P)

The result of the study showed a divergence in P in different rhizospheric soils of medicinal plants such as: Ashwaganda mean- 35.09 kg/ha (range: 32.49-37.47 kg/ha),Cammomile mean-34.6 kg/ha (range: 31.85-36.41kg/ha), Ocimum mean-32.92 kg/ha (range:30.85- 34.68 kg/ha),Salvia mean- 36.43kg/ha (range: 34.48-38.37 kg/ha) in A,Ca,O and S respectively (Table- 4.1). The highest concentration of (P) was found in O and S followed by A and Ca.

4.1.6- Available Potassium (K)

Analysis of K showed a variations in different rhizospheric soils of medicinal plants ranged from medium to high such as: mean-105.28kg/ha (range-104.16- 107.6kg/ha), mean-100.48 kg/ha (range-97.44-102 kg/ha), mean-97.08 kg/ha (range- 94.4-100.4kg/ha), mean-100.76kg/ha (range-97.36-100.8kg/ha) in A,Ca,O and S respectively (Table- 4.1). The highest concentration of K was found in A, and S followed by Ca, O.

The highest concentration of (P) was found in O and S followed by A and Ca.

PARAMETER	ASHWAGANDHA		CAMMOMILE		OCIMUM		SALVIA	
	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
pH	8.69-8.83	8.82	8.47-8.61	8.53	8.25-8.52	8.34	8.04-8.14	8.07
Electrical conductivity (mS/cm)	2.36.1-2.945	2.655	3.384-3.550	3.480	2.221-2.343	2.28	2.18-2.29	2.18
Organic Carbon (%)	0.51-0.66	0.57	045-0.48	0.51	0.21-0.27	0.24	0.75-0.87	0.84
Nitrogen (kg/ha)	172.48-178.92	175.92	221.29-225.79	222.65	200.7-210.11	202.48	215.07-225.79	222.65
Phosphorus (kg/ha)	32.49-37.47	35.09	31.85-36.41	34.6	30.85-34.68	32.92	34.48-38.37	36.43
Potassium (kg/ha)	104.16-107.6	105.28	97.44-102	100.48	944.4-100.4	97.08	97.36-100.8	100.76

4.1- Physico-chemical properties

4.2- Plant available micronutrients

Plants available micronutrients suchas Fe, Mn, Cu ,Zn, B, Ni and Al present in the four different soils are estimated through laboratory analysis by DTPA method using ICP-OES.

4.2.1- - Iron (Fe)

The Fe analysis presented a variation in the different rhizospheric soils of medicinal plants such as: mean-8.32 mg/kg (range- 7.98-8.50 mg/kg), mean-9.06 mg/kg (range-8.48-10.60 mg/kg), mean-7.02 mg/kg (range-6.56-7.46 mg/kg), mean-12.06 mg/kg (range-3.24 -11.44mg/kg) in A,Ca,O and S respectively (Table- 4.2).

4.2.2- Manganese (Mn)

The Mn analysis presented a variation in the different rhizospheric soils of medicinal plants such as: mean-13.6 mg/kg (range-12.64-14.88mg/kg), mean-13.04 mg/kg (range-11.70-13.40 mg/kg), mean-13.18 mg/kg (range-10.96- 14.40 mg/kg), mean- 19.04 mg/kg (range-17.62- 19.38 mg/kg) in A,Ca,O and S respectively (Table- 4.2).

4.2.3 Copper (Cu)

The Cu analysis presented a variation in the different rhizospheric soils of medicinal plant such as: mean-0.658 mg/kg (range-0.638-0.668 mg/kg), mean- 0.692mg/kg (range-0.636-0.730mg/kg), mean-0.470 mg/kg (range-0.420-0.786mg/kg), mean-0.726 mg/kg (range-0.638-0.736mg/kg) in A,Ca,O and S respectively (Table- 4.2).

