CHARACTERISATION OF SOIL AND WATER OF PALAKKAD EASTERN PLAINS IN RELATION TO GROWTH AND NITROGENASE CONTENT OF *AZOLLA* SPP.

By

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(2013-11-142)

THESIS

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DECLARATION

I hereby declare that the thesis entitled "Characterisation of soil and water of Palakkad eastern plains in relation to growth and nitrogenase content of *Azolla* spp." 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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Dedicated to

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ABBREVIATIONS

Sl no.	Abbreviation	Expansion
1	PEP	Palakkad Eastern Plains
2	DOC	Dissolved Oxygen Content
3	N	Nitrogen
4	P	Phosphorus
5	K	Potassium
6	ANOVA	Analysis Of Variance
7	GPS	Geographical Positioning System
8	AEU	Agro Ecological Unit
9	NE	North East
10	EC	Electrical Conductivity
11	C/N	Carbon- Nitrogen ratio

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Introduction

1. INTRODUCTION

Azolla, is a well-known floating pteridophyte which contains the N fixing Cyanobacteria, *Anabaena azollae* as an endosymbiont. This aquatic fern, a genus of seven species belonging to the family Salvinaceae, is one which is extremely reduced in form and specialized with no similarity to other typical ferns. It resembles more the duckweed or home mosses. Considering the immense potential as an aquatic green manure this fern has been widely cultivated in the Asian locations to enrich the rice fields with organic matter and nitrogen. Moreover, its performance as an aquatic green manure has been widely evaluated by many researchers, yielding salient results.

But, obscuring these potentialities reports are on the increase indicating a retreating tendency for Azolla with diminishing populations from many of the wetlands. It is generally postulated to be due to the very low growth pace of the fern, negative environmental factors including incongruous soil and poor water qualities.

The State of Kerala has been appropriated into five agro-ecological zones, based primarily on climate, geomorphology, land use and soil variability, taking block panchayath as the unit for delineation. Accounting panchayats as the delineating unit, 23 agro-ecological units have been identified. An agro-ecological unit is characterized by distinct responses to the macro-elements as reflected in the vegetation, soils and use of land for agriculture.

The Palakkad Eastern Plain (PEP) constitutes the 23rd in the categorization.Palakkad,the most picturesque districts of Kerala often proclaimed as the granary, has probably the biggest mountain pass in the world (30-40 km) separating the two folds of Western Ghats on the border of Kerala and Tamil Nadu and is the gate way to the state of Kerala. Of the district, the PEP region is characterized by typical topography, soil, altitude, rainfall and varying vegetation. It incorporates four Block panchayaths (Alathur,Chittoor,Kollengode and Nenmara)with six Grama panchayaths on an average under the jurisdiction of each block.

The prevalence of Azolla in wetland ecosystem and water bodies is a unique feature as evidenced from literature. Comparable variability observed in the prevalence and/or distribution of Azolla in many parts of the PEP and direct field reports on farmers engaging labourers to discard and eliminate Azolla from paddy fields ignoring its merits ignited interest in gathering solid information on the factors of environment and characteristics of soil and water of the locations favouring Azolla growth. The awareness that Azolla marvels as a 'Green gold mine' because of its nitrogen fixing ability mediated through the nitrogenase enzyme complex and that it would naturally be dependent on all the factors that are conducive for growth also emphasized the need for a detailed analysis, so as to draw a supporting platform to promote appropriate utilization of the fern on a scientific basis.

It was in this background that the present study titled *Characterization of soil and water of Palakkad eastern plains in relation to growth and nitrogenase content of Azolla spp.* was formulated and conducted.

The main objectives of this study were;

[1] to conduct a survey of Azolla species in the rice growing tracts of Palakkad Eastern Plains and [2] to identify the soil and water quality parameters congenial for the growth and nitrogenase content of *Azolla* spp.

Review of Literature

2. REVIEW OF LITERATURE

Nitrogen fixation by microorganisms, symbiotic and non-symbiotic plays an important role in enriching the N economy of soil. The review of work was done focusing the role of *Azolla* as a N fixer. It had been reported that *A. pinnata* fixed 75 kg N/g dry weight/day and produced a biomass of 347 ton fresh weight/ha in a year (Watanabe *et.al.*, 1982) and the average daily N_2 fixing rate was reported to vary between 1.0 to 2.6 kg N/ha (Singh., 1988)

This review is enlisted under the following titles:

- Biological Nitrogen Fixation
- *Azolla*
- *Azolla* species and distribution
- *Azolla-anabaena* relationship
- Chemical composition of *Azolla*
- Factors influencing growth of *Azolla*

2.1 BIOLOGICAL NITROGEN FIXATION

Biological nitrogen fixation could be an important source of nitrogen for supporting aquatic primary productivity(Affourtit *et.al.*, 2001).Nitrogen is one of the most essential elements for all forms of life; a basic material for synthesizing proteins, nucleic acids and other organic nitrogenous compounds. Unfortunately no plant species is able to reduce atmospheric dinitrogen into ammonia and use it directly for its growth. It appears that only a number of prokaryotic microorganisms including bacteria and cyanobacteria have been shown to possess the ability to fix nitrogen (Nghia and Gyurjan, 1987). They occur in the so- called free- living forms e.g. aerobic azotobacter, anaerobic Clostridia or in symbiosis with certain higher plants e.g. Rhizobia with legumes or *Anabaena Azollae* with Azolla. Blue- green algae and Azolla received greater attention in developing countries as a N supplement in rice cultivation.

2.1.1 Azolla

The genus name, Azolla is a conjugation of two greek words, Azo (to dry) and Ollyo (to kill), suggesting the fern is killed by drought. The genus Azolla was established by Lamarck in 1783 (Stevenson, 1944). Azolla is a genus of small aquatic ferns with a world-wide distribution in temperate and tropical locations. Studies on Azolla have generated tremendous interest in the scientific community because of the nitrogen-fixing ability of this fern. The distribution of different Azolla species had been described by Olsen (1970) and Van Hove (1989). Based on the classification proposed by Saunders and Fowler (1992) the most commonly found forms of Azolla are *A. pinnata, A. filiculoides, A. rubra, A. microphylla, A. imbricata* and *A. caroliniana.*

Azolla, an aquatic fern , seen floating on water surface of flooded rice fields, small ponds and canals has a size ranging from 1-5 cm generally except for the giant *A. nilotica*, found mostly in Japan. Generally it multiplies vegetatively and often sexually. The leaf of Azolla consists of two lobes, a thick aerial dorsal lobe and a thin ventral lobe occasionally of a slightly larger size. The dorsal lobe is chlorophyllous and contains the endophytic blue- green algae (*Anabaena azollae*) within a basal cavity connected to the atmosphere by a pore on the adaxial side (Peters *et al.*, 1980). The surface of the dorsal lobe has an epidermis covered with vertical rows of single celled stomata and trichomes of one or more cells (Lumpkin and Plucknett, 1980). The trichomes of the leaf are useful for identification of species. The thin ventral lobe of the leaf is almost achlorophyllous with few stomata and trichomes and several chambers. The ventral lobe probably helps in floating due to its convex surface touching the water (Eames, 1936) and in absorption of water and nutrients by the plant which is devoid of roots and reported to survive for a small period of time. (Lumpkin, 1987)

Azolla utilization

Anabeana azolla association has many uses. It can be utilized as a bio fertilizer for rice and other crops, including taro, wheat and many others, an animal feed, a human food, medicine and water purifier. It may also be used for production of hydrogen fuel and biogas, control of weeds and mosquitoes and in the reduction of ammonia volatilization that accompanies the application of chemical nitrogen fertilizer (Wagner, 1997).

Azolla can help to overcome limitations in plant growth due to nutrient availability, especially N, by enhancing nutrient acquisition. According to the plant species, growing conditions and management practices Azolla provide different benefits to the plants and to the environment as enlisted herewith (Anjuli *et.al.*, 2004)

- Increases yield and crop quality
- Reduces disease incidence
- Enhance flowering and fruiting
- Increase plant establishment and survival at seedling or transplanting
- Improve drought tolerance, allowing water reduction
- Optimize fertilizer use, especially N
- Contribute to maintain soil quality and nutrient cycling

2.1.2 Azolla species and distribution

It is believed that Azolla domestication dates back to the 11th century and was first done in Vietnam. The genus Azolla was botanically established by Lamarck in 1783 (Kannaiyan and Kumar, 2006). The species of Azolla are divided into two subgenera: Euazolla and Rhizosperma Strasb based on the sporocarp characters (Sood *et al.*, 2007). Some other authors use the taxonomic "section" level instead of subgenus (Saunders and Fowler, 1992). Subgenus (section) Azolla has 5 species, namely *A. caroliniana Wild, A. filiculoides Lam., A. mexicana Presl, A. microphylla Kaulf* and *A. rubra* R.Br. while section Rhizosperma has only 2 species called *A. nilotica* Decne ex Mett. (NI) and *A. pinnata* R. Br. (Raja *et al.*, 2012). The most commonly found forms of Azolla are *A. pinnata*, *A. filiculoides*, *A. rubra*, *A. microphylla*, *A. imbricata* and *A. caroliniana*

According to Kannaiyan and Kumar (2006), Azolla species distribution corresponds to fresh water ecosystems of temperate and tropical locations. *Azolla pinnata* is the most widely distributed species in India and throughout the world in both tropical and temperate locations. It is generally found to inhabit in paddy fields, canals, ditches and rivers. In India *A. pinnata* has been found to be distributed widely in stagnant and shallow waters

2.1.3 Azolla- Anabaena Relationship

The Azolla – Anabaena system is a symbiotic association between pteridophyta and the blue green algae *Anabaena azollae*. The host Azolla contains chlorophyll a, b and carotenoids whereas the symbiont *Anabaena azollae* contains chlorophyll a, phycocyanin, allophycocyanin and phycoerythrocyanin. The *Anabaena azollae* association exhibits Calvin cycle (Tyagi *et al.,* 1980).

In *Anabaena azollae* symbiosis, N_2 is fixed by the symbiont and then transported to the host. The nitrogen fixing capacity of the *Anabaena azollae* system is due to the presence of a symbiotic cyanobacterium *Anabaena azollae* which inhabits the dorsal lobe of the leaves. The atmospheric nitrogen is reduced to ammonia by the Nitrogenase enzyme in the heterocyst and this newly fixed N is incorporated in to glutamine through glutamine synthetase (also present within heterocyst) of *Anabaena Azollae* (Ladha and Watanabe, 1984). The normal route of N assimilation in Anabaena is by glutamine synthetase and it has very little of this enzyme and about 90% of the activity of this enzyme is in the host. Thus the ammonia that Anabaena cannot use leaks into the cavity and is used by the host (Peters *et al,* 1986).The host Azolla incorporates this newly fixed N_2 into amino acids. Probably these amino acids, along with reductant and photosynthate, are then supplied to the symbiont. A study with ^{15}N (Peters *et al.*, 1980) revealed that the isolated symbiont exudes up to 50% of its fixed N_2 into incubation medium as ammonia and this observation paved way to several studies on the enzymes in the Anabaena and Azolla that are involved in ammonia assimilation. It was found that both organisms are capable of assimilating ammonia via the enzymes glutamate synthetase, glutamate synthase and glutamate dehydrogenase. On the other hand, the enzyme nitrogenase which is believed to occur in heterocyst of the *Anabaena* symbiont is capable of reducing N_2 and other substrates such as acetylene (Ray *et al.*, 1978 and Ladha and Watanabe, 1984).

