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Characterization of rhizospheric soil of selected Aromatic plants

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SAFIYA KHATOON

Enrollment No. 1700101664

M.Sc. Biotechnology, IVth Semester

Department of Biosciences

Integral University, Lucknow

Under the Supervision of

Dr. B. Shivanna

(Scientist)



CSIR-CIMAP

AGRONOMY AND SOIL SCIENCE DEPARTMENT
CSIR-CENTRAL INSTITUTE OF MEDICINAL AND AROMATIC PLANTS
LUCKNOW-226015 (INDIA)



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P.O.-CIMAP, Near Kukrail Picnic Spot, LUCKNOW-226 015 (U.P.)

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<i>Name of the Candidate</i>	: Miss Safiya Khaton
<i>Institution</i>	: Integral University, Lucknow
<i>Category of Training</i>	: 4 months
<i>Training Department</i>	: Department of Agronomy and Soil Science
<i>Title of Training</i>	: Characterization of rhizospheric soil of selected Aromatic Plants.
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Supervisor

Head of the Department/Division



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Phone No.: +91 (0552) 2890812, 2890730, 3296117, 6451039,

Fax No.: 0522-2890809

Kursi Road, Lucknow-226026, Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Miss Safiya Khatoon**, a student of M.Sc. Biotechnology (IV semester), Integral University has completed his four months dissertation work entitled “**Characterization of rhizospheric soil of selected aromatic 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)

Head,

Department of Biosciences,
Integral University, Lucknow

E-mail: info@integraluniversity.ac.in

Web: www.integraluniversity.ac.in

DECLARATION

I hereby declare that the embodied in this dissertation entitled “**Characterization of rhizospheric soil of selected aromatic 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.

Safiya Khatoon

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(Safiya Khatoon)

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ABBREVIATION

ABBREVIATION	DESCRIPTION
%	Percentage
μ	Mu
°C	Degree Celcius
M	Meter
Mm	Millimeter
l	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
V _{max}	Maximum rate of reaction
K _M	Michaelis constant
EC	Electrical conductivity
OC	Organic carbon
OM	Organic matter
N	Nitrogen
P	Phosphorus
K	Potassium
S	Sulphur
Al	Aluminium
As	Arsenic
Ag	Silver
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

In 1904 the German agronomist and plant physiologist Lorenz Hiltner first coined the term "rhizosphere" to describe the plant-root interface, a word originating in part from the Greek word "rhiza", meaning root (Hiltner, 1904; Hartmann *et al.*, 2008). Hiltner described the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influenced, he postulated, by the chemicals released from plant roots. In the years since, the rhizosphere definition has been refined to include three zones which are defined based on their relative proximity to, and thus influence from, the root .

According to a general view, the rhizosphere includes plant roots and the surrounding soil. This is a wide and wise definition, already coined more than Hundred years ago by Hiltner (1904). In that particular environment, very important and intensive interactions take place between the plant, soil, and microfauna. Biochemical interactions and exchanges of signal molecule between plants and soil microbes have been described and reviewed (Pinton *et al.*, 2007). The rhizosphere inhabiting microorganisms compete for water, nutrients and space and sometimes improve their competitiveness by developing an intimate association with plant (Hartmann *et al.*, 2009). These microorganisms play important roles in the growth and ecological fitness of their host. An understanding of the basic principle of rhizosphere microbial ecology, including the function and diversity of microorganisms that reside there, is necessary before soil microbial technology can be applied in the rhizosphere..

The rhizosphere, which is the volume of soil surrounding the plant root, is influenced by root activities such as exudation of reactive carbon compounds and uptake of mobile nutrients and water (George *et al.*, 2006; Hartmann *et al.*, 2008). Roots have evolved to adapt to their surrounding environment by optimizing their functional architecture to use resources in heterogeneous soils (Hinsinger *et al.*, 2005; Pierret *et al.*, 2007). Thus, the co-evolution of rhizosphere and plant roots play a major role in soil physical, chemical, and biological processes that sustain biodiversity, provide soil carbon sequestration, and cycle nutrients in natural and

agricultural systems (Hinsinger et al., 2009; Lambers et al., 2009). The plant-rhizosphere system also affects the biomass and activity of soil microorganisms that is generally enhanced due to root exudates (Raaijmakers et al., 2009). Different soil types harbor particular indigenous microorganisms that control the influence of plant root activity on rhizosphere microbial communities (Singh et al., 2007; Berg and Smalla, 2009).

The soil samples were collected from farmlands at a depth of 1-12 cm and within 15 cm circumference of the plant stem during the rabi season of 2014 and 2015 by random soil sampling methods. 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-Black titration method.

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 growth (Robert and Berthelin, 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 in rhizospheric soils of aromatic 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

RHIZOSPHERIC SOIL

Rhizosphere - or more appropriately the rhizospheric area - was first defined by L. Hiltner (1904) as the volume of soil influenced by plant roots and their exudates. It is classically distinguished from bulk soil, which corresponds to the area located outside of the rhizosphere, therefore non-adhering to roots and not under its influence. The rhizospheric area forms a hot-spot of microbial abundance and activity due to the presence of plant exudates and rhizodeposits (Kamaludeen and Ramasamy 2008, Zhuang et al. 2007). This microenvironment is a dynamic niche containing complex microbial communities and it may participate in a variety of beneficial interactions with plants such as water and nutrient uptake and may as well contribute to plant growth and health (Canbolat et al. 2006, Yang et al. 2009, Bais et al. 2006). This zone can be separated in two distinct fractions: rhizosphere *sensu stricto* and rhizoplan.

Rhizoplan is defined as the thin layer of soil covering the roots and strongly adhering to them (Cleyet-Marel and Hinsinger 2000, Seguin et al. 2005). It forms an interface between roots and rhizosphere, which corresponds to the rest of the rhizospheric area. Therefore, the term rhizosphere refers to the distal fraction of rhizospheric area that is adjacent to rhizoplan, still under roots influence, but without direct contact with them. It is thus not surprising that bacterial diversities of bulk soil, rhizosphere and rhizoplan significantly differ from each other and it may be of considerable importance to precisely identify the strains belonging to each fraction. However, a totally defined methodology for microbial extraction in the rhizospheric area does not exist. This may be attributed to the difficulty to define exactly the zone influenced by root exudates, which depends on plant and its root system (Angle et al. 1994). Consequently, it is difficult to compare rhizospheric population between different studies.

PHYSICO- CHEMICAL PROPERTIES OF RHIZOSPHERIC SOIL ANALYSIS

pH

Introduction

Soil pH is fundamental to the understanding of soil systems, because it is an indicator of many reactions in the soil (Moore and Loeppert 1987). Measurement of soil pH is highly technique dependent, in that many soil parameters and experimental factors affect the values obtained.

systems, because it is an indicator of many reactions in the soil (Moore and Loeppert 1987). Measurement of soil pH is highly technique dependent, in that many soil parameters and experimental factors affect the values obtained

Soil pH is a measure of the acidity or basicity (alkalinity) of a soil. Soil pH is a key characteristic that can be used to make informative analysis both qualitative and quantitatively regarding soil characteristics. Soil pH is fundamental to the understanding of soil systems, because it is an indicator of many reactions in the soil (Moore and Loeppert 1987). Measurement of soil pH is highly technique dependent, in that many soil parameters and experimental factors affect the value sobtained.