PARAMETERS	ASHWAGANDHA		CAMMOMILE		OCIMUM		SALVIA	
	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
Fe(mg/kg)	7.98-8.50	8.32	8.48-10.60	9.06	6.56-7.46	7.02	11.44-3.24	12.06
Mn (mg/kg)	12.64-14.88	13.56	11.70-13.40	13.04	10.96-14.40	13.18	17.62-19.38	19.04
Cu(mg/kg)	0.638-0.668	0.658	0.636-0.730	0.692	0.420-0.786	0.470	0.638-0.736	0.726
Zn(mg/kg)	0.056-0.060	0.058	0.036-0.058	0.052	0.048-0.076	0.062	0.050 - 0.060	0.058
B(mg/kg)	0.074-0.112	0.112	0.044-0.108	0.082	0.056-0.086	0.078	0.012-0.050	0.014
Co(mg/kg)	0.074-0.122	0.084	0.078-0.098	0.080	0.078-0.104	0.082	0.124-0.176	0.142
Ni(m/kg)	0.056-0.068	0.066	0.034-0.042	0.038	0.042-0.048	0.046	0.058-0.102	0.098
Al(mg/kg)	0.336-0.358	0.298	0.390-0544	0.448	0.302-0.69	0.558	0.428-0.490	0.456

4.2- DTPA-extractable plant available micronutrients

4.3- Enzymes Activities

4.3.1 Acid Phosphotase

The analysis of acidphosphotase activity of different medicinal plants range low to high such as : Ashwandha-3.095 ,Cammomile-2.100,Ocimum- 3.537, Salvia-1.998.

4.3.2 Alkaline Phosphotase

The analysis of alkalinephosphotase activity of different medicinal plants range low to high such as : Ashwandha-5.611 ,Cammomile-5.365,Ocimum- 4.331, Salvia-4.663.

4.3.3 Dehydrogenase Activity

The analysis of alkalinephosphotase activity of different medicinal plants range low to high such as : Ashwandha-3.6864 $x10^{-4}$,Cammomile-2.2504$x10^{-4}$,Ocimum- 1.99210^{-4}, Salvia-2.4058$x10^{-4}$.

4.3- Enzyme activity of different rhizospheric soils of medicinal plants.

Samples	Dehydrogenase enzyme (μ mol $g^{-1}min^{-1}$)	Alkaline phosphotase(μ mol $g^{-1}min^{-1}$)	Acidic phosphotas(μ mol $g^{-1}min^{-1}$)
Ashwagandha	$3.6864x10^{-4}$	5.611	3.095
Cammomile	$2.2504x10^{-4}$	5.365	2.100
Ocimum	$1.9928x10^{-4}$	4.331	3.537
Salvia	$2.4058x10^{-4}$	4.6630	1.998

CHAPTER-5

DISCUSSION

The experimental finding of the investigation entitled "**Chracterization of rhizospheric soil of selected medicinal plants**" are discussed below under the following headings.

5.1- Soil physico-chemical properties

5.1.1- pH

The pH of four differentrhizospheric soils of medicinal plants was ranged from slightly moderate to strongly alkaline.Soil sample of Ashwagandha has the highest ph. Ph was strongly alkaline. The pH of CIMAP farm soil was high, ie., strongly alkaline (Figure 5.1) because of the presence of common base- forming cations include Ca^{2+}, Mg^{2+}, and K^{+}, as well as the composition of N, were highly available within alkaline soil reported by Huang et al. 2001. In the present investigation, it was reported that the concentration of Ca, Mg, K, and N were higher and much higher concentration might be the reason for the alkaline pH of the soil. In the year 2013, Kumar et al. ,also reported that the relatively high pH of the soils might be due to the high degree of base saturation. Only those plants that can adapt to such higher pH are preferred for plantation suggest by Singh et al., 2018.

