ENZYME CHARACTERIZATION OF THE ACID SULPHATE SOILS OF KUTTANAD

by

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I, hereby declare that this thesis entitled **"ENZYME CHARACTERIZATION OF THE ACID SULPHATE SOILS OF KUTTANAD"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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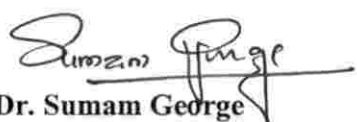Assistant Professor (Soil Science & Agrl. Chemistry)

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LIST OF ABBREVIATIONS

%	-	Per cent
β	-	Beta
μg	-	Microgram
Al	-	Aluminium
B	-	Boron
C	-	Carbon
C D	-	Critical Difference
Ca	-	Calcium
CFU	-	Colony Forming Unit
cm	-	Centimeter
CRD	-	Completely Randomized Design
Cu	-	Copper
dS	-	Deci Siemen
EAN	-	Enzyme Activity Number
EC	-	Electrical Conductivity
ECEC	-	Effective Cation Exchange Capacity
ENVIS	-	Environment and Related Issues Center
et al.	-	And others
Ex. Acidity	-	Exchangeable acidity
Ex. Al^{3+}	-	Exchangeable aluminium

Ex. Ca	-	Exchangeable calcium
Ex. H^+	-	Exchangeable hydrogen
Ex. K	-	Exchangeable potassium
Ex. Mg	-	Exchangeable magnesium
Ex. Na	-	Exchangeable sodium
Fe	-	Iron
Fig.	-	Figure
g	-	Gram
GIS	-	Geographical Information System
h	-	Hour
ha	-	Hectare
i.e.,	-	That is
ICAR	-	Indian Council of Agricultural Research
ISRIC	-	International Soil Reference and Information Centre
K	-	Potassium
KAU	-	Kerala Agricultural University
KF	-	Potassium Flouride
kg	-	Kilogram
MBC	-	Microbial Biomass Carbon
mg	-	Milligram

Mg	-	Magnesium
Mg m^{-3}	-	Megagram per meter cube
mM	-	Millimolar
mm	-	Millimeter
Mn	-	Manganese
MSL	-	Mean Sea Level
MSSRF	-	M. S. Swaminathan Research Foundation
MUB	-	Modified Universal Buffer
N	-	Nitrogen
Na	-	Sodium
NBSS & LUP	-	National Bureau of Soil Survey and land Use Planning
O.C	-	Organic carbon
O.M	-	Organic matter
P	-	Phosphorus
PGPR	-	Plant Growth Promoting Rhizobacteria
pnp	-	Para nitro phenyl phosphate
ppm	-	Parts per million
S	-	Sulphur
S. E	-	Standard Error
TPF	-	Triphenyl Formazon

TTC	-	Triphenyl tetrazolium chloride
viz.,	-	Namely
V_{max}	-	Maximum Velocity
Zn	-	Zinc

Introduction

1. INTRODUCTION

Kuttanad has long been known as the "Granary of Kerala" or the "Rice bowl of Kerala" is a unique tropical wetland ecosystem that spreads over the districts of Alappuzha, Kottayam and Pathanamthitta in Kerala state, India. The Kuttanad tract is a low-lying area extending over 874 sq. km in which, about 500 sq. km is lying 0.6 to 2.2 m below MSL with backwaters, canals, and stream networks. Geographically this tract extends from 9^0 17' N to 9^0 40' N latitude and 75^0 19' E to 76^0 33' E longitude (Chattopadhyay and Sidharthan, 1985). Saline water intrusion due to seasonal inflow of tides from the Arabian sea makes the area saline. During the monsoons, the rivers and rivulets cause intrusion of fresh water into the area. As the North East monsoon recedes, seawater again enters the Vembanad Lake and the whole area becomes saline. But this has been prevented by constructing a barrage at Thaneermukkom so as to protect the punja crop from salinity. The Kuttanad soils which forms the typical water logged soils, are completely different in their morphological, physical and chemical properties compared to normal well-drained soils (ENVIS Center, 2014).

The Kuttanad wetland system, inclusive of the Vembanad lake, is a major agriculture tract in Kerala comprising of 32 Panchayats of Alappuzha district, 27 Panchayats of Kottayam district and 5 Panchayats of Pathanamthitta. The predominant livelihoods in these areas are farming and allied sectors like fishing, animal husbandry etc. (MSSRF, 2007). It is believed that, in the geologic past the entire area of Kuttanad was a part of the shallow coastal area adjoining the Arabian Sea. The silt deposition at the river mouths contributed to the present coast, converting it into an extensive lake-lagoon-backwater system from a shallow bay. Sedimentary formations were occured by the silting up of lagoons and lakes gradually and due to continuous reclamation processes, they eventually converted

into garden lands and wet lands that presently contribute to the unique characteristics of Kuttanad (Pillai *et al.*, 1983).

The three major regions of Kuttanad are Upper Kuttanad, Lower Kuttanad and Kayal-lands (Vijayan and Ray, 2015). Based on the physical, chemical, biological and also by geological features, Kuttanad soils are classified into three groups namely Kari, Karappadam and Kayal soils. Large part of the upper Kuttanad region is characterized by Karappadam soils which extend over an area of 33,000 hectares. These soils, which are seen mostly towards the interior areas of Kuttanad, exhibit less salinity compared to the soils of Kari and Kayal land and are river-borne alluvial in nature.

Reclaimed wetlands from the Vembanad lake beds, which occupy an area of about 13,000 hectares in the districts of Alappuzha and Kottayam represent the Kayal land soils and are deep and dark brown in colour. These poorly drained soils exhibit low fertility status due to low content of inorganic matter and nutrient availability. Even though most of the nutrients are deficient, the kayal lands are fairly rich in calcium. The soils are predominantly silty loam to silty clay loam in texture with slightly acidic to neutral in soil reaction.

Kari soils are characterized by high acidity and salinity with comparatively higher content of organic matter that imparts the deep black colour of the particular soils. The upper and lower Kuttanad regions, comprising the acid sulphate soils have properties similar to the submerged and burned mangrove forest areas. The peculiarity of the acid sulphate soils is the natural formation of sulphuric acid. The oxidation of sulphur compounds present in the wood fossils which are seen at various stages of decomposition occur embedded in this soil leads to the production of free sulphuric acid (Money and Sukumaran, 1973). Beena (2005) delineated six soil series *viz.,* Ambalapuzha, Kallara, Purakkad, Thakazhi, Thottapalli, and

Thuravur that represents the acid sulphate soils. The total area comes about 14,227 ha. The main problem pertaining to this area is the presence of jarosite mineral.

A declining trend is observed in rice production in Kuttanad for the past few decades despite the use of high yielding varieties and modern farming techniques. This could be attributed to loss of soil health and fall in cropped area. Another major problem is the low inflow into Kuttanad during summer months (February-May) which leads to an increase in salinity, acidity and lack of water. Since the acid sulphate soils can exert severe effects on surrounding ecosystems, immediate steps should be taken to improve these soils further. Extreme acidity affects the various biological processes including the activity of agriculturally significant enzymes and other microbes.

Therefore, to increase the production in the Kuttanad tract, especially in the acid sulphate soils, there is a dire need to manage the acid sulphate condition. Generating research knowledge on the physical, chemical and biological characteristics of the soils will help to develop appropriate management strategies. Hitherto several studies have been carried out to characterize the soil physically and chemically, only limited number of research works have been undertaken to characterize the acid sulphate soils in terms of the biological characteristics that is very important for nutrient cycling and microbiologically mediated transformations. This study will help to add to the existing knowledge about the fate of the microflora and various enzymes in severe acidic conditions. Moreover, the characterization of enzymes in these soils would provide a database for the future researcher to investigate in this field of study. Hence the present project was envisaged to characterize the acid sulphate soils in terms of enzyme status with the following objectives

- To assess the chemical and biological parameters of the already identified acid sulphate soil series of Kuttanad.

- To evaluate the biological fertility of the acid sulphate soils of Kuttanad in terms of enzyme activity number.

- To prepare maps using Geographical Information System (GIS) based on various themes *viz.*, enzyme status and microbial population in the acid sulphate soils of Kuttanad.

Review of
Literature

2. REVIEW OF LITERATURE

To feed the escalating population in the country, there is a high need to increase the agricultural production either by means of area expansion under farming or by increasing the productivity. Area expansion is not at all a possible option in the present scenario. Here comes the importance of maximization of production from unit area and the proper exploitation each and every single cultivable land in India, including even the problem soils. In Kerala, the acid sulphate soils of Kuttanad is one of the unique and most problematic soils. Kuttanad has around 14,227 ha area of acid sulphate soils. A handful of research works were carried out on the aspects of physical and chemical characterization and management of acid sulphate soils to increase the production in those areas and in this chapter, an attempt was made to review some of the related works in this context under various subheadings.

2.1 KUTTANAD

Extreme acidity facilitating the solubility of chlorides and sulphates of iron are reported to be the chief source of toxicity in the Kuttanad (Money, 1961).

The replenishment of soils by silt brought by rivers in Kuttanad made the area highly suitable for rice production since early days, but the reclamation practices and prevention of sea water intrusion was a major constraint (Pillai and Paniker, 1965). Aravindakshan and Joseph (1990) opined that Kuttanad that extends over 53,600 ha, is the largest unique wetland ecosystem in the world contributing 18% of the area and 25% of total production of rice in Kerala.

Kuttanad soil contains organic matter in the range of 3 to 7 % and the soil is found to be acidic (Muralidharan et al., 1999). For the prevention of saline water intrusion into the rice fields, Thanneermukkom regulator was built. According to Thampatti and Jose (2000), during the past 23 years closure of the regulator during summer months had affected several important chemical characteristics of the acid sulphate soils of Kuttanad and it was found that most of the physical properties remained unaffected during this period.

Seasonal fluctuations of salinity together with intermingling of fluviatile and estuarine silts have further modified the chemical and biological characteristics of the soil (Padmanabhan et al., 2001). Punja lands which are the cultivated wetlands of Kuttanad lying below MSL is

categorized into Kari, Karappadam and Kayal lands based on variations in physico-chemical properties of the soils (Panicker and Sebastian, 2002).

Kuttanad region extends over Alappuzha, Kottayam and Pathanamthitta districts comprises of ten taluks. More than 75 per cent of the area has silt deposits brought by the rivers named Meenachil, Manimala, Pamba and Achencoil (Thomas, 2002). The quality of irrigation water as well as drinking water in the area is deteriorated due to the presence of pollutants from coir industries, pesticide residues and also by the sea water inflow. These include most of the threats for biodiversity of Kuttanad. Therefore, upgradation of irrigation systems, protection of channel bunds, mechanization in farming can increase yield and production of rice in Kuttanad (NBSS & LUP, 2004).

Kuttanad, comprising of about 53,639 ha is a unique wetland system formed by the deltaic formation of five rivers (Sudhikumar *et al.*, 2005). There are many geotechnical failures reported in Kuttanad region (Bindu and Vinod, 2008) and hence improving the properties of the soil have been a matter of intense research. The soils of the Kuttanad area are typical water-logged soils and fall under the acid sulfate group. However, the soil acidity, salinity intrusion and accumulation of toxic salts in the soils make this area less fertile for rice (Prabha *et al.*, 2013).

Sasidharan and Padmakumar (2012) reported that the rice fields in Kuttanad are underutilized and mostly single cropped. Therefore, there is considerable scope for improvement for farming system approach by growing 2000 fish fingerlings, 300 broiler ducks, 1-2 buffaloes, 20 coconut palms, 40 banana plants, 20-40 yams/cassava in one acre paddy land.

Kuttanad rice agricultural ecosystem is a unique tropical ecosystem as it is lying 0.6-2.2 m below mean sea level. The wetlands of Kuttanad are subjected to flooding during monsoon season and the inundated fields are drained out to the canals and backwaters after rain. Also the northern part of the system is influenced by brackish water during the summer season (Kannan *et al.*, 2014).

Generally the soils of Kuttanad are deep black with high clay content and thereby having high organic and mineral matter with limited variability among different regions of Kuttanad (Ray *et al.*, 2014).

2.2 ACID SULPHATE / KARI SOILS

Beye (1973), Kanapathy (1973) and Yin and Chin (1982) reported that in potential acid sulphate soils, the crop yield was low due to deleterious effects of oxidation of iron pyrites when there is a decrease in level of water table.

Patnaik and Mandal (1982) reported the occurrence of acid sulphate soils in Kari and Kayal lands of Kuttanad. Van Breeman (1982) reported that acidity in acid sulphate soils is directly or indirectly caused by sulphuric acid formed by the oxidation of pyrite (FeS_2). Based on principal component analysis, Marykutty (1986), classified the soil properties of Kuttanad kari soils into various clusters. Kari soils of Kuttanad have been grouped in first and second clusters having an aluminium saturation of effective CEC above 75 per cent. A few acidic Kari soils of Kuttanad have been grouped under third cluster together with Kole soils having an aluminium saturation of effective CEC 70.1 to 75 per cent.

Sen (1988) reported that, regardless of the water table, acidity and pH at 0-30 cm depth, potential acid sulphate soil columns did not show much variation under lower water tables through enhanced oxidation of pyritic materials. Subsoil layers of actual acid sulphate soils were characterized by the presence of sulfidic depositions or yellow mottles of jarosite within a depth of 30 cm (Van Mensvoort, 1988). Approximately 0.2 million hectares of acid sulphate soils were found in the coastal area of Kerala in west belts (Iyer, 1989).

Konsten *et al.* (1990) in Kalimantan, detected no signs of further acidification when the watertable dropped and exposed the sulphuric horizon of acid sulphate soils to the air during the dry season. Sterk (1991) calculated that a rainfall intensity of 36.3 mm in 30 minutes could leach 143.6 mmol $^{(+)}$ m^{-1} of acidity from the topsoil of a new (three months) raised bed of acid sulphate soil. Mapping of acid sulphate soils in Finland has been conducted by conventional soil sampling (Palko, 1994).

The profile and surface samples of Kari soil of Thakazhi and Pokkali soil of Njarakkal recorded the highest active acidity with a soil pH around 3.2 (Usha,1995). Morphological and physicochemical properties of the soils showed great degree of variation. Soils were dark brown to black in colour, sticky and plastic, subangular blocky in structure and sandy to clayey in texture, with random deposits of lime shells and humus. Presence of faint to prominent reddish

yellow or brown mottles and root canals were some of the special characteristics observed in the soil profiles of acid sulphate soils of Kuttanad (Thampatti, 1997).

Variations in depth of groundwater table is a controlling factor of pH and redox potential in acid sulfate soils. Strongly acidic conditions by pyrite oxidation due to deepening of the water table deteriorates agricultural fields in the dry season. On the other hand, toxicity due to reduced Fe under reductive conditions is caused by the high groundwater table in the wet season. Usually, both Al and Fe toxicity do not appear simultaneously. Constraints of rice growth due to excessive Fe and Al can be avoided by choosing an optimal time (moderately reduced condition) for sowing rice in actual acid sulfate soils (Husson *et al.*, 2000).

Land reclamation of potential acid sulfate soils accompanied by water table depletion affects both agricultural and aquacultural productivity because of the deterioration of groundwater quality and soil acidity (Shamshuddin *et al.*, 2004, Gosavi *et al.*, 2004).

A total of 24 million ha of acid sulfate soils exits worldwide (Sullivan, 2004). One of the major impact of acidification is solubilization of the soil matrices that releases toxic products to the soil (Nordmyr *et al.*, 2006). Whenever there is an occurance of sudden and profound drop in water table in the acid sulphate soils, that contribute to the production of sulphuric acid and thereby extreme acidity and release of toxic metals into the adjoining areas (Roos and Astrom, 2006).

The acid sulphate soils covered an area of 14277.51 ha, comprisied of six soil series *viz.*, Ambalpuzha, Purakkad, Thottapally, Thuravur, Kallara and Thakazhi out of the total 54,000 ha of wetlands in Kuttanad. Soils of the region are extremely acidic, deep, poorly drained and waterlogged for most part of the year. Among the different soil series, the largest area is occupied by the Kallara series (Beena *et al.*, 2007).

According to Janjirawuttikul *et al.* (2010) acid sulfate soils are divided into five groups *viz.*, potential acid sulphate soils, active acid sulphate soils, post-active acid sulphate soils, non-acid sulphate soils and transitional soils.

Soils that are strongly acidified by oxidation of pyrite is actual acid sulphate soil, which substantially reduces biological activity and plant productivity. Acid sulfate soil usually emerges

following land degradation after inadequate land reclamation or land use management (Kawahigashi *et al.*, 2012). Sadiq and Babagana (2012) opined that food production in the tropical areas can be increased by bringing the acid sulphate soils, which are riverborne alluvium in origin for cultivation. Eventhough the acid sulpahte soils are fertile in origin, the extreme acidity causes the escape of fixed heavy metals and also anaerobic conditions prevailing in these areas are not at all beneficial for surroundings (Michael, 2013).

2.2.1 Physical Properties

The textural analysis of the soils indicated that the predominant soil texture of acid sulphate soils were clay to silty clay loam. Soil water content at field condition is low in the surface horizons and high in the subsurface horizon (Rahman *et al.*, 1993).

There were contradictions about the moisture content at various depths in the acid sulphate soils, *i. e.*, with depth the moisture content remains conastant (Sait *et al.*, 2002) or the moisture content could increase or decrease with soil depth (Schulze *et al.*, 1996). Kuttanad clay have a natural moisture content of 90 per cent and an optimum moisture content of 33 per cent with specific gravity 2.02 Mg m^{-3} and maximum dry density 1.36 Mg m^{-3} (Bindhu and Ramabhadran, 2011).

The long term incubation study of acid sulphate soil series done by Ara *et al.* (2013) reported that these soils had a bulk density of 1.03 Mg m^{-3} and moisture content under field condition was 49 per cent and the textural class is silty clay loam. Kannan *et al.* (2014), reported that the soils of Kayal lands, Lower Kuttanad, Purakkad Kari and Upper Kuttanad are loamy sand and sandy clay loam.

2.2.2 Chemical Properties

2.2.2.1 *pH, EC and Organic Matter Content.*

According to Datta and Srivastava (1963), organic matter content of the flooded soils help in the reduction of ferric form of iron and thereby improving the bacterial dynamics in the wetlands.

Nhung and Ponnamperuma (1966) opined that oxidation of iron pyrites lead to the production of extreme acidity which decrease the crop growth. The same deleterious effect of low pH was also recorded by Brinkman in 1982.

Negative charges provided by organic matter content of the soils facilitate the adsorption of cations from the soil solution (Ponamperuma, 1972). Hesse (1982) reported that high content of organic matter in soils helps in minimizing the release of toxic elements into the soil environment.

Potential acid sulphate soils can be identified by incubating moist samples. Here the pH drops rapidly and may continue to drop for at least one year if the sample is kept moist (Dent, 1986). Even with continuous field submergence for long period, the pH cannot be raised above 4.5 due to varying nutrient dynamics in the acid sulfate soils and also the soils showed steady and steep decline in EC and organic matter content (Kuruvila and Patnaik, 1994). Storage of soil carbon by the deposition of organic matter is an important characteristic of a wetland ecosystem (Schlensinger, 1997). The pH of the area decreased to more than 0.5 units on incubation, indicating the presence of sulphidic materials and thus confirms the acid sulphate condition (Beena, 2005).

Acid sulphate Kuttanad soils have pH 3-4.5 and an EC of 6 dS m^{-1} during summer and this high EC does not cause any exhaustive effects on surface layers because of continuous surface drainage practices followed by farmers in the area (Mathew $et\ al.$, 2001). Application of organic matter is an important reclamation practice for improving the acid sulphate soils (Kaderi, 2004).

Mahvi $et\ al.$ (2005) and Sitio $et\ al.$ (2007) reported the toxic metal removal capacity of rice husk and hence improving the rice production. High organic matter content also influences the characteristics of acid soils by raising the pH and cation exchange capacity (El Sharkawi $et\ al.$, 2006). Use of various amendments like fly ash, paper factory sludge and rice husk ash for improving the physical and chemical properties of acid soils was studied by Karkamar $et\ al.$ (2009).

According to Sylas *et al.* (2010), the pH of the Kuttanad ranged from 2.4 to 4.8 with a high conductivity of 6.31 dS m^{-1}. He also observed that the organic carbon is enriched during pre-monsoon season and attributed this to the luxuriant growth and decaying of the macrophytes.

2.2.2.2 *Nutrient Status*

The available N and P content in the soils reported to be deficient for plant growth. As the soils are highly acidic, the available P is obviously low as most of the P get fixed. Slightly higher content of available K in the upper horizons may be due to bio-cycling of K (Black, 1968). The availability of phosphorus was high while the land get submerged (Patrick and Mahapatra, 1968) and low pH at extreme level leads to deficiencies and toxicities of some other elements simultaneously (Rorison 1973).

The submerged wet-land rice soils of Kerala showing a pH value of less than 6 were found to contain appreciable amounts of exchangeable aluminium (Abraham, 1984). Keeping the soils under submergence help to maintain the Fe and Mn in soluble form. Again by the prevelance of low pH in these soils, the water soluble and extractable Al content were also high (Raju, 1988; KAU, 1994). Phosphorus availability is usually restricted in acid sulfate soils as added P is strongly absorbed (Belmehdi and Nyiri, 1990).

Because of its marine origin Mg^{2+} content in the acid sulphate soils were relatively higher than Ca^{2+} (Ahmed and Wilson, 1992). The high aluminium content in soils inhibited the enzyme activities mainly phosphatase (Minggang, 1997).

Ramesh and Chhonkar (2001b) reported that the Kuttanad acid sulphate soils had an available P content of 7.13 mg kg^{-1} and available K content of 105 mg kg^{-1}. In a field study to evaluate the nutrient status of Kerala, Usha and Varghese (2002) reported a high status of available N, P and K. Acid and acid sulphate soils were characterized by low P status while the contents of aluminium, iron and manganese were toxic (Onthong *et al.*, 2007).

Aluminum toxicity decreases the availability of P by producing iron and aluminium complexes and at very low pH the toxicity of Al leads to the inhibition of rice growth (Ward *et al.*, 2008).

Disintegration of silicate minerals in acid sulphate soils released the metals like Al and Fe under the existing soil and environmental conditions (Shazana *et al.*, 2011).

The available nitrogen content was high in Kayal and Kari lands and available phosphorus and potassium contents were high in Lower Kuttanad (Bindu, 2012). Organic carbon content of soil samples of the acid sulphate soils were high. Due to high P fixation, these soils were deficient in available P. The potassium content of acid sulphate soils were high and ranged from 142.1 mg kg^{-1} to 326.4 mg kg^{-1}. Ca, Mg, Na and S content were also high. Fe, Mn and Al toxicity were prevalent in the region (Beena and Thampatti, 2013). Zin *et al.* (2015) reported that the rice production in acid sulphate soils was limited by low pH, excess Al, Fe and Mn and also deficiency of P.

2.2.2.3 Acidity Characteristics

Marsh and Grove (1992) reported that one of the important limitations of acid sulphate soils was subsoil acidity that inhibited the crop yield. Approximately 75 per cent of the global acid soils exhibiting subsoil acidity (Eswaran *et al.*, 1997).

Potential acidity and hydrolytic acidity recorded higher values during rainy season while exchangeable acidity was greater during summer. Among the components of exchangeable acidity exchangeable Al^{3+} dominated during rainy season, and exchangeable H^+ during summer (Thampatti, 1997).

Soil samples from acid sulphate region of Kuttanad recorded a positive significant correlation between pH and exchangeable calcium content of 325 mg kg^{-1} along with an exchangeable aluminium concentration of 293 mg kg^{-1} (Ramesh and Chhonkar, 2001a).

The potential acidity of most soils ranged from 13.32 to 112.1 cmol kg^{-1}. The subsoil showed higher potential acidity compared to surface soils. In the surface horizon, potential acidity varied from 32.87 to 110.5 cmol kg^{-1}. The subsurface samples were highly acidic. The exchangeable acidity varied from 1.78 to 9.83 cmol kg^{-1}. The exchangeable Al^{3+} content varied from 0.67 cmol kg^{-1} to 6.64 c mol kg^{-1} (Beena and Thampatti, 2013).

2.2.3 Biological Properties

2.2.3.1 Enzyme Status of the Soil.

Soil organic matter content affects the enzyme activity as reported by Dalal, (1975). According to Jha *et al* . (1992), the activity of major enzymes could be correlated with microbial population in soils. Enzyme activity of soil was contributed by the accumulated enzymes and also by the enzymes produced from proliferating microorganisms (Kiss *et al.,* 1975).

Various enzyme activities *viz.,* urease, amidase, phosphatase, arylsulphatase and glucosidase activities were inhibited by waterlogging as reported by Pulford and Tabatabai (1988). Dick (1992) and Visser and Parkinson (1992) were reported that the changes in enzyme activities is considered as an important index of changes in soil quality. Similar reports of inhibitory effect of waterlogging on enzyme status of soils were also reported by Freeman *et al.* (1996).

An increase in soil pH was positively related to most of the enzyme status in soils except acid phosphatase enzyme as it was sensitive to high pH (Martinez and Tabatabai, 2000). Activities of different enzymes helped to specify a particular group of microflora in soils (Hofrichter, 2002; Baldrian, 2008). Cycling of major nutrients also related with the enzyme activities (Dinesh *et al.,* 2004).

Reports by Wittmann *et al.* (2004) and Nannipieri *et al.* (2012) pointed that the enzyme activities of a soil was crucial for its fertility and Renella *et al.* (2006) opined that each enzyme possess different range of pH for its activities.

2.2.3.1.1 Urease

Urease activity in wetland rice fields was mainly affected by pH and not by moisture content (De Laune and Patrick, 1970) while Singh *et al.* (1991) studied some more factors by correlating the relationship between enzyme status of the soil with its pH, organic carbon content, CEC and texture.

Klose and Tabatabai (1999) studied the relationship between urease and microbial biomass C and N and revealed highly significant relationship between urease activity and

microbial biomass C and N. It was noted that urease activity of the microbial biomass, expressed as per cent of total urease activity ranged from 37.1 to 73.1 % and the remaining 26.9 to 62.9% was extracellular. Urease activity was influenced mostly by soil factors, management practices, and other environmental factors there by it used to be known as biological indicator (Yang *et al.*, 2006).

Corstanje *et al.* (2007) suggested that activity of urease was influenced by both physical and chemical properties of soil while its stability could be controlled by several factors including complexes formed by organic and mineral compounds and humic substances (Makoi and Ndakidemi, 2008). Based on available soil nitrogen content, incorporation of various carbon sources positively influenced the urease activity (Rajashekhararao and Siddaramappa, 2008).

2.2.3.1.2 Phosphatase

Soil pH was the most important factor related with the synthesis, stability and release of phosphatases (Juma and Tabatabai, 1977).

According to Tarafdar and Chhonkar (1979), mostly soil bacteria and fungi were involved in the production of phosphatases in the soils. But the contribution by actinomycetes was negligible.

Killham *et al.* (1983) reported that acid rain and the subsequent drop in soil pH increased the activity of acid phosphatase. Organic P became available to plants by the action of acid phosphatase, mainly the phosphatase of microbial origin (Sarapatka, 2003).

There was a strong relationship between pH, clay content and soil organic P status with phosphatase activity as observed by Turner and Haygarth (2005) in temperate grassland.

2.2.3.1.3 Aryl sulphatase

Hydrolysis of the O–S bond was the important function of aryl sulphatases (Spencer, 1958) and this enzyme also mineralizes the sulphate esters in soil (Tabatabai, 1994).

Study by Deng and Tabatabai (1997), explored the strong bond between the organic carbon status and activity of aryl sulphatases in soil and Knights *et al.* (2001) suggested that one among the key factors that affect the aryl sulphatase enzyme was carbon availability in soils.

Other factors related to status of aryl sulphatase in soil were the population of microbes (Klose and Tabatabai, 1999) and sulphur immobilization (Vong *et al.*, 2003). Turner in 2010, reported that a pH of 3 was found to be optimum for the arylsulphatase activity.