Soil reaction (pH), in particular, can be considered a key variable due to its influence on many other soil proprieties and processes affecting plant growth. Indeed, microorganism activity as well as nutrients solubility and availability are some of the most important processes that depend on pH. For instance, in acid soils, most micronutrients are more available to plants than in neutral-alkaline soils, generally favoring plant growth (Loncari ˇ c et al., 2008 ´). However, some of these micronutrients, along with non-essential elements, can become toxic when their concentration is too large. In contrast, in alkaline soils, although the availability of most

macronutrients is increased, phosphorus and micronutrient availability is generally reduced and their lower levels can adversely affect plant growth. Specifically, many plant characteristics (i.e., traits) such as height, lateral spread, biomass, flower size and number, pollen production, etc., are influenced by pH (Jiang et al., 2017).

Soil pH is a measure of H^+ ion activity and is an important chemical property that affects vegetation growth, microbial activity, decomposition rates, and nutrient availability (nitrogen, phosphorus, calcium). In particular, acidic (low pH) soils may be toxic to vegetation and microbes, reducing inputs of organic matter to the soil and slowing decomposition and other microbial processes. Soil pH depends on concentration of acidic and non-acidic-forming cations. H^+ and Al^{3+} are acid-forming while elements such as Ca^{2+} , Na^+ , and K^+ are base-forming (Jackson et al., 2014). Soil pH is influenced by parent material of the soil, oxidation-reduction reduction (redox) reactions, and the dominant water source. For example, wetlands that are primarily fed by precipitation, such as bogs, generally are more acidic while wetlands such as fens that are fed by groundwater tend to be more neutral (Jackson et al., 2014).

ELECTRICAL CONDUCTIVITY (EC)

Efficient and accurate methods of measuring within-field variations in soil properties are important for precision agriculture (Bullock & Bullock 2000). The apparent profile soil electrical conductivity is one sensor-based measurement that can provide an indirect indicator of the important soil physical and chemical properties. Soil salinity, clay content, cation exchange capacity (CEC), clay mineralogy, soil pore size and distribution, soil moisture content, and temperature all affect EC (McNeill 1992; Rhoades et al. 1999). In saline soil most of the variations in EC can be related to the salt concentration (Williams & Baker 1982). In non saline soils, conductivity variations are primarily a function of soil texture, moisture content, and CEC (Rhoades et al. 1976; Kachanoski et al. 1988). Rhoades et al. (1989) modeled EC as a function of soil water content (both the mobile and the immobile fractions), the electrical conductivity (EC) of the soil water, soil bulk density, and EC of the soil solid phase. The measurements of EC can be used for providing indirect measures of the soil properties listed above if the contributions of

other soil properties affecting the EC measurement are known or can be estimated. If the EC changes due to one soil property are much greater than those attributable to other factors, then EC can be calibrated as a direct measurement of that dominant factor. Lesch et al. (1995a, b) used this direct-calibration approach to quantify the variations in soil salinity in a field where the water content, bulk density, and other soil properties were reasonably homogeneous. The mapped EC measurements were found to be related to a number of soil properties of interest in precision agriculture, including soil water content (Sheets & Hendrickx 1995), clay content (Williams & Hoey 1987), CEC, and exchangeable Ca and Mg (McBride et al. 1990). Because EC integrates texture and moisture availability, two soil characteristics that affect productivity, it can help to interpret spatial grain yield variations, at least in certain soils (Jaynes et al. 1993; Sudduth et al. 1995; Kitchen et al. 1999). Other uses of EC in precision agriculture include refining the boundaries of the soil map units and creating subfield management zones (Fraisse et al. 2001)

ORGANIC CARBON (OC)

Soil Organic Matter (SOM) and soil fertility are critical components of production in cropping and forestry systems (Doran and Smith, 1987). Although SOM is not a requirement for plant growth per se (Follet et al., 1987), it is of fundamental importance in chemical, physical and biological aspects of soil fertility. Depletion of soil organic carbon (SOC) has been recognized for a long time to be a major process of soil degradation in tropical environments where shifting cultivation is practised (Nye and Greenland, 1960, 1964; Van Noordwijk et al., 1997; Drechsel and Gyiele, 1999). As in the case of nutrient depletion, SOC depletion is linked mostly to a disturbed balance of carbon inputs and outputs through cultivation. Most of the objectives for managing soil fertility, namely (i) obtain high production level, (ii) obtain high quality products, (iii) improve and maintain soil fertility and (iv) safeguard environmental quality; directly (iii and iv) or indirectly (i and ii) help manage SOC. In both cropping and forestry production systems, organic matter may be directly or indirectly managed as organic inputs. Management of both components for production of food adequate to meet the needs of an increasing population in

Eastern and Southern Africa, is a major challenge for researchers, extension workers, farmers and policy makers. To meet the demands of these production systems, it is inevitable that productivity of the systems in the region must be improved and sustained through appropriate adaptive strategies based on culturally acceptable, economically feasible, environmentally friendly and agronomically superior options, technologies and practices. This calls for a holistic approach in SOM research and development that involves multi-stakeholders, multidisciplines and multi-strategies, if the 250% increase in grain production from the present 1 billion t yr⁻¹ to 2.5 billion t yr⁻¹ required by 2030 in the less developed countries is to be achieved (Walker et al., 1999)

Soil quality, defined as the ability of the soil to function (Karlen et al., 2008), is largely depending on the topsoil SOC content (Bünemann et al., 2018). SOC decrease strongly impacts soil quality (Lal, 2006), in particular its physical resistance and resilience (Kay, 1998). Owing to the multiple benefits of SOC to soil quality, in particular soil fertility in a broad understanding (King et al., 2020), increasing SOC content is an environmental and agronomical emergency regardless of climate questions. The requirements in SOC content with respect to soil physical quality were recently defined by Johannes et al. (2017), who showed that soil structure vulnerability is proportional to the SOC to clay ratio. This ratio should not be smaller than 10% for acceptable structure vulnerability.

NITROGEN (N)

Nitrogen is an essential mineral nutrient for crop growth and yield (Xu et al., 2012).

During the past three decades, N application rates have increased rapidly and excessive quantities of N fertilizers have been used to enhance crop yields. However, Excessive N application could lead to soil acidification as well as worsen the soil environment thus, ultimately has a negative impact on crop growth and yield (Guo et al., 2010; Schroder et al., 2011).

Nitrogen fertilization can significantly affect soil properties. Soil factors are closely associated with soil nutrient cycling and plant nutrient uptake, and therefore affect productivity. Urease,

phosphatase and invertase play key roles in soil N, P, and C cycles (Zhao et al., 2009). These enzyme activities are directly involved in various biochemical reactions in the soil. Soil available nutrients can be directly absorbed by crops and contribute to soil fertility. Soil organic matter (SOM) is responsible for some important soil processes such as soil respiration, soil aggregate stability and water holding capacity (Herencia et al., 2011). These soil properties are considered as important factors determining soil quality (Gong et al., 2015).