5.1.2- Electrical Conductivity (EC)

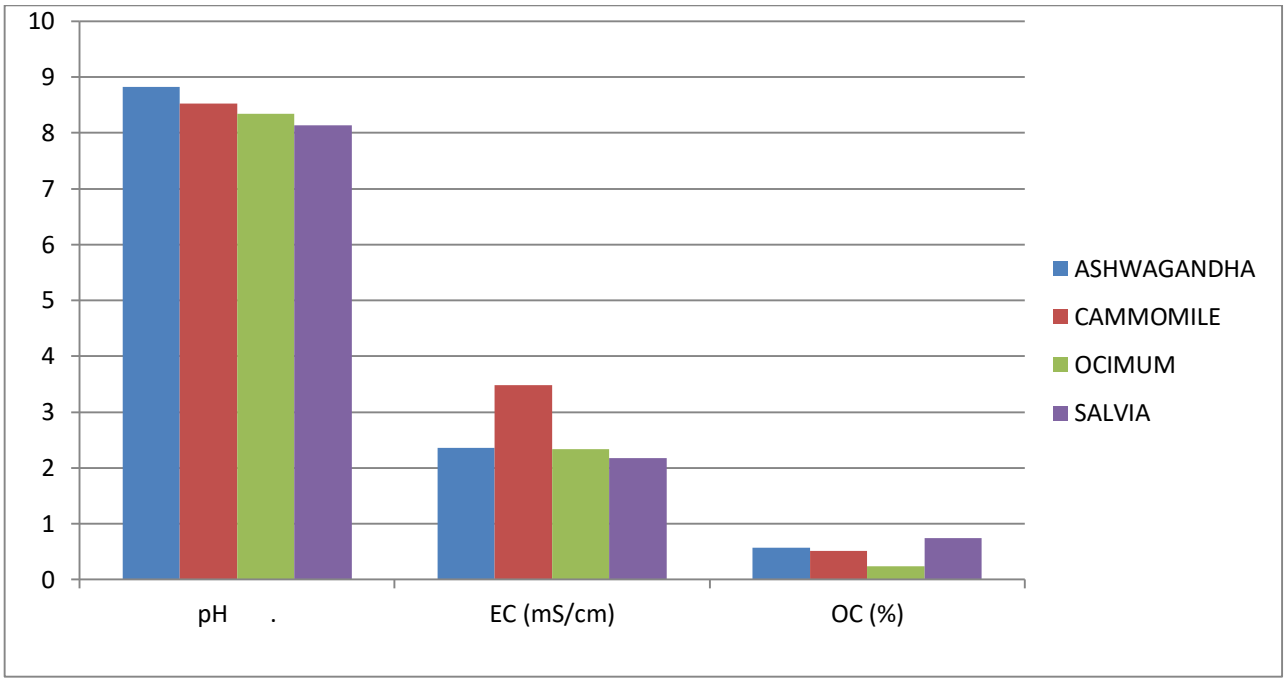
The determination of EC of the differentrhizospheric soil of medicinal plants ranged fron neutral to higher; the EC of L and P was found to be lower than the EC of G and C (Figure 5.1).

The EC ofCammomile wasthe highest as compared to neutral range, and EC of Ca showed a higher range , which might be critical for crops. Mukerjee and Lal. 2014, reported that the soil has a higher salt level will have an adverse effect. As the concentration of electrolytes (salts) increase in soil water, it will dramatically increase soil EC. Walker and Bernal, 2008, suggested that the salinity was associated with EC, and the lower EC may be because of the low concentration of Ca or Mg ions.

5.1.3- Organic Carbon (OC)

The determination of OC of four differentrhizospheric soil of medicinal plants was ranged from low to high. The result showed a low level of OC inOcimum, however, Salviahas the highest OC Levels followed by ashwagandha and cammomile respectively. (Figure 5.1).

In the present investigation, the OC of Ocimumwas lower. It might be because of an increase in heavy metals concertration in the soil, similarly stated by Tahar and Keltoum, 2011. 2019, Singh et al.reported similar studies and suggested that the lower content of organic carbon result from high temperature, which induced the rapid rate of organic matter oxidation and decomposition. The OC of Salvia and Ashwagandha was higher. Singh et al., 2019 stated that the increase in OC level might be the result of the decomposition of organic matter.