*Anabaena azolla*e has three kinds of cells: vegetative cell, heterocyst and akinetes. The heterocyst is the actual site of N_2–fixation. A remarkable feature of symbiotic *Anabaena azollae* is the very high heterocyst frequency. The distance between two heterocyst is about 3-5 vegetative cells. In free living blue green algae, this distance is 15-30 vegetative cells. The blue green algae *A. azollae* consists of unbranched trichomes. In very young leaves, trichomes lack heterocyst. As the leaf matures, *Anabaena* increases its number and heterocyst frequency and equips itself to fix atmospheric N symbiotically and supplies the fixed N to the fern. Heterocyst gradually increases in frequency until they comprise 30-40% of the algal cells. On an average mature trichomes consist of 60.9% vegetative cells, 23.1% heterocyst and 16% akinetes (Hill, 1977; Ladha and Watanabe, 1982; Van Hove, 1989 and Maejima *et al.*, 2002)

Nitrogenase activity

Nitrogen is among the most important macronutrient required for the synthesis of nucleic acids, proteins, phospholipids and many secondary metabolites which play an important role in the overall growth of the plants. The ability of Azolla to fix atmospheric N allows this fern to grow successfully in aquatic habitats lacking or having low levels ofN and even to proliferate in polluted waters as well. (Pabby *et al.* 2003; Sood *et al.* 2007).

The symbiotic association between the Azolla and *Anabaena azollae* resulted in the fixation of atmospheric N through a process involving reduction of atmospheric dinitrogen mediated by an enzyme nitrogenase (Moore, 1969).Since most of the energy is supplied by photosynthates in host Azolla and the presence of a characteristically low levels of ammonia assimilating enzymes in the endosymbiont *A. azollae*, the N fixation process is more efficient only in the cells of *A. azollae* which are in active symbiotic state (Ladha and Watanabe, 1987 and Kannaiyan, 1992)

2.1.4 Chemical composition of Azolla

Chemical composition of Azolla is considered as a selective criterion for its growth, adaptation and functioning which varies not only according to species and ecotypes but also with the ecological conditions and phase of growth. Various aspects of the chemical composition have to be considered depending on the use to which it is put to viz; N bio fertilizer, green manure or food. In general Azolla contains 94 to 96 percent moisture content, 5 to 7 percent dry matter of different Azolla species. Azolla requires a minimum concentration of about 0.2-0.3 percent phosphorus on a dry weight basis for a normal N concentration of 3.0-4.0 percent and the crude protein content is about 19-30 percent of the dry matter during optimum growth conditions. Under natural conditions, protein content near 20-22 percent are frequent. The protein content of Azolla species is comparable to or higher than that of most other aquatic macrophytes. It contains 2-3.5 percent potassium approximately (Van Hove, 1989; Singh and Subudhi, 1978; Peters *et al.*, 1978; Becking, 1978; Herzalla *et al.*, 2001)

2.1.5 Factors influencing growth of Azolla

Environmental factors

Water

The fern cannot survive without water as evidenced from the etymology of Azolla, derived from Greek origin- azo and ollyo which means "killed by drought" (Carrapiço *et al.*, 2000). In other words, water is a vital and important factor for the survival of Azolla. This small aquatic fern should float on the water surface to stay alive. When enough water is available, it can form a layer with a height of around 2-3 cm. Though it can grow on a wet mud surface or wetted peat litter, this fern prefers to grow in free-floating conditions (Serag *et al.*, 2000; Liu *et al.*, 2008) on calm water surfaces and may thus be found on the surface of ponds, canals, and lakes as well as on some slow-moving rivers (Ghorbanzadeh and Tajer , 2009). Sadeghi *et al.*, (2012a) reported a close relationship between depth of water and the optimal growth and biomass production of Azolla in the Anzali wetland. Very low water depths might slow down the growth and hence reduce its biomass production (Biswas *et al.*, 2005).

Peters (1990) reported that the growth of Azolla is promoted by a fairly shallow depth of water in which there can be little turbulence. Kushari and Watanabe (1992) found that the availability of nutrients to Azolla depends largely on the amount of nutrients present in water rather than in the soil. Hechler and Dawson (1995) proved from experimets that nitrogenase activity in *A. caroliniana* was maximum when the moisture content of the tissue was 82-95 percent of the fresh mass; but under moisture stress, when the moisture content dropped to 80 percent, nitrogenase activity decreased to less than 1/5 th of its maximum.

Dissolved oxygen

Dissolved oxygen is probably the single most important water quality parameter that influences Azolla growth and proliferation. Combination of Azolla and cyanobacterial systems recorded a dissolved oxygen content of 7.4 ppm. Azolla that grow on the soil surface as a floating mass act as aerators in paddy field ecosystem. The oxygen released during the photosynthetic activity gets liberated as minute air bubbles thus aerating the water impounded in paddy field resulting in increased dissolved oxygen content (Muthukumar *et al.*, 2007)

Temperature

Temperature is one of the most important factors determining growth rates of free floating macrophytes in the field. The optimum temperature range for Azolla growth has been shown to be between 18 and 28°C. (Tuan and Thuyet, 1979). In fact, higher temperatures (35°C) can inhibit or even be harmful for its growth. However, different Azolla species, strains or varieties have different temperature sensitivities (Uheda *et al.*, 1999). Optimum temperature ranges for *A. caroliniana, A. filiculoides* and *A. pinnata* are between 25 and 30°C. *A. mexicana* will not tolerate frost, while *A. filiculoides* will survive it (Talley and Rains, 1980; Ashton and Walmsely, 1984; Van Der Heide *et al.*, 2006). Debusk and Reddy (1987) studied growth and nutrient uptake of *A. caroliniana* Wild, and *Salvinia rotundifolia* under controlled conditions at constant temperatures (from 10 to 30°C). Maximum plant density was obtained at a temperature range of 15-20°C. Net primary productivity was highest within the 20-

30°C range. There have been numerous studies about the differences in temperature response of Azolla species and their eco-physiological strains indicating that a very high (above 30°C) or very low temperature (below -4°C) could play an inhibitory role in the growth of Azolla (Liu *et al.*, 2008; Fernandez-Zamudio *et al.*, 2010 Sadeghi *et al.*, 2012a).

Light intensity

Photosynthetic activity, growth and N fixation of Azolla and its symbiont were all affected by light intensity. Sporulation was regulated by the interacting effects of light intensity, photoperiod, temperature and other factors such as pH, N and P supply (Pabby *et al.*, 2003). When light intensity was high and amount of nutrients in water is low, Azolla turns red. During hot summer or cold winter also it turned red or brownish-red especially under shaded conditions, whereas in nutrient-rich medium, it became green. Irradiance interacts with temperature in influencing growth of Azolla species (Janes, 1998). Growth rate and N fixation activity of Azolla change with increasing light. Pabby *et al.* (2003) demonstrated that growth rate and N fixation activity of Azolla changed with increasing light. Tuan and Thuyet (1979) pointed out that at pH 5; high light intensity increased Azolla growth whereas at pH 6 and 7 it inhibited growth. High light intensities (above 90 Klux) inhibited N_2 fixation whereas low light intensities or shading had a good effect on Azolla growth and multiplication.

Growing seasons and day length

The length of the growing season and day length were other climatic factors which regulated production of aquatic plants (Serag *et al.*, 2000). Biomass production and growth of Azolla were also dependent on the specific growing season. The growing seasons in Azolla, however, are linked with other factors such as nutrients, pH, salinity and wind. A water body with rich phosphorus and neutral pH is better than one with poor phosphorus and acidic conditions. Water temperature has a close relationship with air temperature. Therefore, any changes in weather conditions over different seasons have an important effect on Azolla growth (Ferdoushi *et al.*, 2008). On the whole production in summer is higher than in other seasons (Speelman *et al.*, 2009).

Physico-Chemical Factors

pH

Among soil properties, soil reaction or pH was reported to be very important factor in growth, establishment and diversity of cyanobacteria, which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth (Singh, 1992).As regards the quality of water; it should have a suitable pH for growth and N fixation. The effects of pH on Azolla also occured indirectly as its affects the availability of nutrients in floodwater. At very low pH, the solubility of Al, Fe and Mn may reach toxic levels in acid soils and may interfere with the absorption of Ca, Mg and other basic cations, and reduce the solubility of some of the micronutrients like Mo (Lumpkin and Plucknett., 1982). In alkaline pH, the availability of Ca, Mg and P decreases due to their decreased solubility and most of the micronutrients (except Mo) like Fe, Mn, Zn, Cu and B become less available. The availability of P depends on pH. Available phosphate ions are more predominant between 4.5 to 7.5. P fixation occurs with Fe, Al, Mn and Mg at lower pH while at higher pH it is mostly with calcium (Brady, 1984; Kaushik, 1994)

Salinity

Various studies have shown the negative effect of salinity on growth of different Azolla species. There are still no in depth studies about variation in salt tolerance of different species of Azolla. Overall, however, this genus is considered to be extremely sensitive to NaCl (Rai and Rai, 2000; Fernandez-Zamudio *et al.*, 2010). Masood *et al.* (2006) stated that salinity inhibits growth of *A. pinnata* and *A. filiculoides* leading to a significant decrease in dry weight. Therefore, the growth of *A. pinnata* and *A. filiculoides* plants gradually decreased when NaCl concentration increased. *A. filiculoides* was more sensitive to high salt concentration than *A. pinnata*. Salt concentrations above 10mM NaCl inhibited growth of *A. filiculoides*, but growth of *A. pinnata* stopped only at 40mM NaCl. The presence of nutrients like nitrate in the saline habitat plays a key role in conveying salt tolerance to Azolla (Mishra and Singh, 2006; Singh *et al.*, 2008). In other words, high salinity level can inhibit Azolla growth (Arora and Singh 2003; Fernandez- Zamudio *et al.*, 2010) leading to a decrease in removal of heavy metals by the species. (Sadeghi *et al.*, 2012a)

Nitrogen

In contrast to other minerals, availability of N and to some extent dissolved oxygen are not considered as limiting factors for Azolla growth, but growth of algae-free plants relies on combined N. An increase in nitrate concentration resulted in a low coverage of Azolla in the Anzali wetland study (Sadeghi *et al.*, 2012a), The reason is that through its symbiosis with Anabaena, Azolla can have free access to atmospheric nitrogen (N_2) for fulfilling all of its N_2 requirements (Costa *et al.*, 2009), while algae free plants are deprived of such opportunities. The upper lobes of Azolla have free contact with air, which is important for N fixation. Based on a study by Costa *et al.* (2009), high ammonium concentrations in wastewater had no effect on N fixation dynamics. Fang *et al.* (2007) surveyed nutrient fluxes along the roots of four wetland plants (*Azolla* spp., *Vallisneria natans*, *Bacopa monnieri* and *Ludwigia repens*) in order to see the dynamics of nutrient removal from eutrophicated water systems When different ions (e.g. NH_4^+, NO_3^-) were tested, *Azolla* spp. showed a preference for NO_3- uptake.