2.2.3.1.4 *β-D-glucosidase*

According to Esen (1993) glucosidase is a rate limiting enzyme in microbial degradation of cellulose. Most of the enzymes including glucosidase, urease and phosphatases were sensitive to field management practices that induced significant alterations in soil quality (Yakovchenko *et al.*, 1996; Adam and Duncan, 2001).

As mentioned in the case of most enzymes, the watelogging practices or situations caused decrease in β –D-glucosidase activity (Wang and Lu , 2006). The β - glucosidase activity ranged in the soils from 1.04 to 63.4 mg kg^{-1} soil h^{-1} (Acosta-Martinez *et al.*, 2007).

Various enzymes have different pH range for optimum activity and in the case of glucosidase it preferred acidic pH while the pH optima showed a wide variation among various soils (Turner, 2010).

2.2.3.1.5 *Dehydrogenase*

Because of its anaerobic origin, there was an increase in the activity of dehydrogenase enzyme under flooded conditions (Chendrayan *et al.*, 1980; Orten and Neuhaus, 1970). Dehydrogenase activities in soil are biological indicators of overall microbial respiratory activity of soils (Bolton *et al.*, 1985). Ray (1985) reported the negative impact of drainage conditions on dehydrogenase activity.

Flooded soils under rice recorded comparatively higher activity of dehydrogenase enzyme than the non flooded lettuce grown soils in the acid sulphate soils of Kuttanad and the dehydrogenases exhibit a pH optimum at 7 (Ramesh and Chhonkar, 2000).

Direct relationship between dehydrogenase status and evolution of CO_2 and N_2O from the wetland flooded soils were reported by Wlodarczyk *et al.* (2002). From the experiment conducted by Simek *et al.* (2011) it was observed that acid sulphate soils have highest dehydrogenase activity in the range of $14.3 \pm 0.1 \mu g$ TPF g^{-1} h^{-1}.

Hinjosa (2008) recorded dehydrogenase activity in non polluted, reclaimed and pyrite sludge polluted soils and the values were $71.4 \pm 5.2\mu g$ TPF g^{-1} 24 h^{-1}, $53.0 \pm 0.1\mu g$ TPF g^{-1} 24 h^{-1} and $2.9 \pm 0.1\mu g$ TPF g^{-1} 24 h^{-1} respectively.

Activity of dehydrogenases in soil system assures the exact cycling of nutrients and all other bio-geochemical activities were going on smoothly. It was used as an index for deterioration of soil by pesticides and heavy metals or by management practices followed in soils and also known as the direct indicator of microbial activity in soil (Kumar *et al.*, 2013).

2.2.3.2 Enzyme Kinetics and Enzyme Activity Number

The estimation of enzyme activity in soils only provides an indication of its quantity present in the soil, while the kinetic parameters of enzymes provide most important details regarding the origin, status and catalytic properties (Perez-Mateos and Gonzales- Carcedo, 1985). In terms of Michaelis-Menten enzyme kinetics, potential enzyme activity is an empirical measurement of V_{max} and K_m (Michaelis and Menten, 1913). V_{max}/K_m value of soil enzymes was affected by soil physicochemical properties, available substrate, organic matter and microbial activity (Garcia *et al.*, 1993)

Farrell *et al.* (1994) and Tabatabai *et al.* (2002) opined that the protective influence of various soil components could be assumed by determining the kinetic parameters of soil enzymes and it could also be used for differentiating enzyme sources. One of the parameter, K_m, a constant used to measure affinity of an enzyme for its specific substrate (Palmer, 1995).

According to Nanniperi *et al.* (1996), the reduction in enzyme activity in enzyme-clay complexes as compared to its free form could be indicated by its kinetic parameters. Kinetic parameters (V_{max}, K_m) of a specific enzyme under fixed conditions were constant, but may vary independently (Marx *et al.*, 2005).

In soil, the constant K_m varied from 0.62 to 1.00 mM (Marinari *et al.*, 2008). Kujur and Patel (2014) reported that the kinetic parameters could be used as markers for measuring the changes occurred in microbial dynamics of soil.

2.2.3.3 Microbial Biomass

The living portion of soil organic matter was constituted by microbial biomass which represented the pool of carbon, available nitrogen, phosphorus and sulphur in soils. Microbial dynamics in a soil could be realted to its microbial biomass activity (Jenkinson and Ladd, 1981).

Diaz *et al.* (1993) reported that immobilization of nutrients as a result of increase in microbial biomass. Alternate flooding and drainage imparted significant effect on microbial biomass and thereby influenced the nutrient cycles in soils (Bossio and Scow, 1995; Noll *et al.*, 2005).

Approximately 80–90 per cent soil processes were mediated by microbes (Nannipieri and Badalucco, 2003). The availability of most of the nutrients especially P was strongly related to the microbe mediated processes (Wakelin *et al.*, 2004; Vassilev *et al.*, 2006).

Increase in rice production and maintenance of soil fertility in wetlands were the two important contributions by microbial community by mineralization and decomposition processes (Kikuchi *et al.*, 2007). In a rice field, all the important basic processes were mediated by microbes (Jianping *et al.*, 2008).

Mineralization and immobilization along with oxidation and reduction reactions resulted in the transformations of sulphur in soil (Vidyalakshmi *et al.*, 2009). Growth of rice was directly and indirectly affected by microorganisms, *i. e.*, directly by symbiosis and indirectly by facilitating nutrient cycling in flooded soils (Kogel-Knabner *et al.*, 2010).

2.2.3.3.1 Bacteria

The optimum pH for *Thiobacillus thiooxidans* in culture is about 2.5, with a range from 0.9 to 4.5 (Rao and Berger, 1971).

The major sulphur oxidizers were heterotrophic organisms and hence the changes in heterotrophic microbial activity also caused changes in sulfur oxidation potential of sulphate enriched soils (Lawrence and Germida, 1988). Chapman (1990) and Lawrence and Germida (1991) reported that there were only scarce population of *Thiobacilli* in most agricultural soils. But during 1991 itself, Grayston and Germida under their study isolated around 273 bacterial

species and among them 133 were sulphur oxidizers. The major sulphur oxiidzers in soil were *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*.

Till now only isolation of these major sulphur oxidizers were carried out extensively (Wood and Kelly, 1991; Johnson *et al.*, 1992), while importance should be given for the activity studies too. Among the known species of sulphur oxidising bacteria, most of them comes under *Thiobacillus, Thiothrix, Thiomicrospira, Achromatium* and *Desulfuromonas* genera (Das *et al.*, 1996). Drop in pH of the media by the sulphur oxidizers was reported by Donati *et al.* (1996).

Alam *et al.* (2002) stated that bacteria were the most predominant organisms in phosphorus solubilisation as compared to fungi. Regarding the total microbial load, phosphorus-solubilizing bacteria comprised 1 to 50 per cent whereas fungi were 0.1 to 0.5 per cent only (Chen *et al.*, 2006). PGPR/PGPB associated abundantly with the rhizosphere soil of rice and produced more quantity of organic acids thereby increasing availability of P and also alleviating the Al toxicity (Nakkeeran *et al.*, 2005). Soil pH was the most important factor that contributed the bacterial activity in soil (Fierer and Jackson, 2006; Hartman *et al.*, 2008; Jenkins *et al.*, 2009; Lauber *et al.*, 2009).

Thiobacillus, an important chemo - lithotrophic bacteria mediated the sulphur transformations primarily (Vidyalakshmi and Sridar, 2007). In the rice – fish rotational farming system, there was an increase in microbial activity because of the the bacterial flora released from the fish excreta and body parts (Smily *et al.*, 2012).

2.2.3.3.2 Fungi

Bewley and Parkinson (1984 and1985) reported a low fungal population in sulphur polluted soils. Fungal species could grow in a wide pH range from 5 – 9 without inhibition of their growth (Wheeler *et al.*, 1991; Nevarez *et al.*, 2009).

Beales (2004), opined that the fungus had less connection with pH compared to bacteria. Total bacterial and actinomycetes population in the acidic soils were found to be higher than fungal populations (Panhwar *et al.*, 2014).

2.2.3.3.3 Actinomycetes

The predominant roles of actinomycetes in soil nutrient cycling and in increasing agricultural productivity was well known (Elliot and Lynch, 1995). Actinomycetes population was reported to possess similarities with fungi than bacteria with regard to the habitat as seen in the case of an increase in actinomycetes count after conversion of land use from forest to agriculture (Burck et al., 1989; Fierer et al., 2009).

Greiner et al. (2007) reported actinomycetes population were 10^5 cfu g^{-1} soil in most studied soils, thus inferred that the actinomycetes population had no influence by the salt content. As the actinomycetes prefer dry soils than the wet soils, water logging or flooding the lands provided an unfavourable condition for their growth (Zenova et al, 2007).

P solubilization capacity of actinomycetes was reported by Ghorbani-Nasrabadi et al. (2012) and Ghorbani-Nasrabadi et al. (2013) opined that the actinomycetes population were affected by soil salinity when comparing two landuses, i. e., the irrigated and rainfed areas.

2.2.3.4 Soil Respiratory Activity and Microbial Biomass Carbon

The rate of CO_2 evolution is an indicator of soil microbial respiration as suggested by Xu and Zheng (1986). Accumulation of CO_2 is also an index of activity of microbial load predominantly soil respiration (Stotzky et al., 1993).

Some of the indices of soil quality included microbial activity, soil respiratory activity, and soil enzymatic activities (Wardle and Ghani, 1995; Ross et al., 2003; Bastida et al., 2008). According to Anderson and Domsch (1989), microbial biomass carbon comprises 1-4 per cent of organic matter in soils and it contributed the most active component of soil organic carbon which facilitates nutrient cycling in all ecosystems (Paul and Clark, 1996).

For estimating various biological processes in different ecosystems basal respiration is crucial (Islan and Weil, 2000). A general direct relation was seen between soil respiration and microbial biomass carbon as indiacted by a decrease in soil respiration upon reduction in microbial biomass (Klose and Ajwa, 2004) or increase in respiration indicated the improved growth of bacterial population (Haney et al., 2000). Chen et al. (2004) reported the relationship between soil respiration and soil physical attributes like soil temperature and soil moisture.

Ghosh (2003) reported that agricultural surface soils had MBC in a range of $24 - 252$ mg kg^{-1} whereas subsurface soil samples recorded a range of $20 - 150$ mg kg^{-1}.

There was a positive significant correlation exist between microbial biomass carbon status in soil with organic carbon content, total culturable microbial load, N content and moisture availability (Velmourougane *et al.*, 2014).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Enzyme characterization of the acid sulphate soils of Kuttanad" was carried out by collecting soil samples from six soil series *viz.,* Ambalapuzha, Kallara, Purakkad, Thakazhi, Thottapalli and Thuravur representing the acid sulphate soils. Analysis were carried out in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during March 2014 to June 2016. The study was envisaged with four phases.

1. First Phase   -   Collection of soil samples
2. Second Phase   -   Incubation studies
   a. Confirmation of acid sulphate condition
   b. Enzyme kinetics.
3. Third Phase   -   Generation of maps using GIS
4. Fourth Phase   -   Computation of biological fertility index.

The present study was envisaged to characterize the acid sulphate wet land soils of Kuttanad on the basis of chemical and biological parameters and to assess the biological fertility status of the soil.

3.1   LOCATION DETAILS

The study area, covering the acid sulphate soils of Kuttanad is spread over three taluks *viz.,* Cherthala, Vaikom and Ambalapuzha. Beena (2005) delineated the acid sulpahte soils comprising an area of 14,227 ha of Kuttanad into six series *viz.,* Ambalapuzha, Kallara, Purakkad, Thakazhi, Thottapalli and Thuravur.

The soil series under acid sulphate soils in Kuttanad from where the geospatial soil samples from surface and subsurface layer representing 0-15 cm and 15-30 cm were collected are presented in Table 1.

Fig. 1. Details of soil sampled locations (GIS map).

Table 1. Location details

Sl. No.	Locations	GPS Coordinates	
		Latitude	Longitude
1	Ambalapuzha	9°21'30"N to 9°23'0"N	76°22'0"E to 76°23'0"E
2	Kallara	9°41'0"N to 9°42'30"N	76°27'0"E to 76°28'30"E
3	Purakkad	9°21'30"N to 9°22'0"N	76°22'0"E to 76°23'0"E
4	Thakazhi	9°21'30"N to 9°22'30"N	76°23'0"E to 76°24'30"E
5	Thottapalli	9°19'30"N to 9°21'0"N	76°22'0"E to 76°24'0"E
6	Thuravur	9°45'0"N to 9°46'30"N	76°17'0"E to 76°18'30"E

The two other major soil types of Kuttanad *i.e.*, Karappadam and Kayal lands were also included in the study for the purpose of data generation and general comparison. Karappadam and Kayal soils were collected from Nedumudi (Alappuzha series) and Kainakari (Champakulam series) respectively.

3.2   EXPERIMENT DETAILS

Design   :   Completely Randomized Design (CRD)

No. of locations   :   6

No. of samples / location   :   20

Total number of samples   :   120

Table 2. Treatment details

Treatments	Details
T_1	Ambalapuzha
T_2	Kallara
T_3	Purakkad
T_4	Thakazhi
T_5	Thottapalli
T_6	Thuravur

3.3 EXPERIMENTAL METHODS

3.3.1 Collection and Preparation of Soil Samples

The soil samples from both surface (0- 15 cm) and subsurface (15- 30 cm) were collected by stratified random sampling technique and was geocoded. Ten samples each were collected from both levels. The samples were collected in polythene bags and a part of the sample stored in deep freezers to ensure the viability of microorganisms. The rest of the samples were dried in shade, powdered with wooden mallet, sieved using 2 mm sieve and stored in polythene bags for carrying out physico-chemical analysis.

3.3.2 Characterization of Soils

3.3.2.1 *Physical and Chemical Characterization*

Table 3. Standard analytical methods followed in the soil analysis

Sl. No.	Soil Properties	Method	Reference
	Physical Properties		
1	Bulk density	Core method	Gupta and Dakshinamurthy (1980)
2	Particle density	Pycnometer method	Black *et al.*, 1965
3	Water holding capacity	Core method	Gupta and Dakshinamurthy (1980)
	Chemical Properties		
4	pH	pH meter (soil and water taken in a ratio of 1:2.5 w/v)	Jackson, 1973
5	EC	Conductivity meter (soil and water taken in a ratio of 1:2.5 w/v)	Jackson, 1973
6	O. C	Walkley and Black wet digestion.	Walkley and Black, 1934
7	Available N	Alkaline potassium permanganate method	Subbiah and Asija, 1956

8	Available P	Bray No.1 extraction and estimation using spectrophotometer.	Bray and Kurtz, 1945
9	Available K and Na	Neutral Normal NH_4OC extraction and estimation using flamephotometer.	Jackson, 1973
10	Available Ca and Mg	Versanate titration method	Jackson, 1973
11	Available S	$CaCl_2$ extraction and estimation using spectrophotometer.	Massoumi and Cornfield, 1963
12	0.5 N HCl extractable Fe, Mn, Zn and Cu	Atomic Absorption Spectrophotometer.	Sims and Jhonson, 1991
13	Hotwater extractable B.	Spectrophotometer	Jackson, 1973

3.3.2.1.1 Exchangeable Acidity (cmol kg^{-1} soil)

Exchangeable acidity was determined using extraction with 1 M KCl from dried soil samples as described by Page (1982).

Ten grams of dried finely ground soil was transferred into a dry filter paper placed in a funnel. Leach this soil with ten equal portions of 10 ml 1M KCl solution into a 100 ml volumetric flask at sufficient time intervals. After percolation, made

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upto 100 ml and pipetted out 25 ml aliquot into a conical flask. Added 3-5 drops of phenolphthalein indicator and titrated against 0.02 N NaOH.

3.3.2.1.2 Exchangeable Aluminium (cmol kg^{-1} soil)

Exchangeable aluminium was determined by titration method using dried soil sample with 1 N Potassium flouride. After titration for exchangeable acidity, 10 ml 1N KF was added and titrated with 0.02 N HCl until pink colour disappeared. After 30 minutes, additional HCl was added for a clear end point (ISRIC, 1992).

3.3.2.1.3 Exchangeable Hydrogen (cmol kg^{-1} soil)

Exchangeable hydrogen was calculated by deducting exchangeable aluminium from exchangeable acidity (ISRIC, 1992).

3.3.2.1.4 Exchangeable K, Na, Ca and Mg (cmol kg^{-1} soil)

It was estimated by taking the difference between available and water soluble K, Na, Ca and Mg (Hesse, 1971).

Water soluble extract was taken by shaking 10 g soil with 50 ml water for half an hour and the filtrate was collected. The filtrate was fed to flamephotometer for the determination of exchangeable K and Na while versanate titration was done for determination of exchangeable Ca and Mg.

3.3.2.1.5 Effective CEC (cmol kg^{-1} soil)

ECEC of soil samples were determined by addition of exchangeable Na, K, Ca, Mg and exchangeable acidity (ISRIC, 1992).

3.3.2.2 Biological Activity

3.3.2.2.1 Urease Enzyme Activity (ppm of urea hydrolyzed g^{-1} soil h^{-1})

Broadbent *et al.* (1964) described the method of estimation of urease enzyme activity in the soil samples.

1 g soil was taken in a 50 ml conical flask and 20 ml of 500 ppm urea solution was added. Then kept for incubation at 37 ^{0} C for 4 hours. After that, the flasks were taken out and add 100 mg $CaSO_4$ to stop the urease activity in the sample. To the filtrate 10 ml of colouring agent, p – dimethyl amino benzaldehyde was added and the volume was made upto 50 ml. Colour developed after 5 minutes and the resulted greenish yellow solution was read by spectrophotometer at 420 nm. Urea standards of known concentration was also prepared.

3.3.2.2.2 Acid Phosphatase Activity (µg of p-nitrophenol released g^{-1} of soil h^{-1})

The acid phosphatase activity of soils was determined using the procedure outlined by Tabatabai and Bremner (1969). The enzyme activity was expressed in µg of p-nitrophenol released g^{-1} soil h^{-1} on dry weight basis at 37 ^{0} C at pH 6.5.

In a 100 ml volumetric flask, 1gm soil was weighed. Toluene (0.2 ml) and modified universal buffer having pH 6.5 (4 ml) were added ito the flask, followed by 1 ml of 0 .05 M p- nitrophenyl phosphate and kept in the incubator after swirling the flask for one hour at 37 ^{0} C. After incubation period, 1 ml $CaCl_2$ (0.5 M) and 4 ml NaOH (0.5 M) were added. The filtrate with yellowish colour was collected and read in spectrophotometer at 420 nm. The concentration of pnp in the filtrate was determined by plotting standard curve.

3.3.2.2.3 Alkaline Phosphatase Activity (µg of p-nitrophenol released g⁻¹ soil h⁻¹)

The procedure for alkaline phosphatase assay was outlined by Tabatabai and Bremner (1969). The enzyme activity was expressed in µg of p-nitrophenol released g^{-1} of soil h^{-1} at 37 ^{0} C at pH 11. The procedure followed was same as acid phosphatase assay except for the use of modified universal buffer at pH 11.

3.3.2.2.4 Arylsuphatase Activity (µg of p-nitrophenol released g⁻¹ soil h⁻¹)

The assay was done on the basis of procedure outlined by Tabatabai and Bremner in 1970.

One gram of soil was taken and added toluene (0.2 ml), 0.5 M acetate buffer having pH 5.8 (4 ml), followed by 1 ml of 0.05 M p- nitrophenyl sulphate and kept in the incubator after swirling the flask for one hour at 37 ^{0} C. After incubation period add 0.5 M $CaCl_2$ (1 ml) and 0.5 M NaOH (4 ml). Filtrate was collected and the intensity of developed yellowish colour was read in spectrophotometer at 400 nm.

3.3.2.2.5 Dehydrogenase Activity (µg of TPF released g⁻¹ soil 24 h⁻¹)

The dehydrogenase activity was measured by the procedure described by Casida *et al.,* 1964.

One gram of air dried sample blended with 0.2 g $CaCO_3$ and add 1 ml of 3 per cent 2, 3, 5 - triphenyl tetrazolium chloride (TTC) and distilled water (2.5 ml), mixed well and kept for incubation (24 hours) at room temperature. After 24 hours, add methanol (10 ml) and shake for one minute. The sample was then filtered using a glass funnel plugged with absorbent cotton and the whole amount of soil in the tube should be transferred into the funnel by washing with methanol. The tube was washed and the soil was transferred into the funnel. The reddish colour in the absorbant cotton was vanished while washing with methanol. Filtrate, which is red

in colour, was made up to 100 ml with methanol and the colour intensity was measured using spectrophotometer at 485 nm. The concentration of dehydrogenase in the sample was obtained by plotting standard graph drawn by using tri phenyl formazon (TPF) as standard.

3.3.2.2.6 β-glucosidase Activity (μg pnp D- glucosidase g⁻¹ soil h⁻¹ x 10⁻⁴)

Eivazi and Tabatabai (1988) outlined procedure for estimating activity of glucosidase in soil samples.

One gram of soil was taken and added modified universal buffer of pH 6 (10 ml) and 1 ml p- nitrophenyl beta - glucopyranoside (0.5 M) into it, mixed thoroughly and incubated for one hour at 37 ^{0} C. After incubation, added 1 ml of 0.5 M CaCl$_2$, then 4 ml Tris buffer (pH 10). Yellow colour of the filtrate was read at 405 nm in spectrophotometer.

3.3.2.2.7 Soil Respiratory Activity (μg of CO$_2$ evolved g⁻¹soil h⁻¹)

Jenkinson and Powlson (1976) described the procedure for estimation of soil respiratory activity. The CO$_2$ evolved from the soil was trapped in 1.0 N NaOH kept in the vial and quantified by titrating against 1.0 N HCl.

3.3.2.2.8 Microbial Biomass Carbon (μg g⁻¹ soil)

Biomass carbon was determined by the fumigation – incubation technique outlined by Jenkinson and Ladd (1981).

3.3.2.2.9 Michaelis – Menten Constants (V $_{max}$ and K$_m$)

The collected soil samples were incubated at 37 °C in the laboratory for estimating V $_{max}$ and K$_m$. Soils were added with varying concentrations of substrates and the activity of the major enzymes such as dehydrogenase, glucosidase, aryl

sulphatase, acid phosphatase and urease were estimated by constructing the Line-Weaver-Burk plot.

When 1/V is plotted against 1/S, a straight line graph is obtained in Line-Weaver-Burk method, where V is the velocity and S is the substrate concentration. Here the slope is K_m/V_{max}, the intercept on the ordinate is $1/V_{max}$ (Vaughan and Ord, 1991).

Six concentrations each of urea solution (0.005, 0.010, 0.015, 0.020, 0.035 and 0.040 mol l^{-1}), p-nitrophenyl phosphate solution (0.0005, 0.001. 0.0025, 0.005, 0.015 and 0.050 mol l^{-1}) and TTC solution (0.003, 0.007, 0.010, 0.020, 0.030 and 0.050 mol l^{-1}) were the substrates for different enzymes viz., urease, phosphatise, dehydrogenase respectively and incubation study was carried out.

3.3.2.2.10 Microbial load (log cfu g^{-1} soil)

Microbial counts in the soil samples were enumerated by serial dilution technique given by Timonin (1940).

Table 4. Media used for estimation of microbial population.

Sl No.	Microflora	Media used	Reference
1	Bacteria	Nutrient Agar medium	Atlas and Parks, 1993
2	Fungi	Martin's Rose Bengal Agar	Martin, 1950
3	Actinomycetes	Ken knight's agar medium	Cappuccino and Sheman, 1996.
4	*Thiobacillus* spp.	Thiobacillus differential agar	Atlas and Parks, 1993
5	Nitrogen fixers	LGI agar	Cavalcante and Dobereiner, 1988

| 6 | Phosphorus solubilizers | Phosphorus solubilizers differential agar (Pikovskaya's media) | Rao and Sinha, 1963 |

3.3.2.3 Incubation Study.

The soil samples collected from the different soil series from the study area were incubated at 37 ° C in the laboratory for three months to confirm the acid sulphate condition. Here the initial pH was recorded and then pH readings were taken in fortnightly intervals (Beena, 2005).

3.3.3 Generation of Thematic Maps

GIS (ARC VIEW) software ArcGIS 10.3 was used to prepare thematic maps on various themes such as population of microflora and enzyme status of the study area using the spatial intrapolation technique IDW (Inverse Distance Weighted).

3.3.4 Computation of Biological Fertility Index Using Enzyme Activity Number (EAN)

Biological Fertility Index, for the different soils collected from the identified soil series were computed based on the activity of five different enzymes *viz.,* Phosphatase (Acid and Alkaline), Aryl sulphatase, Dehydrogenase and Glucosidase following the procedure outlined by Beck (1984).

EAN = 0.2[TPF + (Acid phosphatase/40) + (Alkaline phosphatase/40) +

(Aryl sulphatase/20) + (β-glucosidase/10)]

3.4 STATISTICAL ANALYSIS

The data generated from the above mentioned experiments were subjected to analysis of variance as per the design, CRD and their significance was tested using F

test (Snedecor and Cochran, 1975). If the treatments were found to be significant, critical difference (C D) was calculated using standard techniques at 0.05% probability levels.

Results

4. RESULTS

The study entitled "Enzyme characterization of acid sulphate soils of Kuttanad" has been carried out by collecting geocoded samples from six acid sulphate soil series of Kuttanad *viz.*, Ambalapuzha, Kallara, Purakkad, Thakazhi, Thottapalli and thuravur along with two other soil types from Karappadam (Alappuzha series) and Kayal areas (Chambakulam series) for the purpose of general comparison among the soil types. The soil samples were analyzed in the lab for various physical, chemical and biological parameters for the computation of biological fertility index. Results were expressed based on statistically analyzed data pertaining to the experiment conducted during the course of investigation and are presented in this chapter.

4.1 PHYSICAL PROPERTIES OF THE SOIL SAMPLE

The physical properties like bulk density, particle density and water holding capacity of the surface and subsurface soil were analyzed and the results are presented in the Tables 5 and 6 respectively.

4.1.1 Bulk Density

The mean values of bulk density at surface soils ranged from 0.61 to 1.06 Mg m^{-3}. Among the six different acid sulphate soil series, the highest bulk density was reported in Ambalapuzha series (L$_1$-1.06 Mg m^{-3}) which significantly from other locations. The lowest value was noticed at Kallara series (L$_2$-0.61 Mg m^{-3}) and was on par with Thottapalli (L$_5$-0.67 Mg m^{-3}) and Thuravoor series (L$_6$ - 0.68 Mg m^{-3}). At 0-15 cm level, the Karappadam soils recorded a bulk density of 1.18 Mg m^{-3} and the Kayal lands had a bulk density value of 1.01 Mg m^{-3}.

Regarding the subsurface soil, the bulk density values ranged from 0.63 to 1.07 Mg m^{-3} and the the highest value for bulk density was recorded at Purakkad series (L$_3$ − 1.07 Mg m^{-3}) and the lowest bulk density of 0.63 Mg m^{-3} was noticed at Thuravur series (L$_6$) which was on par with Kallara series (L$_2$ − 0.68 Mg m^{-3}). Here the Karappadam soils were found to have a bulk density value of 1.12 Mg m^{-3} and the Kayal lands recorded a bulk density of 0.95 Mg m^{-3}.