5.1.Physico chemical Properties

5.1.4- Mineralizable Nitrogen (N)

The estimation of nitrogen of the four different rhizospheric soil of medicinal plants was ranged from low to high. The result showed a low level of N in Ashwagandha. (Figure-5.2)

A low concentration of N was present in Ashwagandha , as analyzed in this study. Low N concentration can be attributed to prevailing soil acidity conditions demonstrated by Palwe and Yelwe, 2018 and Sethy et al, 2019.

In Salvia, the concentration was higher. Kumar et al. reported that continuous mineralization of organic matter in surface soil might be responsible for the higher values of N.

5.1.5- Available Phosphorus (P)

The assessment of P of the four different rhizosphericsoil of medicinal plants was ranged from low to high. The concentration of phosphorus present in Salvia was extremely higher concentration, followed by >Ocimum>cammomile>Ashwagandha. (Figure-5.2)

The concentration of phosphorus in salvia was found to be higher similarly reported by Amos-Tautua et al, 2013. In 2019, Sethy et al . suggested that available Phosphorus content greatly dependent on organic carbon status and microbial activity of the soils. The pH of the soil was somewhat affected by the Phosphorus content. In the present analysis the lower pH has higher Phosphorus concentration as compared to higher ph soils have lower Phosphorus, a similar statement has been given by Lemanowicz et al., 2016. Phosphorus in the pesticides industries might be the cause of higher accumulation Phosphorus in the Salvia.

5.1.6- Available Potassium (K)

The assessment of potassium of the four differentrhizospheric soil of medicinal plants was ranged from Low to high. Potassium present in Ashwagandha . soil was in higher concentration ,in salvia slightly high concentration and cammomile and ocimum possesses a low level as compared (Figure 5.2).

The concentration of K in Salvia was higher. And it was slightly high in ashwagandha>cammomile>ocimum.

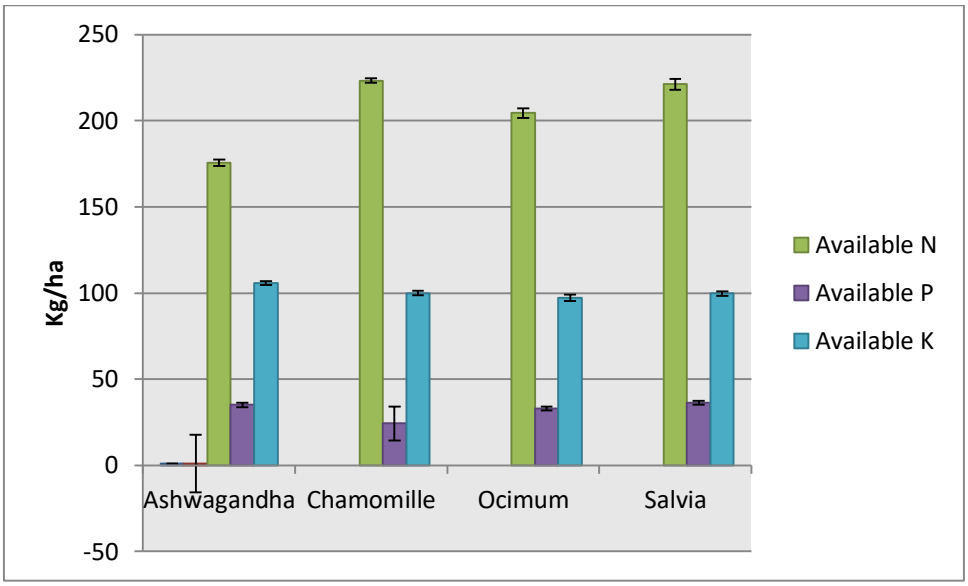


Figure 5.2- Available NPK .