The response of soil properties and crop physiological attributes to N fertilization depends on soil type, climate conditions and other factors (Lupwayi et al., 2012; Giacometti et al., 2013). Therefore, optimal N fertilization strategies must be based on specific site and conditions. Various studies have focused on the single effects of N fertilization on soil properties or crops physiology.

Presently, there is no generally adopted and completely reliable method for establishing the availability of nitrogen in soil (Goh and Haynes, 1986). This statement is based on the well-known fact that soil nitrogen is found primarily in its organic form and that it is available to plants only after its mineralization has been completed. The purpose of this research was to establish whether the total and easily hydrolyzed nitrogen content in the soil in which the experiment was made could be used to indicate nitrogen availability of soil.

PHOSPHORUS (P)

Phosphorus (P) is an essential nutrient for all living organisms (White and Hammond, 2008). As weathering of minerals and the deposition of atmospheric dust are minor sources of P (Walker and Syers, 1976; Vitousek et al., 2010; Wang et al., 2015), the recycling of organic P from litter and soil organic matter is of utter importance for plant growth and microbial activity in terrestrial ecosystems. In P-poor ecosystems, limited P recycling may dampen the response of plant growth to elevated CO₂ concentration (Ellsworth et al., 2017). Yang et al. (2014) reported in a modeling study that the effect of elevated CO₂ on plant productivity in the Amazon Basin critically dependent on assumptions regarding the P-recycling efficiency within soils, which was strongly related to the parameterization of phosphatase production in their model. The rate at which

ecosystems can recycle P from litter and soil organic matter is poorly quantified by observation (Gill and Finzi, 2016). Soil phosphatases secreted by fungi, bacteria, and plant roots play an important but poorly quantified role in transforming complex and unavailable forms of organic P into assimilable phosphate (Caldwell, 2005). Potential phosphatase activity in soils, which can be measured in the lab from soil samples, is an indicator of the capacity of enzyme communities to cleave organic molecules containing P (Krämer and Green, 2000), and serves as a surrogate for the lacking measurements of P mineralization in the soil.

POTASSIUM (K)

Soil potassium (K⁺) exists in solution, exchangeable, and non-exchangeable forms that are in dynamic equilibrium with each other (Cox et al., 1999). Exchangeable K is widely used for evaluating the soil K status and prediction of crop K requirements (Samaadi, 2006; Krauss, 2003) while in other countries the K saturation index (%) is used for the assessment of soil K status (Mutscher, 1995). However, research has shown that solution and exchangeable K⁺ are replenished by non-exchangeable K⁺ when they are depleted by plant removal or leaching. Bansal et al. (2002) showed that exchangeable K alone cannot be used as the basis for evaluating K availability under intensive cropping; as those soils considered sufficient in exchangeable K were not able to maintain that condition for long under intensive cropping with high yielding varieties. Similarly, Mohammed (2006) observed that complications arise in the use of neutral normal ammonium acetate (NH₄OAc) pH 7.0, as some of the non-exchangeable K was utilized by plants. Thus, it is considered important to study methods of available K extraction for better understanding of the fertility status of agricultural soils. The choice of the particular extractant is however decided by establishing a correlation between the amounts extracted and crop growth or yield. In order to make accurate predictions of crop response to potassium, soil test methods should have a high correlation with crop performance (Ekpete, 1972). Also, the economically optimum fertilizer rate, an estimate calculated by fitting a regression model with crop yield as the response variable and the amount of applied nutrient as the predictor variable have been used as bench marks for fertilizer needs (National Academy of Sciences – National Research Council,

1961; Stanford, 1973; Vanotti and Bundy, 1994a,b; Rehm et al., 2006). Dean (1954) obtained available phosphorus values in soils where the yield- of- phosphorus curves fitted the equation $Y = a + bX$ by extrapolating the curves to the X-axis. The values approximated the A-values which Fried and Dean (1952) had conceived in the measurement of available soil nutrients (Ekpete, 1972). Dilute mineral acids, exchangeable potassium and potassium extracted with very dilute CaCl_2 solution were employed in extracting potassium from the soils used in this study. This knowledge is required for a soil-testing service which is very important in the programme of increasing food production through fertilizer application.

DTPA-EXTRACTABLE MICRONUTRIENTS

The methods most commonly used to extract the available micronutrient cations Fe, Mn, Cu, and Zn include the diethylene triamine pentaacetic acid (DTPA) method, ethylene diamine tetraacetic acid (EDTA) method, and 0.1 M HCl method. The DTPA soil test was originally developed by Lindsay and Norvell, as outlined in 1978, for micronutrient extraction of near-neutral and calcareous soils. The detailed theoretical basis for the DTPA soil test and reviews on its use have been published by Lindsay and Norvell (1978), Cox (1987), and O'Connor (1988). DTPA has been selected among a number of chelating agents because it has the most favorable combination of stability constants for the simultaneous complexing of Fe, Mn, Cu, and Zn. The extractant which is highly pH dependent is buffered at pH 7.30 with triethanolamine (TEA). The addition of 0.01 M CaCl_2 enables the extract to attain equilibrium with CaCl_2 , so that it can minimize the dissolution of CaCO_3 from calcareous soils.

The DTPA method is inexpensive, reproducible, and easily adaptable to routine operations, if standardized procedures for preparation and extraction of the samples are established (Soltanpour et al. 1976). Various reports showed that DTPA extracts micronutrient metals from labile pools in soil (Wallance and Mueller 1968, Rule and Graham 1976) and that the level of DTPA-extractable metals correlate significantly with plant uptake (Haq and Miller 1972, Randall et al. 1976). However, the method is not suitable for sludge-amended soils (Barbarick and Workman 1987, Bidwell and Dowdy 1987).

SOIL ENZYMES ACTIVITIES

The conceptual rationale for soil enzyme activity as a soil quality indicator is that enzyme activities: (i) are often closely related to important soil quality parameters such as organic matter, soil physical properties and microbial activity or biomass (Dick, 1994); (ii) can begin to change much sooner (1 to 2 yr) than other properties (e.g., soil organic C) thus providing an early indication of the trajectory of soil quality with changes in soil management; (iii) can be an integrative soil biological index of past soil management; and (iv) involve procedures that are relatively simple compared to other important soil quality properties (e.g., physical and some biological measurements) and therefore have potential to be done routinely by soil testing or analytical environmental laboratories.