Growth and N fixation was estimated at about 3.1 to 4.6 kg N ha^{-1} day^{-1} in *A. pinnata* under field conditions (Kulasooriya and Hirimburegama, 1982). When field conditions are optimal for this species, its N_2 accumulation rate is estimated to be 5.0-9.0 mg N g^{-1} dry weight day^{-1}. This can result in a dry matter increase of 0.135-0.290 g g^{-1} Azolla dry weight day^{-1}. This value is consistent with a doubling time of 2.5-5.5 days. (Cary and Weerts, 1992; Biswas *et al.*, 2005; Costa *et al.*, 2009)

Phosphorus

Phosphorus (P) is an important nutrient to yield a successful and rapid growth of Azolla species (Katony *et al.*, 1996). The effect of this important nutrient on the overgrowth of Azolla has also been confirmed in the Anzali wetland (Sadeghi *et al.*, 2012a, b). In laboratory experiments, Janes (1998) found that increasing P supply and/or plant density led to increased sporulation. If there is enough P in the aquatic environment, Azolla will be able to grow without the need to provide combined nitrogen such as NH_4NO_3 (Costa *et al.*, 1999)

Azolla absorbs P from the floodwater and makes it available to the plant. The threshold concentration of P in Azolla tissue is probably about 0.2-0.3% on dry weight basis. P stressed plants are usually smaller, pink to red, less vigorous and have a low concentration of total nitrogen. Under severe stress, the plants become highly compact and dark red and often develop very long curled roots. The essentiality of P is emphasized from these findings. (Kobayashi *et al.*, 2008)

For alleviating P deficiency , P loaded Azolla grown with a smaller amount of P application into a smaller area of nursery will lead to higher concentration of P in the plant and this loaded P continue its multiplication for several times in the field conditions. Secondly instead of application of large amount of P at one time, split application in smaller dose (2-5 kg/ha) was considered more economical and efficient (Watanabe and Espinas, 1985; Watanabe, 1994). Field cultivation of Azolla usually requires P application, therefore the amount of P intended for direct application to rice can be applied to Azolla for increasing its growth and N_2- fixation and the same P will become available to rice after Azolla is incorporated into soil (Lumpkin, 1987).The ability of different Azolla species to grow under P deficient conditions indicates that species like *A. pinnata* may be used in low P soils (Watanabe, 1994)

Potassium

Apart from N and P, Azolla requires other macro nutrients like K, Ca and Mg for its growth. K and organic compound of N and P in the biomass of Azolla can be good indicators for its use as a bio fertilizer in domestic wastewaters or natural environments (Malavolta *ét al.*, 1981; Watanabe, 1982; Costa *et al.,* 1999). Azolla is able to take up K from the soil and make it available to the rice plants. In K- deficient plants, fronds become yellowish brown, roots become dark brown and their growth is stunted (Serag *et al.*, 2000)

Micronutrients

The importance of some micronutrients such as Fe and Mo has long been recognized for a successful and quick growth of Azolla, particularly in relation with its N fixation metabolism. It was observed that the threshold levels of the micronutrients such as Fe, Mn, Mo and B for Azolla growth were 50, 20, 0.3, and 30 μgL^{-1}, respectively (Wagner, 1997; Biswas *et al.*, 2005). Nitrogen fixation by Azolla-Anabaena requires Co and Mo (Zahran *et al.*, 2007). Singh *et al.*, 2010 studied the effect of micronutrients ($Mo^{6+,}$ $Mn^{2+,}$ $Zn^{2+,}$ Cu^{2+} and $Fe^{2+)}$ on cellular and extracellular activities of two Azolla species (*A. microphylla* and *A. filiculoides*) exposed to a P-deficient, saline (20mL M NaCl) medium. At lower concentrations (0-0.01mLM), the micronutrients showed a significant enhancement in the given activity whereas higher concentrations (e.g. at 10 mLM) played an inhibitory role. Sadeghi *et al.* (2012 b) reported a moderate effect of Fe on the growth of *A. filiculoides* in the Anzali wetland. The availability of Fe to be accessed by Azolla itself is influenced by pH value. When pH value is high, Fe is precipitated as hydroxide so that it becomes less available to Azolla. When Azolla has no access to Fe, it turns yellow.

Jain *et al.* (1992) studied the toxic effect of some elements on Azolla. Their study showed that iron and manganese did not have any toxic effect on the anaerobic fermentation of Azolla while Cu, Co, Pb and Zn showed toxicity.

Heavy metals

Among various water pollutants, heavy metals are of major concern because of their persistent and bio-accumulative nature (Lokeshwari and Chandrappa 2007; Chang *et al.* 2009; Yadav *et al.* 2009). A definite need exists to develop a low cost and eco-friendly technology to remove pollutants particularly heavy metals, thereby improving water quality. Phytoremediation offers an attractive alternative. Among these, Azolla, a free-floating, fast growing, and N fixing pteridophyte seems to be an excellent candidate for removal, disposal, and recovery of heavy metals from the polluted aquatic ecosystems (Arora *et al.* 2006; Umali *et al.* 2006). Rai (2008) reported that *A. pinnata* removed 70–94% of heavy metals (Hg and Cd) from ash slurry and chlor-alkali effluent in Singrauli region of U.P. (India) and the concentration of these heavy metals ranged between 310 and 740 mg kg^{-1} (l g g^{-1}) dry mass in tissues of Azolla. Jafari *et al.* (2010) observed the highest bio concentration potential of Pb, Cu, Mn, and Zn Was 94% in *A. microphylla*, 96% in *A. filiculoides*, 71% in *A. pinnata*, and 98% in *A. microphylla*, respectively. Rai (2010) recorded a marked reduction in concentrations of heavy metals like Cu, Cr, Fe, Mn, Ni, Pb, Zn, Hg, and Cd. Sarkar and Jana (1986) observed that the treatment of *A. pinnata* with As, Pb, Cu, Cd, and Cr (2 and 5 mg L^{-1} each), decreased Hill- activity, chlorophyll content, protein and dry weight, and increased tissue permeability with respect to control. The effects were most pronounced with the highest treatment of (5 mg L^{-1})

Carbon and Nitrogen metabolism

The rate of mineralization is influenced by the C: N ratio. Azolla species with a low C: N ratio mineralized in 2 days whiles the species with high C: N ratio mineralized in 5 days (Wang *et al.*, 1987).

Some of the ammoniacal (fixed) N is released directly into the water. The N fixed by Azolla-Anabaena symbiosis is mainly available only after its decomposition and mineralization (Moore, 1969 ;Watanabe *et.al.*, 1981). Azolla biomass, when incorporated into soil, decomposes and its N along with other nutrients is released during different stages of rice growth (Alejar 1982). The non-incorporated Azolla also decomposes after dense mat formation. Overcrowding stimulated its decomposition (Singh and Singh 1990). However its decomposition is reported to be faster when incorporated into soil than left as cover (Singh, 1992; Talley *et.al.*, 1977 and Watanabe *et.al.,* 1981). Lumpkin and Plucknett (1980) determined that Azolla pinnata had a C/N ratio of 15:1. Reports indicated an increase in N recovery when Azolla was incorporated into soil rather than allowed to decompose in water. N in Azolla was released slowly and its availability was only about 2/3 rd of ammonium sulfate. Most of the studies had revealed that two thirds of the N in Azolla was released after six weeks. Kannaiyan (1992) also reported that Azolla species had a low C/N ratio ranging from 10.14-13.79:1.

Materials and methods

3. MATERIALS AND METHODS

This investigation on the "Characterisation of soil and water of Palakkad eastern plains in relation to growth and Nitrogenase content of Azolla spp." was carried out with the two objectives in view achieved through two different experiments as detailed below:

Experiment 1: Survey of areas in Palakkad Eastern Plains (AEU 23)

Experiment 2: Collection and characterisation of soil, water and Azolla samples

3.1 EXPERIMENT-1: SURVEYING OF AREAS IN PALAKKAD EASTERN PLAINS (AEU 23)

An initial survey was carried out to identify the areas where Azolla was prevailing in Palakkad eastern plains (AEU23) (Plate 1) with the help of GPS

3.1.1 Methodology adopted

Two stages random sampling procedure was adopted for the survey. Two block panchayaths namely Chittoor and Kollengode were selected on the basis of Azolla prevalence. From Chittoor block panchayath four grama panchayath were identified. From Kollengode block panchayath the identification was limited to five grama panchayaths.

3.2 COLLECTION AND CHARACTERIZATION OF SOIL, FLOOD WATER AND AZOLLA SAMPLES

The collection was done during August- December 2014. Ten samples each of soil, water and Azolla were collected from the growing locations of the identified places making a total number of 270. From the non-growing locations the collection was restricted to soil and water samples making a total of 180. After the collection, the samples were processed and preserved for individual analysis.