Table 5. Physical properties of acid sulphate soils of Kuttanad– surface soils (0-15 cm)

SOIL SERIES	Bulk density (Mg m^{-3})	Particle density (Mg m^{-3})	Water holding capacity (%)
L$_1$-Ambalapuzha	1.06	2.04	48.44
L$_2$-Kallara	0.61	1.19	48.31
L$_3$-Purakkad	0.97	1.87	48.39
L$_4$-Thakazhi	0.88	1.71	48.36
L$_5$-Thottapalli	0.67	1.30	48.35
L$_6$-Thuravur	0.68	1.31	48.34
S.E (M)*	0.03	0.03	0.04
C.D(0.05)	0.06	0.13	NS
Karappadam	1.18	2.18	45.75
Kayal	1.01	2.18	53.81

*- Standard Error (Mean)

Table 6. Physical properties of acid sulphate soils of Kuttanad– subsurface soils (15-30 cm)

SOIL SERIES	Bulk density (Mg m^{-3})	Particle density (Mg m^{-3})	Water holding capacity (%)
L$_1$-Ambalapuzha	0.96	1.87	48.39
L$_2$-Kallara	0.68	1.31	48.31
L$_3$-Purakkad	1.07	2.09	48.39
L$_4$-Thakazhi	0.73	1.42	48.29
L$_5$-Thottapalli	0.73	1.43	48.36
L$_6$-Thuravur	0.63	1.24	48.31
S.E (M)	0.02	0.06	0.03
C.D(0.05)	0.07	0.15	NS
Karappadam	1.12	2.09	46.11
Kayal	0.95	2.05	53.61

4.1.2 Particle Density

Among the six acid sulphate series of Kuttanad from where the soils are collected, the highest particle density was recorded from samples of Ambalapuzha (L_1) at surface soils with 2.04 Mg m^{-3} value and the lowest at Kallara series (L_2 – 1.19 Mg m^{-3}) which was on par with Thottapalli (L_5-1.30 Mg m^{-3}) and Thuravur (L_6-1.31 Mg m^{-3}). The Karappadam and Kayal lands recorded same values for particle density at surface soils, ie. 2.18 Mg m^{-3}.

Considering the subsurface soils, the highest value for particle density was recorded in Purakkad (L_3-2.09 Mg m^{-3}) and the lowest at Thuravur series (L_6- 1.24 Mg m^{-3}). The lowest value noticed in Thuravur (1.24 Mg m^{-3}) was on par with the particle density of Kallara series (L_2-1.31 Mg m^{-3}). The Karappadam soils recorded a value of 2.09 Mg m^{-3} for particle density and 2.05 Mg m^{-3} at Kayal lands.

4.1.3 Maximum Water Holding Capacity

The water holding capacity of acid sulphate soils did not vary significantly at both the surface and sub surface soils. The water holding capacity of six acid sulphate soils didnot show much variation between locations and the mean values varied from 48.31 to 48.44 per cent at surface and from 48.29 to 48.39 per cent at sub surface soils. Among the Karappadam and Kayal lands, Kayal lands recorded a higher water holding capacity of 53. 81 per cent at surface and 53.61 per cent at subsurface levels.

4.2 ELECTROCHEMICAL PROPERTIES OF THE SOIL

4.2.1 Soil Reaction (pH)

From Table 7, it was observed that pH of the soils are found to have significant effect among locations at surface soils. All the samples show pH below 4.5 except Thakazhi soils (L_4) and the mean values ranged between 2.43 to 4.67. Comparing various locations, the Thuravur series (L_6) recorded the lowest pH of 2.43 at surface soils. Thakazhi recorded the highest pH (L_4-4.67) which was on par with Ambalapuzha series (L_1). Regarding the pH of Karappadam and Kayal lands, Karappadam soils recorded the highest pH among all locations, i.e. a pH of 5.25 and the Kayal lands showed an extremely low pH of 2.94.

Table 7. Electrochemical properties of acid sulphate soils of Kuttanad-surface soils (0-15 cm)

SOIL SERIES	pH	EC (dS m^{-1})
L$_1$-Ambalapuzha	4.45	0.71
L$_2$-Kallara	4.18	1.01
L$_3$-Purakkad	4.07	1.59
L$_4$-Thakazhi	4.67	0.23
L$_5$-Thottapalli	4.09	0.14
L$_6$-Thuravur	2.43	7.52
S.E (M)	0.08	0.26
C.D(0.05)	0.22	0.73
Karappadam	5.25	0.14
Kayal	2.94	0.72

Table 8. Electrochemical properties of acid sulphate soils of Kuttanad-subsurface soils (15-30 cm)

SOIL SERIES	pH	EC (dS m^{-1})
L$_1$-Ambalapuzha	4.40	0.19
L$_2$-Kallara	4.22	0.74
L$_3$-Purakkad	4.04	1.08
L$_4$-Thakazhi	4.33	1.59
L$_5$-Thottapalli	4.04	0.15
L$_6$-Thuravur	2.89	6.53
S.E (M)	0.07	0.09
C.D(0.05)	0.19	0.25
Karappadam	4.76	0.22
Kayal	4.51	0.91

From Table 8, it was clear that at 15-30 cm depth, the pH showed a significant effect among locations and at subsurface level the Thuravur series recorded a lowest pH of 2.89 and the mean values ranged from 2.89 to 4.40. The highest pH was seen at Ambalapuzha series (L_1). Karappadam soils registered a pH of 4.76 and Kayal lands had a pH of 4.51 at subsurface level.

4.2.2 Soluble Salt Content (EC)

In the case of EC (Tables 7 and 8) both the surface and subsurface soils were highly significant and among different locations the highest EC of 7.52 dS m^{-1} was recorded from the Thuravur (L_6) area value which was significantly different from others. The lowest value was recorded by the samples of Thottapalli (L_5) with the lowest EC of 0.14 dS m^{-1}which was on par with Thakazhi (L_4- 0.23 dS m^{-1}) and Ambalapuzha series (L_1-0.71 dS m^{-1}). Karappadam and Kayal lands recorded EC values of 0.14 dS m^{-1} and 0.72 dS m^{-1} respectively at surface soils.

With regard to the EC values of subsurface soils, the highest EC of 6.53 dS m^{-1} was seen at Thuravur series (L_6) and the lowest at Thottapalli (L_5) with a value of 0.14 dS m^{-1} which was on par with Ambalapuzha series (L_1- 0.19 dS m^{-1}). EC values of 0.22 dS m^{-1} and 0.91 dS m^{-1}was recorded from the subsurface soils of Karappadam and Kayal lands respectively.

4.3 CHEMICAL PROPERTIES OF SOIL

4.3.1 Organic Carbon

Statistical analysis of the data indicated that the organic carbon contents of the soils were significant in all the acid sulphate series both at surface and sub surface soils. The mean values of organic carbon content at surface soils ranged from 1.28 to 3.01 per cent. The highest organic carbon content was recorded in the Kallara series (L_2-3.01 per cent) and the lowest organic carbon per cent was shown by the Purakkad series (L_3-1.28 per cent). Both Karappadam and Kayal lands have significant influence on the organic carbon content of the area. Kayal lands had a higher organic carbon content of 3.13 per cent while the Karappadam area recorded an organic carbon content of 2.73 percent at surface soils.

Table 9. Organic carbon and organic matter content of acid sulphate soils of Kuttanad-surface soils (0-15 cm), %.

SOIL SERIES	O.C	O.M
L_1-Ambalapuzha	1.93	3.33
L_2-Kallara	3.01	5.18
L_3-Purakkad	1.28	2.20
L_4-Thakazhi	1.94	3.35
L_5-Thottapalli	2.79	4.82
L_6-Thuravur	2.00	3.45
S.E (M)	0.09	0.15
C.D(0.05)	0.25	0.42
Karappadam	2.73	4.70
Kayal	3.13	5.39

Table 10. Organic carbon and organic matter content of acid sulphate soils of Kuttanad – subsurface soils (15-30 cm), %.

SOIL SERIES	O.C	O.M
L_1-Ambalapuzha	1.17	2.01
L_2-Kallara	2.96	5.10
L_3-Purakkad	1.01	1.74
L_4-Thakazhi	1.92	3.31
L_5-Thottapalli	2.41	4.15
L_6-Thuravur	1.69	2.91
S.E (M)	0.10	0.18
C.D(0.05)	0.29	0.51
Karappadam	3.06	5.26
Kayal	2.68	4.62

On considering the subsurface soil organic carbon content, the Kallara series soils again recorded highest organic carbon content (L_2-2.96 per cent) and the lowest organic content of 1.01 percent at Purakkad area (L_3) which was on par with the Ambalapuzha series (L_1-1.17 per cent). At the sub surface level, Karappadam soils recorded an organic carbon content of 3.06 per cent and 2.68 per cent in Kayal lands.

4.3.2 Organic Matter

From the Tables 9 and 10, it was clear that the all the locations vary significantly with respect to the organic matter content in the Kuttanad area. Among various locations the highest value of organic matter content was recorded in the Kallara series at surface (L_2-5.18 per cent) and was significantly superior than all other soil series. The lowest mean value was seen at Purrakad series (L_3-2.20 per cent) and was significantly different from all other locations

In the case of sub surface soil analysis, again the Kallara series (L_2-5.10 per cent) recorded highest organic matter content and lowest by the Purakkad area (L_3- 1.74 per cent). Here the lowest value was on par with the organic matter content of Ambalapuzha series (L_1-2.01 per cent). The Kayal lands registered an organic matter percent of 5.26 at sub surface and the Karappadam soils had an organic matter content of 4.62 per cent.

4.3.3 Available Nutrient Status in the Soil

The soils collected were analyzed for the available nutrient contents and the resuls are presented in Tables 11 and 12.

4.3.3.1 Available Nitrogen

The available nitrogen status clearly depicts that the locations had a highly significant effect on available nitrogen status of the areas at surface level (Table 11). In general, the soils of Kuttanad recorded high available nitrogen content at surface soil layers. The available nitrogen status vary from a mean value of 296.94 to 674.02 kg ha^{-1} in various locations and among them the highest available nitrogen status was reported by the Kallara series (L_2-674.02 kg ha^{-1}). The Purakkad (L_3 - 296.94 kg ha^{-1}) series which was significantly different from all other sample means recorded lowest available nitrogen status among the six acid sulphate series. While

comparing the distribution of available nitrogen in the surface samples of Karappadam and Kayal lands, Kayal lands showed a higher value of 701.34 kg ha^{-1} than the surface samples of Karappadam soils where the available nitrogen status was 611.52 kg ha^{-1}.

Regarding the contents in subsurface soil, all the locations showed a wide variation in the availability of nitrogen and the mean values varied from 226.69 to 663.26 kg ha^{-1}. The highest available nitrogen status was noticed at Kallara series (L$_2$-663.26 kg ha^{-1}) and the lowest at Purakkad series (L$_3$- 226.69 kg ha^{-1}) at the sub surface level. The lowest value was on par with the available nitrogen status of the Ambalapuzha series (L$_1$-271.18 kg ha^{-1}). The Karappadam soils recorded an available nitrogen content of 684.54 kg ha^{-1} and the Kayal lands registered a value of 600.77 kg ha^{-1}.

4.3.3.2 Available Phosphorus

Analysis of the data showed that the variations in available phosphorus content in the six acid sulphate series in Kuttanad are significant and the phosphorus availability varied widely in these soils. The highest phosphorus content at surface soils was noticed at Ambalapuzha series (L$_1$-78.60 kg ha^{-1}). The Purakkad series (L$_3$) with available phosphorus content of 4.09 kg ha^{-1}, reported lowest available phosphorus status. Among Karappadam and Kayal lands, Karappadam recorded a higher phosphorus availability of 60.36 kg ha^{-1} than the Kayal lands (33.65 kg ha^{-1}).

At subsurface level, the mean values for the available phosphorus content registered mean values ranged from 11.95 to 66.73 kg ha^{-1}. The highest phosphorus availability was recorded at Purakkad series (L$_3$-66.73 kg ha^{-1}) and lowest at Ambalapuzha series (L$_1$-11.95 kg ha^{-1}). The Karappadam soils had a higher phosphorus availability than Kayal lands with a mean of 72.01 kg ha^{-1} while Kayal lands recorded a value of 45.64 kg ha^{-1} for available phosphorus content.

4.3.3.3 Available Potassium

Analysis of the data in the Tables 11 and 12, in respect of the surface and subsurface soils of Kallara series have showed a significant effect on the availability of potassium from the soils of the investigated area. The mean values for available potassium ranged from 65.40 to 148.49

kg ha^{-1}. The highest value of 148.49 kg ha^{-1} was recorded in the surface soils of Kallara series (L$_2$) and the lowest availability at Thuravur series (L$_6$) having a mean value of 65.40 kg ha^{-1}. Karappadam soils recorded an available potassium content of 301.11 kg ha^{-1} while the Kayal lands recorded a value of 384.57 kg ha^{-1} for available potassium content.

Considering the sub surface soil availability of potassium, the mean values ranged from 51.87 to 154.14 kg ha^{-1}. The highest available potassium content at sub surface soils was recorded at Kallara series (L$_2$-154.14 kg ha^{-1}) and the Thuravur series (L$_6$) registered a lowest potassium availability of 51.87 kg ha^{-1}. The comparison among the Karappadam and Kayal lands showed that Karappadam recorded a higher value for potassium availability with a mean value of 343.33 kg ha^{-1} while the Kayal lands recorded 290.63 kg ha^{-1} available potassium content at subsurface soils.

4.3.3.4 Calcium

Data presented in Tables 11 and 12 have shown that all the locations were found to have significant effect on the availability of calcium in the soils of Kuttanad both at surface and subsurface soils. The mean values of calcium availability at surface soils varied from 634.00 to 912.80 mg kg^{-1}. Among the six acid sulphate series, the highest calcium availability was recorded at Kallara series (L$_2$-912.80 mg kg^{-1}) and lowest at Thuravur series (L$_6$ - 634.00 mg kg^{-1}). The Karappadam and Kayal lands recorded calcium content of 935.80 to 900.10 mg kg^{-1} respectively in surface samples.

With respect to the sub surface samples, the highest calcium content was noticed at Kallara series (L$_2$) with a mean of 959.21 mg kg^{-1} while Thuravur series (L$_6$) recorded lowest calcium content with a mean value of 715.30 mg kg^{-1}. The lowest value was on par with the Thottapalli series (L$_5$) with a mean of 729.00 mg kg^{-1}. The Karappadam soils recorded a value of 977.60 mg kg^{-1} for calcium while Kayal lands recorded a value of 1005.70 mg kg^{-1}.

4.3.3.5 Magnesium

Critical appraisal of the data in Tables 11 and 12, revealed that the availability of magnesium was significantly influenced by the locations. Considering the effect on surface soils on the available magnesium content, the highest mean value of 669.20 mg kg^{-1} was recorded in

Table 11. Available nutrient status of acid sulphate soils of Kuttanad- surface soils (0-15 cm)

SOIL SERIES	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	S (mg kg^{-1})
L$_1$-Ambalapuzha	432.99	78.60	121.93	743.10	507.40	284.14
L$_2$-Kallara	674.02	9.59	148.49	912.80	537.30	657.49
L$_3$-Purakkad	296.94	4.09	142.91	865.40	669.20	389.54
L$_4$-Thakazhi	435.01	37.04	116.18	789.90	524.00	286.77
L$_5$-Thottapalli	626.75	9.33	125.39	662.80	446.10	883.86
L$_6$-Thuravur	448.89	11.46	65.40	634.80	498.70	435.70
S.E (M)	17.49	1.49	14.98	8.14	11.01	59.50
C.D(0.05)	49.61	4.21	42.46	23.07	31.226	168.71
Karappadam	611.52	60.36	301.11	935.80	512.20	114.93
Kayal	701.34	33.65	384.57	900.10	695.60	179.94

Table 12. Available nutrient status of acid sulphate soils of Kuttanad- subsurface soils (15-30 cm)

SOIL SERIES	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	S (mg kg^{-1})
L$_1$-Ambalapuzha	271.18	11.95	77.28	800.50	591.70	695.82
L$_2$-Kallara	663.26	36.78	154.14	959.20	621.20	485.31
L$_3$-Purakkad	226.69	66.73	75.62	889.90	706.40	553.59
L$_4$-Thakazhi	430.30	32.18	77.00	820.30	618.60	364.59
L$_5$-Thottapalli	539.84	22.67	77.71	729.00	581.80	374.52
L$_6$-Thuravur	378.56	19.81	51.87	715.30	539.40	728.53
S.E (M)	22.02	2.53	7.34	14.86	13.79	104.68
C.D(0.05)	62.44	7.17	20.80	42.12	39.10	NS
Karappadam	684.54	72.01	343.33	977.60	482.70	85.56
Kayal	600.77	45.64	290.63	1005.70	623.30	82.37

the soil samples of Purakkad series (L_3). The lowest mean value was 446.10 mg kg^{-1} and it was recorded in the samples of Thottapalli series (L_5). The Karappadam soils recorded a magnesium content of 512.20 mg kg^{-1} whereas Kayal lands had a magnesium content of 695.60 mg kg^{-1}.

At subsurface level the available magnesium content of the six acid sulphate series varied from 539.40 to 706.40 mg kg^{-1}. The highest mean value for magnesium content at sub surface soils was recorded at Purakkad series (L_3- 706.40 mg kg^{-1}) and the lowest values was seen at the soils of Thuravur series (L_6- 539.40 mg kg^{-1}). Karappadam soils recorded a magnesium content of 482.70 mg kg^{-1} and the Kayal lands recorded a mean value of 623.30 mg kg^{-1}.

4.3.3.6 Sulphur

It was obvious from the data presented in Table 11 that available sulphur in the surface as well as other soil types were significantly influenced by the effect of locations. The effect was highly significant and the mean values ranged from 284.14 to 883.86 mg kg^{-1}. The highest mean of 883.86 mg kg^{-1} was recorded in the surface samples of Thottapalli (L_5). The lowest sulphur availability was recorded by the Ambalapuzha series (L_1- 284.14 mg kg^{-1}) and was on par with Thuravur (L_6 – 435.70 mg kg^{-1}), Purakkad (L_3- 389.54 mg kg^{-1}) and Thakazhi series (L_4 – 286.77 mg kg^{-1}). The Karappadam soil registered a mean value of 114.93 mg kg^{-1} for available sulphur and the Kayal lands recorded an available sulphur content of 179.94 mg kg^{-1}.

The available sulphur content in subsurface soils of different series showed a non significant effect. Even though the effect was non significant, the highest available sulphur content was recorded by the Thuravur series (L_6-728.53 mg kg^{-1}) and the lowest value at Thakazhi (L_4- 364.52 mg kg^{-1}). Karappadam soils had an available sulphur content of 85.56 mg kg^{-1} and Kayal lands recorded an available sulphur content of 82.37 mg kg^{-1} at sub surface soils.

4.3.4 Ammonium Acetate Extractable Sodium Content

The NH_4OAc extractable sodium content in the six acid sulphate soils of Kuttanad was significant both at surface and sub surface soils. In surface soils, the mean values for available sodium content varied from 140.02 to 408.53 kg ha^{-1}. The highest sodium availability was noticed at Purakkad series (L_3 – 408.53 kg ha^{-1}) which was on par with the Thuravur series (L_6-

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Table 13. NH$_4$OAc extractable sodium content in the acid sulphate soils – surface (0-15 cm) and subsurface soils (15-30 cm), kg ha^{-1}.

SOIL SERIES	NH$_4$OAc extractable sodium content	
	Surface	Subsurface
L$_1$-Ambalapuzha	286.59	161.73
L$_2$-Kallara	338.01	354.41
L$_3$-Purakkad	408.53	276.49
L$_4$-Thakazhi	140.02	164.19
L$_5$-Thottapalli	148.98	139.44
L$_6$-Thuravur	391.88	392.21
S.E (M)	27.63	22.50
C.D(0.05)	78.33	63.79
Karappadam	196.39	242.15
Kayal	168.33	137.39

391.88 kg ha^{-1}) and Kallara series (L$_2$ – 338.01 kg ha^{-1}). The lowest value was recorded by the Thakazhi series (L$_4$-140.02 kg ha^{-1}) and was on par with the surface samples of Thottapalli series (L$_5$-148.98 kg ha^{-1}). Regarding the Karappadam and Kayal lands, the Karappadam registered an NH$_4$OAc extractable sodium content of 196.39 kg ha^{-1} while Kayal lands, a value of 168.33 kg ha^{-1} at surface soils.

At subsurface soil samples, the highest NH$_4$OAc extractable sodium content was noticed at the soil samples of Thuravur (L$_6$-392.21 kg ha^{-1}) and was on par with Kallara series (L$_2$-354.41 kg ha^{-1}). The lowest NH$_4$OAc extractable sodium content was seen at Thottapalli (L$_5$ -139.44 kg ha^{-1}). The lowest value was on par with the NH$_4$OAc extractable sodium content at Thakazhi (L$_4$- 164.192) and Ambalapuzha (L$_1$-161.73 kg ha^{-1}). Among the Karappadam and Kayal lands the highest NH$_4$OAc extractable sodium content was recorded by Karappadam soils, i. e., a mean value of 242.15 kg ha^{-1} . The Kayal lands recorded a mean value of 137.39 kg ha^{-1} for NH$_4$OAc extractable sodium content.

4.3.5 Available Micro Nutrient Status

Tables 14 and 15, shows the availability of micronutrients in the Kuttanad soils at surface and sub surface level.

4.3.5.1 Iron

The location effects were highly significant with respect to the availability of iron in acid sulphate series (Table 14 and 15). While analyzing the available iron content in the six acid sulphate soils at surface level, the mean values ranged from 145.78 to 1024.23 mg kg^{-1}. The highest available iron content was noticed at Thottapalli series (L$_5$-1024.23 mg kg^{-1}) and the lowest at Thakazhi series (L$_4$-145.78 mg kg^{-1}). Regarding the comparison among Karappadam and Kayal lands, the highest available iron content was noticed at Kayal lands (840.35 mg kg^{-1}) while the Karappadam soils have an iron content of 485.85 mg kg^{-1}.

Regarding the subsurface soil iron availability, the mean values ranged from 84.15 to 924.50 mg kg^{-1}. The highest value was recorded at Thuravur series (L$_6$-924.50 mg kg^{-1}). The Purakkad series recorded the lowest value for available iron content (L$_3$-84.15 mg kg^{-1}). At

Karappadam area the available iron content was 607.85 mg kg^{-1} and the Kayal lands recorded 738.85 mg kg^{-1} of iron availability.

4.3.5.2 Manganese

From the Tables 14 and 15, it was clear that the location effect was highly significant and the mean values varied between 2.57 to 31.69 mg kg^{-1}. Among locations, the highest mean value was recorded by Thakazhi series (L$_4$ – 31.69 mg kg^{-1}) and the lowest by Ambalapuzha samples (L$_1$-2.57 mg kg^{-1}), both the highest and the lowest location effects were significantly different from others. Considering the Karappadam and Kayal lands available manganese content, the highest manganese content of 18.40 mg kg^{-1} was seen at Karappadam and the lowest at Kayal lands (11.37 mg kg^{-1}).

Considering the subsurface soil manganese availability, the highest mean value of was observed at Thottapalli series (L$_5$-33.92 mg kg^{-1}) and the lowest at Purakkad (L$_3$-8.38 mg kg^{-1}) among the acid sulphate soils. The Karappadam soils registered a value of 16.68 mg kg^{-1} for available manganese content and the Kayal lands had manganese content of 11.62 mg kg^{-1} at sub surface level.

4.3.5.3 Zinc

All the locations have a significant influence on the availability of zinc in the surface soils and the values varied between a wide range of 4.89 to 19.56 mg kg^{-1}. The highest mean value of 19.56 mg kg^{-1} was registered in Thuravur series (L$_6$) and the lowest at Purakkad series (L$_3$-4.89 mg kg^{-1}). Karappadam soil recorded a value of 23.91 mg kg^{-1} available zinc content at surface level while the Kayal lands registered a value of 9.49 mg kg^{-1}.

Among the six acid sulphate series in sub surface soils, the highest available zinc content of 20.58 mg kg^{-1} was noticed at Thuravur (L$_6$) which was superior than all other locations. The lowest value was seen at Purakkad (L$_3$-8.3 mg kg^{-1}) and was also significantly different from others. The Karappadam soils recorded a value of 17.84 mg kg^{-1} for available zinc content. The Kayal soils were found to have the available zinc content of 15.43 mg kg^{-1} at sub surface.

4.3.5.4 Copper

The location effects significantly influenced the copper availability in the surface soils and the highest mean value was recorded in Ambalapuzha series (L_1-26.21 mg kg^{-1}). The lowest value was 1.68 mg kg^{-1} and was noticed at Thuravur (L_6) and was significantly different from other soil series. Karappadam and Kayal lands registered an available copper content of 13.05 and 7.94 mg kg^{-1} respectively.

At sub surface soils, the highest copper availability was seen at Ambalapuzha series (L_1-13.16 mg kg^{-1}) which was superior than all other acid sulphate soil series and the lowest at Purakkad area (L_3-0.75 mg kg^{-1}) (Table 15). The lowest mean value was also singnificantly different from other locations. The Karappadam soils registered a copper content of 13.48 mg kg^{-1} and the Kayal lands had a copper content of 7.83 mg kg^{-1} at sub surface soils.

4.3.5.5 Boron

On scrutinizing the data in Table 14, it was observed that the locations had imparted a significant effect on the boron availability in the surface soils. The mean values ranged from 6.07 to 13.45 mg kg^{-1}. Among the six locations of acid sulphate soils, the highest available boron content was noticed at Thuravur (L_6 -13.45 mg kg^{-1}) and the lowest was seen at Thakazhi (L_4-6.07 mg kg^{-1}) which was on par with Purakkad area ($L3$- 7.05 mg kg^{-1}). The Karappadam soils recorded an available boron content of 7.31 mg kg^{-1} while the Kayal lands had 7.26 mg kg^{-1} boron content.

The sub surface soils of all the series recorded significant influence on the availability of boron content (Table 15). The mean values ranged from 6.07 to 11.64 mg kg^{-1}. The highest value was noticed at Thuravur (L_6 – 11.64 mg kg^{-1}) and the lowest at Ambalapuzha (L_1-6.07 mg kg^{-1}). The lowest content was on par with Purakkad (L_3-6.24 mg kg^{-1}) and Thakazhi (L_4-6.76 mg kg^{-1}). Karappadam area had registered an available boron content of 6.13 mg kg^{-1} while the Kayal lands recorded a value of 5.71 mg kg^{-1} for available boron content.

Table 14. Available micronutrient status of acid sulphate soils of Kuttanad- surface soils (0-15 cm), mg kg^{-1}.

SOIL SERIES	Fe	Mn	Zn	Cu	B
L$_1$-Ambalapuzha	283.13	2.57	6.06	26.21	7.12
L$_2$-Kallara	637.50	14.35	7.41	1.96	10.86
L$_3$-Purakkad	229.85	10.65	4.88	2.98	7.05
L$_4$-Thakazhi	145.78	31.69	5.93	2.67	6.07
L$_5$-Thottapalli	1024.23	18.56	7.51	4.93	7.54
L$_6$-Thuravur	406.63	18.47	19.56	1.68	13.45
S.E (M)	3.78	0.03	0.03	0.05	0.35
C.D(0.05)	10.72	0.09	0.08	0.15	0.99
Karappadam	485.85	18.40	23.91	13.05	7.31
Kayal	840.35	11.37	9.49	7.94	7.26

Table 15. Available micronutrient status of acid sulphate soils of Kuttanad-subsurface soils (15-30 cm), mg kg^{-1}.

SOIL SERIES	Fe	Mn	Zn	Cu	B
L$_1$-Ambalapuzha	156.18	9.91	4.25	13.16	6.07
L$_2$-Kallara	100.53	30.41	7.48	1.90	8.93
L$_3$-Purakkad	84.15	8.38	3.83	0.76	6.24
L$_4$-Thakazhi	440.00	10.09	8.30	2.47	6.76
L$_5$-Thottapalli	317.03	33.92	6.30	6.07	8.29
L$_6$-Thuravur	924.50	24.04	20.59	2.69	11.64
S.E (M)	2.67	0.02	0.02	0.04	0.29
C.D(0.05)	7.56	0.09	0.06	0.10	0.84
Karappadam	607.85	16.68	17.84	13.48	6.13
Kayal	738.85	11.62	15.43	7.83	5.71

4.3.4 Acidity Parameters

The acidity parameters like exchangeable aluminium, exchangeable hydrogen and exchangeable acidity are presented in Tables 16 and 17 for surface and sub surface soils respectively.