5.3- Plants available micronutrients

5.3.1- Iron (Fe)

The assessment of Fe of the four differentrhizosperic soil of medicinal plants was ranged from Low to high. Fe present in S soil was in higher concentration, as compared to A,C,O. (Figure 5.3).

5.3.2- Manganese (Mn)

The assessment of Mn of the four differentrhizospheric soil of medicinal plants was ranged from Low to high. Mn present in Salvia soil was in higher concentration, as compared to A,C, and O. (Figure 5.3).

5.3.3 - Copper (Cu)

The assessment of Cu of the four different rhizosperic soil of medicinal plants was ranged from Low to high. Cu present in S soil was in higher concentration, as compared to A,Ca,O. (Figure 5.3).

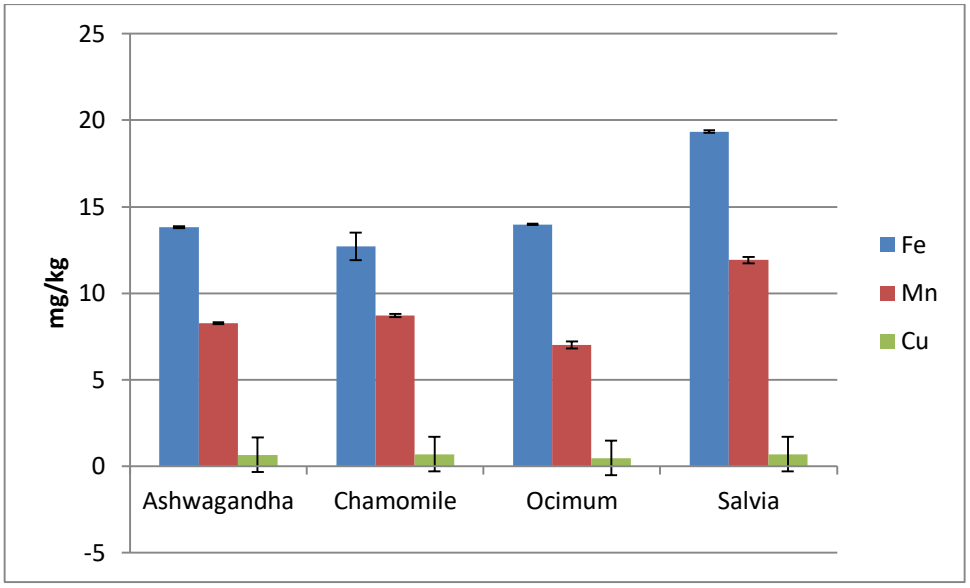


Figure 5.3- Plants available micronutrients

5.2 Soil Enzymes

5.2.1 Phosphotase Activity

Acid Phosphotase activity (AlkP) was found predominantly in acid soils and alkalinephosphotase activity was found in neutral or alkaline soils.

Alkaline Phosphotase

The highest concentration was found in Ashwagandha >Cammomile>Salvia >Ocimum.

Acidic Phosphotase

The highest concentration was found in Ocimum>Ahwagandha>Salvia.