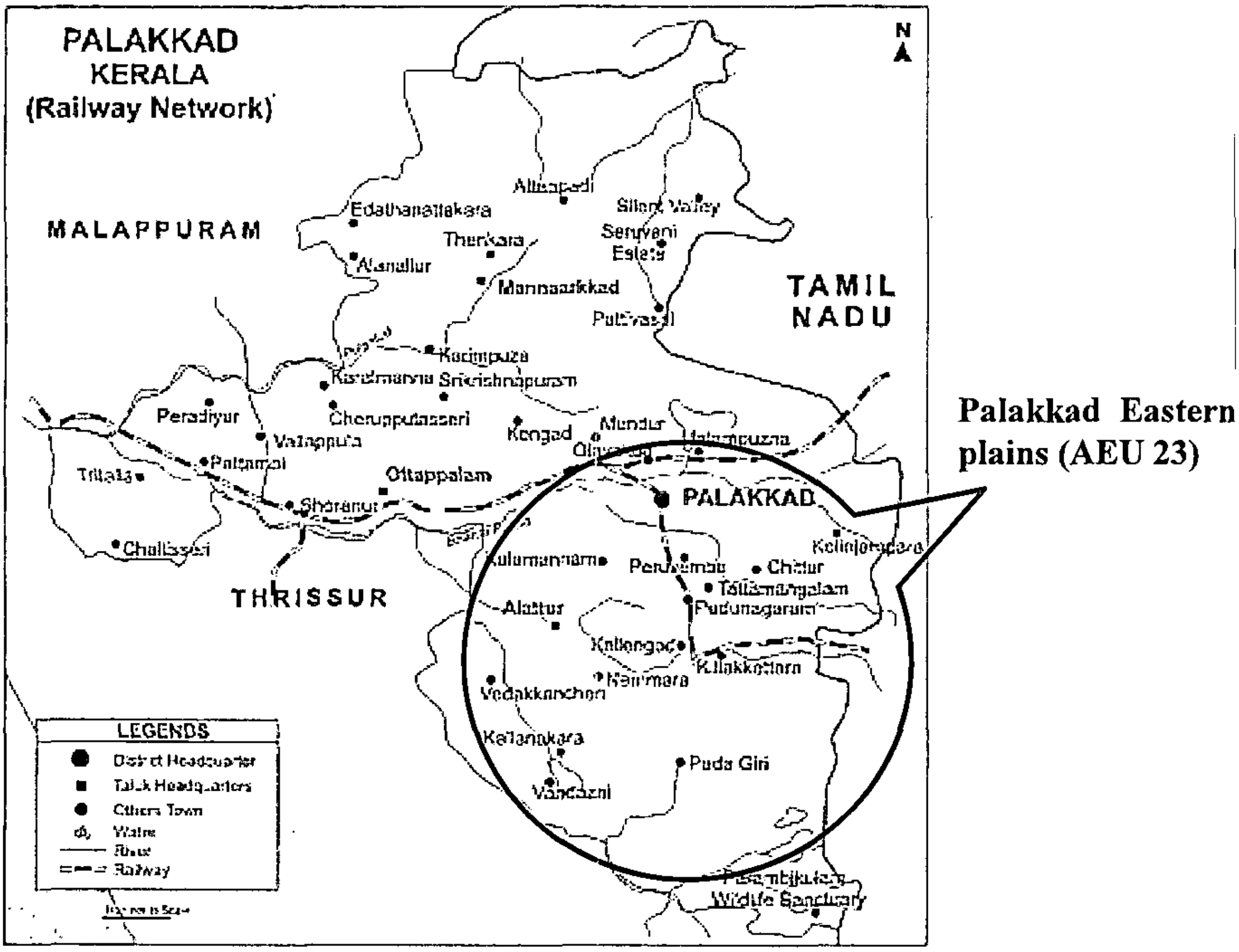


Plate 1: Map showing the experimental site

Plate 2: Collection of soil, floodwater and Azolla samples from different locations of Chittoor block

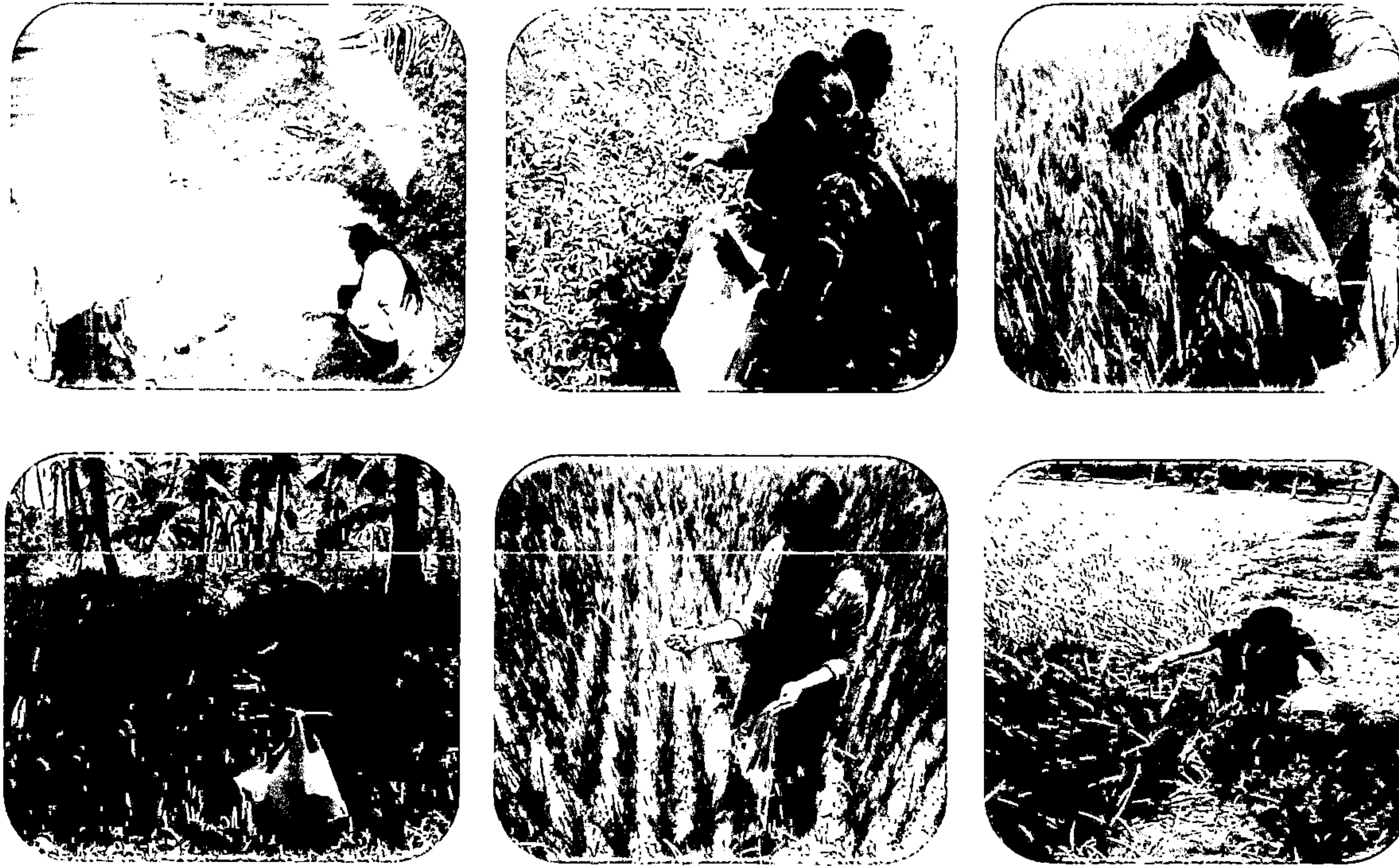


Plate 3: Collection of soil, flood water and Azolla samples from different locations of Kollengode block

a) Soil:

The collected soil samples were oven dried and sieved using 2mm sieve and subjected to analysis employing standard procedure as shown in Table 1. For organic carbon estimation the samples were passed through a 0.5 mm sieve.

Table 1. Methods of soil analysis

Parameter	Extraction and Estimation Methods	Reference
pH	1: 2.5 soil water suspension – pH Meter.	Jackson (1958)
Electrical conductivity	Supernatant liquid used for pH determination- conductivity meter	
Organic carbon	Wet oxidation method	Walkley and Black (1934)
Available N	Alkaline permanganate method	Subbiah and Asija (1956)
Available P_2O_5	Olsen's extractant (0.05 *N*aHCO3)	Olsens *et al.*, 1954
Available K_2O	Neutral normal ammonium acetate method using flame photometer.	Jackson (1958)
Total Heavy metals (Fe, Mn, Zn, Cu, Cr, Cd, Ni, Pb)	Di acid digestion (HNO_3:$HCLO_4$ in 9:4) followed by estimation using Atomic Absorption Spectrophotometry	Hesse (1994)

b) Water:

Flood water samples were collected from the field in bottles of 500 mL capacity .After bringing to the laboratory they were filtered using Whatmann No: 1 filter paper and stored in sample bottles of 100 mL capacity. In order to avoid contamination two to three drops of toluene were added. Various estimations were done using standard methods as shown in Table 2

Table 2: Methods of flood water analysis

Parameter	Methods	Reference
pH	pH meter and conductivity meter	Wilcox , (1950)
Electrical conductivity (EC)		
Dissolved oxygen	Dissolved Oxygen meter	–
Temperature (in-situ))	In- situ Thermometer	–
Water soluble heavy metals (Fe, Mn, Zn, Cu, Cr, Cd, Ni, Pb)	Atomic Absorption Spectrophotometer	HACH (1997)

Plate 4: In-situ measurement of pH using portable pH meter

Plate 5: In-situ measurement of water temperature using thermometer

c) Azolla

Before the initiation of the experiment, moisture content of Azolla was determined and then Azolla samples were thoroughly washed, put in brown paper cover and oven dried at 50 to 70° C. The dried samples were ground, properly mixed and used for estimating C, N, P, K and crude protein as per the methods mentioned in Table 3

Table 3: Composition of Azolla – Methodology adopted

Parameter	Methods	Reference
Moisture	Gravimetry	Piper, 1942
Total carbon	CHNS analyzer	Model: Elementar's vario EL cube
Total Nitrogen		
Total Phosphorus	Diacid Extract; Spectrophotometry	Piper, 1942
Total Potassium	Diacid Extract; Flame photometry	

Crude protein content was calculated by multiplying N with the factor 6.25

C.1 identification of species

The species of Azolla collected from different locations were identified using the facilities available at NARDeP (National Agricultural and Rural Development Policy Center) Kanyakumari.

d) Nitrogenase activity of Azolla

The Nitrogenase activity of Azolla was quantified using Acetylene reduction assay (Hardy et al., (1968).The enzyme Nitrogenase reduces acetylene (C_2H_2) to ethylene (C_2H_4) and the ethylene formed can be very sensitively detected by gas chromatography.

Methodology

The Methodology of Acetylene reduction assay (Plate 6) is detailed below:

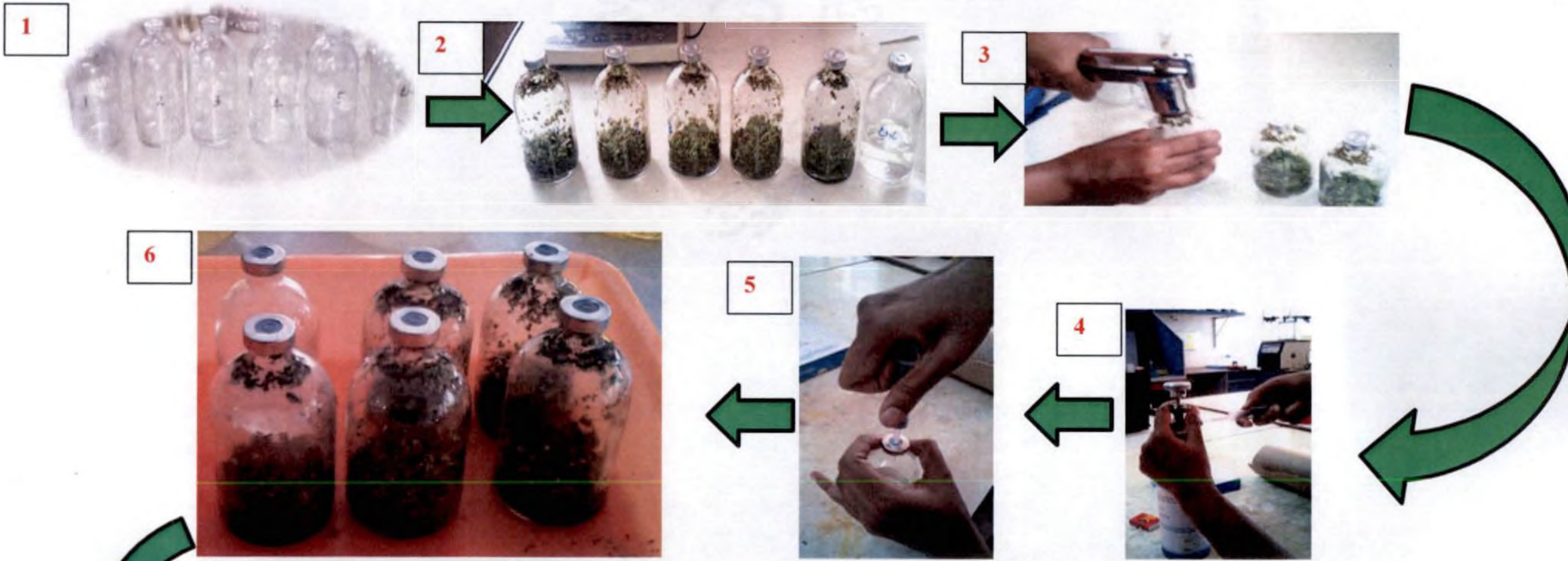
1. Five grams of Azolla Samples were placed into 500 ml capacity serum bottle.
2. The bottles were sealed with rubber stoppers
3. The acetylene was injected @ 10% to replace an equal volume of air displaced from the bottle
4. Then incubated at 25°C for 24 h.
5. A quantity of 0.5 mL of gas inside the jar was drawn out using a syringe and the concentration of ethylene in it was analyzed by GC (gas chromatography) equipped with FID . The column used was Porapack N (GL Sciences).
6. The results were expressed as nmol C_2H_4 produced g^{-1} h^{-1}