DEHYDROGENASE ENZYME (DHA)

Measurement of the activity by dehydrogenase systems is attractive because these systems are an integral part of microorganisms and their apparent role in oxidation of organic matter (Casida et al., 1964); however, its activity does not consistently correlate well with other biological properties such as O₂ uptake, CO₂ evolution or microbial biomass (Skujins, 1978; Frankenberger & Dick, 1983; Ross, 1973; Howard, 1972). Dehydrogenase activity may be confounded by extracellular phenol oxidases, which are known to exist in soils (Howard, 1972) or by inorganic compounds in soils (Bremner & Tabatabai, 1973) that can catalyze the dehydrogenase reaction. Also, Chander and Brookes (1991) found there was an abiological reaction between triphenylformazan (the end product measured in dehydrogenase activity) and Cu, which could result in underestimating of dehydrogenase activity in soils that have been contaminated with various soil pollutants. Nonetheless other studies have found dehydrogenase to be correlated with microbial biomass and other measures of biological activity (Steven-son, 1959; Skujins, 1973; Ladd & Paul, 1973). Although dehydrogenase has been shown to be sensitive to soil management effects (Martens et al., 1992; Dick et al., 1988a; Doran, 1980), it is less suited to project permanent changes in soil quality because it cannot accumulate in a complexed form in soils and is best used as an indication of the viable microbial population.

PHOSPHOTASE ENZYME

Phosphatases are important in the P cycle because they provide P for plant uptake by releasing PO₄. Phosphomonoesterases are classified as acid (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) or alkaline (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) according to the optimal activity at acid and alkaline pH, respectively (Tabatabai & Bremner, 1969; Eivazi & Tabatabai, 1977). Phosphatase activity is strongly influenced by soil pH (Eivazi & Tabatabai, 1977; Juma & Tabatabai, 1977, 1978). Repeated additions of PO₄ fertilizer suppresses phosphatase activity (Dick, 1994). None-the-less, we have included phosphatase activity because it is important in the P cycle and acid phosphatase has been widely studied and it provides a potential index for a soil to mineralize organic P. The phosphatase activity procedures are based on a similar principle to β-glucosidase in that hydrolysis of p-nitrophenyl phosphate releases p-nitrophenol, which is extracted quantitatively from soil and measured colorimetrically.

CHAPTER-3

MATERIALS AND METHODS

The present investigation entitled “**Characterization of rhizospheric soil of selected aromatic plants**” at CIMAP farm, Lucknow was conducted to determine the physico-chemical properties in rhizosphere soil of selected aromatic plants. This work was carried out in the Department of Agronomy and Soil Sciences at CSIR-Central Institute of Medicinal and Aromatic plants (CSIR-CIMAP), Lucknow, Uttar Pradesh, India. The study includes the collection of rhizospheric soil samples from four aromatic plants: Geranium, Lemongrass, Citronella, and Palmarosa. The details of Method and Materials followed in this investigation are described in this chapter.

3.1. Location of the study area

CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, Uttar Pradesh. Located at 26°5' N latitude 80°5' E longitude with an elevation of about 120 meters above mean sea level under the sub-tropical plains of North India. (Nilofer et al; 2018). The soil was collected from four aromatic plants: Geranium, Lemongrass, Citronella, and Palmarosa respectively.

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



Figure-3.1: Map location of the CSIR-CIMAP.



Figure- 3.2: Satellite map location of CSIR-CIMAP, Lucknow, Uttar Pradesh

3.2- Collection of soil samples and soil sampling

The rhizospheric soil samples of aromatic plants were taken from the agricultural area of CIMAP farm from four different aromatic plants: (a) Geranium, (b) Lemongrass ,(c) Citronella, (d) Palmarosa .



(a) GERANIUM

(b) LEMONGRASS



(c) CITRONELLA

(d) PALMAROSA

The soil samples 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

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 a buffer

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 determine soil Organic carbon (OC) from which one of the most convenient methods is oxidation with $K_2Cr_2O_7$ in the presence of sulfuric acid (H_2SO_4)(Walkley and Black,1934).

0.5 g of soil sample was taken in a conical flask. 10ml of 1N $K_2Cr_2O_7$ and 20ml of H_2SO_4 was added to it. Then it was swirled 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:

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

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 $KMnO_4$, 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 :-

$$\text{Available N (kg ha}^{-1}\text{)} = \frac{(\text{Sample}) \times \text{N of H}_2\text{SO}_4 \times 0.014 \times 2 \times 10^8}{\text{Wt. of soil}}$$

3.3.5- Determiration 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:

$$\text{Available P}_2\text{O}_5 = \frac{\text{Graph ppm} \times \text{Vol. of extractant} \times \text{Vol. made} \times 2.24 \times 2.29 \text{ (kg ha}^{-1}\text{)}}{\text{Wt. of soil} \times \text{Aliquot}}$$

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:

$$\text{Available K}_2\text{O} = \frac{\text{Graph ppm} \times \text{vol. of extractant} \times 2.24 \times 1.20 \text{ (kg ha}^{-1}\text{)}}{\text{Wt. of soil}}$$

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:

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

3.5- Determination of Soil enzymes activity

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 are used 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 mL of 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 Phosphatase 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 or 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 calibration 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-nitrophenol analysis of the incubated soil sample (i.e., add 1 mL of 0.5 M CaCl₂ and 4 mL of 0.5 M 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 β-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

RESULTS

The present investigation was undertaken to study “**Characterization of rhizospheric soil of selected aromatic 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. The physico-chemical of the four different rhizospheric soils of aromatic plants were estimated through laboratory analysis and are presented below.

4.1.1- pH

The pH analysis showed a great variations in the four different rhizospheric soils of aromatic plants ranged from Slightly neutral to alkaline such as mean- 7.95 (range: 7.45-8.26), mean-8.17 (range:8.12-8.27), mean- 8.24 (range: 8.10-8.30), and mean-8.05 (range:8.00-8.22) in G, L, C, and P respectively (Table- 4.1). The results indicate that G was neutral and L, C, P was alkaline.

4.1.2- Electric Conductivity (EC)

Analysis of EC, presented variation in the four different rhizospheric soils of aromatic plants such as: mean- 2.15 mS/cm (range: 1.21-2.76 mS/cm), mean- 1.80 mS/cm (range: 1.56-2.33), mean-2.64 mS/cm (range:2.54-2.74), and mean-1.75 mS/cm (range:1.80-2.97) in G, L, C, and P respectively (Table- 4.1). The EC of G and C higher compared to the EC value of L and P. And it is probably critical for crop growth.

4.1.3- Organic Carbon (OC)

Analysis of OC, exhibited a dissimilarity in the four different rhizospheric soils of aromatic plants such as :mean-0.56 % (range:0.45-0.99), mean-0.36% (range:0.33-0.72%), mean-0.66% (range:0.63-0.75%), mean- 0.72% (range: 0.69-0.75%) in G, L, C, and P respectively (Table- 4.1). An excellent build up OC was seen C and P as compared to the OC values of G and L.

4.1.4- Mineralizable Nitrogen (N)

The determination of N in four different rhizospheric soils of aromatic plants ranged from low to medium such as: mean- 178.7 kg/ha (range -172.4-228.9 kg/ha), mean- 210.1 kg/ha (range –

197.5-213.2 kg/ha), mean – 225.7 (range- 213.2- 235.2),mean – 200.7(range – 191.2-242.6) in G, L, C, and P respectively (Table- 4.1). Among these L, C and P had highest mean then G.