4.3.4.1 Exchangeable Aluminium

The results revealed that all the location effects varied significantly for exchangeable aluminium content. The mean values of exchangeable aluminium content ranged from 0.18 to 5.46 cmol $^{(+)}$ kg^{-1}. The highest mean was recorded by the Thuravur series (L_6-5.46 cmol $^{(+)}$ kg^{-1}) which was significantly different from others. Thakazhi soils (L_4 – 0.18 cmol $^{(+)}$ kg^{-1}) recorded the lowest mean value among the locations and was on par with the suface soils of Ambalpauzha (L_1-0.19 cmol $^{(+)}$ kg^{-1}) and Kallara (L_2-0.32 cmol $^{(+)}$ kg^{-1}). Comparing the surface soils of Karappadam and Kayal lands, in the surface soils, higher exchangeable aluminium was recorded by Kayal lands with a value of 0.82 cmol$^{(+)}$ kg^{-1}. The Karappadam soils recorded 0.78 cmol $^{(+)}$ kg^{-1}exchangeable aluminium content.

The exchangeable aluminium in the subsurface soils recorded highest mean value for Thuravur series (L_6-3.59 cmol $^{(+)}$ kg^{-1}) and the lowest at Purakkad (L_3 – 0.05 cmol$^{(+)}$ kg^{-1}) and the value was on par with Ambalapuzha series (L_1 -0.13 cmol $^{(+)}$ kg^{-1}). Among Karappadam and Kayal lands, Karappadam recorded a higher value of 0.94 cmol $^{(+)}$ kg^{-1} than the Kayal lands which recorded a value of 0.31 cmol $^{(+)}$ kg^{-1}.

4.3.4.2 Exchangeable Hydrogen

The location effects on exchangeable hydrogen varied significantly with mean values ranged from 0.69 to 14.06 cmol $^{(+)}$ kg^{-1} at surface and sub surface level (Tables 14 and 15). The highest mean was recorded by Thuravur (L_6- 14.06 cmol $^{(+)}$ kg^{-1}) followed by Kallara series (L_2) with a mean value of 8.66 cmol$^{(+)}$ kg^{-1} and both were significantly different from others. The lowest value was recorded in Thottapalli series (L_5 – 0.69 cmol $^{(+)}$ kg^{-1}) which was on par with Purakkad ($L3$-0.96 cmol $^{(+)}$ kg^{-1}). Among Karappadam and Kayal lands, the higher value was noticed at Kayal lands with a mean of 0.22 cmol $^{(+)}$ kg^{-1} while Karapapdom soils recorded a mean of 0.15 cmol $^{(+)}$ kg^{-1}.

Table 16. Acidity parameters of acid sulphate soils of Kuttanad- surface soils (0-15 cm), cmol$^{(+)}$ kg^{-1}.

SOILS SERIES	Ex. Al^{3+}	Ex. H$^+$	Ex. Acidity
L$_1$-Ambalapuzha	0.19	1.71	1.90
L$_2$-Kallara	0.32	8.67	8.99
L$_3$-Purakkad	0.72	0.96	1.69
L$_4$-Thakazhi	0.19	2.62	2.80
L$_5$-Thottapalli	1.07	0.69	1.77
L$_6$-Thuravur	5.46	14.06	19.52
S.E (M)	0.06	0.10	0.08
C.D(0.05)	0.18	0.29	0.24
Karappadam	0.78	0.14	0.92
Kayal	0.82	0.22	1.04

Table 17. Acidity parameters of acid sulphate soils of Kuttanad-subsurface soils (15-30 cm), cmol$^{(+)}$ kg^{-1}.

SOILS SERIES	Ex. Al^{3+}	Ex. H$^+$	Ex. Acidity
L$_1$-Ambalapuzha	0.13	1.37	1.49
L$_2$-Kallara	0.31	7.09	7.39
L$_3$-Purakkad	0.05	2.35	2.39
L$_4$-Thakazhi	0.50	2.10	2.61
L$_5$-Thottapalli	1.72	1.90	3.62
L$_6$-Thuravur	3.59	9.09	12.68
S.E (M)	0.08	0.11	0.07
C.D(0.05)	0.20	0.32	0.22
Karappadam	0.94	0.13	1.07
Kayal	0.31	0.61	0.92

At sub surface soils, the Thuravur series again recorded highest exchangeable hydrogen conent of 9.09 cmol $^{(+)}$ kg^{-1}. Among the six acid sulphate series the lowest was noticed at Ambalapuzha series (L$_1$- 1.37 cmol $^{(+)}$ kg^{-1}) and was significantly different from others. The Karappadam soils recorded an exchangeable hydrogen content of 0.13 cmol $^{(+)}$ kg^{-1} and the Kayal lands had recorded an exchangeable hydrogen of 0.61 cmol$^{(+)}$ kg^{-1}.

4.3.4.3 Exchangeable Acidity

Exchangeable acidity of the Kuttanad soils were analyzed and it was noticed from the Tables 16 and 17, that the exchangeable acidity of soils were significantly influenced by the locations. The highest mean value was recorded in the case of location L$_6$, i. e., Thuravur with a mean of 19.52 cmol $^{(+)}$ kg^{-1} followed by Kallara surface samples (L$_2$ – 8.99 cmol$^{(+)}$ kg^{-1}), which was significantly different from the former one. The lowest mean value was noticed in Purakkad series (L$_3$ – 1.69 cmol $^{(+)}$ kg^{-1}). The Karappadam soils recorded a value of 0.92 cmol $^{(+)}$ kg^{-1} and the Kayal lands registered an exchangeable acidity of 1.04 cmol $^{(+)}$ kg^{-1} at surface soils.

The subsurface samples of acid sulphate series of Kuttanad registered mean values ranged from 1.49 to 12.68 cmol $^{(+)}$ kg^{-1}. The highest mean was recorded at Thuravur series (L$_6$- 12.68 cmol $^{(+)}$ kg^{-1}) and the lowest at Ambalapuzha series (L$_1$-1.49 cmol $^{(+)}$ kg^{-1}). The Karappadam soils recorded an exchangeable acidity of 1.07 cmol $^{(+)}$ kg^{-1} while the Kayal lands had a mean of 0.92 cmol $^{(+)}$ kg^{-1} in the sub surface soil samples.

4.3.5 Soil Exchangeable Cations

Tables 18 and 19, represent the mean values of exchangeable cations including exchangeable calcium, magnesium, sodium, potassium and then the calculated effective CEC.

4.3.5.1 Exchangeable Potassium

From the data it is inferred that the locations does not influence significantly on the exchangeable potassium content in the surface soils of acid sulphate series of Kuttanad while the effect of locations were significant at 15-30 cm depth. Table 18 shows that, among the six locations the highest value was recorded by the sub surface soil samples of Kallara series (L$_2$- 0.22 cmol $^{(+)}$ kg^{-1}). The lowest content of exchangeable potassium was recorded by sub surface

soils of Thottapalli series (L_5-0.09 cmol $^{(+)}$ kg^{-1}). The lowest exchangeable potassium content at Thottapalli was on par with Ambalapuzha (L_1- 0.13 cmol $^{(+)}$ kg^{-1}), Purakkad (L_3 – 0.11 cmol $^{(+)}$ kg^{-1}), Thakazhi (L_4 -0.14 cmol $^{(+)}$ kg^{-1}) and Thuravur (L_6 – 0.13 cmol $^{(+)}$ kg^{-1}). Karappadam soils recorded exchangeable potassium content of 0.82 cmol $^{(+)}$ kg^{-1} at subsurface layer and Kayal lands showed a value of 0.69 cmol $^{(+)}$ kg^{-1}.

4.3.5.2 Exchangeable Sodium

Data presented in Tables 18 and 19, revealed that the effects of locations were significant in the content of exchangeable sodium. At surface layer, the highest mean of 0.91 cmol $^{(+)}$ kg^{-1} was recorded at Purakkad series (L_3) and was on par with Ambalapuzha (L_1- 0.70 cmol $^{(+)}$ kg^{-1}) and Thuravur (L_6 -0.75 cmol $^{(+)}$ kg^{-1}). The lowest exchangeable sodium was noticed at Thottapalli series with a mean of 0.13 cmol $^{(+)}$ kg^{-1} and was on par with Kallara (L_2 – 99.61 0.43 cmol $^{(+)}$ kg^{-1} and Thakazhi (L_4 – 0.20 cmol $^{(+)}$ kg^{-1}). The Karappadam soils recorded an exchangeable sodium content of 0.25 cmol $^{(+)}$ kg^{-1} and 0.28 cmol $^{(+)}$ kg^{-1} at Kayal lands.

At sub surface level, the exchangeable sodium content ranged from 0.20 to 1.02 cmol $^{(+)}$ kg^{-1}. The highest value was seen at Thuravur (L_6 – 1.02 cmol $^{(+)}$ kg^{-1}) and the lowest at Ambalapuzha series (L_1-0.20 cmol $^{(+)}$ kg^{-1}). Among Karappadam and Kayal lands, the Karappadam soils recorded a value of 0.50 cmol $^{(+)}$ kg^{-1} and Kayal lands showed a value of 0.22 cmol $^{(+)}$ kg^{-1}.

4.3.5.3 Exchangeable Calcium

Locations had significant effect on the exchangeable calcium content in the surface layers as observed from Tables 18. Among the different locations the highest mean value was recorded in Purakkad series (L_2 – 8.09 cmol $^{(+)}$ kg^{-1}) and was significantly different from others. The lowest value of 1.41 cmol $^{(+)}$ kg^{-1}, it was noticed in Thakazhi soils (L_3). The Karappadam soils had recorded an exchangeable calcium content of 5.71 cmol $^{(+)}$ kg^{-1} while the Kayal lands recorded a value of 5.72 cmol $^{(+)}$ kg^{-1}.

The subsurface soils had also influenced significantly on the availability of exchangeable calcium content. At sub surface soils, the highest value was seen at Thakazhi (L_4 -6.61 cmol $^{(+)}$ kg^{-1}) and the lowest at Thuravur (L_3-3.78 cmol $^{(+)}$ kg^{-1}). The lowest value was on par

with Kallara series (L_2-4.15 cmol $^{(+)}$ kg^{-1}). The Karappadam and Kayal lands recorded an exchangeable calcium content of 4.72 and 3.57 cmol $^{(+)}$ kg^{-1} respectively.

4.3.5.4 Exchangeable Magnesium

Various locations significantly influenced the exchangeable magnesium of the surface and sub surface soils (Tables 18 and 19). Purakkad series (L_3) recorded the highest mean of 10.89 cmol $^{(+)}$ kg^{-1} followed by Thottapalli (L_5-6.50 cmol $^{(+)}$ kg^{-1}) which was significantly different from the former. The lowest mean was showed by Thuravur series (L_6-1.41 cmol $^{(+)}$ kg$^-$ ^{1}) which was on par with Thakazhi series (L_5-1.81 cmol $^{(+)}$ kg^{-1}). Among Karappadam and Kayal lands, the Kayal soils had higher exchangeable magnesium content of 6.90 cmol $^{(+)}$ kg^{-1} than the Karappadam soils which recorded exchangeable magnesium content of 3.55 cmol $^{(+)}$ kg^{-1}.

Among the six acid sulphate series of Kuttanad at sub surface level, the highest value was recorded by Thakazhi series (L_4-8.65 cmol $^{(+)}$ kg^{-1}) which was on par with Kallara series (L_2-8.27 cmol $^{(+)}$ kg^{-1}). The Thottapalli series (L_5) recorded a lowest mean of 2.33 cmol $^{(+)}$ kg^{-1}. Karappadam and Kayal lands, recorded an exchangeable magnesium content of 5.80 and 8.13 cmol $^{(+)}$ kg^{-1} respectively.

4.3.5.5 Effective CEC (ECEC)

ECEC of the ranged from 4.36 to 22.38 cmol $^{(+)}$ kg^{-1} among various locations at surface level and from 7.02 to 17.77 cmol $^{(+)}$ kg^{-1} in the case of sub surface soils. The influence of locations on ECEC of the soil was significant. The highest ECEC was recorded at Thuravur (L_6– 17.77 cmol $^{(+)}$ kg^{-1}) and was superior than all other soil series. The lowest value was seen at Thottapalli (L_5-7.02 cmol $^{(+)}$ kg^{-1}) which was significantly different from other locations. The Karappadam soils had recorded an ECEC value of 6.25 cmol $^{(+)}$ kg^{-1} at surface level and the Kayal lands recorded 6.49 cmol $^{(+)}$ kg^{-1} ECEC values.

At sub surface soils, the highest ECEC was recorded at Thuravur (L_6-17.77 cmol $^{(+)}$ kg^{-1}) and the lowest value was seen at Ambalapuzha series (L_1-7.13 cmol $^{(+)}$ kg^{-1}). The lowest value was on par with the ECEC values of Purakkad (L_3–7.32 cmol $^{(+)}$ kg^{-1}) and Thakazhi series (L_4– 9.54 cmol $^{(+)}$ kg^{-1}). Among the Karappadam and Kayal lands, the Karappadam soils recorded an ECEC value of 6.25 cmol $^{(+)}$ kg^{-1} while Kayal lands registered a mean of 6.49 cmol $^{(+)}$ kg^{-1}.

Table 18. Exchangeable cations in acid sulphate soils of Kuttanad – surface(0-15 cm), (cmol(+) kg^{-1})

SOIL SERIES	Ex. K	Ex. Na	Ex. Ca	Ex. Mg	ECEC*
L$_1$-Ambalapuzha	0.16	0.70	5.88	3.37	12.01
L$_2$-Kallara	0.13	0.43	7.01	3.46	20.02
L$_3$-Purakkad	0.18	0.91	8.09	10.89	21.76
L$_4$-Thakazhi	0.19	0.20	1.41	1.81	6.41
L$_5$-Thottapalli	0.17	0.13	5.72	6.50	14.29
L$_6$-Thuravur	0.16	0.75	4.44	1.41	26.28
S.E (M)	0.08	0.13	1.14	1.01	0.14
C.D(0.05)	NS	0.33	2.07	2.23	0.40
Karappadam	0.72	0.25	5.71	3.55	11.15
Kayal	0.92	0.28	5.72	6.90	14.86

*ECEC- Effective Cation Exchange Capacity

Table 19. Exchangeable cations in acid sulphate soils of Kuttanad-subsurface soils (15-30 cm), (cmol(+) kg^{-1})

SOIL SERIES	Ex. K	Ex. Na	Ex. Ca	Ex. Mg	ECEC
L$_1$-Ambalapuzha	0.13	0.20	4.74	7.67	14.23
L$_2$-Kallara	0.22	0.86	4.15	8.27	20.89
L$_3$-Purakkad	0.11	0.52	5.35	5.29	13.66
L$_4$-Thakazhi	0.14	0.29	6.61	8.65	18.30
L$_5$-Thottapalli	0.09	0.26	5.07	2.33	11.37
L$_6$-Thuravur	0.13	1.02	3.78	6.95	24.56
S.E (M)	0.31	0.75	1.86	1.79	0.16
C.D(0.05)	0.64	1.73	2.82	3.10	0.47
Karappadam	0.83	0.50	4.72	5.80	12.92
Kayal	0.69	0.22	3.57	8.13	13.53

4.4 BIOLOGICAL PROPERTIES OF SOIL

4.4.1 Enzyme Status of the Soil

Effect of locations on the enzyme activity of acid sulphate soils along with Karappadam and Kayal lands are presented in Tables 20 and 21. The major enzymes that were assessed in the present study included Urease, Phosphatase (acid and alkaline), β-glucosidase, Arylsulphatase and Dehydrogenase.

4.4.1.1 Urease Activity

Regarding the urease activity, the data showed that the urease activity varied significantly with the locations at surface soils (Table 20). The mean values ranged from 57.51 to 75.78 ppm of urea hydrolyzed g^{-1} of soil h^{-1} and the highest mean value was recorded by the Thakazhi series (L_4 – 75.78 ppm of urea hydrolyzed g^{-1} of soil h^{-1}). The lowest mean value was recorded by the Ambalapuzha series (L_1-57.51 ppm of urea hydrolyzed g^{-1} of soil h^{-1}) which was on par with surface samples of Thottapalli (L_5- 59.59 ppm of urea hydrolyzed g^{-1} of soil h^{-1}). The Karappadam soils recorded a value of 52.89 ppm of urea hydrolyzed g^{-1} of soil h^{-1} for urease activity and the Kayal lands had an activity rate of 50.77 ppm of urea hydrolyzed g^{-1} of soil h^{-1}.

In sub surface soils, the effect of various locations on urease activity was significant. The Thuravur series (L_6) recorded highest urease activity of 77.33 ppm of urea hydrolyzed g^{-1} of soil h^{-1} and was on par with the urease activity of Kallara series (L_2- 72.25 ppm of urea hydrolyzed g^{-1} of soil h^{-1}). The lowest activity was seen at Thakazhi series (L_4-52.36 ppm of urea hydrolyzed g^{-1} of soil h^{-1}) which was on par with the urease activity of acid sulphate soils of Ambalapuzha (L_1-53.57 ppm of urea hydrolyzed g^{-1} of soil h^{-1}), Purakkad (L_3-56.44 ppm of urea hydrolyzed g^{-1} of soil h^{-1}) and Thottapalli series (L_5- 58.22 ppm of urea hydrolyzed g^{-1} of soil h^{-1}). Comparing the urease activity of sub surface soils of Karappadam and Kayal lands, the Kayal lands recorded higher activity of 58.66 ppm of urea hydrolyzed g^{-1} of soil h^{-1} than the Karappadam soils which registered activity of 49.05 ppm of urea hydrolyzed g^{-1} of soil h^{-1}.

4.4.1.2 Acid Phosphatase Activity

Activity of acid phosphatases varied significantly with different locations (Tables 20 and 21). The mean values of various acid sulphate series locations on acid phosphatase activity ranged from 24.59 to 57.58 µg of p-nitrophenol released g^{-1} soil h^{-1}. Among the locations, the highest activity was recorded in the samples from Purakkad series (L_2- 57.58 µg of p-nitrophenol released g^{-1} soil h^{-1}) which was on par with the acid phosphatase activity of Thakazhi (L_4 – 54.13 µg of p-nitrophenol released g^{-1} soil h^{-1}) and also on par with Ambalapuzha series (L_1) having an acid phosphatase activity of 52.99 µg of p-nitrophenol released g^{-1} soil h^{-1}. The lowest mean value was reported at Thuravur series (L_6-24.59 µg of p-nitrophenol released g^{-1} soil h^{-1}) which was significantly different from other locations. At Karappadam soils at surface level, the acid phosphatase activity was 45.58 µg of p-nitrophenol released g^{-1} soil h^{-1} while Kayal lands recorded an activity of 22.62 µg of p-nitrophenol released g^{-1} soil h^{-1}.

The sub surface soils of acid sulphate series in Kuttanad recorded mean values in a range from 11.02 to 71.88 µg of p-nitrophenol released g^{-1} soil h^{-1}. Among the six locations the highest acid phosphatase activity at sub surface was recorded at Purakkad series (L_3-71.88 µg of p-nitrophenol released g^{-1} soil h^{-1}) which was significantly different from other locations. The Thuravur series (L_6) with an activity of 11.02 µg of p-nitrophenol released g^{-1} soil h^{-1} recorded the lowest mean for acid phosphatase activity in sub surface soils. At Karappadam soils the acid phosphatase activity was 31.97 µg of p-nitrophenol released g^{-1} soil h^{-1} and Kayal lands recorded a value of 32.67 µg of p-nitrophenol released g^{-1} soil h^{-1} activity.

4.4.1.3 Alkaline Phosphatase Activity

It is obvious from the data that the locations have significant influence on the alkaline phosphatase activity of the analyzed surface and sub surface soils. The highest value was reported in the samples from Thakazhi series (L_3) with a mean value of 6.41 µg of p-nitrophenol released g^{-1} soil h^{-1} and was significantly different from other series. The lowest mean was 6.04 µg of p-nitrophenol released g^{-1} soil h^{-1} at Thuravur series (L_6) which was also significantly different from other locations. The samples of Karappadam soils recorded a value of 30.97 µg of p-nitrophenol released g^{-1} soil h^{-1} for alkaline phosphatase activity and the Kayal lands reported an activity rate of 14.77 µg of p-nitrophenol released g^{-1} soil h^{-1}.

At sub surface soils, the mean values of alkaline phosphatase activity ranged from 5.34 to 44.67 µg of p-nitrophenol released g^{-1} soil h^{-1} and among the six acid sulphate soil series the highest value was recorded Kallara series (L_2-44.67 µg of p-nitrophenol released g^{-1} soil h^{-1}) and the lowest value of 5.34 µg of p-nitrophenol released g^{-1} soil h^{-1} by Thuravur series (L_6). Both the highest and lowest values are significantly different from other locations. Comparing the alkaline phosphatase activity of Karappadam and Kayal lands, the Karappadam soils recorded higher activity of 19.60 µg of p-nitrophenol released g^{-1} soil h^{-1} than Kayal lands which reported a value of 17.23 µg of p-nitrophenol released g^{-1} soil h^{-1}.

4.4.1.4 β-glucosidase Activity

All the locations within the six acid sulphate series of Kuttanad had significant influence on the activity of β-glucosidase in the surface and subsurface soils and in the case of location effect of surface soils on particular enzyme activity, the mean value of enzyme status varied between 1.65 to 3.04 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}. The highest enzyme status among the locations was reported from the samples of Purakkad area (L_3- 3.04 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}), which was on par with Kallara (L_2-2.95 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}) and Thuravur series (L_6-2.94 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}). The lowest activity was noticed in the Thakazhi series (L_4) with a mean of 1.65 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}. The lowest mean was on par with the Ambalapuzha series (L_1- 1.67 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}). Among Karappadam and Kayal lands, both recorded an activity of 2.26 and 3.37 µg pnp D - glucosidase g^{-1} soil h^{-1} x 10^{-4} respectively.

At 15 – 30 cm soil depth, the highest β-glucosidase activity was registered by the Thuravur series (L_6 – 6.04 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}) and lowest by Thakazhi soils (L_4-3.04 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}). These two are significantly different from other locations. The other soil types Karappadam and Kayal lands recorded an activity of 1.86 and 2.59 µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4} respectively at sub surface soils.

4.4.1.5 Aryl sulphatase Activity

It is inferred from the Table 20, that the locations imposed highly significant effect on the aryl sulphatase activity of the surface soils of Kuttanad acid sulphate soil series. Regarding the

location effect, the highest mean was recorded in the Thottapalli series (L_5- 56.96 µg of p-nitrophenol released g^{-1} soil h^{-1}) and lowest in the Purakkad series (L_3-17.23 µg of p-nitrophenol released g^{-1} soil h^{-1}). Karappadam and Kayal lands registered an aryl sulphatase activity of 53.29 and 34.49 µg of p-nitrophenol released g^{-1} soil h^{-1} respectively.

While analyzing the sub surface soil samples of acid sulphate series of Kuttanad, the aryl sulphatase activity registered mean values ranged from 32.11 to 71.88 µg of p-nitrophenol released g^{-1} soil h^{-1}. Among the six locations, the highest activity was noticed at Thakazhi series (L_4-71.88 µg of p-nitrophenol released g^{-1} soil h^{-1}) while the lowest value of 32.11 µg of p-nitrophenol released g^{-1} soil h^{-1} was recorded by Purakkad series (L_3). Considering the Karappadam sub surface soil samples, which recorded a mean activity of 56.72 µg of p-nitrophenol released g^{-1} soil h^{-1} while the Kayal lands registered a mean aryl sulphatase activity of 35.57 µg of p-nitrophenol released g^{-1} soil h^{-1}.

4.4.1.6 Dehydrogenase Activity

Critical approach of the data showed that the locations affect the dehydrogenase activity of the soils varied significantly. An appraisal of the data on the influence of locations on dehydrogenase activity at surface soil depth, the Thuravur series (L_6) with a mean of 115.74 µg of TPF released g^{-1} soil 24 h^{-1} recorded the highest activity which was on par with Purakkad series (L_3-115.09 µg of TPF released g^{-1} soil 24 h^{-1}). Ambalapuzha series (L_1- 68.06 µg of TPF released g^{-1} soil 24 h^{-1}) surface samples had the lowest mean among various locations. While comparing the dehydrogenase activity of Karappadam and Kayal lands, Karappadam soils recorded an activity rate of 145.73 µg of TPF released g^{-1} soil 24 h^{-1} while the Kayal lands recorded a dehydrogenase activity rate of 114.80 µg of TPF released g^{-1} soil 24 h^{-1}.

Regarding the sub surface soil analysis records, it was observed that the highest mean of 55.39 µg of TPF released g^{-1} soil 24 h^{-1} was recorded at Kallara series (L_2) and the lowest by Thuravur series (L_6-13.19 µg of TPF released g^{-1} soil 24 h^{-1}) and both the values were significantly different from others. At sub surface soils, Karappadam soils recorded dehydrogenase activity of 21.60 µg of TPF released g^{-1} soil 24 h^{-1} and the Kayal lands had an activity of 42.53 µg of TPF released g^{-1} soil 24 h^{-1}.

Table 20. Enzyme activity in the acid sulphate soils of Kuttanad –surface soils (0-15 cm)

SOIL SERIES	Urease (ppm of urea hydrolyzed g^{-1} soil h^{-1})	Acid phosphatase (μg of p-nitrophenol released g^{-1} soil h^{-1})	Alkaline phosphatase (μg of p-nitrophenol released g^{-1} soil h^{-1})	β-Glucosidase (μg pnp D-glucosidase g^{-1} soil h^{-1} x 10^{-4})	Aryl sulpahtase (μg of p-nitrophenol released g^{-1} soil h^{-1})	Dehydrogenase (μg of TPF released g^{-1} soil 24 h^{-1})
L₁-Ambalapuzha	57.51	52.99	30.81	1.67	35.69	68.06
L₂-Kallara	64.28	35.78	20.12	2.95	43.12	96.04
L₃-Purakkad	67.54	57.58	29.93	3.04	17.23	115.09
L₄-Thakazhi	75.78	54.13	46.41	1.65	35.24	96.55
L₅-Thottapalli	59.59	42.98	20.09	2.85	56.96	92.66
L₆-Thuravur	67.53	24.59	6.04	2.94	22.67	115.74
S.E (M)	1.90	2.30	3.00	0.05	0.24	1.76
C.D(0.05)	5.37	6.52	8.51	0.14	0.69	4.99
Karappadam	52.89	45.58	30.97	2.26	53.29	145.73
Kayal	50.77	22.62	14.77	3.37	34.49	114.80

Table 21. Enzyme activity in the acid sulphate soils of Kuttanad-subsurface soils (15-30 cm)

SOIL SERIES	Urease (ppm of urea hydrolyzed g^{-1} soil h^{-1})	Acid phosphatase (μg of p-nitrophenol released g^{-1} soil h^{-1})	Alkaline phosphatase (μg of p-nitrophenol released g^{-1} soil h^{-1})	β-Glucosidase (μg pnp D-glucosidase g^{-1} soil h^{-1} × 10^{-4})	Aryl sulpahtase (μg of p-nitrophenol released g^{-1} soil h^{-1})	Dehydrogenase (μg of TPF released g^{-1} soil 24 h^{-1})
L_1-Ambalapuzha	53.57	39.13	29.77	3.40	54.19	50.67
L_2-Kallara	72.25	23.52	44.67	5.39	67.02	55.39
L_3-Purakkad	56.44	71.88	33.31	3.21	32.11	44.90
L_4-Thakazhi	52.36	51.04	25.54	3.04	71.88	33.86
L_5-Thottapalli	58.22	32.73	27.33	5.77	61.83	21.07
L_6-Thuravur	77.33	11.02	5.34	6.04	65.59	13.19
S.E (M)	3.01	3.08	4.37	0.02	0.22	0.58
C.D(0.05)	8.54	8.74	12.39	0.07	0.62	1.66
Karappadam	49.05	31.97	19.60	1.86	56.72	21.60
Kayal	58.66	32.67	17.23	2.59	35.57	42.53

Plate 1. Enzyme assay of dehydrogenase

Plate 2. Enzyme assay of urease

Plate 3. Enzyme assay of phosphatase

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4.4.2 Soil Respiratory Activity and Microbial Biomass Carbon

The effect of locations on the soil respiration and microbial biomass carbon at surface and sub surface samples of acid sulphate series of Kuttanad, is presented in Tables 22 and 23.