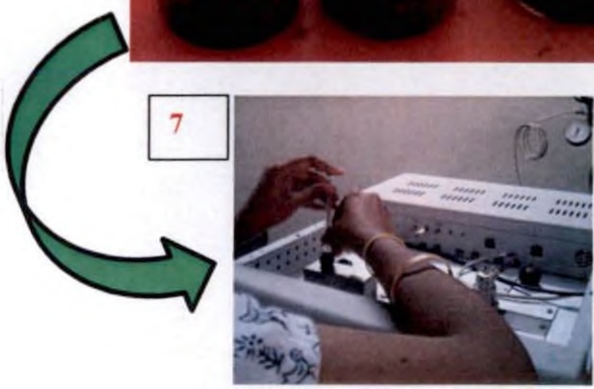
$$\text{Nitrogenase activity} = \frac{\text{peak length in mm P x attenuation x range x 0.0006 x vol. of gas}}{\text{Hour of incubation x vol. of gas sample injected in to Gas chromatography x dry weight of the sample}}$$

3.3 STATISTICAL ANALYSIS

Data were subjected to analysis of variance (ANOVA) (Panse and Sukhatme, 1985) using statistical package 'SPSS' package. Whenever the t test was significant (at 5 % level) multiple comparison among the treatments were done with Duncan's Multiple Range test (DMRT).

Plate 6: Schematic representation on acetylene reduction assay





Stages:

1- Serum bottles (500 mL) for containing Azolla
2- Added 20g of Azolla into each bottle
3- Capped the containers with rubber stoppers and then sealed
4- Preparatory evacuation of the containers
5- Injection of acetylene gas replacing an equal volume of air
6- Incubation for 24 h.
7- Analysed using gas chromatography

Results

4. RESULTS

The results of the investigations conducted on "Characterisation of soil and water of Palakkad eastern plains in relation to growth and Nitrogenase content of *Azolla* spp." are presented in this chapter.

4.1 Experiment 1: Surveying the Azolla growing areas in Palakkad Eastern Plains (AEU 23)

4.2 Experiment 2: Collection and characterization of soil, water and Azolla samples

4.1 SURVEYING THE AZOLLA GROWING AREAS IN PALAKKAD EASTERN PLAINS (AEU 23)

An initial survey was carried out with the help of GPS to identify the areas where Azolla is prevalent in Palakkad eastern plains (AEU23). After the comprehensive survey conducted in four blocks viz. Alathur,. Chittoor, Kollengode and Nenmara, the prevalence of Azolla could be seen only in two blocks namely Chitoor and Kollengode. The GPS reading of the selected sites are given in Table 4

Table 4: GPS readings of Azolla prevalent areas in Palakkad eastern plains

Areas	GPS readings
<u>Kollengode</u>	
Spot 1	76° 43' 31.15" E (longitude) 10° 36' 57.48" N (latitude)
Spot 2	76° 43' 31.12" E 10° 36' 57.46" N
Spot 3	75° 43'42.02" E 10° 36'44.15" N
Spot 4	76° 45' 58.37" E 10° 36' 11.23" N
Spot 5	76° 45' 58.39" E 10° 36' 11.20" N

Vadavannur	
Spot 1	76° 42' 01.17" E 10° 39' 25.94" N
Spot 2	76° 42' 01.95" E 10° 39' 25.96" N
Spot 3	76° 41' 58.89" E 10° 39' 26.51" N
Spot 4	76° 40' 00.29" E 10° 39' 26.54" N
Spot 5	76° 40' 00.27" E 10° 39' 26.52" N
Muthalamada	
Spot 1	76° 45' 58.37" E (longitude) 10° 36' 11.53" N (latitude)
Spot 2	76° 45' 58.39" E 10° 36' 11.20" N
Spot 3(pond)	75° 43' 42.02" E 10° 36' 44.15" N
Spot 4	75° 43' 41.04" E 10° 36' 43.17" N
Spot 5	76° 45' 58.36" E 10° 36' 11.15" N
Nalleppily	
Spot 1	76° 48' 08.69" E 10° 43' 11.60" N
Spot 2	76° 46' 59.72" E 10° 43' 41.57" N
Spot 3	76° 47' 54.6" E 10° 43' 30.02" N
Spot 4	76° 47' 36.86" E 10° 43' 46.65" N
Spot 5	76° 46' 59.72" E 10° 43' 41.57" N
Kozhinjampara	
Spot 1	76° 49' 45.70" E 10° 42' 52.83" N
Spot 2	76° 49' 45.56" E 10° 42' 54.04" N

Spot 3	76° 49' 45.75" E 10° 42' 52.88" N
Spot 4	76° 49' 45.65" E 10° 42' 52.76" N
Spot 5	76° 49' 45.71" E 10° 42' 52.80" N
Elappully	
Spot 1	76° 45' 10.57" E (longitude) 10° 45' 37.54" N (latitude)
Spot 2	76° 45' 09.9" E 10° 45' 37.0" N
Spot 3	76° 45' 08.2" E 10° 45' 36.0" N
Spot 4	76° 45' 38.1" E 10° 45' 53.5" N
Spot 5	76° 45' 35.8" E 10° 45' 51.2" N
Perumatty	
Spot 1	76° 44' 48.27" E 10° 40' 50.98" N
Spot 2	76° 44' 45.0" E 10° 40' 50.3" N
Spot 3	76° 44' 49.8' E 10° 40' 48.8" N
Spot 4	76° 44' 39.3" E 10° 40' 45.4" N
Spot 5	76° 44' 39.7" E 10° 40' 49.0" N
Pattencheri	
Spot 1	76° 43' 42.90" E 10° 38' 46.30" N
Spot 2	76° 43' 53.6" E 10° 38' 45.7" N
Spot 3	76° 43' 54.6" E 10° 38' 03.6" N
Spot 4	76° 43' 51.8" E 10° 38' 40.6" N
Spot 5	76° 43' 53.8" E 10° 38' 45.8" N

Pudunagaram	
Spot 1	76° 40' 57.7" E 10° 40' 41.6" N
Spot 2	76° 41' 13.8" E 10° 38' 48.7" N
Spot 3	76° 41' 09.8" E 10° 38' 44.4" N
Spot 4	76° 41' 07.1" E 10° 38' 45.0" N
Spot 5	76° 40' 59.3" E 10° 38' 47.4" N

4.2 COLLECTION AND CHARACTERIZATION OF SOIL, FLOOD WATER AND *AZOLLA* SAMPLES

Samples of soil, water and Azolla were collected from the selected sites and characterized based on electro-chemical properties (for soil and flood water). In case of Azolla the composition was analyzed. These results are presented under different headings

A. SOIL SAMPLE

4.2.1 pH

pH is considered to be one of the important factor deciding the growth and functioning of Azolla. The mean values on soil pH of soil samples collected both from growing as well as non- growing locations are given in Table 5

Table 5: Soil pH of samples collected from different places of Azolla growing and non-growing locations

pH / Places of collection	Growing	Non-growing
Vadavannur	7.12[cd]	7.42[ab]
Muthalamada	7.23[abc]	7.48[ab]
Nalleppilli	7.34[abc]	7.55[ab]
Kollengode	**7.47**[a]	7.59[a]
Pudunagaram	7.4[abc]	7.45[ab]
Pattencheri	7.42[abc]	7.50[ab]
Elappully	7.44[b]	**7.61**[a]
Perumatty	**6.84**[d]	**7.17**[c]
Kozhinjampara	7.13[bcd]	7.39[b]
CD (0.01)	**0.425**	**0.266**
(0.05)	**0.317**	**0.198**
t-value	**3.779****	

** significance at 1% level

The data on soil pH revealed a significant difference between places under each category. The mean pH value of Azolla growing areas ranged from 6.84 (Perumatty) to 7.47 (Kollengode) whereas the range was 7.17 (Perumatty) to 7.61 (Elappully) in the non- growing locations. In general a significant difference was noticed between growing and non-growing locations.

4.2.2 Electrical Conductivity

Electrical conductivity which is the measure of the soluble salts present in soil was determined in order to understand the tolerance of Azolla to salinity. Mean values of soil Electrical Conductivity obtained in growing and non-growing locations are shown in Table 6.

Table 6: Soil Electrical Conductivity (dS m^{-1}) of samples collected from different places of Azolla growing and non-growing locations

EC / Places of collection	Growing	Non-growing
Vadavannur	**0.17**^{c}	**0.29**^{e}
Muthalamada	0.27^{ab}	0.37^{cd}
Nalleppilli	0.30^{a}	0.40^{bc}
Kollengode	0.29^{ab}	0.40^{c}
Pudunagaram	0.30^{a}	0.39^{bc}
Pattencheri	**0.30**^{a}	0.42^{b}
Elappully	0.29^{ab}	**0.47**^{a}
Perumatty	0.25^{b}	0.34^{d}
Kozhinjampara	0.27^{ab}	0.39^{c}
CD (0.01)	**0.064**	**0.045**
(0.05)	**0.048**	**0.034**
t- value	**9.927****	

** significance at 1% level

In general the EC values of Azolla growing and non- growing locations differed significantly. The mean value showed considerable variation in the growing and non-growing sites. Significant difference was there within places under each category. The EC values in the growing locations ranged from 0.17 dS m^{-1} (Vadavannur) to 0.30 dS m^{-1} (Pattencheri) as against from 0.29 dS m^{-1} (Vadavannur) to 0.47 dS m^{-1} (Elappully) recorded in the non-growing sites.