4.1.5- Available Phosphorus (P)

The result of the study showed a divergence in P in the four different rhizospheric soils of aromatic plants such as: mean-161.7kg/ha (range:131.9-236.9 kg/ha), mean- 204.0 kg/ha (range: 203.2-208.8 kg/ha), mean- 195.1 kg/ha (range:174.2-230.5 kg/ha), mean- 160.8 kg/ha (range:147.9- 182.5 kg/ha) in G, L, C and P respectively (Table- 4.1). The highest concentration of (P) was found in L followed by C, G and P.

4.1.6- Available Potassium (K)

Analysis of K showed a variations in the four different rhizospheric soils of aromatic plants ranged from medium to high such as: mean-140.0 kg/ha (range- 120.9-142.2kg/ha), mean- 155.6 kg/ha (range- 153.4- 173.6 kg/ha), mean- 196.0 kg/ha (range- 187.0-260.9 kg/ha), mean-150.0 kg/ha (range-135.5-161.2 kg/ha) in G, L, C, and P respectively (Table- 4.1). The highest concentration of K was found in C followed by L, P and G.

PARAMETERS	GERANIUM		LEMONGRASS		CITRONELLA		PALMAROSA	
	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
pH	7.45-8.26	7.95	8.12-8.27	8.17	7.45-8.26	7.95	8.12-8.27	8.17
Electrical conductivity (mS/cm)	1.21-2.76	2.15	1.56-2.33	1.80	1.21-2.76	2.15	1.56-2.33	1.80
Organic Carbon (%)	0.45-0.99	0.56	0.33-0.72	0.36	0.45-0.99	0.56	0.33-0.72	0.36
Nitrogen (kg/ha)	172.4-228.9	178.7	197.5-213.2	210.1	172.4-228.9	178.7	197.5-213.2	210.1
Phosphorus (kg/ha)	131.9-236.9	161.7	203.2-208.8	204.0	131.9-236.9	161.7	203.2-208.8	204.0
Potassium (kg/ha)	120.9-142.2	140.0	153.4-173.6	155.6	187.0-260.9	196.0	135.5-161.2	150.0

Table:- 4.1- Physico-chemical properties of four different rhizospheric soils of aromatic plants.

4.2- Plant available micronutrients

Plants available micronutrients such as Al, B, Co, Cu, Fe, Ni, Mn, and Zn 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 four different rhizospheric soils of aromatic plants such as: mean-14.8 mg/kg (range-13.2-24.0 mg/kg), mean- 18.86 mg/kg (range-18.26-19.02 mg/kg), mean- 18.54 mg/kg (range- 18.36-19.46 mg/kg), mean- 19.56 mg/kg (range- 18.56-20.0 mg/kg) in G, L, C, and P respectively (Table- 4.2). The highest concentration in Fe was found in L and P followed by G and C.

4.2.2- Manganese (Mn)

Analysis of Mn, showed variations in the four different rhizospheric soils of aromatic plants such as: mean- 22.8 mg/kg (range-21.0-27.6 mg/kg), mean-16.04 mg/kg (range-15.62-16.86 mg/kg), mean- 16.86 mg/kg (range- 15.62-18.86 mg/kg), mean- 17.4 mg/kg (range- 14.02-17.94 mg/kg) in G, L, C, and P respectively (Table- 4.2). The highest concentration in Mn was found in G followed by L, C, P.

4.2.3- Copper (Cu)

The Cu analysis presented a disparity in the four different rhizospheric soils of aromatic plants such as: mean- 1.516mg/kg (range:1.434-1.648 mg/kg), mean- 0.94 mg/kg (range: 0.872-1.008 mg/kg), mean-0.936 mg/kg (range:0.926-0.97 mg/kg), and mean-0.998 mg/kg (range:0.87-1.0 mg/kg) in G, L, C and P respectively (Table-4.2). the highest concentration of Cu was found in G and L followed by C and P.

4.2.4 - Zinc (Zn)

The Zn analysis presented a variation in the four different rhizospheric soils of aromatic plants such as: mean-(-0.04) mg/kg (range-(0.06-0.10mg/kg), mean-(-0.05) mg/kg (range-(-0.07-0.09 mg/kg), mean- (-0.08)mg/kg (range- (-0.08)-(-0.05) mg/kg), mean- (-0.04) mg/kg (range-(-0.06)-0.08 mg/kg) in G, L, C, and P respectively (Table- 4.2).

4.2.5- Boron (B)

The B analysis presented a variation in the four different rhizospheric soils of aromatic plants such as: mean- 0.045 mg/kg range- 0.040-0.050mg/kg), -mean- 0.085 mg/kg (range-0.080-0.090mg/kg), mean- 0.048 mg/kg (range- 0.046-0.056 mg/kg), mean- 0.056 mg/kg (range-0.054-0.060 mg/kg) in G, L, C, and P respectively (Table- 4.2). The highest concentration in B was found in L and P followed by C and G.

4.2.6- Cobalt (Co)

The Co analysis presented a variation in the four rhizospheric soils of aromatic plants such as: mean-0.14 mg/kg (range-0.01-0.11mg/kg), mean-0.095 mg/kg (range-0.093-0.12 mg/kg), mean-0.98 mg/kg (range-0.09-0.11 mg/kg), mean- 0.10 mg/kg (range- 0.08-0.11 mg/kg) in G, L, C, and P respectively (Table- 4.2).

4.2.7-Nickel (Ni)

The Ni analysis presented a variation in the rhizospheric soils of four aromatic plants such as: mean- 0.048 mg/kg (range-0.01-0.15 mg/kg), mean- 0.15 mg/kg (range-0.07-0.17 mg/kg), mean-0.12 mg/kg (range-0.11-0.16 mg/kg), mean- 0.14 mg/kg (range- 0.13-0.16 mg/kg) in G, L, C, and P respectively (Table- 4.2).

4.2.8- Aluminium (Al)

The Al analysis presented a variation in the four rhizospheric soils of different aromatic plants such as: mean- 0.49 mg/kg (range-0.41-0.58mg/kg), mean- 0.66 mg/kg (range-0.49-0.32 mg/kg), mean-0.48 mg/kg (range- 0.45-0.49mg/kg), mean-1.33 mg/kg (range- 0.38-0.87mg/kg) in G, L, C, and P respectively (Table- 4.2). The highest concentration in Al was found in P followed by L, G and C.