4.4.2.1 Soil Respiratory Activity

The data clearly indicates that the influence of locations were highly significant in the soil respiratory activity of surface samples (Table 22). Considering the effect of locations, the mean values ranged from 1.02 to 1.24 µg of CO_2 evolved g^{-1} soil h^{-1} with the highest mean at Thottapalli series (L_5-1.24 µg of CO_2 evolved g^{-1} soil h^{-1}) which was on par with the soil respiratory activity of Kallara series (L_2-1.18 µg of CO_2 evolved g^{-1} soil h^{-1}). The lowest mean was seen at Thakazhi series (L_4-1.02 µg of CO_2 evolved g^{-1} soil h^{-1}) which was on par with Purakkad series (L_3-1.06 µg of CO_2 evolved g^{-1} soil h^{-1}). Among the Karappadam and Kayal lands, the Karappadam soils was found to have respiratory activity of 1.60 µg of CO_2 evolved g^{-1} soil h^{-1} while the Kayal lands had a mean of 1.58 µg of CO_2 evolved g^{-1} soil h^{-1}.

Regarding the soil respiratory activity at sub surface soil depth, the highest respiratory activity of 1.24 µg of CO_2 evolved g^{-1} soil h^{-1} was again noticed at Thottapalli series (L_5) and the value was on par with Ambalapuzha series (L_1-1.19 µg of CO_2 evolved g^{-1} soil h^{-1}). The lowest respiratory activity was noticed at Thakazhi series (L_4) with a mean of 1.02 µg of CO_2 evolved g^{-1} soil h^{-1} which was on par with the respiratory activity of Purakkad area (L_3-1.06 µg of CO_2 evolved g^{-1} soil h^{-1}). Karappadam and Kayal lands recorded the value of 1.63 and 1.15 µg of CO_2 evolved g^{-1} soil h^{-1} respectively for respiratory activity at $15 - 30$ cm depth.

4.4.2.2 Microbial Biomass Carbon (MBC)

Comparing the effect of various locations on microbial biomass carbon content in the surface soils of acid sulphate soil series, the Purakkad soils (L_3- 357 µg g^{-1}) recorded the highest mean value and lowest by Thuravur series (L_5-43 µg g^{-1}). The mean values for microbial biomass carbon at Karappadam and Kayal lands are 406 and 390 µg g^{-1} respectively.

Considering the effect of sub surface soils samples of various locations on the microbial biomass carbon at Kuttanad acid sulphate soil series, it was observed that the mean values ranged

Table 22. Soil respiratory activity and microbial biomass carbon of acid sulphate soils of Kuttanad-surface soils (0-15 cm)

SOIL SERIES	Soil Respiratory Activity (μg of CO_2 evolved g^{-1} soil h^{-1})	Microbial Biomass Carbon ($\mu g\ g^{-1}$)
L_1-Ambalapuzha	1.20	273
L_2-Kallara	1.18	149
L_3-Purakkad	1.06	357
L_4-Thakazhi	1.02	203
L_5-Thottapalli	1.24	137
L_6-Thuravur	1.13	43
S.E (M)	0.03	4.31
C.D(0.05)	0.06	9.86
Karappadam	1.60	406
Kayal	1.58	390

Table 23. Soil respiratory activity and microbial biomass carbon of acid sulphate soils of Kuttanad-subsurface soils (15-30 cm)

SOIL SERIES	Soil Respiratory Activity (μg of CO_2 evolved g^{-1} soil h^{-1})	Microbial Biomass Carbon ($\mu g\ g^{-1}$)
L_1-Ambalapuzha	1.19	259
L_2-Kallara	1.12	131
L_3-Purakkad	1.06	100
L_4-Thakazhi	1.02	128
L_5-Thottapalli	1.24	287
L_6-Thuravur	1.13	26
S.E (M)	0.02	13.30
C.D(0.05)	0.09	29.32
Karappadam	1.63	367
Kayal	1.15	237

Plate 4. Soil respiratory activity studies

from 26 to 287 µg g^{-1}. Thottapalli series with mean value of 287 µg g^{-1} recorded highest microbial biomass carbon and was on par with the values of Ambalapuzha (L$_1$) series with mean values of 259 µg g^{-1}. The lowest value was noticed at Thuravur series (L$_6$) with a mean of 26 µg g^{-1}. The Karappadam soils recorded a mean of 367 µg g^{-1} at sub surface samples and a mean of 237 µg g^{-1} in Kayal lands.

4.4.3 Microbial Population

The population of bacteria, fungi and actinomycetes at the surface and sub surface samples of six acid sulphate soil series of Kuttanad were presented as total viable count and also among the bacteria *Thiobacillus* spp., nitrogen fixers and phosphorus solubilizers were shown in the Tables 24 and 25.

4.4.3.1 Bacteria

All the locations had a significant influence on the total count of bacterial colonies and the mean values varied from 9.13 to 9.30 log cfu g^{-1} soil. Thottapalli series (L$_5$- 9.30 log cfu g^{-1} soil) recorded the highest total bacterial count at surface soils. The lowest count was noticed at Thakazhi series (L$_4$ – 9.13 log cfu g^{-1} soil). The Karappadam soils and Kayal lands recorded an total bacterial population of 9.15 and 9.19 log cfu g^{-1} soil respectively.

Total bacterial population recorded highest count of 9.23 log cfu g^{-1} soil in sub surface level at Thottapalli series (L$_5$) while the Thakazhi series (L$_4$) with bacterial population 9.07 log cfu g^{-1} soil registered the lowest count. The highest and lowest count were on par with the Ambalapuzha series (L$_1$- 9.20 log cfu g^{-1} soil) and Purakkad series (L$_3$ – 9.11 log cfu g^{-1} soil) respectively. Among the other soil types, Karappadam soils registered a total bacterial population of 9.02 log cfu g^{-1} soil and a population of 9.18 log cfu g^{-1} soil in Kayal lands.

4.4.3.1.1 Thiobacillus spp.

From the Table 24, it is clear that the locations have significant influence on the *Thiobacillus* spp. count at surface soils. Regarding the location effect, Kallara series (L$_2$) with 9.37 log cfu g^{-1} soil registered the highest mean value which was significantly different from others. The lowest mean of 8.86 log cfu g^{-1} soil was seen at Purakkad soils (L$_3$) and was on par

with the samples from Ambalapuzha (L_1 - 9.04 log cfu g^{-1} soil), Thakazhi (L_4-8.91 log cfu g^{-1} soil) and Thuravur series (L_6 - 9.04 log cfu g^{-1} soil). Through the appraisal of the data of Karappadam and Kayal lands the population of *Thiobacillus* spp. were 8.94 and 9.02 log cfu g^{-1} soil respectively.

At sub surface soils the *Thiobacillus* spp. showed non significant effect among various acid sulphate series of Kuttanad soils.

4.4.3.1.2   Nitrogen Fixers

It was observed from the data (Tables 24 and 25) that the locations influenced the nitrogen fixers population in the surface soil significantly, however at sub surface soils the locations had no significant influence on the nitrogen fixers population. The highest mean value for location effect at surface soils was noticed by the samples from both Thottapalli (L_5) and Thuravur (L_6) series with a mean of 3.91 log cfu g^{-1} soil. The lowest mean was recorded at Ambalapuzha series (L_1 - 3.61 log cfu g^{-1} soil) and was on par with the samples from Kallara (L_2 – 3.63 log cfu g^{-1} soil) and Purakkad (L_3 – 3.70 log cfu g^{-1} soil). With respect to the analysis of the data of surface soils from Karappadam and Kayal lands, the population of nitrogen fixers were 3.81 and 3.73 log cfu g^{-1} soil respectively.

4.4.3.1.3   Phosphorus Solubilizers

The statistical analysis of the data on the count of phosphorus solubilizers inferred that the locations had significant influence on the population both at surface and sub surface soils. Regarding the analysis of the surface samples higher mean of 3.97 log cfu g^{-1} soil was recorded by the Ambalapuzha series (L_1) and was on par with Kallara series (L_2-3.96 log cfu g^{-1} soil). While the lowest count was seen at Purakkad series (L_3 - 3.72 log cfu g^{-1} soil) which was on par with samples from Thakazhi (L_4-3.83 log cfu g^{-1} soil) and Thuravur series (L_6-3.78 log cfu g^{-1} soil). At surface level the Karappadam soil recorded phosphorus solubilizers count of 3.92 log cfu g^{-1} soil and the Kayal lands registered a count of 3.82 log cfu g^{-1} soil.

The sub surface samples from the acid sulphate series of Kuttanad had a mean value ranged from 3.63 to 3.84 log cfu g^{-1} soil for the phosphorus solubilizers population. The highest count was seen at the Ambalapuzha series (L_1-3.63 log cfu g^{-1} soil) which was on par with

Kallara series (L$_2$-3.72 log cfu g^{-1} soil). The lowest population was recorded at Thuravur series (L$_3$-3.63 log cfu g^{-1} soil) which was on par with Purakkad (L$_3$-3.66 log cfu g^{-1} soil) and Thakazhi series (L$_4$-3.73 log cfu g^{-1} soil). The Karappadam soils recorded a mean of 3.71 log cfu g^{-1} soil and the Kayal lands had a population of 3.66 log cfu g^{-1} soil.

4.4.3.2 Fungi

The analysis of the data inferred that the location effects were significant and the mean values ranged from 5.38 to 6.03 log cfu g^{-1} soil at surface soil depth and from 5.23 to 5.86 log cfu g^{-1} soil at sub surface level.

The highest mean value at surface level was reported by the samples from Thottapalli series (L$_5$-6.03 log cfu g^{-1} soil) which was on par with Purakkad (L$_3$-5.98 log cfu g^{-1} soil) and Thakazhi (L$_4$-5.88 log cfu g^{-1} soil) series. Thuravur series (L$_6$) with mean of 5.28 log cfu g^{-1} soil recorded the lowest fungal population at surface soils which was on par with Kallara series (L$_2$-5.64 log cfu g^{-1} soil). Among Karappadam and Kayal lands, Karappadam soils had recorded a higher fungal population of 6.09 log cfu g^{-1} soil than the Kayal lands which registered a count of 5.97 log cfu g^{-1} soil.

At sub surface level the highest fungal population was noticed at Thottapalli series (L$_5$-5.86 log cfu g^{-1} soil). The lowest population was seen at Thuravur (L$_6$-5.23 log cfu g^{-1} soil) and the count was on par with the Thakazhi (L$_4$-5.37 log cfu g^{-1} soil), Purakkad (L$_3$-5.40 log cfu g^{-1} soil) and Kallara series (L$_2$-5.38 log cfu g^{-1} soil). The Karappadam and Kayal lands recorded the values of 5.92 and 5.88 log cfu g^{-1} soil respectivey for fungal population at sub surface soil samples.

4.4.3.3 Actinomycetes

Critical appraisal of the data shows that the locations have significant effect at sub surface soils for the actinomycetes count whereas in surface soils the count showed non significant effect. The highest mean value for location effect at sub surface was recorded by the Thuravur series (L$_6$) with mean population of 7.01 log cfu g^{-1} soil and the count was on par with Ambalapuzha (L$_1$-6.87 log cfu g^{-1} soil), Purakkad (L$_3$-7.00 log cfu g^{-1} soil) and Kallara series (L$_2$-6.82 log cfu g^{-1} soil). Thakazhi series recored the lowest count of 6.49 log cfu g^{-1} soil. The

Table 24. Microbial populations in acid sulphate soils of Kuttanad-surface soils (0-15 cm), log cfu g^{-1} soil.

SOIL SERIES	Total Bacterial Count	Thiobacillus Spp.	N Fixers	P Solubilizers	Fungi	Actino-mycetes
L_1-Ambalapuzha	9.23	9.04	3.61	3.97	5.76	6.95
L_2-Kallara	9.24	9.08	3.63	3.96	5.64	7.04
L_3-Purakkad	9.17	8.86	3.70	3.72	5.98	7.12
L_4-Thakazhi	9.13	8.91	3.81	3.83	5.88	6.96
L_5-Thottapalli	9.30	9.06	3.91	3.91	6.03	7.11
L_6-Thuravur	9.25	9.04	3.91	3.78	5.38	7.09
S.E (M)	0.02	0.07	0.06	0.04	0.09	0.05
C.D(0.05)	0.03	0.21	0.17	0.12	0.26	NS
Karappadam	9.15	8.94	3.81	3.92	6.09	7.19
Kayal	9.19	9.02	3.73	3.82	5.97	6.78

Table 25. Microbial populations in acid sulpahte soils of Kuttanad-subsurface soils (15-30 cm), log cfu g^{-1} soil.

SOIL SERIES	Total Bacterial Count	Thiobacillus Spp	N Fixers	P Solubilizers	Fungi	Actinom-ycetes
L_1-Ambalapuzha	9.20	8.98	3.54	3.84	5.54	6.87
L_2-Kallara	9.15	8.91	3.53	3.82	5.37	6.82
L_3-Purakkad	9.11	8.89	3.62	3.66	5.39	7.00
L_4-Thakazhi	9.07	8.82	3.62	3.73	5.37	6.49
L_5-Thottapalli	9.23	9.03	3.84	3.73	5.86	6.75
L_6-Thuravur	9.12	8.87	3.61	3.63	5.28	7.01
S.E (M)	0.02	0.05	0.07	0.05	0.08	0.08
C.D(0.05)	0.04	NS	NS	0.14	0.24	0.22
Karappadam	9.02	8.80	3.62	3.71	5.92	7.16
Kayal	9.18	8.94	3.60	3.66	5.88	6.72

(A)

(B)

(C)

Plate 5. Microbial load in acid sulphate soils of Kuttanad – (A) *Thiobacillus* spp. (B) N fixers (c) P solubilizers.

Karappadam soils registered an actinomycetes count of 7.12 log cfu g^{-1} soil and the Kayal lands recorded a population of 6.73 log cfu g^{-1} soil.

4.4.4 Enzyme Activity Number

Enzyme activity number, the index of biological fertility of the soils, as presented in the Tables 26 and 27, showed the mean values of the enzyme activity number for the locations vary from 14.42 to 23.69 at surface soils and from 3.49 to 12.20 at sub surface level. Regarding the surface soils of acid sulpahte series and the enzyme activity number the highest value was noticed at Purakkad series (L_3-23.69) which was on par with Thuravur series (L_6), $i.e.,$ 23.59. Ambalapuzha series (L_1-14.42) recorded the lowest value for enzyme activity number. Both Karappadam and Kayal lands recorded an enzyme activity number of 30.10 and 23.56 respectively at surface soils.

At the sub surface soil level, the highest enzyme activity number was seen at Kallara series with a value of 12.20 and the Thuravur series (L_6-3.49) recorded the lowest enzyme activity number. Both the highest and lowest mean values were significantly different from other locations. Among Karappadam and Kayal lands at sub surface soils, Kayal lands recorded a higher enzyme activity number of 9.16 than the Karappadam soils which recorded a value of 5.18.

4.4.5 Enzyme Kinetics of the Soil

Tables 28 and 29, represents the data obtained from determination of V_{max} and K_m in fortnightly intervals using various substrates for the enzymes like urease, acid phosphatase, aryl sulphatase, dehydrogenase and β-D-glucosidase.

From the Table 28, it was clear that the for enzyme urease, the mean values for V_{max} varied from 2.6 x 10^{-3} to 3.77 x 10^{-3} moles of urea hydrolyzed g^{-1} soil h^{-1}. The maximum value was reported at 8th week after incubation and lowest at 2nd week. The K_m values ranged from 0.98 x 10^{-4} to 7.2 x 10^{-4} moles of urea hydrolyzed g^{-1} soil h^{-1} with highest value at 4th week after incubation.

Table 26. Enzyme activity number of acid sulphate soils of Kuttanad-surface soils (0-15 cm)

SOIL SERIES	Enzyme Activity Number
L_1-Ambalapuzha	14.42
L_2-Kallara	19.98
L_3-Purakkad	23.69
L_4-Thakazhi	20.19
L_5-Thottapalli	19.47
L_6-Thuravur	23.59
S.E (M)	0.35
C.D(0.05)	1.01
Karappadam	30.10
Kayal	23.56

Table 27. Enzyme activity number of acid sulphate soils of Kuttanad-subsurface soils (15-30 cm)

SOIL SERIES	Enzyme Activity Number
L_1-Ambalapuzha	11.09
L_2-Kallara	12.20
L_3-Purakkad	9.89
L_4-Thakazhi	7.93
L_5-Thottapalli	5.25
L_6-Thuravur	3.49
S.E (M)	0.12
C.D(0.05)	0.35
Karappadam	5.18
Kayal	9.16

Table 28. Enzyme kinetic parameter – V_{max}

Period	V_{max}				
	Urease	Phosphatase	Aryl sulphatase	Dehydrogenase	β-D-Glucosidase
2nd week	2.6×10^{-3}	11.9×10^{-3}	1.3×10^{-3}	4.2×10^{-3}	21
4th week	3.4×10^{-3}	11.9×10^{-3}	2.7×10^{-3}	5.2×10^{-3}	34
6th week	3.0×10^{-3}	14.8×10^{-3}	2.8×10^{-3}	7.7×10^{-3}	38
8th week	3.7×10^{-3}	15.4×10^{-3}	2.9×10^{-3}	4.2×10^{-3}	12
10th week	2.9×10^{-3}	12.8×10^{-3}	1.8×10^{-3}	5.1×10^{-3}	34
12th week	1.9×10^{-3}	19.4×10^{-3}	2.8×10^{-3}	1.6×10^{-3}	150

V_{max} of Urease : ppm of urea hydrolyzed g^{-1} soil h^{-1}

V_{max} of Phosphatase : µg of p-nitrophenol released g^{-1} soil h^{-1}

V_{max} of aryl sulphatase : µg of p-nitrophenol released g^{-1} soil h^{-1}

V_{max} of Dehydrogenase : µg of TPF released g^{-1} soil 24 h^{-1}

V_{max} of β-glucosidase : µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}

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Table 29. Enzyme kinetic parameter : Michaelis – Menten constant -K_m

Period	K_m (mM)				
	Urease	Phosphatase	Aryl sulphatase	Dehydrogenase	β-D-Glucosidase
2nd week	1.92×10^{-4}	2.8×10^{-4}	1.16×10^{-5}	2.2×10^{-4}	3.95
4th week	7.2×10^{-4}	2.8×10^{-4}	1.31×10^{-5}	1.02×10^{-4}	4.20
6th week	1.95×10^{-4}	7.1×10^{-4}	1.19×10^{-5}	2.98×10^{-4}	3.90
8th week	5.8×10^{-4}	0.4×10^{-4}	$1.34 \times 10{-5}$	2.74×10^{-4}	5.9
10th week	0.98×10^{-4}	3×10^{-4}	1.23×10^{-5}	2.86×10^{-4}	2.12
12th week	2.2×10^{-4}	14×10^{-4}	1.43×10^{-5}	3.72×10^{-4}	2.74

With respect to the enzyme acid phosphatase, the V_{max} varied between 11.9 x 10^{-3} to 19.4 x 10^{-3} μg of p-nitrophenol released g^{-1} soil h^{-1} having the highest mean at 12th week after incubation and the K_m values varied from 0.3 x 10^{-4} to 14 x 10^{-4} μg of p-nitrophenol released g^{-1} soil h^{-1} and after 12th week of incubation the highest value was noticed.

Regarding the enzyme kinetic parameters of aryl sulphatase, the V_{max} was the highest after 8th week of incubation with the mean values varied from 1.31 x 10^{-3} to 2.9 x 10^{-3} μg of p-nitrophenol released g^{-1} soil h^{-1} and the lowest mean at 2nd week of incubation. The K_m values showed a variation from 1.16 x 10^{-5} to 1.43 x 10^{-3} μg of p-nitrophenol released g^{-1} soil h^{-1} with the highest mean at 12th week.

In the case of dehydrogenase enzyme, the V_{max} ranged from 1.56 x 10^{-3} to 7.79 x 10^{-3} μg of TPF released g^{-1} soil 24 h^{-1}. Among the mean values the highest was noticed after 6th week of incubation and the lowest at 12th week of incubation. With respect to K_m values, the means ranged between 1.02 x 10^{-4} to 3.72 x 10^{-4} μg of TPF released g^{-1} soil 24 h^{-1} with the highest mean of 3.72 x 10^{-4} μg of TPF released g^{-1} soil 24 h^{-1} at 12th week after incubation.

It is inferred from Table 28, that the V_{max} value for glucosidase varied from 12 to 150 μg pnp D- glucosidase g^{-1} of soil h^{-1} x 10^{-4} with the highest mean at 12th week and the lowest at 8th week. The K_m values ranged from 2.12 to 5.9 μg pnp D- glucosidase g^{-1} of soil h^{-1} x 10^{-4} and the highest K_m was noticed at 8th week after incubation and the lowest at 10th week.

4.5 CORRELATION STUDIES

Correlation of chemical characteristics and biological characteristics were determined for surface and sub surface soils and the correlated values are presented in Tables 30 to 33 respectively.

From the correlation matrix (Table 30.) it was clear that the surface soil pH was positively correlated with the availability of nitrogen, phosphorus and potassium, activity of enzymes like acid phosphatase, aryl sulphatase and also with the biomass of fungi in the soil. Among these, the correlation of acid phosphatase enzyme status with soil acidity was highly significant compared to others (0.8060*). The soil properties including status of urease, dehydrogenase, β -glucosidase and the biomass of bacteria and actinomycetes recorded negative

Table 30. Correlation studies of the chemical and biological properties of acid sulphate soils of Kuttanad- surface soils (0-15 cm)

	Soil Acidity	N	P	K	Urease	Acid phos.	Gluco-sidase	Aryl sulpahtase	Dehydro-genase	Bacteria	Fungi	Actino-mycetes
Soil Acidity	1											
N	0.061	1										
P	0.419	-0.204	1									
K	0.778	0.189	-0.049	1								
Urease	-0.024	-0.355	-0.280	-0.192	1							
Acid phos.	0.806*	-0.509	0.397	0.590	0.089	1						
β-Gluco-sidase	-0.556	0.218	-0.883*	0.049	-0.123	-0.501	1					
Aryl sulphatase	0.391	0.842*	0.054	0.293	-0.437	-0.092	-0.095	1				
Dehyd-rogenase	-0.604	-0.274	-0.837*	-0.276	0.573	-0.333	0.694	-0.542	1			
Bacteria	-0.392	0.632	-0.151	-0.187	-0.772	-0.591	0.422	0.569	-0.189	1		
Fungi	0.722	-0.111	-0.018	0.651	-0.065	0.791	-0.128	0.329	-0.195	-0.173	1	
Actino-mycetes	-0.562	0.025	-0.848*	-0.060	-0.111	-0.355	0.932	-0.129	0.719	0.414	0.057	1

Table 31. Correlation studies of the chemical and biological properties of acid sulphate soils of Kuttanad-subsurface soils (15-30 cm)

	Soil Acidity	N	P	K	Urease	Acid phos.	Gluco-sidase	Aryl sulp-ahtase	Dehyd-rogenase	Bacteria	Fungi	Actino mycetes
Soil Acidity	1											
N	0.072	1										
P	0.105	-0.201	1									
K	0.357	0.702	0.186	1								
Urease	-0.782	0.422	-0.127	0.231	1							
Acid phos.	0.558	-0.548	0.709	-0.134	-0.773	1						
Gluco-sidase	-0.700	0.613	-0.367	0.137	0.797	-0.838*	1					
Aryl sulphatase	0.046	0.685	-0.653	0.200	0.317	-0.690	0.421	1				
Dehyd rogenase	0.616	-0.003	0.323	0.686	-0.279	0.391	-0.521	-0.309	1			
Bacteria	-0.026	0.222	-0.512	0.107	-0.059	-0.325	0.406	-0.011	0.011	1		
Fungi	0.235	0.195	-0.284	-0.056	-0.429	0.041	0.182	-0.041	-0.146	0.820*	1	
Actino mycetes	-0.729	-0.397	-0.206	-0.156	0.488	-0.129	0.286	-0.608	-0.014	0.149	-0.214	1

Table 32. Correlation between organic carbon content and enzyme activities of the acid sulphate series – surface soils (0-15 cm)

	O.C	Urease	Acid phosphatase	Alkaline phosphatase	β-Glucosidase	Aryl sulpahtase	Dehydro genase
O.C	1						
Urease	-0.351	1					
Acid phosphatase	-0.513	0.089	1				
Alkaline phosphatase	-0.338	0.407	0.864*	1			
β-Glucosidase	0.203	-0.123	-0.501	-0.719	1		
Aryl sulphatase	0.842*	-0.437	-0.092	0.008	-0.095	1	
Dehydrogenase	-0.282	0.573	-0.333	-0.352	0.694	-0.541	1

*.- Significant

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Table 33. Correlation between organic carbon content and enzyme activities of the acid sulphate series – sub surface soils (15-30 cm)

	O.C	Urease	Acid phosphatase	Alkaline phosphatase	β-Glucosidase	Aryl sulpahtase	Dehydro Genase
O.C	1						
Urease	0.427	1					
Acid phosphatase	-0.544	-0.773	1				
Alkaline phosphatase	0.334	-0.289	0.381	1			
β-Glucosidase	0.615	0.797	-0.838*	-0.291	1		
Aryl sulphatase	0.682	0.317	-0.690	-0.218	0.421	1	
Dehydrogenase	-0.013	-0.279	0.391	0.858*	-0.521	-0.309	1

* -Significant

correlation with soil acidity. Intercorrelation among other characters showed that aryl sulphatase activity had a negative significant correlation with availability of nitrogen in surface soils (0.8416*). The activity of β-glucosidase (-0.8834*), dehydrogenase (-0.8371*) and actinomycetes population (-0.8484*) had a negative significant correlation with phosphorus availability of the surface soils.

The correlation matrix for sub surface soil pH with chemical and biological characteristics at sub surface (Table 31) clearly depicts that the soil pH was positively correlated with availability of nitrogen, phosphorus and potassium, activity of acid phosphatase, aryl sulphatase and dehydrogenase and also with the fungal population. Soil pH showed negative correlation with urease and β-glucosidase activity along with population of bacteria and actinomycetes in the sub surface soils. Also the β-glucosidase (-0.8375*) has recorded a significant correlation with acid phosphatase activity of sub surface soils.

From Tables 32 and 33, it was clear that the organic carbon content of the surface and sub surface soils of acid sulphate series in Kuttanad had recorded positive correlation between organic carbon content and activities of β-glucosidase and aryl sulphatase in surface soil and in sub surface level also a positive correlation exists between these parameters along with correlation between organic carbon and alkaline phosphatase activity. Among this, aryl sulpahatse at surface soils had recorded a positive significant correlation with organic carbon content.

4.6 INCUBATION STUDY

From the Table 34, it was clear that the surface and sub surface soils from six acid sulphate soil series recorded a drastic drop in soil pH after three months of incubation at 37 ° C in the laboratory. All the soils recorded a drop in pH of more than 0.5 units and that confirmed the acid sulphate condition in the study area.

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Table 34. Incubation study of acid sulphate soils – surface (0-15 cm) and subsurface soils (15-30 cm)

SOIL SERIES	pH of surface soils		pH of subsurface soils	
	Initial	Final*	Initial	Final*
L$_1$-Ambalapuzha	4.45	3.09	4.40	3.12
L$_2$-Kallara	4.18	2.24	4.22	2.21
L$_3$-Purakkad	4.07	3.15	4.04	3.02
L$_4$-Thakazhi	4.67	2.98	4.33	3.02
L$_5$-Thottapalli	4.09	2.93	4.04	2.92
L$_6$-Thuravur	2.43	1.51	2.89	1.33

* After three months of incubation at room temperature.