4.2.3 Organic carbon

Carbon content of the soil as an index of soil fertility was determined and Table 7 shows the values obtained in the Azolla growing and non-growing locations.

Table 7: Soil Organic carbon content (%) of samples collected from different places of Azolla growing and non-growing locations.

OC / Places of collection	Growing	Non-growing
Vadavannur	**1.64**[a]	**1.4**[a]
Muthalamada	1.56[a]	1.24[b]
Nalleppilli	1.56[a]	1.23[b]
Kollengode	1.41[b]	1.14[bcd]
Pudunagaram	1.36[bc]	1.11[cd]
Pattencheri	1.32[bc]	1.32[bc]
Elappully	1.3[bc]	**1.06**[d]
Perumatty	1.28[bc]	1.20[bc]
Kozhinjampara	**1.23**[c]	1.11[cd]
CD (0.01)	**0.187**	**0.132**
(0.05)	**0.139**	**0.099**
t- value	**7.19****	

** significance at 1% level

Organic carbon content was significantly different in the Azolla growing and non- growing locations, also among the places significant difference was noticed under each category. The content range from 1.23 (Kozhinjampara) to 1.64% (Vadavannur) in the growing sites whereas it was between 1.06 (Elappully) and 1.40% (Vadavannur). The mean soil organic carbon of Azolla growing locations was more in Azolla growing locations.

4.2.4 Available nitrogen

The data on available N is furnished in Table 8.In general a significant difference was noticed between growing and non-growing locations and within each category. It is clear from the values that irrespective of the growth of Azolla, N content was in the low category . The available N status in all the places under the Azolla growing category was higher than in the non-growing category. The highest value of 161.8 kg ha^{-1} was obtained in Elappully panchayath under the growing areas whereas it was only 142.4 kg ha-1 in the non-growing tracts of the same location: In the non-growing locations maximum value (142.4 kg $ha-^{1}$) was recorded in Nalleppilly panchayath and minimum (105.8 kg ha^{-1}) in Kozhinjampara panchayath.

Table 8: Available N (kg ha^{-1}) of soil samples collected from different places of Azolla growing and non-growing locations.

Soil N / Places of collection	Growing	Non-growing
Vadavannur	**129.18** ^{d}	115.9 ^{a}
Muthalamada	144.2 ^{c}	108.4 ^{a}
Nalleppilli	153.6 ^{b}	149.9 ^{b}
Kollengode	154.3 ^{ab}	**142.4** ^{a}
Pudunagaram	153.0 ^{b}	112.1 ^{bc}
Pattencheri	152.0 ^{bc}	114.7 ^{bc}
Elappully	**161.8** ^{a}	141.1 ^{a}
Perumatty	157.1 ^{ab}	112.1 ^{bc}
Kozhinjampara	159.1 ^{ab}	**105.8** ^{c}
CD (0.01) (0.05)	**10.759** **8.024**	**12.819** **9.561**
t- value	**9.474****	

** significance at 1% level

4.2.5 Available Phosphorus

Phosphorus is considered to be the most limiting nutrient for the growth of Azolla. The available soil P status of the Azolla growing and non-growing locations are given in Table 9

Table 9: Available P content (kg ha^{-1}) of soil samples collected from different places of Azolla growing and non-growing locations.

Soil P / Places of collection	Growing	Non-growing
Vadavannur	61.2^{ab}	80.7^{ab}
Muthalamada	57.3^{c}	79.1^{b}
Nalleppilli	**63.3**^{a}	**84.2**^{a}
Kollengode	61.4^{ab}	83.6^{a}
Pudunagaram	52.4^{d}	77.7^{b}
Pattencheri	52.4^{d}	78.1^{b}
Elappully	61.3^{ab}	80.1^{ab}
Perumatty	58.6^{bc}	79.2^{b}
Kozhinjampara	**50.2**^{d}	**71.7**^{c}
CD (0.01)	**5.195**	**5.583**
(0.05)	**3.875**	**4.164**
t- value	**20.9****	

** significance at 1% level

There was significant difference in the available P status among the Azolla growing and non-growing locations. In general the P status was under high category as per soil fertility rating. Soil samples from Azolla growing locations of Nalleppilli panchayath recorded a value of 63.32 kg ha^{-1} available P whereas the non-growing locations of the same location recorded 84.2 kg ha^{-1} of available P.

4.2.6 Available Potassium

The data on available K is furnished in Table 10. In general a significant difference was noticed between growing and non-growing locations. Available K status ranges from 140 to 194 kg ha^{-1} in growing areas and it ranged from 136 to 186 kg ha^{-1} in non-growing areas. The Maximum reading of 194.3 kg ha^{-1} was noticed in Vadavannur panchayath under growing and among non-growing locations the highest value of 186.3 kg ha^{-1} was reported to be at Pattencheri panchayath.

Table 10: Available K readings (kg ha^{-1}) of soil samples collected from different places of Azolla growing and non-growing locations.

Soil K / Places of collection	Growing	Non- growing
Vadavannur	**194.3**^{a}	182.4^{ab}
Muthalamada	148.1^{de}	**136.0**^{e}
Nalleppilli	191.2^{a}	179.4^{abc}
Kollengode	193.3^{a}	181.7^{abc}
Pudunagaram	188.9^{ab}	176.6^{bc}
Pattencheri	178.7^{bc}	**186.3**^{a}
Elappully	158.9^{d}	173.6^{c}
Perumatty	173.2^{c}	150.8^{d}
Kozhinjampara	**140.3**^{e}	138.9^{e}
CD (0.01)	**15.481**	**11.557**
(0.05)	**11.547**	**8.620**
t- value	**1.577**	

4.2.7 Total Heavy metals

Soil samples collected from selected sites were characterized for heavy metals viz. Fe, Mn, Zn, Cu, Cr, Cd, Ni and Pb. The results of individual element are presented in the Tables 11, 12, 13 and 14.

a) Iron

Table 11: Iron content (mg kg^{-1}) of soil samples collected from different places of Azolla growing and non-growing locations.

Soil Fe / Places of collection	Growing	Non-growing
Vadavannur	**1506.1**[a]	1678.9
Muthalamada	1625.6[bc]	1660.0
Nalleppilli	1646.2[bc]	1680.0
Kollengode	1631.6[bc]	1659.0
Pudunagaram	1646.2[bc]	1657.0
Pattencheri	1648.0[bc]	1652.7
Elappully	1622.4[b]	1641.9
Perumatty	**1653.9**[c]	1688.4
Kozhinjampara	1623.2[b]	1665.6
CD (0.01)	**35.77**	
(0.05)	**26.68**	NS
t- value	**5.268****	

** significance at 1% level

Fe is considered to be important for the functioning of Nitrogenase enzyme. Significant difference was noticed between places under Azolla growing category. In growing areas the highest value of 1653.9 mg kg^{-1} was observed at Perumatty panchayath and in the non-growing areas, the maximum value of 1688.4 mg kg^{-1} was reported at the same location.

b) Manganese

The data for total Mn content in soil is furnished in Table 12. A difference of 99.2 mg kg^{-1} was noticed in the mean values between growing and non-growing locations. The highest value of 309.6 mg kg^{-1} was recorded at Kollengode panchayath among the growing location and it was 461.7 mg kg^{-1} at Kozhinjampara among non-growing locations. Significant difference was noticed within and between growing and non-growing locations.

Table 12: Manganese content (mg kg^{-1}) of soil samples collected from different places of Azolla growing and non-growing locations.

Element - Mn / Places of collection	Growing	Non-growing
Vadavannur	**228.1** ^{d}	332.4 ^{c}
Muthalamada	267.4 ^{b}	304.5 ^{c}
Nalleppilli	234.0 ^{cd}	330.2 ^{c}
Kollengode	**309.6** ^{a}	394.9 ^{b}
Pudunagaram	301.7 ^{a}	369.5 ^{b}
Pattencheri	247.9 ^{c}	402.9 ^{b}
Elappully	231.8 ^{cd}	**266.3** ^{d}
Perumatty	278.6 ^{b}	377.7 ^{b}
Kozhinjampara	248.5 ^{c}	**461.7** ^{a}
CD (0.01)	**23.20**	**47.05**
(0.05)	**17.30**	**35.09**
t- value	**9.700****	

** significance at 1% level

c) Zinc

Zinc content of the collected soil samples are given in Table 13. Zn also revealed a similar trend, like Fe and Mn, with respect to mean values which was the highest at non-growing locations as against in growing locations. Among the non-growing category highest value of 27.9 mg kg^{-1} was reported at Vadavannur panchayath and samples from Kollengode panchayath recorded the highest value of 24.3 mg kg^{-1} among the Azolla growing locations. Zn content among the locations shows significant difference only in growing areas.

Table 13: Zinc content (mg kg^{-1}) of soil samples collected from different places of Azolla growing and non-growing locations.

Element-Zn / Places of collection	Growing	Non-growing
Vadavannur	21.7^{bc}	27.9
Muthalamada	23.1^{ab}	26.3
Nalleppilli	23.3^{ab}	26.1
Kollengode	**24.3^{a}**	26.9
Pudunagaram	22.4^{abc}	24.2
Pattencheri	21.5^{bc}	24.4
Elappully	20.8^{c}	27.7
Perumatty	**16.6^{d}**	26.8
Kozhinjampara	22.4^{abc}	27.5
CD (0.01) (0.05)	**2.733** **2.038**	NS
t- value	**15.04****	

** significance at 1% level

d) Copper

The results in Table 14 revealed a significant difference among the locations with respect to Cu. The values ranged from 16.9 mg kg^{-1}(Muthalamada) to 36.22 mg kg^{-1} (Kozhinjampara) in the growing locations whereas it varied between 22.44 mg kg^{-1} (Kollengode) and 29.1 mg kg^{-1} (Kozhinjampara) in the non-growing locations.

Table 14: Copper content (mg kg^{-1}) soil samples collected from different places of Azolla growing and non-growing locations

Element- Cu / Places of collection	Growing	Non-growing
Vadavannur	18.3^{fg}	26.3^{bc}
Muthalamada	**16.9^{g}**	24.4^{cde}
Nalleppilli	24.8^{c}	24.9^{cd}
Kollengode	20.8^{ef}	**22.4^{e}**
Pudunagaram	29.7^{b}	26.5^{bc}
Pattencheri	21.8^{de}	27.8^{ab}
Elappully	24.3^{cd}	23.7^{de}
Perumatty	24.2^{cd}	25.8^{bcd}
Kozhinjampara	**36.2^{a}**	**29.1^{a}**
CD (0.01)	**3.873**	**2.955**
(0.05)	**2.889**	**2.204**
t- value	**1.604**	

e) Cd, Cr, Ni & Pb

In both growing and non-growing locations and among different locations under each category the content of the heavy metals viz. Cd, Cr, Ni and Pb were below the detectable limits hence it is not presented in tabular form

B. FLOOD WATER SAMPLE

Flood water samples were collected in plastic containers from rice fields and other water bodies based on the presence or absence of Azolla. These samples were analyzed in the laboratory for various parameters and the results are presented in the following tables.