PARAMETERS	GERANIUM		LEMONGRASS		CITRONELLA		PALMAROSA	
	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
Fe (mg/kg)	13.2-24.0	14.8	18.26-19.02	18.86	18.36-19.46	18.54	18.56-20.0	19.54
Mn (mg/kg)	21.0-27.6	22.8	15.62-16.86	16.04	15.62-18.86	16.86	14.02-17.94	17.4
Cu (mg/kg)	1.434-1.648	1.516	0.872-1.008	0.94	0.926-0.11	0.98	0.08-0.11	0.10
Zn (mg/kg)	0.05-0.10	0.07	0.07-0.09	0.05	0.08-0.10	0.09	0.06-0.08	0.05
B (mg/kg)	0.040-0.050	0.045	0.080-0.090	0.085	0.046-0.056	0.048	0.054-0.060	0.056
Co (mg/kg)	0.01-0.11	0.14	0.093-0.12	0.095	0.094-0.11	0.98	0.08-0.11	0.10
Ni (mg/kg)	0.10-0.15	0.11	0.07-0.17	0.15	0.11-0.16	0.12	0.13-0.16	0.14
Al (mg/kg)	0.41-0.58	0.49	0.36-0.55	0.55	0.45-0.49	0.48	0.38-0.87	0.50

Table 4.2- DTPA-extractable plant available micronutrients in four different rhizospheric soils of aromatic plants.

4.3- Enzyme activity of rhizospheric soil of aromatic plants.

4.3.1- Dehydrogenase enzyme -

Analysis of dehydrogenase enzyme activity showed a variations in different types of soils such as: Geranium- 2.1680×10^{-4} , Lemongrass- 3.1656×10^{-4} ,Citronella- 4.1156×10^{-4} , Palmarosa- 2.4023×10^{-4} .The highest concentration of DHA was found in C followed by P, G and L.

4.3.2 - Alkaline Phosphatase-

Analysis of alkaline enzyme activity showed a variations in different rhizospheric soils such as: Geranium-7.1023, Lemongrass- 7.2728 ,Citronella-6.9287, Palmarosa-6.9412 .

4.3.2- Acidic phosphatase-

Analysis of acidic enzyme activity showed a variations in different rhizospheric soils such as: Geranium-4.2729, Lemongrass- 2.6306 ,Citronella-3.1128, Palmarosa-2.3726 .

SAMPLES	DEHDROGENASE ENZYME (μ mol g⁻¹.min⁻¹)	ALKALINE PHOSPHOTASE ENZYME (μ mol g⁻¹.min⁻¹)	ACIDIC PHOSPHOTASE ENZYME (μ mol g⁻¹.min⁻¹)
GERANIUM	2.1680X10 ⁻⁴	7.1023	4.2729
LEMONGRASS	3.1656X10 ⁻⁴	7.2728	2.6306
CITRONELLA	4.1156x10 ⁻⁴	6.9287	3.1128
PALMAROSA	2.4023x10 ⁻⁴	6.9412	2.3726

Table 4.3- Enzyme activity of four rhizospheric soil of aromatic plants.

CHAPTER-5

DISCUSSION

The experimental finding of the investigation entitled “**Characterization of rhizospheric soil of selected aromatic plants**” are discussed below under the following headings.

5.1- Soil physico-chemical properties

5.1.1- pH

The pH of different rhizospheric soils of four aromatic plants was ranged from slightly neutral to strongly alkaline. The pH of CIMAP farm soil was high, i.e., 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 rhizospheric soils of four aromatic plants ranged from 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 of G and C was high compared to neutral range, and G and C 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 rhizospheric soils of four aromatic plants was ranged from low to high. The result showed a low level of OC in C and P, however, at G and L demonstrated medium build-up of OC (Figure 5.1).

In the present investigation, the OC of G and L was lower. It might be because of an increase in heavy metals concentration 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 C and B was a higher. Singh et al., 2019 stated that the increase in OC level might be the result of the decomposition of organic matter.

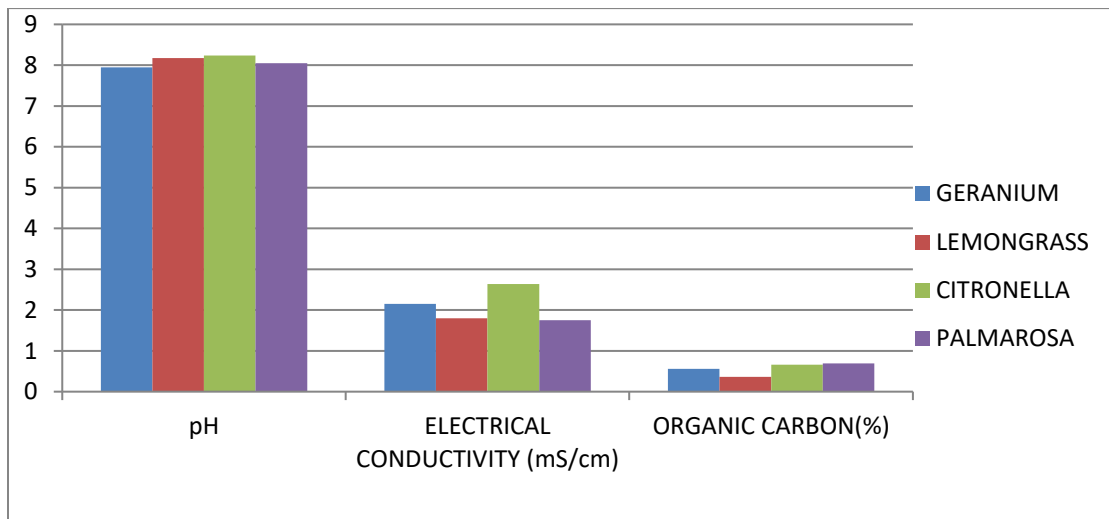


Figure 5.1- Soil physico-chemical properties (pH, Electrical conductivity, and Organic Carbon).

5.1.4- Mineralizable Nitrogen (N)

The estimation of nitrogen of rhizospheric soils of four aromatic plants the was ranged from low to high. The result showed a low level of N in G. (Figure-5.2)

A low concentration of N was present in G , 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. A similar result was seen in the present study, i.e, the N concentration in acidic soil was lower comparatively in alkaline soil.

In L, C and P, 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 rhizospheric soils of four aromatic plants seen was from low to high the concentration of phosphorus present in L extremely higher concentration, P possessed medium concentration and in G and C possessed low concentration. (Figure-5.2)

The concentration of phosphorus in L 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 have lower Phosphorus are similar statement has been given by Lemanowicz et al., 2016. Phosphorus in the pesticides industries might be because of higher accumulation Phosphorus in the L.

5.1.6- Available Potassium (K)

The assessment of potassium of rhizospheric soils of four aromatic plants was ranged from Low to high. Potassium present in C soil was in higher concentration, in L slightly high concentration and P and G possesses a low level (Figure 5.2).