4.7 PREPARATION OF GIS MAPS BASED ON ENZYME STATUS AND MICROFLORA OF THE ACID SULPHATE SOILS

From the prepared GIS maps, it was evident that the highest urease activity was seen in Thakazhi series at surface layers. In Thakazhi series, around 22.97 ha area exhibits the highest urease activity while in the subsurface soils, Thuravur recorded highest activity in 18.25 ha area. Acid phosphatase activity was highest at Purakkad series both in surface and subsurface layers and the area which recorded highest acid phosphatase activity was approximately 7.02 and 11.21 ha respectively. Regarding dehydrogenase activity, Kallara and Thuravur series recorded the highest activity among all the six locations. Considering the microbial population from the GIS maps, the total bacterial count and the fungal population have recorded the highest population range in Thottapalli series (surface soils) and the highest range was spread over an area of nearly 15.26 ha and 22.32 ha respectively.

Discussion

5. DISCUSSION

The present investigation "Enzyme characterization of the acid sulphate soils of Kuttanad" was envisaged during the year 2014-16. Kuttanad popularly known as the rice bowl of Kerala, located in the central Kerala, is a large wetland habitat comprising of paddy fields, marshes, lakes and rivers. It is separated from the Arabian Sea by a strip of land and is a deltaic formation of four river systems namely Meenachil, Pampa, Manimala and Achenkovil together with low lying areas in and around the Vembanad lake.

The puncha lands of Kuttanad are classified under three categories based on elevation, geographical formation and soil characteristics into Karappadam, Kayal lands and Kari lands. The acid sulphate soils of Kuttanad region are highly complex and characterized by the acid sulphate mineral, "Jarosite" in the sub surface layer. This reflects the unique soil properties which are entirely different from the other soils of Kerala. Hence this study in the acid sulphate soil series *viz.*, Ambalapuzha, Purakkad, Thottapalli, Kallara, Thakazhi and Thuravur along with Karappadam and Kayal land soil samples for data generation and general comparison was carried out to characterize the physical, chemical and biological parameters and the results obtained are discussed in the following paragraphs.

5.1 PHYSICAL PROPERTIES OF THE SOIL

The physical properties like bulk density, particle density and water holding capacity of the soil revealed significant difference among the locations. With respect to bulk density, as observed from the Tables 5 and 6, significant difference was observed between the locations while the effect of locations on water holding capacity of the soils was non significant. Even though the water holding capacity was non significant, most of the locations recorded a mean value of 48 per cent and the highest mean value was seen at Kayal lands with 53 per cent. The high water

Fig. 2. Physical properties of acid sulphate soils of Kuttanad–surface (0-15 cm).

Fig. 3. Physical properties of acid sulphate soils of Kuttanad –subsurface (15–30 cm)

holding capacity might be attributed to the clay content of the locations. Beena (2005) also reported that the texture of the acid sulphate soils in Kuttanad showed varied textural properties from sandy clay to clayey texture with high clay content. The highest value for bulk density was noticed in the Ambalapuzha series in surface samples and Purakkad series at sub surface level. From the study it is observed that the bulk density values were generally low compared to the optimum bulk density of 1.33 Mg m^{-3}, which might be attributed to the high organic carbon content of 1.93 per cent and 1.01 per cent respectively. Similar findings were reported by Thampatti and Jose (2000).

With respect to particle density, a similar trend was observed as inferred from the Figs. 2 and 3. It is observed that the effect of locations were dominant in determining the particle density of the soil and the highest value was recorded in Ambalapuzha and Purakkad series for surface and sub surface soils respectively. The role of organic matter in influencing the bulk density and particle density cannot be ignored despite of the fact that particle density is dependent upon the soil mineral characteristics. This is revealed by the higher values for particle density at Kainakary (Kayal lands) where the organic carbon content was 3.13 per cent and 2.68 per cent at surface and sub surface depth respectively. The influence of organic carbon on soil particle density was earlier reported by Usha (1995).

5.2 ELECTROCHEMICAL PROPERTIES

Acid sulphate soils are well known groups of problem soils containing iron sulphide and generating sulphuric acid on oxidation. But these soils are usually manageable in waterlogged conditions. One of the important impacts of excess acidity in the Kuttanad soils is the iron toxicity (Neue *et al.*, 1998). Under the conditions prevailing in the acid sulphate soils silicate minerals in them disintegrate and weather, thereby releasing iron and aluminium. From the present study it is inferred that both the surface and sub surface samples from the locations had

significant effect on soil pH. All the soil samples showed a pH below 4.5 except Thakazhi soils in 0-15 cm soil depth. The high subsurface soil acidity is also one of the reasons for the extreme acidity in Kuttanad surface soils. The higher values of pH reported in the Thakazhi soils might be due to the presence of water in the fields during the april-may months due to summer rains. The weather parameters for the study area during the year 2014 is presented in the Appendix 1, which provides an insight of rainfall in the area at sample collection periods. Exposure of the surface layers to air through drainage or evacuation where the iron sulphides in the soils react with oxygen and water to produce iron compounds and sulphuric acid lead to the increase in acidity in the area during dry seasons. This acid might release heavy metals from the soils pose damages to the ecological balance. Similar findings were reported by Hinwood *et al.* (2006). Soil acidity has been a major constraint to crop production in general and this acid sulphate soils in particular. The low pH or high acidity is also associated with the toxic range of iron availability in these soils as observed from Tables 14 and 15. These findings corroborated with the findings of Benham (1997) who reported that the higher acidity might be due to the production of sulphuric acid and other organic acids. Increasing acidity in an acid sulphate soil profile is harmful for crop production especially paddy as reported by Cook *et al.* (2006).

Indepth analysis for pH indicates that sub surface soils of Kallara, Thuravur and Kayal lands recorded higher pH than surface soils in present study. The results can be attributed to less exposure or no exposure of subsoil layers to air. A similar trend, of an increase in pH with depth of soil profile was reported by Ajwa *et al.* (1998); Sahu *et al.* (2001); Deenik and Yost (2006) and Kumar and Kishan (2012).

With respect to the electrical conductivity (EC) values significant variations were observed among the different locations of the Kuttanad soils. The highest EC value of 7.52 dS m^{-1} and 6.53 dS m^{-1} were noticed in surface and subsurface soils of

Thuravur series. Similar findings were reported by Ara *et al.* (2013) in a field study on acid sulphate soils. Beena (2005) also reported that among the six acid sulphate series of Kuttanad, Thuravur series had highest EC values. Ramakrishnan *et al.* (1998) reported an EC of 11.5 dS m^{-1} in acid sulphate soils of Kerala. Khan *et al.* (2002) also reported the EC values of an acid sulphate soils to be in the range 10-20 dS m^{-1}. This might be attributed to the high content of salts in the soil due to sea water intrusion in the summer months. Among two depths, the sub surface layer soils belonging to Thakazhi, Karappadam and Kayal lands reported higher EC values than surface soils. Thampatti (1997) also reported the same increasing trend of salinity with increasing depth.

5.3 ORGANIC CARBON AND ORGANIC MATTER CONTENT

Large amounts of woody matter at various stages of decomposition occur embedded in acid sulphate soils. In acid sulphate soils, organic matter is very crucial because in addition to being a source of plant nutrition, organic matter is the major source of negative charge which is important for helping the soil to adsorb cations in the soil solution (Ponnamperuma, 1982).

From the present investigation it is observed that the content of organic carbon in soils is high irrespective of the locations. Various locations have a significant effect on the organic carbon and thereby organic matter content of the soils. The Kallara series reported highest organic carbon and organic matter content of 3.01 and 5.18 per cent respectively at 0-15 cm depth among different locations. At sub surface soils also Kallara series (L_2-2.96 per cent) recorded the highest organic carbon content. The lowest organic carbon and organic matter content were noticed in the Purakkad series (1.28 and 2.20 per cent respectively). With negative charge, which is provided by carboxyl compounds, organic matter is able to minimize toxicity by decreasing the solubility of heavy metals in soil solution. Since the Kuttanad region is influenced by the runoff water during monsoon and brackish

water in other seasons, the land gets fertilized by the organic materials deposited, thereby resulting in high organic matter deposition in the acid sulphate soils. The reason for the increased organic carbon and organic matter content in acid sulphate soils of Kuttanad can also be attributed mainly to the inherent characteristics of the Kuttanad soils due to the accumulation of organic debris from the surrounding rivers. This might be also due to the luxuriant growth and decaying of the macrophytes in the fields. Similar findings were also reported by Kannan *et al.* (2014) from the Kuttanad soils and reported that the organic carbon content ranges from 2.79 to 7.70 per cent. Earlier Sylas *et al.* (2010) also noticed that the organic carbon content is enriched during pre-monsoon season.

Several other scientists observed same status of organic carbon content in Kuttanad acid sulphate soils. Gong and Xu (1990); Neue *et al.* (1998); Lal (2002) and Tanji *et al.* (2003) also reported the large deposits of organic matter in paddy soils via organic fertilizers and plant residues. Waterlogging associated with rice cropping might have enhanced the accumulation of organic carbon. The highest organic matter of 5.18 per cent reported in Kallara soils might be also due to enrichment of weed biomass and paddy straw in the cultivated fallows (Fores and Comin, 1987; Pillai and Subrahmanyan, 1929). This finding is in agreement with the reports of Beena (2005). The results also corroborated with the reports of Usha (1995) and Marykutty (1986), who reported that among the acid sulphate soils of Kuttanad, Kallara (L_2) had the highest organic matter content.

Indepth analysis of surface soils reported a higher organic matter content and organic carbon content than the sub surface soils in soil samples of most locations. This might be due to low organic matter input, low microbial activity and reduced exposure to sunlight with increasing depth. This finding corroborated with the finding of Krishnan *et al.* (2012), who observed that the soil organic carbon content showed a decreasing trend with increase in depth.

Fig. 4. pH of the acid sulphate series of Kuttanad–surface and subsurface soils.

Fig. 5. Organic carbon content of acid sulphate soils–surface and subsurface soils.

5.4 AVAILABLE NUTRIENT STATUS

In the present study, the available nitrogen status varied from 296.94 to 674.02 kg ha^{-1} in surface soils which shows that most of the soils belong to the medium to high nitrogen range. The highest available nitrogen status was observed in Kallara series and lowest in Purakkad series. The highest available nitrogen in Kallara soils might be due to its high organic carbon and organic matter content of this locality as noticed in Tables 9 and 10. Similar report of high nitrogen availability in Kallara series among the acid sulphate series was published by Beena (2005). In Kuttanad after harvesting, approximately 30 per cent of the straw remains in the field which later decomposes to release the nutrients like nitrogen, phosphorus and potassium (Fores and Comin, 1987). The presence of aquatic weeds water hyacinth and Salvinia in aquatic system might have also contributed to the organic carbon status thereby increasing the available nitrogen status upon mineralization.

Acid sulphate soils commonly encounter relatively severe mineral stress. The H^{+} associated with soil acidity has indirect effects on mineral elements in low pH soils, so that deficiencies of phosphorus, calcium, magnesium and potassium commonly appear (Clark *et al.*, 1999). The decaying of organic materials especially the paddy straw and aquatic plant debris increases the organic matter content. Also the absence of sea water intrusion and reduction in outflow due to Thaneermukkom barrage has helped in the accumulation of organic materials in the wetlands of Kuttanad which in turn resulted in a static pool of nutrients within the system.

Regarding the subsurface soils, the highest available nitrogen status was reported in Kallara series soils and lowest under Purakkad samples. This might also be due to the of highest organic carbon content (2.96 per cent) present in both Kallara series and the surface layers. On comparing the two depths in the present study, it was observed that in general, a higher available nitrogen content existed in surface soils (528.43 kg ha^{-1}) than the subsurface soils of Kuttanad region. This

might be due to the high organic carbon and organic matter content in the surface soils as noticed from the Tables 7 and 8. Similar results of depth wise distribution of available nitrogen was also reported by Gill *et al.* (1999) and Kido *et al.* (2010). The high status of available nitrogen in general in these soils might also be attributed to the high mineralization rates and as well as the rich organic pools.

Phosphorus availability is usually restricted in acid sulphate soils as added phosphorus is strongly adsorbed (Tables 11 and 12). Like other nutrients, phosphorus availability also influenced by soil pH. From the present investigation it is observed that phosphorus availability ranged from 9.33 to 78.60 kg ha^{-1} at 0-15 cm depth of soil. It is inferred that the soils showed a wide range of available phosphorus. The results are contradictory to the general trend of phosphorus availability in acid sulphate soils. But this phosphorus values are in consent with the reports of Rajasekharan *et al.* (2013), who reported that the present phosphorus status of Kerala is high to extremely high (35 to 100 kg ha^{-1}) in 61per cent of the selected samples from all the districts. High phosphorus levels of soils are usually attributed to over fertilization or addition of enormous quantity of manures. Repeated application of rock phosphate might have caused the accumulation of residual phosphorus in the soils. Ray *et al.* (2014) also reported available phosphorus ranged from 7.14 to 129 kg ha^{-1} in the acid sulphate soils of Kerala.

Available potassium is one of the important plant nutrients especially for the rice crop. Generally potassium deficiency in wetland soils is less compared to nitrogen and phosphorus. From the present study, it is observed that the variations in locations influenced the availability of potassium significantly. Among six locations, Kallara soils reported the highest potassium availability of 148.49 kg ha^{-1} and 154.14 kg ha^{-1} at surface and sub surface soil depths respectively. The available potassium values varied widely among the samples and have a range of 65.40 to 148.49 kg ha^{-1} at surface level. This might be due to high incorporation of paddy

Fig. 6. Available NPK status of the acid sulphate soils of Kuttanad–surface
(0-15 cm).

Fig. 7. Available NPK status of acid sulphate soils of Kuttanad–subsurface
(15-30 cm).

straw and organic matter deposition in the region. Saline water intrusion also contributed to the high potassium availability in the Kuttanad area. The findings corroborated with findings of Ponnamperuma (1982), who reported that the available potassium content in the Kuttanad region may be due to addition of organic manures and amendments in the soils. The low values in the Thuravur series both at surface and sub surface soils might be due to the low pH values (Tables 7 and 8). Beena (2005) also reported similar trend in available potassium content of Kuttanad soils. The author reported that the available potassium content of Kuttanad soils ranged from 142.1 to 326.4 mg kg^{-1}.

The available calcium content of the acid sulphate soils of Kuttanad at 0-15 cm depth varied from 634.80 to 912.80 mg kg^{-1} (Fig. 8). The location effect was significant both at surface and sub surface soils. Among the six acid sulphate soil series, Kallara series reported the highest available calcium content of 912 mg kg^{-1} and the lowest by Thuravur series at surface level. The result corroborated with the findings of Beena (2005) who reported the similar trend of available calcium content among the acid sulpahte soils of Kuttanad. This might be due to the deposition of marine sediments and the influence of sea water, use of soil amendments like lime which might have contributed for the fair content of available calcium in the acid sulphate soils. As opined by Thampatti (1997), generally acid sulphate soils never deficient in available calcium and the calcium reserves are contributed by its marine origin. The author also had reported an available calcium range of 370-1400 mg kg^{-1} in acid sulphate Kuttanad soils.

Regarding the depth wise distribution of available calcium in acid sulphate soils, subsurface samples registered higher calcium content than the surface soils in all the acid sulphate series. This might be due to the downward movement of water along with the salts present in the surface layers. The results are in corroboration

with the finding of Thampatti (1997) who reported higher levels of calcium content in lower profile layers.

Available magnesium content in the Kuttanad soils at 0-15 cm depth as seen in Fig. 8, ranged from 446.10 to 669.20 kg ha^{-1} and the highest and lowest magnesium content was registered by the Purakkad series and Thottapalli soils respectively. The same reason for calcium availability can also be attributed to the magnesium content in acid sulphate soils. The magnesium reserves in these soils were contributed by the sea water intrusion and liming practices in the region (Thampatti, 1997). The present investigation reflected the findings of Beena (2005), who reported approximately the same range of available magnesium content (486-674 kg ha^{-1}) in the acid sulphate soils of Kuttanad.

In the Kerala scenario, the soils which reported to have an available magnesium content of more than 120 mg kg^{-1} falls under sufficiency range (KAU, 2011). By analyzing the data of magnesium availability in the present investigation, it is noticed that all the soils fall in the sufficiency range. In the depth wise distribution of available magnesium in the Kuttanad soils, the subsurface soils registered a higher value than the surface soils in all locations of acid sulphate soils. This might be attributed to the peculiar hydro-physiography prevalent in the Kuttanad region which aids in the faster water movement in soils and thereby aids in leaching of salts to the lower layers.

In acid sulphate soils, the major source of acidity is the free sulphuric acid formed by the oxidation of sulphur compounds present in the sulfidic materials in the soil. This high available sulphur content may be attributed to the presence of sulphuric horizon within the profiles which is harmful to crops (Simon and Jacob, 2012). In the present investigation, the sulphur availability in acid sulphate soils of Kuttanad was significantly influenced by locations at surface soils and at 15-30 cm depth the available sulphur content showed a non significant effect. The mean
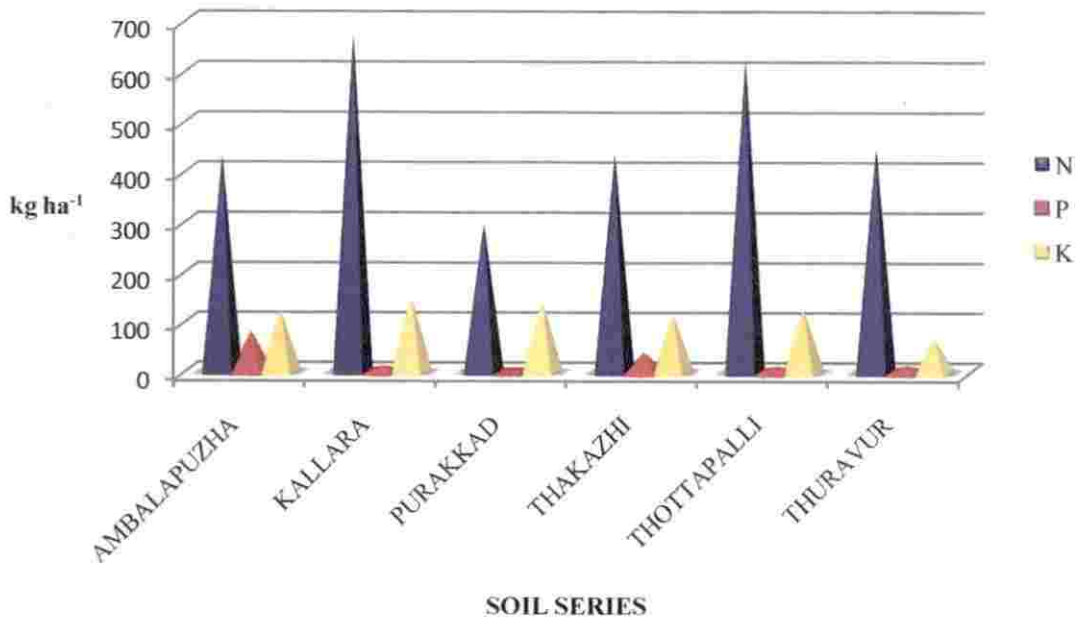
Fig. 8. Available secondary nutrient status of the acid sulphate soils of Kuttanad–
surface (0-15 cm).

Fig. 9. Available secondary nutrient status of acid sulphate soils of Kuttanad –
sub surface (15-30 cm).

content ranged from 286.77 to 883.86 kg ha^{-1} at surface soils. The highest sulphur availability was noticed in Thottapalli series and lowest in Thakazhi area. Beena and Thampatti (2013) also reported higher sulphur availability of around 1000 kg ha^{-1} in the acid sulphate soils. The sulphuric acid released from the sulphuric horizon is attributed to this higher availability of sulphur in these acid sulphate soils. The higher available sulphur content may be also attributed to the presence of H$_2$S in the paddy soils, which can be toxic to crops (Yoshida, 1975; Van Bermeen, 1982). This finding corroborated with the findings of Kames (1973), who reported adverse effects of sulphide toxicity in the acid sulphate soils which was associated with decreased crop yields. The presence of high available sulphur might be due to the accumulation of sulphidic sediments under waterlogged conditions where there is a supply of sulphate, i.e., the higher available sulphur content can be associated with the origin of the acid sulphate soils itself. Acid sulphate soils originated when sea level rose and inundated the land, and sulphate from seawater mixed with iron oxides in sediments, allowing microorganisms to form iron sulphides (Fitzpatrick *et al.*, 2009).

The higher value of sulphur content was noticed in the subsurface soils than the surface samples except in Thottapalli and Kallara series in the present study which can be attributed to the presence of sulphuric horizon in the subsoil layers (Van Bermeen, 1982).

5.5 AVAILABLE MICRONUTRIENT STATUS IN SOIL

Generally in Kuttanad soils, concentration of Fe and Mn are in excess levels which causes deterioration of soil health (Thampatti *et al.*, 2005). Nair and Pillai, (1990) also suggested the presence of excess quantities of Fe and Mn in the Kuttanad soils.

Fig. 10. Available iron content in the acid sulphate soils of Kuttanad – surface and sub surface soils.

While analyzing the micronutrient status from the present investigation, available Fe content was in excess quantity, *i. e.,* at surface soil depth the available Fe content varied from 145.78 to 1024.23 mg kg^{-1}. That implies the higher concentration of available iron in the Kuttanad soils. Kabeerathumma and Patnaik (1978) also reported that there is a tenfold or more concentration of available iron compared to the total amount of other redox elements. Thampatti and Jose (2000) also reported that the total Fe content varied from 2.75 to 7.72 per cent. The toxicity of iron might be due to reduction of iron under conducive conditions caused by the high ground water table in the wet season as reported by Raju (1988). Fe toxicity in acid sulphate soils was also reported by Elisa *et al.* (2011) and Azman *et al.* (2014), with a mean of 525 kg ha^{-1} (surface) and 284.7 kg ha^{-1} (sub surface).

The problems of Fe toxicity is often encountered in these soils since the initial increase in Fe content due to submergence is maintained throughout the period of flooding (Thampatti and Jose, 2006). The reduction of Fe^{3+} to Fe^{2+} and thereby iron content is more dominant than Mn beyond 20 mg kg^{-1}, thereby toxicity of Fe has been reported.

Excess availability of Mn in Kari soils of Kuttanad is yet another factor that limits the crop production in this area. From the present study, it is inferred that the Mn content in the Kuttanad soil ranges from 2.57 to 31.69 mg ha^{-1} among the surface samples of six various locations. The highest available Mn content is reported in the Thakazhi series and lowest in the Ambalapuzha soils. Earlier Rajendran and Iyer (1981) also reported that the total Mn content varied from 28 to 350 mg kg^{-1} in Kuttanad soils. Thampatti and Jose (2006) also reported similar range of available Mn content in the Kuttanad soil, *i.e.,* available Mn content of 1.008 to 13.05 mg kg^{-1}. Compared to available Fe, Mn availability is negligible in these soils. Considering the depth wise distribution, the content of available Mn showed a variation among locations. All others acid sulphate series registered higher quantity of available Mn

in subsurface soils while Purakkad and Thakazhi series reported highest content in surface soils. This might be due to Mn retention in the organic rather than the inorganic colloids. Similar report was put forth by Ilori and Shittu (2015), who opined that Mn distribution decreases down the depth in most of the profiles. Dhaliwal and Singh (2013) also reported that the magnitude of soil fertility parameters and thereby micronutrients generally decreased with depth in profile. Jurjovee *et al.* (2002) earlier reported that the sources of Mn may be either carbonate minerals or substitution from aluminosilicate minerals.

From the present investigation in Kuttanad soils, available Zn content of 4.88 to 19.56 mg kg^{-1} was noticed in surface samples. Lowest available Zn content was registered by Purakkad series while highest by Thurvaur soils. Compared to Fe and Mn availability, available Zn content is low in these soils. In acid sulphate soils, at a pH range of 4-5, the available Zn concentration was controlled by desorption from ferrihydrite or dissolution of ferrihydrite and release of co-precipitated Zn from within the ferrihydrite structure (Jurjovee *et al.*, 2002). The findings are in agreement with Panhwar *et al.* (2014) who reported that the available Zn and Cu content were low in acid sulphate soils compared to high extractable Fe content. Similar results were reported by Zhan-jun *et al.* (2014)

The same trend as seen in available Zn concentration is also noticed in the case of copper availability. Available copper content is highest in surface samples generally than the subsurface soil in the present study and it might also be due to the increase in pH with increasing depth. Similar results of high available copper content in surface soils was also reported by Shittu (2008). Among the various locations, highest value of 26.21 mg kg^{-1} was recorded by Ambalapuzha series and lowest by Thuravur area (1.68 mg kg^{-1}) which shows that copper is adequately distributed in the soils. Most of the copper in acid sulphate soils were found in the residual fraction, and are generally less bioavailable.

Fig. 11. Available micronutrient status of the acid sulphate soils of Kuttanad – surface (0-15 cm).

Fig. 12. Available micronutrient status of the acid sulphate soils of Kuttanad– sub surface (15-30 cm).

Generally acidic soils are rich in available boron content, but in Kerala due to high rainfall leaching losses occurs and thereby boron deficiencies can be seen in some areas. In the present study the available boron content reported a range of 6.07 to 13.45 mg kg^{-1} among six locations of acid sulphate soils at 0-15 cm depth. Among these locations the highest available boron content was reported from the Kallara series and lowest by Thakazhi series. The highest available B content at Kallara might be due to high organic carbon content in the area which minimizes the leaching of B. Depth wise analysis revealed higher available boron content in the surface soils in general than the subsurface samples. These findings can be attributed to the decrease in organic carbon, iron and aluminium content with increase in depth of the soil profile. The result corroborated with the report of Sarkar *et al.* (2005) which reported that the boron content showed a decreasing trend with increasing soil depth. Decrease in extractable boron content with increasing depth was also observed by Mandal & De (1993) in acidic soils.

5.6 NH$_4$OAc EXTRACTABLE SODIUM CONTENT OF THE SOIL

Generally the NH$_4$OAc extractable sodium content is high in acid sulphate soil series. Higher concentration of acidic and basic metals were typically due to the ionic composition of the actual acid sulphate soils (Hartikinen and Yli-Halla, 1986).

In the present study the NH$_4$OAc extractable sodium content ranged from 140.02 to 408.53 kg ha^{-1} in surface soils with the highest content at Purakkad area. Sub surface soils recorded an available sodium content of 139.44 to 392.21 kg ha^{-1} and Thuravur series registered highest content at 15-30 cm depth of soil. This shows the variation in sodium content in acid sulphate soils of different locations and this might be attributed to the seasonal and environmental effects experienced in the area. The available sodium content at high rate in these soils is evidently due to the impact of saline water intrusion in the area. Many scientists have reported varying concentrations of sodium in the acid sulphate soils. Kawahigashi *et al.* (2012) also

reported an available sodium content of 0.1 to 0.5 cmol [(+)] kg^{-1} in acid sulphate soils and again Ara *et al.* (2013) reported sodium availability of 9.14 cmol [(+)] kg^{-1} in acid sulphate soils.

5.7 ACIDITY PARAMETERS OF THE SOIL

Despite of the general uniform appearance of acid sulphate soils, there is a considerable variation in the amount of existing acidity. In the present investigation acidity parameters like exchangeable aluminium, exchangeable hydrogen and exchangeable acidity were determined, since acidity is one of the major yield limiting factor in the acid sulphate soils.