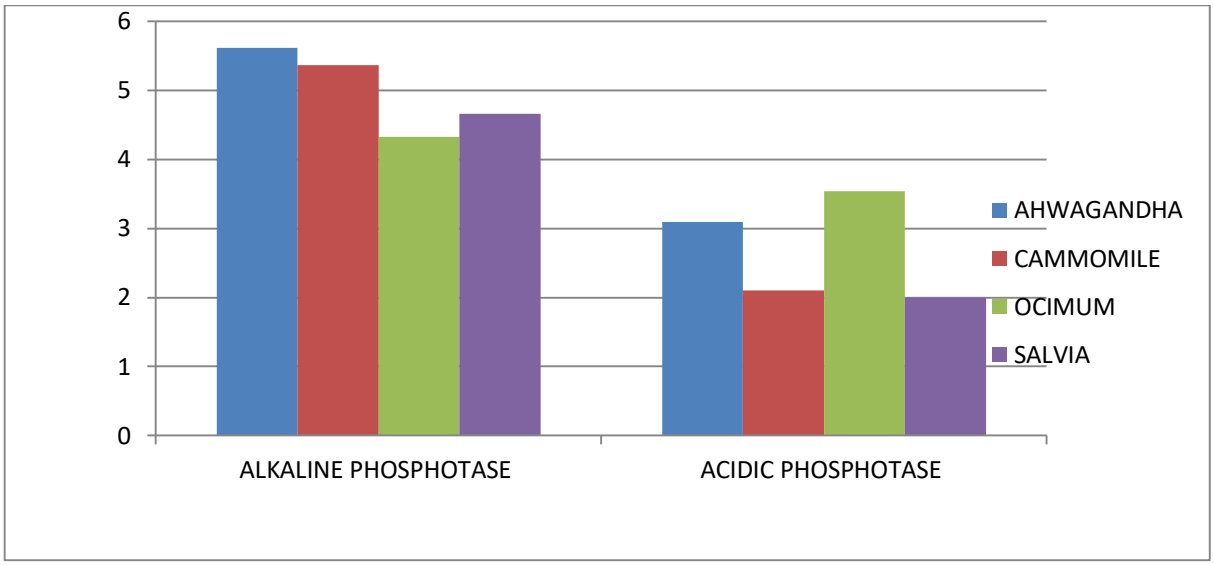


Figure 5.4- Enzyme activity of alkaline and acid phosphotase.

5.3- Dehydrogenase enzyme

The highest enzyme activity was found in Ashwagandha >salvia >Cammomile>Ocimum.

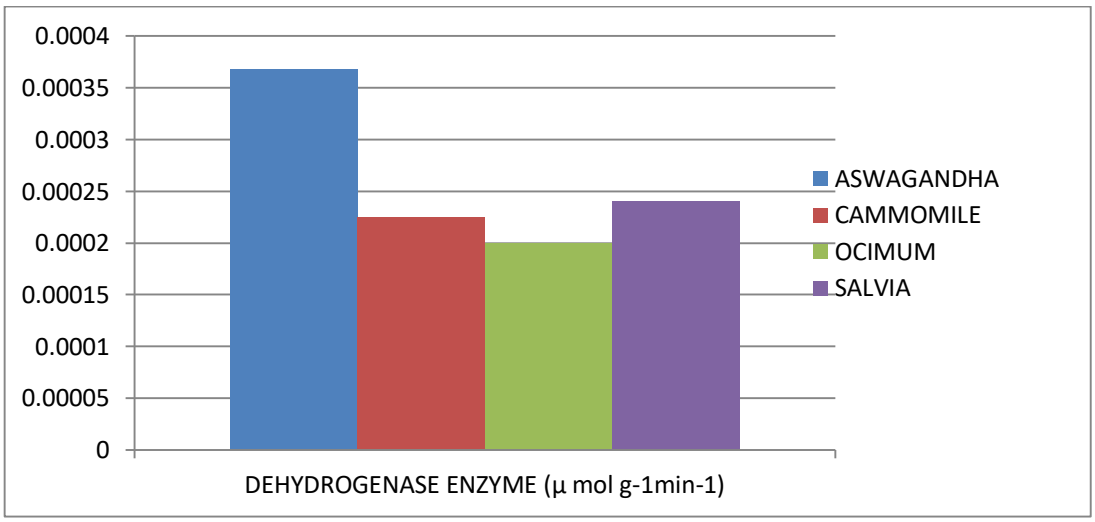


Figure 5.5- Enzyme activity of dehydrogenase .