4.2.8 pH

Results of flood water pH are given in Table 15.In general the pH of water sample was in neutral to alkaline range. The non-growing locations recorded a higher mean pH value as against the growing locations. The location wise value on soil pH was also higher in the non-growing locations. The pH value ranged from 6.5 (Perumatty) to 6.9 (Vadavannur, Elappully, Nalleppilli) in the growing locations compared to values between 6.9(Vadavannur) and 7.3 (Pudunagaram) in the non-growing locations. Effect of pH was significantly different between the categories but not among places under growing and non-growing categories.

Table 15: Flood water pH of samples collected from different places of Azolla growing and non-growing locations.

Flood water pH / Places of collection	Growing	Non-growing
Vadavannur	6.9	6.9
Muthalamada	6.6	7.0
Nalleppilli	6.9	7.1
Kollengode	6.7	7.2
Pudunagaram	6.8	7.3
Pattencheri	6.7	7.2
Elappully	6.9	7.2
Perumatty	6.5	7.2
Kozhinjampara	6.8	7.1
CD (0.05)	NS	NS
t- value	**5.769****	

** significance at 1% level

4.2.9 Electrical conductivity

The electrical conductivity, which indicated the degree of salinity, was recorded in Azolla growing and non-growing sites. The results presented in Table 16 revealed that the mean EC value was exactly the similar in both the sites. In general it ranged from 0.21dSm^{-1} (growing region) to 0.26 dS m^{-1} (non-growing region). Places had a significant effect on EC value among growing and non-growing locations, but in general there is no significant difference between growing and non-growing locations.

Table 16: Electrical conductivity (dS m^{-1}) of flood water samples collected from different places of Azolla growing and non-growing locations

Flood water EC / Places of collection	Growing	Non-growing
Vadavannur	0.25	**0.13**^{b}
Muthalamada	0.22	0.25^{a}
Nalleppilli	0.22	0.21^{a}
Kollengode	0.21	0.22^{a}
Pudunagaram	0.22	**0.26**^{a}
Pattencheri	0.21	0.26^{a}
Elappully	0.25	0.26^{a}
Perumatty	0.22	0.23^{a}
Kozhinjampara	0.26	0.24^{a}
CD (0.01) (0.05)	**NS**	**0.074** **0.055**
t- value	**0.000**	

4.2.10 Flood water temperature

Results for flood water temperature are given in Table 17.Water temperature measured ranged from 29.94^{0}C (Muthalamada) to 31.35^{0}C (Pudunagaram) in the growing locations and from 32.19 ^{0}C (Kozhinjampara) to 33.48^{0}C (Pattencheri) in the non-growing locations. In general a significant difference was not there between growing and non-growing locations but the places was significantly different with respect to temperature recorded in both the sites. However in the non-growing locations there was an increase of 2^{0}C in water temperature was noticed.

Table 17: Flood water temperature (^{0}C) of samples collected from different places of Azolla growing and non-growing locations

Flood water Temperature / Places of collection	Growing	Non-growing
Vadavannur	30.71	33.03
Muthalamada	29.94	33.12
Nalleppilli	30.45	32.98
Kollengode	31.08	32.93
Pudunagaram	31.35	32.30
Pattencheri	31.27	33.48
Elappully	30.90	32.35
Perumatty	31.28	33.01
Kozhinjampara	31.10	32.19
CD (0.01) (0.05)	**NS**	**NS**
t- value	**9.002****	

** significance at 1% level

4.2.11 Dissolved oxygen content

Dissolved oxygen refers to the level of free non-compounds of oxygen present in water or other liquids. It is an important parameter in assessing water quality because of its influence on the organisms living within a body of water. Results of dissolved oxygen content in water are given in Table18. In general a significant difference was noticed between the two categories. In the growing locations the highest value recorded was 7.84 mg L^{-1} (Kollengode) whereas it was 7.52 mg L^{-1} (Pudunagaram) in the non-growing region.

Table 18: Dissolved oxygen content (mg L^{-1}) of flood water samples collected from different places of Azolla growing and non-growing locations

Flood water DOC / Places of collection	Growing	Non-growing
Vadavannur	7.80	7.36
Muthalamada	7.82	7.44
Nalleppilli	7.74	7.44
Kollengode	7.84	7.44
Pudunagaram	7.74	7.52
Pattencheri	7.78	7.46
Elappully	7.76	7.48
Perumatty	7.72	7.50
Kozhinjampara	7.78	7.48
CD (0.01) (0.05)	**NS**	**NS**
t- value	**15.880****	

** significance at 1% level

4.2.12 Heavy metals

The content of soluble form of heavy metals detected in the flood water samples of Azolla growing and non-growing locations are presented in the Tables 19 to 22

a) Flood water soluble iron

Fe content in the flood water sample is shown in Table 19. The average value varied from 10.2 mg L^{-1} (Vadavannur) to 13.7 mg L^{-1} (Perumatty) ppm in the growing locations, whereas it was between 13.7 mg L^{-1} (Kozhinjampara) and 15.8 mg L^{-1} (Elappully) in the non-growing locations. Places had a significant effect at both the levels on Fe present in the flood water sample collected from Azolla growing locations. In the Azolla non-growing locations the Fe content did not show any significant effect due to places of collection. A comparison between Azolla growing and non-growing locations revealed a significant effect of Azolla in deciding the amount of iron present in flood water.

b) Flood water soluble manganese

The data on Mn content of flood water samples collected from Azolla growing and non-growing locations are presented in Table 20. Much similar to iron the level of Mn was significantly influenced by the places only in the presence of Azolla. The highest value of Mn (14.7 mg L^{-1}) was recorded in Perumatty and the lowest value (11.1 mg L^{-1}) was recorded in Pudunagaram. In the non-growing locations at Elappully recorded the maximum content (16.3 mg L^{-1}) of Mn while it was only 12.9 mg L^{-1} in the samples collected from Pudunagaram. In case of Mn significant difference was noticed between Azolla growing and non-growing locations at both the levels of significance.

Table 19:Flood water soluble iron content (mg L^{-1}) of samples collected from different places of Azolla growing and non-growing locations

Flood water Soluble Fe / Places of collection	Growing	Non-growing
Vadavannur	**10.2**^{e}	15.5
Muthalamada	12.1^{bcd}	15.4
Nalleppilli	13.1^{abc}	15.1
Kollengode	12.6^{abcd}	14.7
Pudunagaram	13.0^{abc}	14.6
Pattencheri	13.2^{ab}	15.2
Elappully	11.6^{d}	15.8
Perumatty	**13.7**^{a}	14.4
Kozhinjampara	11.9^{cd}	13.7
CD (0.01) (0.05)	**1.702** **1.270**	**NS**
t- value	**7.919****	

** significance at 1% level

Table 20: Flood water soluble Mn content (mg L^{-1}) of samples collected from different places of Azolla growing and non-growing locations

Flood Water soluble Mn / Places of collection	Growing	Non- growing
Vadavannur	12.3^{cd}	15.3
Muthalamada	13.4^{b}	14.7
Nalleppilli	12.5^{cd}	13.9
Kollengode	12.0^{d}	13.5
Pudunagaram	**11.1^{e}**	12.9
Pattencheri	13.0^{bc}	15.4
Elappully	12.2^{cd}	16.3
Perumatty	**14.7^{a}**	16.2
Kozhinjampara	13.7^{b}	14.3
CD (0.01) (0.05)	**1.319** **0.984**	**NS**
t- value	**3.626****	

** significance at 1% level

c) Flood water soluble Zinc

The results on Zn content of water samples collected from Azolla growing and non-growing locations are given in Table 21. As against Fe and Mn the amount of Zn was low in both the locations, though the places played a significant effect on Zn content in the Azolla non-growing locations. The average value of Zn varied between 0.02 mg L^{-1} and 0.09 mg L^{-1} in the growing locations while the non-growing locations recorded a value between 0.04 mg L^{-1} and 0.26 mg L^{-1}.Significant effect was noticed at both 5% and 1% levels of significance in the Zn content of water present in Azolla growing and non-growing category

Table 21: Flood water soluble Zn content (mg L^{-1}) of samples collected from different places of Azolla growing and non-growing locations

Flood water Soluble Zn / Places of collection	Growing	Non-growing
Vadavannur	0.03	**0.04**[a]
Muthalamada	0.04	0.05[abc]
Nalleppilli	0.09	**0.26**[f]
Kollengode	0.06	0.17[e]
Pudunagaram	0.03	0.09[c]
Pattencheri	0.02	0.08[bc]
Elappully	0.02	**0.04**[a]
Perumatty	0.01	0.13[d]
Kozhinjampara	0.03	0.14[de]
CD (0.01) (0.05)	**NS**	**0.047** **0.035**
t- value	**5.830****	

** significance at 1% level

d) Flood water soluble copper

Copper content of flood water samples collected from Azolla growing and non-growing locations is given in table 22. Locational effect was significant in both the locations with respect to content of Cu. The highest value of 3.03 mg L^{-1} was obtained in the samples collected from Kozhinjampara. Whereas that from Muthalamada panchayath recorded the minimum quantity (1.20 mg L^{-1}). In the non-growing locations the very same place (Kozhinjampara) shows the highest value of 3.198 mg L^{-1} and the lowest value of 1.87 mg L^{-1} was recorded at Kollengode. The Cu content was significantly different at both 5% and 1% levels of significance in the Azolla growing and non-growing locations.

Table 22: Flood water soluble Cu content (mg L^{-1}) of samples collected from different places of Azolla growing and non-growing locations

Flood water soluble Cu / Places of collection	Growing	Non-growing
Vadavannur	1.25^{e}	2.61^{bcd}
Muthalamada	**1.20**^{e}	2.16^{de}
Nalleppilli	2.12^{c}	2.43^{cd}
Kollengode	1.54^{de}	**1.87**^{e}
Pudunagaram	2.55^{b}	2.65^{bc}
Pattencheri	1.72^{d}	2.95^{ab}
Elappully	2.36^{bc}	2.23^{cde}
Perumatty	2.37^{bc}	2.52^{bcd}
Kozhinjampara	**3.03**^{a}	**3.20**^{a}
CD (0.01)	**0.509**	**0.620**
(0.05)	**0.380**	**0.463**
t- value	**4.047****	

** significance at 1% level

e) Cd, Cr, Ni & Pb

In both growing and non-growing locations and among different locations under each category the content of the heavy metals viz. Cd, Cr, Ni and Pb were below the detectable limits hence it is not presented in tabular form

C) AZOLLA SAMPLES

4.2.13 Moisture

Moisture content of Azolla is given in Table 23. The average value ranged from 93.47 to 94.45 percent. However significant difference was not noticed between places with respect to moisture content.

Table 23: Moisture (%) of samples of Azolla collected from different locations

Locations	Moisture (%)
Vadavannur	94.60
Muthalamada	94.56
Nalleppilli	93.81
Kollengode	94.23
Pudunagaram	94.45
Pattencheri	94.03
Elappully	94.15
Perumatty	93.47
Kozhinjampara	93.67
CD (0.01) (0.05)	NS

4.2.14 Dry matter

Dry matter refers to material remaining after removal of water. The dry matter of plant and animal materials would be its solids i.e. all its constituents excluding water.