Aluminium toxicity is widely considered as the most important limiting factor for crop growth in acid sulphate soils (Panhwar *et al.*, 2015). From the present investigation, it was observed that the exchangeable aluminium in the Kuttanad region ranged from 0.18 to 5.45 cmol [(+)] kg^{-1} at surface soils. A wide variation among six locations was noticed and the highest exchangeable aluminium content was reported from Thuravur series while lowest from Thakazhi soils. This might be due to the aluminium rich parent material in acid sulpahte soils as opined by Beena (2005). The author also reported that the exchangeable aluminium in Kuttanad soils were ranged from 0.98 to 6.12 cmol [(+)] kg^{-1}. In this study, surface soils registered high exchangeable aluminium than subsurface samples except in Thakazhi and Thottapalli series, which can be attributed to the increasing pH with soil depth and low organic matter content in the subsoil layers (Fig. 5).

Exchangeable hydrogen contributes to the exchangeable acidity of the soils (Beena, 2005). From the present investigation, it was noticed that the exchangeable hydrogen vary widely among different locations in the study area within a range of 0.69 to 14.06 cmol [(+)] kg^{-1}. The presence of exchangeable hydrogen might be due to the rupture or breakage of silicate clay minerals and release of aluminium at low pH.

Fig. 13. Acidity parameters of acid sulphate soils of Kuttanad- surface (0-15 cm).

Fig. 14. Acidity parameters of acid sulphate soils of Kuttanad-subsurface (15-30 cm).

It can also be attributed to the dissolution of organic carbon and also due to the presence of clay fractions in the soil. Beena (2005) also reported that the exchangeable hydrogen vary significantly among different locations and the mean values ranged from 0.94 to 5.02 cmol $^{(+)}$ kg^{-1}. The depth wise analysis of exchangeable hydrogen in acid sulphate soils in general, reported higher values in surface soils than subsurface soils.

In the present study, the exchangeable acidity of the Kuttanad soils ranged from 1.67 to 19.52 cmol $^{(+)}$ kg^{-1} at 0-15 cm depth and from 1.45 to 12.68 cmol $^{(+)}$ kg^{-1} at 15-30 cm soil depth. This higher exchangeable acidity is contributed by both exchangeable aluminium and exchangeable hydrogen. Similar report was given by Beena *et al.* (2007) on the exchangeable acidity in the range of 1.23 to 8.1 cmol $^{(+)}$ kg^{-1} in Kuttanad soils. With regard to the depth wise comparison, generally exchangeable acidity also showed higher values in surface soils than sub surface soils.

5.8 EXCHANGEABLE CATIONS IN SOIL

In the present investigation all the exchangeable cations show highest content at Purakkad area at surface. At sub surface soils, both exchangeable calcium and magnesium recorded highest content for same series, *i.e.,* Thakazhi while the exchangeable potassium and sodium registered highest values at Kallara and Thuravur respectively. On comparing the content of exchangeable cations in the acid sulphate soils, it was noticed that the highest share was contributed by both exchangeable calcium and magnesium. The mean values ranged from 1.41 to 8.09 cmol $^{(+)}$ kg^{-1} at surface soils regarding the exchangeable calcium. Most of the areas in Kuttanad have calcium carbonate shell deposits in the lake bottoms. The fields reclaimed from these lakes and also due to its marine origin contributed to the highest exchangeable calcium and magnesium in these soils. The higher content of exchangeable calcium might also be due to the intensive liming practiced in this

area. The concentration of all the basic cations present in the area can be attributed to the intrusion of sea water during the summer season in the Kuttanad area. Similar reports of exchangeable bases were reported by Thampatti (1997), who also opined that the exchangeable calcium was the dominant basic cation in the Kuttanad region.

Effective CEC (ECEC) is the sum of all exchangeable cations and exchangeable acidity in a soil sample. From the present study, it was inferred that the ECEC of Kuttanad area ranged from 6.41 to 26.28 cmol $^{(+)}$ kg^{-1} at surface soil depth. The highest ECEC was recorded at Thuravur series and lowest at Thakazhi series. Similar results were reported by Beena (2005) in acid sulphate soils and the author reported that the ECEC values of the region showed wide variation among locations and the highest value was registered in Thuravur series (20 to 24.1 cmol $^{(+)}$ kg^{-1}). The report corroborated with the present findings. Thampatti (1997) also reported similar trend of ECEC in the Kuttanad area and the author reported that the ECEC value of the region were higher as compared to other locations of Kerala.

5.9 ENZYME STATUS OF THE KUTTANAD SOIL

Soil enzymes are considered as the indicators of soil quality which increase the reaction rate at which plant residues decompose and release plant available nutrients. As soil is the part of the terrestrial environment and supports all terrestrial life forms, protection of soil is therefore of high priority and a thorough understanding of soil enzymes activities is a critical factor in assuring that soil health. A better understanding of the role of this soil enzymes activity in maintaining the soil health will potentially provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management.

Enzymes play key role in biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al.* 1991). They are important in catalyzing several vital reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling, hence playing an important role in agriculture (Dick *et al.* 1994; Dick 1997). These enzymes may include amylase, arylsulphatase, β-glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease, and urease. The technique is quite simple and produces reproducible results, and is of practical importance because the influence of agro-chemicals, industrial waste, heavy metals in soil fertility.

5.9.1 Urease

Urea added to soils as fertilizer or as animal urine hydrolyzed enzymatically by soil urease ($NH_2CONH_2 + H_2O \longrightarrow 2NH_3 + CO_2$) and results in the release of ammonia. Urea hydrolysis as in case of any enzymatic reaction may only be needed to reduce activation energy for the formation of intermediate product (Dharmakeerthi and Thenabandu, 1996). Urease thus hydrolyses non peptide C-N bonds in linear amides. Even in the absence of enzymes, urea can be hydrolyzed physico-chemically.

From the present study conducted, it was observed from Fig 15 and 16, that the highest value for urease activity was noticed in Thakazhi series and Thuravur series at surface and sub surface depths respectively. This might be due to the presence of ureolytic bacteria capable of synthesizing urease enzyme (Tejada *et al.,* 2006). It was also observed that the increased organic carbon content in Thuravur and Kallara series (2.00 and 3.01 per cent respectively) might have also contributed to increased urease activity in these soils. More over this may be the reason for the higher activity of urease in the surface soils than in the sub surface soils except in Thuravur and Kallara series. In general, the urease activity is observed to be low in

Fig. 15. GIS map of urease activity in acid sulphate series soils of Kuttanad –

surface (0-15 cm).

Fig. 16. GIS map of urease activity in acid sulphate soils of Kuttanad –

subsurface (15-30 cm).

Kuttanad regions as the deleterious role of soil pH and effect of H^+ ions cannot be evicted (Reddy *et al.,* 1987). The increase in urease activity also suggests that the release of the enzymes was linked to lysis of microbial cells (Kulkarni *et al.,* 2011).

5.9.2 Phosphatase

Phosphatase is an enzyme that remove a phosphate group from its substrate by hydrolyzing phosphoric acid monoesters into a phosphate ion and a molecule with a free hydroxyl group. It has been shown that the activities of phosphatases depends on several factors like soil properties, soil organisms and their interactions, plant cover, leachate, inputs and presence of inhibitors (Speir and Ross, 1978).

From the study, it is observed that the values for acid phosphatase varied from 24.59 to 57.58 µg of p-nitrophenol released g^{-1} soil h^{-1} at surface soils and from 11.02 to 71.88 µg of p-nitrophenol released g^{-1} soil h^{-1} at sub surface soils. The activity of acid phosphatase is thus influenced by the effect of various locations. The highest activity in surface soils were recorded in Purakkad series might be due to the lowest available phosphorus content (4.09 kg ha^{-1}). The enzyme activity was increased by phosphate due to the presence of extracellular acid phosphomonoesterase stabilized by soil colloids or due to the presence of consecutive microbial phosphomonoesterase in these acid sulphate soils (Tabatabai and Bremner, 1969). An inverse relationship exists between soil available phosphorus and acid phosphatase activity. From the study it is inferred that the surface soil exhibited higher activity than sub surface except in Purakkad soils. This finding corroborated with the findings of Bergstrom *et al.* (1998) who opined that there is higher enzyme activities in the surface soil than the sub surface because of the increased organic matter content.

Alkaline phosphatase is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, thus influencing the ability of

Fig. 17. GIS map of acid phosphatase activity in acid sulphate soils of Kuttanad-surface (0-15 cm).

Fig. 18. GIS map of acid phosphatase activity in acid sulphate soils of Kuttanad –

subsurface (15-30 cm).

Fig. 19. GIS map of alkaline phosphatase activity in acid sulphate soils of Kuttanad –
surface (0-15 cm).

Fig. 20. GIS map of alkaline phosphatase activity in acid sulphate soils of Kuttanad –

subsurface (15-30 cm).

plants to cope up with P stress conditions. From the present study, it was inferred that the activity of alkaline phosphatse is high in Thakazhi series (L_4) where the available P content was comparatively high (37.04 kg ha^{-1}). This might be due to the highest microbial phosphorus solubilizers count that might have helped in the solubility of P sources and increasing the availability of substrates for the enzyme to act upon. This corroborated with the findings of Dick *et al.* (2000). The highest activity at the sub surface soils was recorded by Purakkad series (L_3) and the result may be correlated with the low status of available P at this location. This finding may also be due the integration of soil chemical, physical and biological parameters to express a single response (Eivazi and Tabatabai, 1977).

The lowest alkaline phosphatase activity was recorded in Thuravur series both at the surface and sub surface level. This might be associated with the low pH in these two depths in this location, i.e. surface pH was 2.43 and at sub surface pH recorded a value of 2.89. Several studies show that alkaline phosphatase activity is totally derived from microorganisms (Dick *et al.*, 1983). It was also concluded that the production, stability and destruction of phosphatase (either acid or alkaline) are correlated to soil pH. The activity of this alkaline phosphatase can thus be used as an indicator to determine the optimum pH for crop production and the amount of lime required to achieve this optimum (Dick and Tabatabai, 1987).

5.9.3 Aryl sulphatase

There are several types of sulphatases which are classified according to the bond they hydrolyze. Enzymes under this group include aryl sulphatase, alkyl sulphatase, glucosulphatase and myrosulphatase. Aryl sulphatase is an enzyme which plays an important role in sulphur cycling because it releases plant available sulphate ions and can be used as an indicator of fungi (Tabatabai and Bremner, 1970). Aryl sulphatase catalyses the hydrolysis of an aryl sulphatase anion fission of the O-S bond.
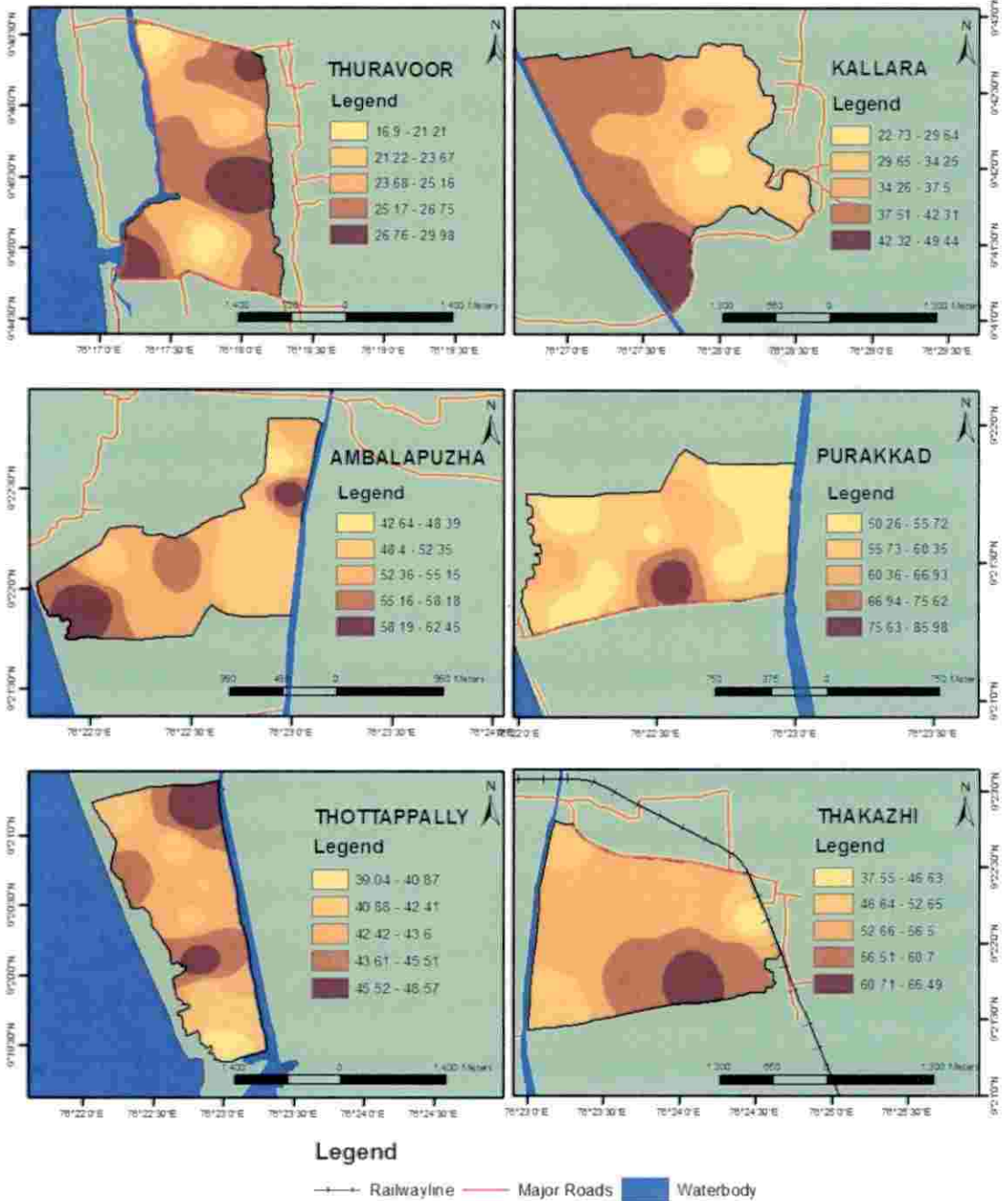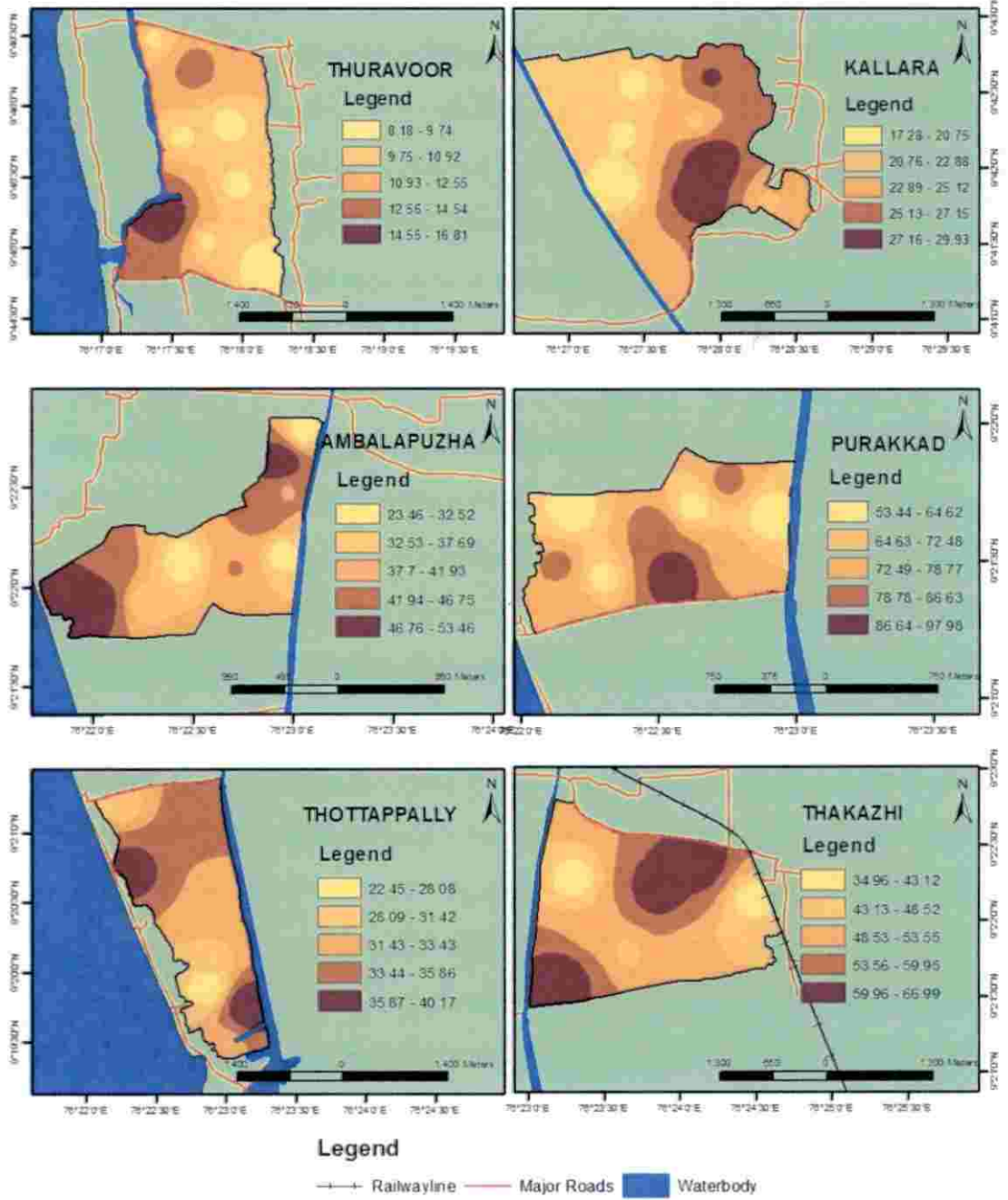
Fig. 21. GIS map of aryl sulphatase activity in the acid sulphate soils of Kuttanad –
surface (0-15 cm).

Fig. 22. GIS map of aryl sulphatase activity in the acid sulphate soils of Kuttanad – subsurface (15-30 cm).

From the present study, it is observed that the highest value for aryl sulphatase activity recorded in Thottapalli series (0-15 cm) and Thakazhi (15-30 cm). This also reflects the high fungal population in Thottapalli at surface level and a reasonably higher value for fungal flora in Thakazhi sub surface soils also. An interesting observation with the study is that the location with highest available sulphur (Thottapalli – 883.86 mg kg^{-1}) has recorded the highest value for aryl sulphatase activity, thus revealing a positive relationship between the availability of substrate and the rate of reaction of this particular enzyme. Similar results have been reported by Bolten *et al.* (1985).

5.9.4   β- Glucosidase

This particular enzyme plays an important role in soils because it is involved in catalyzing the hydrolysis and biodegradation of various β-glucosidase present in plant debris decomposing in the ecosystem (Ajwa and Tabatabai, 1994; Martinez and Tabatabai, 2000). β-glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilize the soil organic matter, and can be used to detect management effect on soils (Bandick and Dick, 1999; Ndiaye *et al.*, 2000).

Results of the β-glucosidase activity of acid sulphate soils under study recorded the mean values within a range of 1.65 to 3.04 μg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4} at surface level and from 3.04 to 6.04 μg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4} at sub surface soils. At surface layers, the highest activity was recorded by Purakkad series (L$_3$) while Thuravur series (L$_6$) recorded highest activity at sub soil layers. Comparing with all other enzyme activities, the β-glucosidase activity registered lowest values in the present study. Similar report was putforth by Acosta-Martinez *et al.* (2007) who reported that among the enzymes studied, acid phosphatase and arylsulfatase activities were more predominant than the activity of the glycosidases like β-glucosaminidase, β-glucosidase and α-galactosidase. The

Fig. 23. GIS map of β-D-glucosidase activity in acid sulphate soils of Kuttanad –
surface (0-15 cm).

Fig. 24. GIS map of β-D-glucosidase activity in acid sulphate soils of Kuttanad –
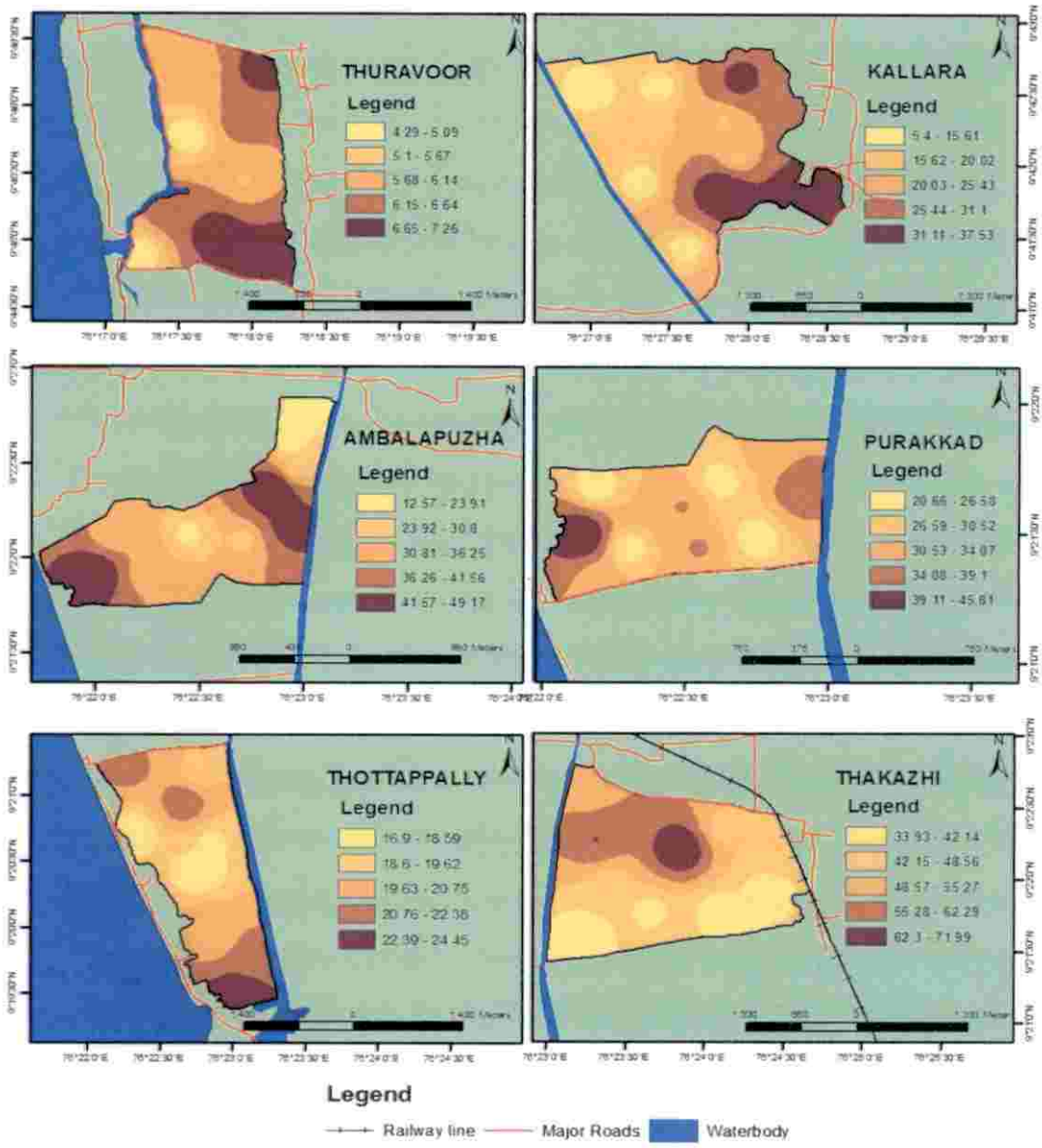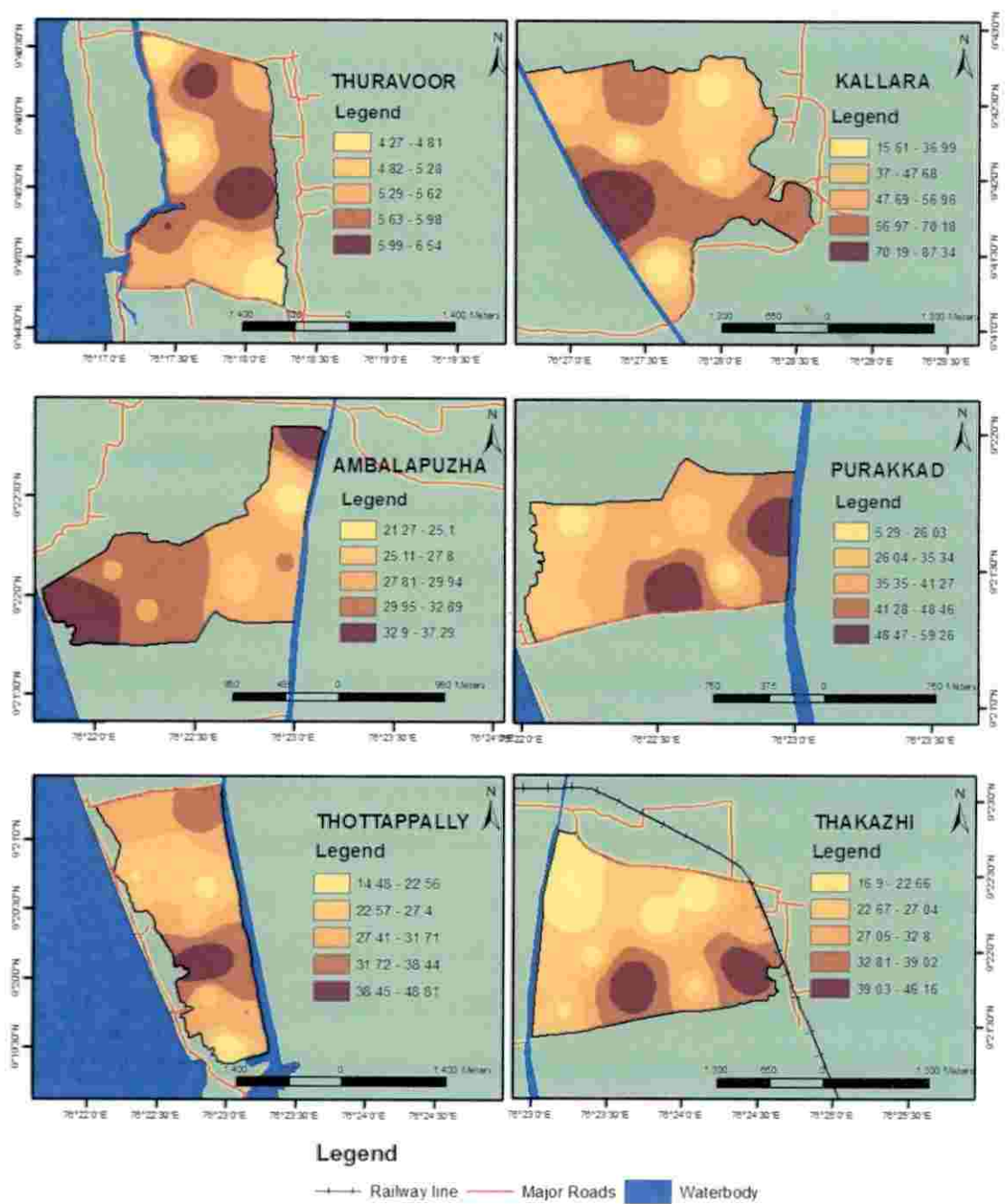
subsurface (15-30 cm).

predominance of soil enzyme activities might be more related to the ecological role and kinetic characteristics of the enzymes studied despite the effects of chemical and physical properties, geology, and land use of the soils studied (Tabatabai, 1994). The highest activity at Purakkad series can be attributed to the soil pH 0f 4.04 since the particular enzyme prefer acidic pH in a range of 4-5.5 and organic matter content (1.28 per cent) also can contributed to the activity rate. Thuravur series at sub surface level had the highest activity and that can be attributed to the low copper availability in the soils. Joachim and Patrick (2008) also reported similar findings and opined that the β - glucosidase enzyme is also known to be inhibited by heavy metal contamination such as Cu, Fe, Cd etc.