CHAPTER-6

SUMMARY AND CONCLUSION

The soil is the most important constituent to fulfilment of all the basic needs of human beings. Soil is an important component of our farming. An eminent position in global cultivation of wheat, rice, jawar, pulses, sugarcane, vegetables and fruits etc. is occupied by Indian agriculture and reason of physical, chemical condition of whatever land is indispensable for proper implementation of the other management practices. Thus the physico-chemical study of territory is very significant because both physical and chemical properties which bear upon the soil productivity. This, physico-chemical study of soil is based on various parameters like pH, electrical conductivity, texture, moisture, temperature, soil organic matter, available nitrogen, phosphorus and potassium. This knowledge will help the farmers to grow nutritional plants.

This assessment was made to determine the properties of rhizospheric soil, available macronutrients and micronutrients. The current reaseachenlitled**" Chracterization of rhizospheric soil of selected medicinal plants"** is necessary for the management of improving the quality of selected medicinal plants by the analysis of rhizospheric soil.

According to objective main concluding points of the present research are as fallow:

- The soil samples were taken from four different crops- Ashwagandha, Cammomile,Ocimum and Salvia for analysis in the research laboratory of the soil science and agronomy department CSIR-CIMAP. Lucknow, Uttar Pradesh.

- There was a significant variation in soil physico-chemical properties concering areas. The physico-chemical properties (pH,Electrcal conductivity, Organic Carbon) of the soil shows high concentration in pH according to the graph.
- The NPK (macronutrients) availability was also higher as compared to another physico-chemical properties.
- Available P content is found to be significantly higher in rhizosphere soils of Ashwagandha followed by Salvia and ocimum.
- Similarly available K content is found to be significantly higher in rhizosphere soils of Ashwagandha followed by Salvia andocimum.
- DTPA extractable Fe content is found to be significantly higher in rhizosphere soils of Salvia followed by Ashwagandha ,ocimum, and chamomile.
-
- Alkaline phosphotase concentration was found to be higher in the soil sample of Ashwagandha followed by Cammomile,Salvia and Ocimum.
- The enzyme activiy of dehydrogenase was higher in Ashwagandha. as compared to another samples.

APPENDIX

I. Chemicals and glassware sources-

Chemical and glassware used were produced from the following sources:

- Applied biosystems, USA
- Eppendof India Ltd.,India
- Genexy Scientific Pvt. Ltd., India
- Himedia Biosciences, India
- Qualigens Fine chemicals, India
- Sigma chemical Co., USA
- SRL Pvt. Ltd., India
- Tarsons product Pvt. Ltd., India
- Thermo Scientific Pvt. Ltd., India

II. Reagents and buffers

1. **Reagents required for determination of organic carbon of soil**
   - **1 N Potassium dichromate:-**49.04 g of AR grade $K_2Cr_2O_7$ in 1 litre distilled water.
   - **Conc.Sulphuric acid.**
   - **0.5 N Ferrous ammonium sulphate :**196 g of Ferrous ammonium sulphate dissolved in distilled water, then added 200 ml of conc. H_2SO_4 and made volume to 1 litre.
   - **Diphenylamine indicator:** 0.5 g of the dye in the maximum 20 ml distilled water and 100 ml of conc. H_2CO_4.
   - **Orthophosphoric acid (85%) or Sodium fluoride.**

2. **Reagent required for the determination of mineralizable nitrogen of soil-**
    - **0.32 % $KMnO_4$**: 3.2 g of $KMnO_4$ in 1 liter distilled water.
    - **0.25% NaOH**: 25 g of Sodium hydroxide pellet in 1 liter distilled water.
    - **2% Boric acid**: 20 g of Boric acid powder dissolved in warm water by stirring and diluted to 1 liter.
    - **Mixed indicator**: Dissolved 0.066 g of methyl red and 0.099 g of bromocresol green in 100 ml of ethyl alcohol. Added 25 ml of mixed indicator to each liter of 2 % boric acid solution. The pH was adjusted to 4.5 with NaOH.
    - **0.1 N Potassium hydrogen phthalate** : 20.422 g of the salt in 1 litre distilled water.
    - **0.1 N NaOH** : 4 g of naoh in 1 litre distilled water.
    - **0.1 N H2SO4**: 2.8 ml of conc. H_2SO_4 to about 990 ml of distilled water. From this 0.02 N H_2SO_4 was prepared by diluting a suitable volume five with distilled water. Standardized it against 0.1 N NaOH solution.