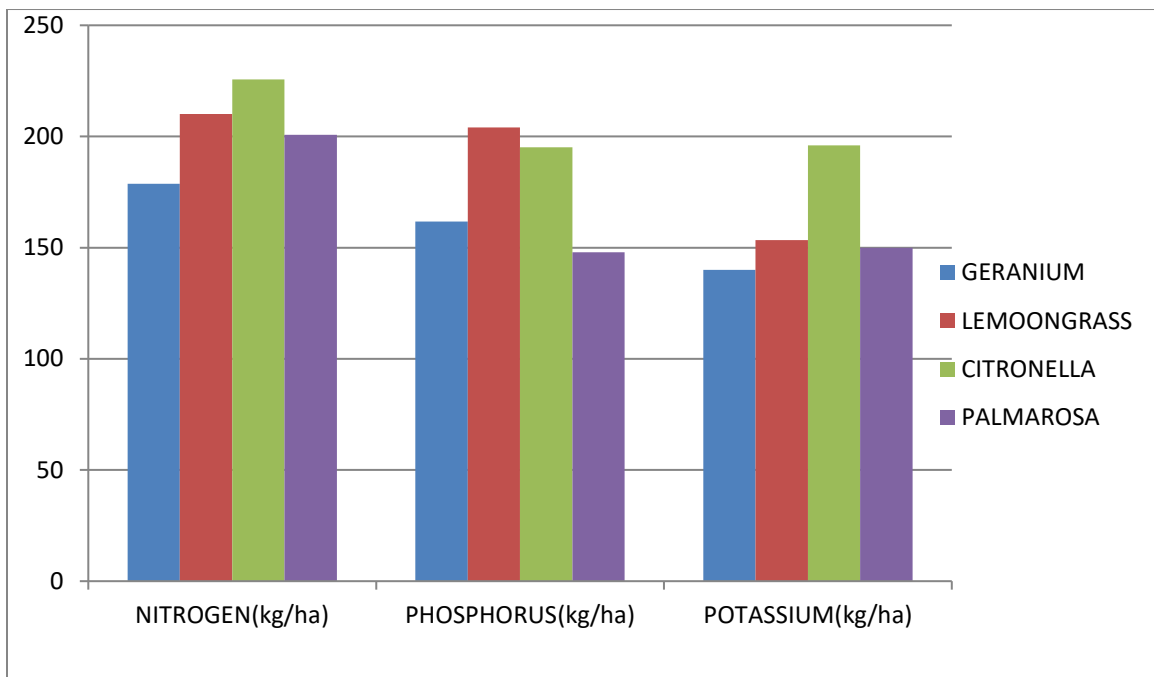


Figure 5.2: Essential major plants available nutrients (Nitrogen, Phosphorus, Potassium)

5.3- Plants available micronutrients

5.3.1- Iron (Fe)

The assessment of Fe of the rhizospheric soils of four aromatic plants was ranged from Low to high. Fe present in L, C and P soil was in higher concentration, as compared to G. (Figure 5.3).

5.3.2- Mangnase (Mn)

The assessment of Mn of the rhizospheric soil of four aromatic plants was ranged from Low to high. Mn present in G soil was in higher concentration, as compared to L, C, and P. (Figure 5.3).

5.3.3- Calcium (Ca)

The assessment of Ca of the rhizospheric soil of four aromatic plants was ranged from Low to high. Ca present in L, C and P soil was in higher concentration, as compared to G. (Figure 5.3).

5.4.1 - Copper (Cu)

The assessment of Cu of the rhizospheric soil of four aromatic plants was ranged from Low to high. Cu present in G soil was in higher concentration, followed by P, C and L. (Figure 5.4).

5.4.2- Zinc (Zn)

The assessment of Zn of the rhizospheric soil of four aromatic plants was ranged from Low to high. Zn present in C soil was in higher concentration ,in L slightly high concentration, P and G possesses a low level (Figure 5.4).

5.4.3- Boron (B)

The assessment of B of the rhizospheric soil of four aromatic plants was ranged from Low to high. B present in G soil was in higher concentration, as compared to L, C, and P (Figure 5.3)..

5.4.4- Cobalt (Co)

The assessment of Co of the rhizospheric soil of four aromatic plants was ranged from Low to high. Co present in G soil was in higher concentration, as compared to L, C, and P. (Figure 5.4).

5.4.5- Aluminium (Al)

The assessment of Al of the rhizospheric soil of four aromatic plants was ranged from Low to high. Al present in L soil was in higher concentration ,in P slightly high concentration and C and G possesses a low level (Figure 5.4).

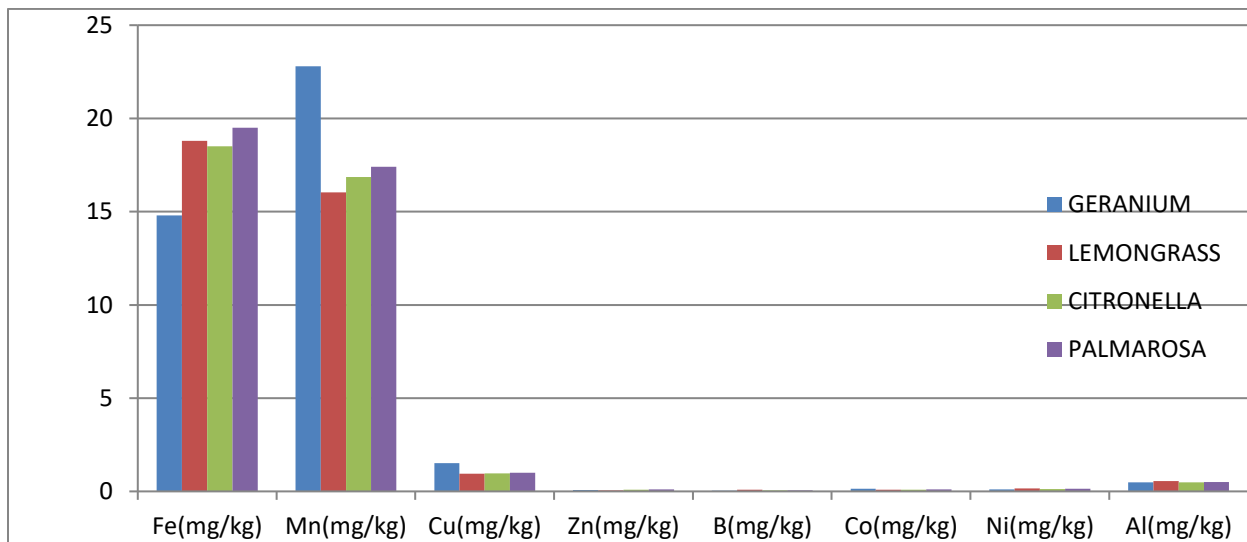


Figure- 5.3- Plants available micronutrients

5.5- Enzyme activity of acidic and alkaline phosphatase

5.5.1- Acidic phosphatase (ACP)

The assessment of ACP of the four different soil was ranged from Low to high. ACP present in G soil was in higher concentration ,in C slightly low concentration, L and P possesses a low level (Figure 5.5).

5.5.2- Alkaline phosphotase (ALP)

The assessment of ALP of the four different soil was ranged from Low to high. ALP present in C soil was in higher concentration ,as compared to L, G and P. (Figure 5.5).