5.9.5   Soil Dehydrogenases

The dehydrogenase activity is considered to be an indicator of the oxidative metabolism in soils and thus of the microbial activity (Trevors, 1984). The present study inferred that the Purakkad series and Kallara series had recorded the highest activity of dehydrogenase at surface and sub surface soils respectively. The highest activities in the two series might be attributed to the high organic carbon content of Thuravur (2.00 per cent) at surface and Kallara (2.96 per cent) at sub surface. Dehydrogenases play a significant role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010). The activity of dehydrogenase was also influenced by the micronutrient content in the soils. The Purakkad soils at surface level had low Fe content and high exchangeable aluminium and these two might be the reason for the highest activity in the series. Similar findings were reported by Venkatesan and Senthurpandian (2006), who also reported the inhibition of dehydrogenase activity by high iron content and low aluminium even though the soil is rich in organic matter and microbial population.

Fig. 25. GIS map of dehydrogenase activity in acid sulphate soils of Kuttanad – surface (0-15 cm).

Fig. 26. GIS map of dehydrogenase activity in acid sulphate soils of Kuttanad –

subsurface (15-30 cm).

5.10 SOIL RESPIRATORY ACTIVITY AND MICROBIAL BIOMASS CARBON

Soil respiration is defined as the total carbon dioxide production per unit area and time, caused by respiration of edaphic organisms, roots, mycorrhiza and by chemical oxidation of organic compounds. As the carbon constitutes the highest weight content of organic matter, the carbon dioxide produced by microbial respiration corresponds to soil mineralization index and thereby provides an index of biological activity of soils.

The present investigation provided the soil respiratory activity of the acid sulphate series which ranged from 1.02 to 1.24 µg of CO_2 evolved g^{-1} soil h^{-1} at 0-15 cm and 15-30 cm depth. This can be attributed to the presence of high organic matter and thereby population of microflora and fauna in the acid sulphate soils at 0-30 cm depth. In the present study, while analyzing the depthwise distribution of microbial community and the respiratory activity, it was evident that both have a direct relation, *i.e.*, both parameters recorded highest mean values at surface soils. The results corroborated with the reports of Simek *et al.* (2011), who reported that basal soil respiration in acid sulphate soils ranged from 0.1 to 1.6 µg of CO_2 evolved g^{-1} soil h^{-1}. The author also quoted that basal respiration was highest in surface horizon and then sharply decreased with increasing depth.

Microbial biomass carbon generally comprises of 1-4 per cent of soil organic matter (Anderson and Domsch, 1989) and is the most active component of soil organic carbon that regulates biogeochemical processes in terrestrial ecosystems (Paul and Clark, 1996). The microbial biomass carbon is one of the most promising indicators of soil quality as it responds to environmental changes much earlier than bulk soil organic matter.

The acid sulphate soils under study reported microbial biomass carbon content within a range of 43 to 357 µg g^{-1} at surface soils and 26 to 287 µg g^{-1} at sub

surface. Among the six locations the highest microbial biomass carbon was noticed at Purakkad and Thottapalli series at 0-15 cm and 15 – 30 cm depth respectively. The variation in surface and surface soils microbial biomass content content at different locations might be due to the total organic carbon content present in that area, *i.e.,* at Purakkad and Thottapalli the native organic carbon content was low (1.28 per cent and 2.79 per cent respectively) thereby recorded higher microbial biomass carbon. Also compared to normal soils, the acid sulphate soils under study recorded low MBC values and that might be due to the toxic levels of Zn, Al, Cu and EC values in the area as suggested by Chander and Brookes (1991).

5.11 MICROBIAL POPULATION IN THE KARI SOILS

Acid sulphate soil support an extensive microbial community that is adapted to utilizing the organic compounds and nutrients accumulated during the formation of these soils, though the current microbial activity can be very low due to low temperatures, long term anaerobiosis and other unfavourable conditions in undrained acid sulphate soils (Simek *et al.,* 2011). The flooded rice soils are the best source for studying the diversity of microbial community in an important ecosystem (Sridevi *et al.,* 2013). However, the relationship between an individual biochemical property and the total microbial activity is not always obvious, especially in the case of complex systems like soils, where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse (Nannipieri *et al.,* 1996).

In the present investigation, the microbial count from the Kuttanad area showed that the total bacterial count varied from 9.13 to 9.30 log cfu g^{-1} soil in 0-15 cm depth and the 9.07 to 9.23 log cfu g^{-1} soil in 15-30 cm depth. Fungal count varied from 5.38 to 6.03 log cfu g^{-1} soil at surface and in the case of total actinomycetes count the values are non significant at surface soil depth. Enumeration of *Thiobacillus* spp., Nitrogen fixers and phosphorus solubilizers were also made under the present study. Regarding *Thiobacillus* spp., the count varied from 8.86 to

9.38 log cfu g^{-1} of surface soil and the highest count was recorded at Kallara series. This can be attributed to the highest sulphur content and organic matter content prevailing in the specific series. While comparing the N fixers (3.61 to 3.91 log cfu g^{-1} surface soil) and P solubilizers count (3.72 to 3.97 log cfu g^{-1} suface soil), the *Thiobacillus* spp. count dominated in these acid sulphate series. Dominant *Thiobacillus* spp. in the region might be due to its significant role in sulphur oxidation and thereby contributing to high sulphur content in the acid sulphate soils.

The findings of the present study corroborated with reports of Panhwar *et al.* (2014), who reported that the total bacterial count and actinomycetes population were higher than fungal populations in acid sulphate soils. The less occurence of fungi might be due to the physico-chemical properties of soil due to continuous use of the fields. Behra *et al.* (2014) also reported that among the thirteen bacterial isolates from mangrove acid sulphate soils of Odisha, twelve isolates were better sulphur oxidizers and they could reduce the pH of the media. Similar findings regarding *Thiobacillus* spp. count was reported by Yang *et al.* (2010), who reported that the corresponding values of *Thiobacillus* spp. count was increased from 5.46 to 8.60 log cfu g^{-1} soil after sulphur application and subsequent drop in pH.

Regarding the depth wise distribution of all the microbial community in the study, the results obtained indicated the higher counts in the surface soils in general than sub surface samples. Similar results were obtained by Krishnan *et al.* (2012), who reported that there was a significant decrease in microbial load while analyzing the depth wise distribution. The finding can be attributed to the lower organic matter and organic carbon content in the sub soil layers of the region. The decreasing trend in microbial abundance was also reported by Trumbore (2000) who opined that this trend is commonly seen and is due to the heterotrophic nature of most of the microbial population in the soil.

Fig. 27. GIS map of total bacterial population in acid sulphate soils of Kuttanad –
surface (0-15 cm).

Fig. 28. GIS map of total bacterial population in acid sulphate soils of Kuttanad –
subsurface (15-30 cm).

Fig. 29. GIS map of fungal population in acid sulphate soils of Kuttanad -

surface (0-15 cm).

Fig. 30. GIS map of fungal population in acid sulphate soils of Kuttanad –

subsurface (15-30 cm).

Fig. 31. GIS map of actinomycetes population in acid sulphate soils of Kuttanad – surface (0-15 cm).

Fig. 32. GIS map of actinomycetes population in acid sulphate soils of Kuttanad –

subsurface (15-30 cm).

5.12 INCUBATION STUDY OF SURFACE AND SUBSURFACE SOILS FOR THE CONFIRMATION OF ACID SULPHATE CONDITION

The soil samples from the entire study area were incubated at room temperature in the lab for three months to confirm the acid sulphate condition in the Kuttanad acid sulphate soils. The soils under study reported a general decrease in the initial pH at all the locations and both at surface and sub surface soil depths that confirmed the acid sulphate condition in the area. Most of the soils recorded more than one unit decrement in the pH of the soil samples.

The result corroborated with the findings of Beena (2005), who also reported a decline of 0.5 units in pH of the acid sulphate soils soils during incubation. Simek *et al.* (2011) reported similar findings that the pH of acid sulphate soils decreased while incubating the samples. This might be due to the oxidation of pyrite layer in the soil and thereby contributing to the production of sulphuric acid and the low pH in the soils.

5.13 ENZYME KINETIC PARAMETERS OF THE MAJOR ENZYMES IN THE SOILS

Kinetic studies indicated that the activity of enzymes in clay–enzyme complexes is greatly reduced when compared to that of the respective free enzyme (Nannipieri *et al.*, 1996). For this reason it has been hypothesised that the determination of kinetic parameters of soil enzymes highlights the protective influence of different soil components and can be also used to differentiate enzyme sources (Farrell *et al.*, 1994; Tabatabai *et al.*, 2002).

From the present study the kinetic parameters, Michaelis-Menten constant K_m and the maximum velocity V_{max} of five enzymes *viz.*, urease, phosphatase, dehydrogenase, aryl sulphatase and β-glucosidase were estimated. And the results inferred that the V_{max} for urease and aryl sulphatase exhibited the highest value after

8th week of incubation (3.77×10^{-3} moles of urea hydrolyzed g^{-1} soil h^{-1} and 2.85×10^{-3} µg of p-nitrophenol released g^{-1} soil h^{-1}), while the phosphatase and β-glucosidase recorded the highest V_{max} after 12th week of incubation and dehydrogenase after 6th week. Considering the constant K_m, the enzymes phosphatase, aryl sulphatase and dehydrogenase exhibited the highest value after 12th week of incubation. The other enzymes like urease and β-glucosidase registered highest values for K_m after 4th and 8th week of incubation. Similar V_{max} and K_m for urease, phosphatase and dehydrogenase was reported by Maharana and Patel (2013).

5.14 ENZYME ACTIVITY NUMBER - INDEX OF BIOLOGICAL FERTILITY

Some soil quality indices have been developed using biochemical properties to address important ecological functions such as decomposition and nutrient cycling. One of the most important index is the enzyme activity number (EAN) (Beck, 1984) which could be suitable technique for studying the physiological reaction of the soil biomass under various stress conditions.

The EAN values, which represent the five enzyme activities that was monitored singularly in the present study, the mean values of the enzyme activity number for the locations varied from 14.42 to 23.69 in surface soils and from 3.49 to 12.20 in sub surface soils. Regarding the surface soils of acid sulphate series and the enzyme activity number, the highest value was noticed at Purakkad series (L_3-23.69). At the sub surface soil, the highest enzyme activity number was seen at Kallara series.

Even though the organic carbon content in the Purakkad series is low compared to Kallara at surface soil, Purakkad recorded highest EAN and the result might be due to the influence of high aluminium content and low iron toxicity in the particular area compared to others. The inhibition of enzyme activities by high iron content and low aluminium even though the soil is rich in organic matter and

microbial population was also reported by Venkatesan and Senthurpandian (2006). Higher enzyme activity number in untilled management system than the tilled management system was reported by Riffaldi *et al.* (2002) and Saviozzi *et al.* (2011) also reported EAN in a range of 11.93 to 18.87 in a salinity affected soil.

5.15 CORRELATION STUDIES

From the correlation matrix (Table 30) it was clear that the surface soil pH was positively correlated with the availability of nitrogen, phosphorus and potassium, activity of enzymes like acid phosphatase, aryl sulphatase and also with the biomass of fungi in the soil. At sub soils (Table 31) soil pH showed negative correlation with urease and β-glucosidase activity along with population of bacteria and actinomycetes in the sub surface soils. Salazar *et al.* (2011) also reported non significant correlation of soil pH with the biological and chemical properties of the soil.

Intercorrelation among other characters showed that aryl sulphatase activity had a positive significant correlation with availability of nitrogen in surface soils (-0.842*). The activity of β-glucosidase (-0.883*), dehydrogenase (-0.837*) and actinomycetes population (-0.848*) had a negative significant correlation with phosphorus availability of the surface soils. Speir and Ross (1978) also reported similar results and the authors reported that there are contradictory relationship between the enzyme activities and the inorganic nutrient content in the soil. Salazar *et al.* (2011) also reported that dehydrogenase enzyme had a positive significant correlation with N, P and K.

The correlation between organic carbon and enzyme activities at surface and sub surface recorded a positive and significant correlation with aryl sulphatase while urease, dehydrogenase and acid phosphatase registered negative correlation both at surface and sub surface levels. Deng and Tabatabai (1997) also reported a strong

correlation between the arylsulphatase activity and organic carbon content, bearing the hypothesis that the enzymes in the soils are limited by the clay and humic colloids and the association with the humic substances is an effective form to protect these enzymes soil environment.

Summary

6. SUMMARY

A study entitled "Enzyme characterization of the acid sulphate soils of Kuttanad" was carried out during 2014-16 in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani. The study was envisaged to characterize the acid sulphate wetland soils of Kuttanad for chemical and biological parameters and to assess the biological fertility of the soil. Six acid sulphate soil series were selected for the study *viz.*, Ambalapuzha (L_1), Kallara (L_2), Purakkad (L_3), Thakazhi (L_4), Thottapalli (L_5) and Thuravur (L_6). Soil samples were also collected from Karappadom (Alappuzha series) and Kayal lands (Champakulam series) for comparison. Both surface (0-15 cm) and sub surface (15-30 cm) soil samples were collected from the six soil series. The salient findings of the chemical and biological characterization of the acid sulphate soils of Kuttanad is presented in this chapter.

Ambalapuzha series (L_1) recorded the highest values of bulk density, particle density, available P and copper at the surface layers, while the sub surface soils registered the highest pH (less acidic), copper content and P solubilizers population among all the acid sulphate soil series.

Regarding the Kallara series (L_2), both surface and sub surface soils recorded the highest organic carbon content, available N, K and the calcium contents. Compared to other series of acid sulphate soils in Kuttanad, the surface soils had recorded the highest population of *Thiobacillus* spp. and P solubilizers while the sub surface soils marked the highest alkaline phosphatase and dehydrogenase activities with the highest enzyme activity number. Sub surface soils of this series also recorded the highest exchangeable K content.

Considering the chemical and biological characterization of Purakkad series (L_3), the chemical properties viz. available Mg and Na, exchangeable Ca, Mg and Na

were registered the highest values in the surface soils. The surface soils also recorded the highest values of β- glucosidase and acid phosphatase enzyme activities with the highest enzyme activity number and microbial biomass carbon content. At sub surface level, this series registered the highest values for bulk density, particle density, available P and Mg content along with the highest acid phosphatase activity.

The surface soils of Thakazhi series (L_4) recorded the highest mean values for pH, available Mn as well as urease and alkaline phosphatise activities. The aryl sulphatase activity, EC, exchangeable Ca and Mg were the highest at sub surface soils of this particular series.

The most important acid sulphate series, Thottapalli series (L_5) recorded the highest available S, Fe content, activity of aryl sulphatase, soil respiration and population of total bacteria, N fixers and fungi at the surface soils. The sub surface soils of the series recorded the highest mean values for soil respiration, microbial biomass carbon, Mn availability and total bacteria and fungal populations.

Thuravur series (L_6) had recorded the highest values of dehydrogenase activity, EC, ECEC, acidity parameters including exchangeable Al^{3+}, exchangeable H^+ and exchangeable acidity along with the highest availability of Zn and B. At sub surface layers, Thuravur series recorded the highest values of available Na, Fe, Zn and B contents, the highest urease and β–glucosidase activities along with highest count of actinomycetes population.

The study also revealed that the V_{max} for urease and aryl sulphatase exhibited the highest value after 8th week of incubation, while the phosphatase and β-glucosidase recorded the highest V_{max} after 12th week of incubation and dehydrogenase after 6th week. Considering the constant K_m, the enzymes phosphatase, aryl sulphatase and dehydrogenase exhibited the highest value after 12th

week of incubation. The other enzymes like urease and β-glucosidase registered the highest values for Km after 4th and 8th week of incubation.

Correlation study results of soil pH with chemical and biological parameters of the soil showed that in the surface soil pH was positively correlated with acid phosphatase enzyme status with high significance. Intercorrelation among other characters showed that aryl sulphatase activity had a negative significant correlation with availability of nitrogen in surface soils. The activity of β-glucosidase, dehydrogenase and actinomycetes population had a negative significant correlation with phosphorus availability of the surface soils. At sub surface soils, β-glucosidase had a negative significant correlation with acid phosphatase activity.

For the confirmation of acid sulphate soil condition in the selected locations of Kuttanad, lab incubation studies were done for three months. The results of the incubation study inferred that there was a drastic decline in pH from initial to final with most of the samples exhibited a decline of more than one unit.

Thematic maps were prepared based on themes viz. microflora and enzyme status of the acid sulphate Kuttanad soils using GIS (ARC VIEW) software by making use of IDW spatial intrapolation technique. Among the six locations, urease activity was the highest at Thakazhi series in the surface layers and the highest activity range was spread over an area of 22.97 ha. Considering the acid phosphatise activity in surface layers and subsurface layers, approximately 7.02 ha and 11.21 ha in the Purakkad area recorded highest activity range. Regarding the microbial population, an area of 15.26 ha and 22.32 ha in the Thottapalli surface layer had the highest population range of total bacteria and fungi.

6.1 CONCLUSIONS

The present study reveals that the acid sulphate soils of Kuttanad are fertile with respect to soil chemical and biological properties even though the soil is highly

acidic. With respect to biological fertility status, Purakkad recorded the highest enzyme activity number at 0-15 cm, while Kallara series recorded the highest enzyme activity number in the subsurface layer and are observed to be biologically sustainable. With regard to the chemical characterization of the acid sulphate series of Kuttanad, Kallara series recorded the highest nutrient status and organic carbon content and thereby highly productive. The study also inferred that inspite of low nutrient status, Purakkad series recorded the highest Enzyme activity number, there by the role of micronutrients in influencing enzyme activities cannot be neglected.

6.2 FUTURE LINE OF WORK

- The relation between micronutrients and enzyme activities in Kuttanad soils can be further investigated.
- Investigations on the soil acidity and its influence on soil biological as well as nutrient management aspects can be initiated in future.

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ENZYME CHARACTERIZATION OF THE ACID SULPHATE SOILS OF KUTTANAD

by

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Abstract of the thesis

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ABSTRACT

A study entitled "Enzyme characterization of the acid sulphate soils of Kuttanad" was carried out during 2014-16 in the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani. The study was envisaged to characterize the acid sulphate wetland soils of Kuttanad on the basis of chemical and biological parameters and to assess the biological fertility of the soil. Six acid sulphate soil series were selected for the study, *viz.,* Ambalapuzha (L_1), Kallara (L_2), Purakkad (L_3), Thakazhi (L_4), Thottapalli (L_5) and Thuravur (L_6). Soil samples were collected from two different depths - surface (0-15 cm) and subsurface (15-30 cm) and also from Karappadam (Alappuzha series) and Kayal lands (Champakulam series) for general comparison.

On evaluating the electro chemical properties of the surface soils, Thakazhi (L_4) recorded the highest value for pH (4.67) while in the case of sub surface samples, Ambalapuzha (L_1) recorded the highest pH value of 4.40. Thuravur series (L_6) recorded the lowest values for pH and the highest EC value of 7.52 dS m^{-1} and 6.53 dS m^{-1} at surface and subsurface soils respectively.

With respect to soil chemical properties, the highest organic carbon content was noticed in Kallara series (L_2), both at surface and subsurface layers with mean values of 3.01 per cent and 2.96 per cent respectively. In the case of available nutrient status, the highest N and K was noticed in Kallara (L_2) being 674.02 kg ha^{-1} and 148.49 kg ha^{-1} respectively in the surface layers. Amabalapuzha series recorded the highest values for available phosphorus (L_1-78.60 kg ha^{-1}). Among the acid sulphate series of Kuttanad, Fe content recorded the highest value of 1024.23 mg kg^{-1} at Thottapalli series (L_5), Thakazhi (L_4) registered the highest Mn availability while Cu content was the highest at Ambalapuzha series (L_1) in surface soils. Thuravur series (L_6) marked the highest Zn and B availability compared to other locations at both surface and sub surface levels.

Considering the distribution of secondary nutrients at surface and subsurface levels, Ca and Mg recorded the highest content at Kallara (L_2) and Purakkad (L_3) series respectively, while the highest mean value for available S content was recorded by Thottapalli series (L_5) at surface layers.

Regarding the enzyme status of the acid sulphate soils, the activity of urease was the highest in Thakazhi (L4-75.78 ppm of urea hydrolyzed g^{-1} soil h^{-1}) in the surface layer. Acid and alkaline phosphatase activities were found to be the highest in Purakkad (L_3-57.58 µg pnp released g^{-1} soil h^{-1}) and Thakazhi series (L4-46.41 µg pnp released g^{-1} soil h^{-1}) respectively in the surface layers. The highest activity of dehydrogenase was noticed in Thuravur series (L_6-115.74 µg of TPF released g^{-1} soil 24 h^{-1}) in the surface and Kallara series in the subsurface layers (L_2-55.39 µg of TPF released g^{-1} soil 24 h^{-1}). Karappadam and Kayal recorded the values of 145.73 and 114.80 µg of TPF released g^{-1} soil 24 h^{-1} for dehydrogenase activity respectively at surface layers.

With regard to the microbial population, Thottappalli (L_5) recorded the highest total bacterial count and fungal population in both surface and subsurface layers. *Thiobacillus* spp. recorded the highest population of 9.08 log cfu g^{-1} of soil at Kallara series (L_2) while P solubilizers recorded the highest count at Ambalapuzha series (L_1). Thuravur series recorded the highest actinomycetes population in subsurface soils.

Micahelis – Menten constant, K_m and the maximum velocity V_{max} for different enzymes were determined and it was observed that for dehydrogenase V_{max} value was found to be the highest in 6th week and for phosphatase only after 12th week of incubation. Thematic maps were prepared based on themes like microflora and enzyme status using GIS (ARC VIEW).

Hence the study conclude that, with respect to biological fertility status of the study area, Purakkad recorded the highest enzyme activity number (23.69) at 0-15 cm, while Kallara series recorded the highest enzyme activity number in the subsurface layer and are observed to be biologically sustainable.

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സംഗ്രഹം

കേരളത്തിലെ കുട്ടനാട് മേഖലയിലെ കരി മണ്ണിനത്തിൽ ഉൾപ്പെട്ട ആറു മണ്ണു സീ രീസ്സിൽ, നെല്ലധിഷ്ഠിത കൃഷി സമ്പ്രദായം അവലംബിച്ചിട്ടുള്ള കർഷകരുടെ പാട ശേഖരങ്ങളിൽ നിന്ന് ശേഖരിച്ച മേൽമണ്ണിന്റെയും അടിമണ്ണിന്റെയും ഭൗതിക, രാസ ജൈവ സ്വഭാവഗുണങ്ങളാണ് ഈ പഠനത്തിന് (2014-16) വിധേയമാക്കിയത്. കരി മണ്ണുസീരീ സ്സിൽ ഉൾപ്പെട്ട അമ്പലപ്പുഴ, പുറക്കാട്, കല്ലറ, തുറവൂർ, തോട്ടപ്പള്ളി, തകഴി എന്നീ സ്ഥല ങ്ങളിൽ നിന്നു 0 - 15 സെ.മീ ഉം 15-20 സെ.മീ ഉം താഴ്ചയിൽ നിന്ന് ആകെ 120 സാമ്പി ളുകളാണ് ശേഖരിച്ചത്. ഇതിനു പുറമെ കരപ്പാടം, കായൽ എന്നീ മണ്ണിനങ്ങളും കുട്ടനാ ട്ടിൽ നിന്നു ശേഖരിച്ചു.

മണ്ണിന്റെ ജൈവ ആരോഗ്യസൂചികകളായ ബാക്ടീരിയ, ഫംഗസ്, ആക്റ്റിനോമൈ സറ്റ്, തയോബാസിലസ്, നൈട്രജൻ ഫിക്സേഴ്സ്, ഫോസ്ഫറസ് സോളുബിലൈസേഴ്സ് എന്നീ സൂക്ഷ്മാണു ജീവികളുടെ എണ്ണം, യൂറിയേസ്, ഫോസ്ഫറ്റേസ്, ഡിഹൈഡ്രോജി നേസ്, അറൈൽസൾഫറ്റേസ്, ഗ്ലൂക്കോസിഡേസ് എന്നീ എൻസൈമുകളുടെ അളവ്, മണ്ണിന്റെ ശ്വസന തോത്, കാർബൺ, നൈട്രജൻ, അമ്ലത, സൂക്ഷമ മൂലകങ്ങൾ എന്നീ ഘടകങ്ങളുടെ അളവ് എന്നിവ പഠിച്ചതിൽ നിന്നും പുറക്കാട് സീരീസിൽ നിന്നുള്ള മേൽമണ്ണും കല്ലറ സീരിസ്സിൽ ഉൾപ്പെട്ട അടിമണ്ണ് സാമ്പിളുകളുമാണ് ഏറ്റവും മികച്ച ജൈവ സന്തുലിതാവസ്ഥ രേഖപ്പെടുത്തിയത്.

Appendices

APPENDIX 1

WEATHER PARAMETERS AT STUDY AREA DURING COLLECTION OF SOIL SAMPLES

(January 2014 to December 2014)

Month	Maximum Temperature (°C)	Minimum Temperature (°C)	Rainfall (cm)
January	32.10	21.65	0.00
February	32.95	22.74	0.44
March	34.10	23.80	0.46
April	33.92	24.31	3.83
May	33.24	24.98	5.60
June	30.90	24.09	13.87
July	29.98	22.80	14.19
August	30.41	22.96	22.60
September	31.69	23.43	6.96
October	31.80	23.32	10.83
November	31.56	22.98	4.08
December	31.80	22.67	1.16

APPENDIX II

COMPOSITION OF MEDIA FOR MICROBIAL ENUMERATION

1. Enumeration of Bacteria

Media   :   Nutrient Agar

Composition   :

1. Peptone   -   5 g
2. NaCl   -   5 g
3. Beef Extract   -   3 g
4. Agar   -   20 g
5. pH   -   7.0
6. Distilled water   -   1000 ml

2. Enumeration of Fungi

Media   :   Rose Bengal Agar

Composition   :

1. Glucose   -   3.0 g
2. $MgSO_4$ -   0.2 g
3. K_2HPO_4 -   0.9 g
4. Rose Bengal   -   0.5 g
5. Streptomycin   -   0.25 g
6. Agar   -   20 g
7. Distilled water   -   1000 ml

3. Enumeration of Actinomycetes

Media   :   Kenknight's Agar

Composition   :

1. Dextrose   -   1.0 g
2. KH_2PO_4 -   0.1 g
3. $NaNO_3$ -   0.1 g
4. KCl   -   0.1 g
5. $MgSO_4$ -   0.1 g
6. Agar   -   15 g
7. Distilled water   -   1000 ml

4. Enumeration of *Thiobacillus* spp.

Media   :   Thiobacillus Differential Agar

Composition   :

1. $(NH_4)_2SO_4$:   0.4 g
2. KH_2PO_4 :   4.0 g
3. $CaCl_2$:   0.25 g
4. $FeSO_4$:   0.01 g
5. $MgSO_4$:   0.5 g
6. $Na_2S_2O_3$:   5 g
7. Agar   :   15 g
8. Distilled water   :   1000 ml

5. Enumeration of Nitrogen Fixers

Media   :   LGI Agar

Composition   :

1. K_2HPO_4 :   0.2 g
2. KH_2PO_4 :   0.2 g
3. $MgSO_4$:   0.6 g
4. $CaCl_2$:   0.2 g
5. Na_2MoO_4 :   0.02 g
6. $FeCl_2$:   0.002 g
7. Agar   :   15 g
8. Distilled water   :   1000 ml

6. Enumeration of Phosphorus Solubilizers

Media   :   Phosphate Solublizers Differential Agar

Composition   :

1. Yeast extract   :   0.5 g
2. Dextrose   :   10 g
3. $Ca_3(PO_4)_2$:   5.0 g
4. $(NH_4)_2SO_4$:   0.5 g
5. KCl   :   0.2 g
6. $MgSO_4$:   0.1 g
7. $MnSO_4$:   0.0001 g
8. $FeSO_4$:   0.0001 g
9. Agar   :   15 g
10. Distilled water :   1000 ml