3. Reagents required for the determination of available phosphorus of soil-

- **Bray's P-1 Extractant:** 1.110 g of AR grade ammonium fluoride in 1 litre of 0.025N HCL.
- **1.5% Dickman and Bray's reagent:** 15 g AR grade ammonium molybdate in 300 ml of warm water, allowed to cool and then added exact 350 ml of 10 N HCL. Made the volume to 1 litre.
- **40% $SnCl_2$ stock solution:** 10 g pure stannous chloride in 25 ml of conc. HCL and dissolved by heating. Allowed to cool, the transferred to an amber coloured bottle and store in dark after adding a small piece of Zn metal (AR grade) to prevent oxidation. From this, prepared a dilute $SnCl_2$ solution (0.5 ml diluted to 66 ml) immediately before use.
- **Standard stock solution:** 0.439 g of AR grade KH_2PO_4 dried in oven 60^0C for one hour in a 1 Litre beaker added about 500 ml of distilled water. Then added 25 ml of approx 7 N H_2SO_4 and made the volume to 1 litre. This is 100 mg solution L^{-1} solution.
- **Standard working solution :**Diluted a suitable volume of 100 mg L^{-1} P solution 50 times to get 2 mg L^{-1} P Solution.
- **0.**5 M $NaHCO_3$:Dissolved 42 gm of P- free sodium bicarbonate in about 500 ml of hot water and then diluted to 1 litre . Adjusted the pH to 8.5 using diluted NaOH solution or dilute HCL.
- **Activated Charcoal:** Washed pure activated charcoal or commercially available Darco G-60 with acid to make P-free.
- **1.5% Ammonium molybdate solution**: Dissolve 50 gram of AR grade ammonium molybdate in 300 ml of warm

water,allowed to cool and then added exact 400 ml of 10 N HCL.Made the volume to 1 litre.

4. **Reagent required for the determination of Potassium of soil-**

- **1N Ammonium aceate**: Dissolved 77.08 gm of ammonium acetate in about 500 ml of distilled water and made the volume to 1 litre.The pH was adjusted to 7.0 with acetic acid +

- **Standard Potassium Solution**: 1.908 gm of AR grade of KCL salt(Oven dried at 70^0 C for two hours) in 1 litre distilled water.Diluted suitable volumes of the solution to get 100 ml of working standard containing 5,10,15,20,25,30 and 40mg K L^{-1}.

5. **Reagents required for the determination of Enzyme activity of soil-**

Dehydrogenase:

- Methanol, analytical reagent grade.
- Calcium carbonate (CaCO3), reagent grade.
- 2,3,5-Triphenyltetrazolium chloride (TTC), 3%:, dissolve 3g of TTC Calbiochem, Los Angeles) in 80 mL of water and adjust the volume to 100 mL with water.
- Triphenyl formazan (TPF) standard solution: In a 100 mL volumetric flask dissolve 100 mg of TPF (Calbiochem, Los Angeles) in about 80 mL of methanol and adjust the volume to 100 mL with methanol. Mix thoroughly.

Phosphotase:

- Toluene, MUB pH 6.5 and 11 (use stock MUB and titrate with 0.1 M HCl or 0.1 M NaOH, respectively), calcium chloride (CaCl2; 0.5 M), and standard p-nitrophenol solution as described in ß-glucosidase section.
- p-Nitrophenyl phosphate solution (PNP), 0.05 M: Dissolve 0.840 g of disodium p-nitrophenyl phosphate tetrahydrate (Sigma 104, Sigma Chemical Co., St. Louis, MO) in about 40 mL of MUB pH 6.5 (for acid phosphatase activity), or pH 11 (alkaline phosphatase activity), and take to 50 mL with the same buffer. Store the solution at 4°C.

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