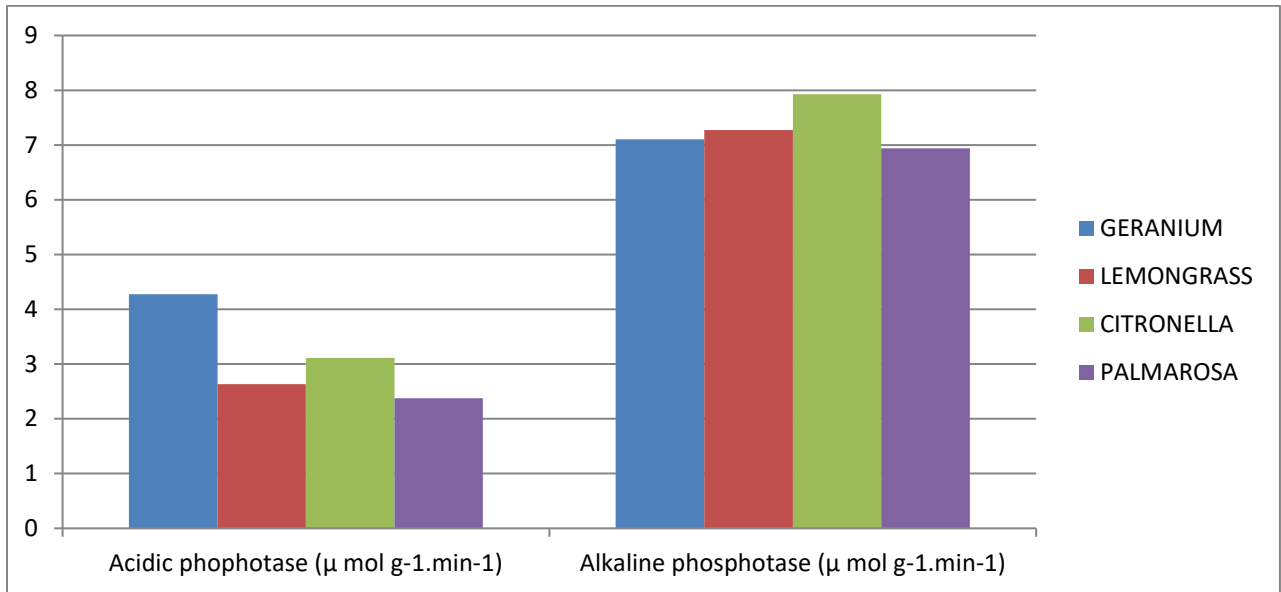


Figure- 5.4- Enzyme activity of acidic and alkaline phosphatase

5.6- Dehydrogenase enzyme (DHA)

The assessment of DHA of the four different rhizospheric soil of aromatic plants was ranged from Low to high. DHA present in C soil was in higher concentration as compared to G,L and P. (Figure 5.6)

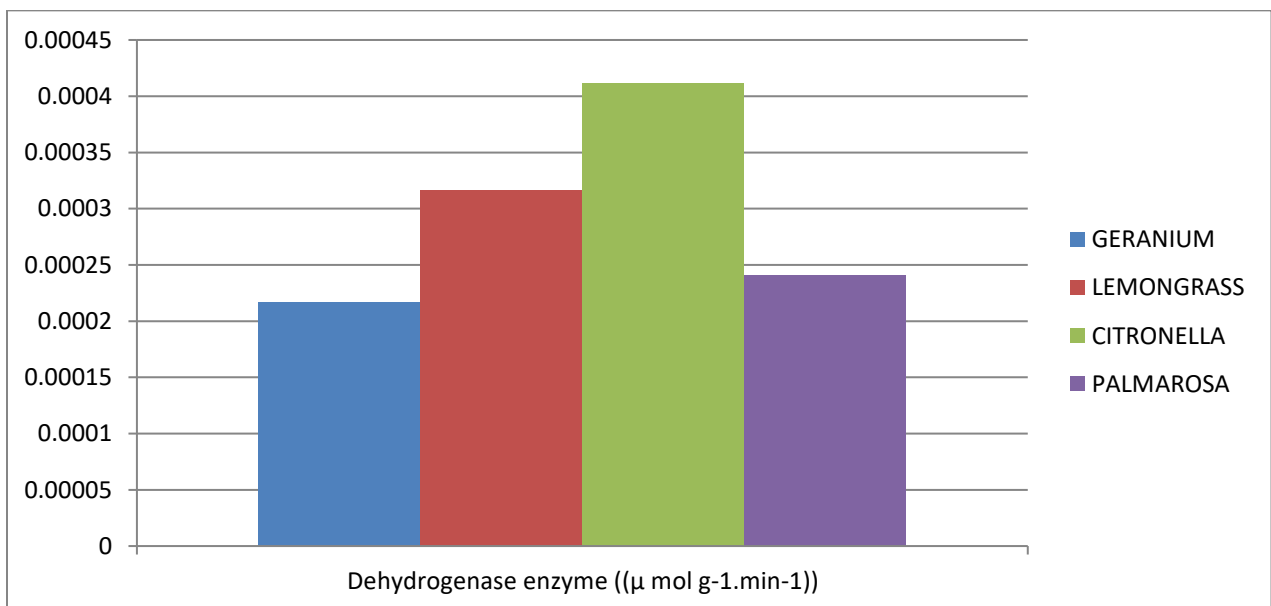


Figure 5.5- Enzyme activity of dehydrogenase enzyme

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 to the people who are interested to work in agricultural field.

This assessment was made to determine the properties of rhizospheric soil, available macronutrients and micronutrients. The current research entitled “ **Characterization of rhizospheric soil of selected aromatic plants**” is necessary for the management of improving the quality of selected aromatic plants by the analysis of rhizospheric soil.

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

- The soil samples were taken from four different crops- Geranium, Lemongrass, Citronell, Palmarosa for analysis in the research laboratory of the soil science and agronomy department CSIR-CIMAP, Lucknow, Uttar Pradesh.
- The results of the study revealed that the physico chemical properties like pH, EC, OC and available nutrient and enzyme status in rhizospheric soils as influenced by different aromatic plants.
- The physico chemical properties of rhizospheric soils like pH, EC and organic carbon content
- Plant available nitrogen content in all the rhizospheric soils was low.

- Iron and Manganese content was recorded in higher content in all the selected aromatic plants compared to other elements.
- Alkaline phosphatase enzyme was found to be higher compared to acid phosphatase and dehydrogenase in all soils of aromatic plants.

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APPENDIX

I. Chemicals and glassware sources-

Chemical and glassware used were produced from the following sources:

- Applied biosystems, USA
- Eppendorf 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. H₂SO₄ to about 990 ml of distilled water. From this 0.02 N H₂SO₄ 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% SnCl₂ 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 SnCl₂ solution (0.5 ml diluted to 66 ml) immediately before use.
- **Standard stock solution:** 0.439 g of AR grade KH₂PO₄ dried in oven 60⁰C for one hour in a 1 Litre beaker added about 500 ml of distilled water. Then added 25 ml of approx 7 N H₂SO₄ 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 NaHCO₃ :** 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 acetate:** 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 70⁰ 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.

Phosphatase:

- 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 (CaCl_2 ; 0.5 M), and standard p-nitrophenol solution as described in β -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.