Table 24: Dry matter (%)of samples of Azolla collected from different locations

Locations	Dry matter (%)
Vadavannur	5.94
Muthalamada	5.46
Nalleppilli	6.18
Kollengode	5.81
Pudunagaram	5.60
Pattencheri	5.94
Elappully	5.62
Perumatty	5.88
Kozhinjampara	6.36
CD (0.05)	NS

Dry matter content of Azolla is given in Table 24. Places did not exert any significant effect on dry matter. The values ranged from 5.6 to 6.36 percent.

4.2.15 Carbon

The carbon content of Azolla samples are shown in Table 25. The values ranged from 22.9 (Perumatty) to 39.5 percent (Pattencheri). Locations had a significant effect on carbon content.

Table 25: Carbon (%) of samples of Azolla collected from different locations

Locations	Carbon (%)
Vadavannur	27.6^{e}
Muthalamada	34.1^{c}
Nalleppilli	38.6^{a}
Kollengode	34.5^{c}
Pudunagaram	38.6^{a}
Pattencheri	**39.5^{a}**
Elappully	37.2^{b}
Perumatty	**22.9^{f}**
Kozhinjampara	32.5^{d}
CD (0.01) (0.05)	**1.539** **1.148**

4.2.16 Nitrogen

Nitrogen content of Azolla is given in Table 26. On an average values ranged from 2.6 (Kozhinjampara) to 3.6 percent (Nalleppilli). In addition the location effect was also significant with respect to N content.

Table 26: Nitrogen (%)of samples of Azolla collected from different locations

Locations	Nitrogen (%)
Vadavannur	2.9^{b}
Muthalamada	2.7^{bc}
Nalleppilli	**3.6**^{a}
Kollengode	3.4^{a}
Pudunagaram	3.0^{bc}
Pattencheri	3.0^{b}
Elappully	3.3^{a}
Perumatty	2.7^{bc}
Kozhinjampara	**2.6**^{c}
CD (0.01)	**0.332**
(0.05)	**0.248**

4.2.17 C/N ratio

The ratio between carbon and nitrogen of Azolla samples given in Table 27 showed that the maximum C/N ratio of 14.09 was recorded in the samples from Pattencheri and the lowest value of 8.55 was recorded in the samples collected from Perumatty. Locations had a significant role in the C/N ratio of Azolla.

Table 27: C/N ratio of samples of Azolla collected from different locations

Locations	C/N ratio
Vadavannur	9.62[e]
Muthalamada	12.58[b]
Nalleppilli	10.80[cd]
Kollengode	10.27[de]
Pudunagaram	14.08[a]
Pattencheri	**14.09**[a]
Elappully	11.30[c]
Perumatty	**8.55**[f]
Kozhinjampara	12.79[b]
CD (0.01) (0.05)	**1.266** **0.944**

4.2.18 Crude protein

Crude protein, which indicates nitrogenous substances along with protein was estimated in the Azolla samples collected from different places (Table 28). The contents ranged from 15.92 (Kozhinjampara) to 22.31 percent (Nalleppilli). Locational effect was significant at both the levels.

Table 28: Crude protein (%) of samples of Azolla collected from different locations

Locations	Crude protein (%)
Vadavannur	17.94[b]
Muthalamada	16.95[bc]
Nalleppilli	**22.31**[a]
Kollengode	21.02[a]
Pudunagaram	17.11[bc]
Pattencheri	17.50[b]
Elappully	20.78[a]
Perumatty	17.00[bc]
Kozhinjampara	**15.92**[c]
CD (0.01) (0.05)	**2.063** **1.539**

4.2.19 Phosphorus

Phosphorus content of Azolla samples given in Table 29.Locational effect was significant at both levels. The average value ranged from 0.156 (Kozhinjampara) to 0.224 percent (Nalleppilli)

Table 29: Phosphorus (%) of samples of Azolla collected from different locations

Locations	Phosphorus (%)
Vadavannur	0.210[b]
Muthalamada	0.174[c]
Nalleppilli	**0.224[a]**
Kollengode	0.218[c]
Pudunagaram	0.180[c]
Pattencheri	0.184[c]
Elappully	0.216[ab]
Perumatty	0.172[c]
Kozhinjampara	0.156[d]
CD (0.01) (0.05)	**0.018** **0.013**

4.2.20 Potassium

Compared to phosphorus, Azolla contained more of K which was significantly influenced by locations. The highest value (1.55 percent) recorded for the samples collected from Nalleppilli whereas the lowest (1.32 percent) was in those samples collected from Kozhinjampara.

Table 30: Potassium (%) of samples of Azolla collected from different locations

Locations	Potassium (%)
Vadavannur	1.48^{abc}
Muthalamada	1.39^{de}
Nalleppilli	**1.55^{a}**
Kollengode	1.54^{a}
Pudunagaram	1.51^{ab}
Pattencheri	1.41^{cd}
Elappully	1.41^{cd}
Perumatty	1.42^{bcd}
Kozhinjampara	**1.32^{e}**
CD (0.01) (0.05)	**0.108** **0.081**

4.2.21 Nitrogenase activity

Nitrogenase activity was measured by Acetylene Reduction Assay (ARA) and the results are given in Table 31. Through this assay the reducing power of the enzyme from acetylene to ethylene was determined and results were represented in nmoles of ethylene g^{-1} h^{-1} and the values were ranged between 192.5 (Pattencheri) to 235.9 (Vadavannur) nmoles ethylene g^{-1} h^{-1}.

Table 31: Nitrogenase activity (nmoles ethylene g^{-1} h^{-1}) of samples of Azolla collected from different locations

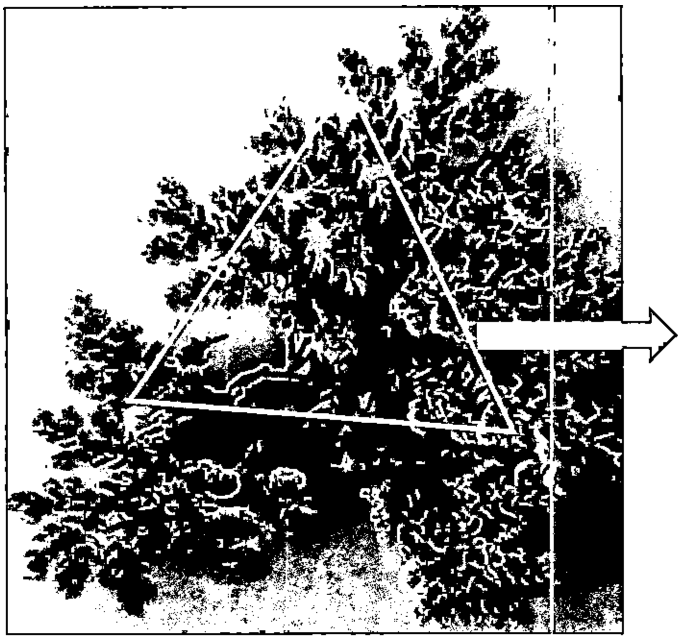
Locations	Nitrogenase activity
Elappully	208.2^{c}
Nalleppilli	226.3^{b}
Pattencheri	**192.5^{d}**
Perumatty	227.9^{b}
Vadavannur	**235.9^{a}**
CD (0.01) (0.05)	**4.150** **3.043**

4.2.22 Species identification

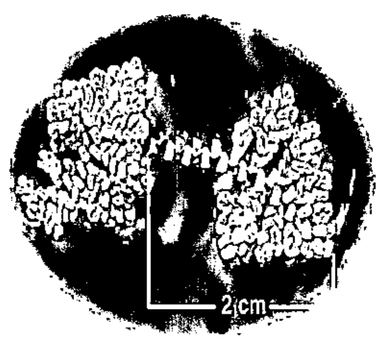
The morphological features of Azolla samples collected from the experimental sites are furnished in Table 32 ; Plate 7

Table 32: Species identification of samples of Azolla collected from different locations

Characters	Features
Size and shape	Small 1.5-2.5cm long, with straight main axis with pinnately arranged side branches, progressively longer towards the base, thus roughly triangular in shape.
Roots	Fine lateral rootlets giving a feathery appearance in the water.
Leaves:	Minute 1-2mm long
Lobe features	Upper lobe green, brownish green or reddish, lower lobe translucent brown. Minute, short pillae +/- cylindrical unicellular hairs often present on the upper lobes.



Triangular shaped Lobes



Fine lateral roots

Fronds of size 2 cm

Plate 7: Species identification

4.2.23 Total effect of soil and water quality parameters on composition of Azolla and Nitrogenase enzyme content

The soil and water quality parameters studied was correlated with the composition of Azolla, collected from all the locations and with nitrogenase activity of Azolla collected from five locations. The results are presented in Tables 33 and 34

Table 33: Correlation between Nitrogenase activity and soil parameters

Soil / Nitrogenase activity	pH	EC
Nitrogenase activity	-.674**	-.732*

Nitrogenase activity showed a negative but significant correlation with pH and EC of soil at an optimum range. Though all other parameters were also considered, neither positive nor negative correlation could be obtained with nitrogenase activity.

Table 34: Correlation between composition of Azolla and soil parameters

Soil/water parameters / Azolla composition	pH	EC	P	K
Moisture	-	-	-	-
Dry matter	-	-	-	-
Carbon	.903**	.747*	-	-
Nitrogen	-	-	.990*	-
Crude protein	-	-	.790*	-
C/N ratio	-	-	-.746*	-
Phosphorus	-	-	.842**	-
Potassium	-	-	-	.897**

*Significance at 5% level

** Significance at 1% level

On correlating the soil and water parameters with the composition of Azolla, it was seen that a significant and positive correlation existed between the carbon content of Azolla and the electro chemical soil properties like pH and EC. The content of nitrogen, crude protein and phosphorus of Azolla revealed a significant positive correlation with soil phosphorus status. But the C/N ratio exhibited a significant negative correlation with soil P. The potassium content in Azolla showed a significant positive correlation with the potassium content of soil.

4.2.23 Quality of Azolla in relation to different locations.

In order to find an ideal location, the quality parameters of Azolla has obtained from different locations was made and the results are presented in table 35

Table 35: Quality of Azolla in relation to different locations.

Locations	Carbon (%)	Nitrogen (%)	C/N ratio	Crude protein (%)	Phosphorus (%)	Potassium (%)
Vadavannur	27.6^{e}	2.9^{b}	9.62^{e}	17.94^{b}	0.210^{b}	1.48^{abc}
Muthalamada	34.12^{c}	2.7^{bc}	12.58^{b}	16.95^{bc}	0.174^{c}	1.39^{de}
Nalleppilli	38.6^{a}	3.6^{a}	10.80^{cd}	22.31^{a}	0.224^{a}	1.55^{a}
Kollengode	34.5^{c}	3.4^{a}	10.27^{de}	21.02^{a}	0.218^{ab}	1.54^{a}
Pudunagaram	**38.6^{a}**	3.0^{bc}	14.08^{a}	17.11^{bc}	0.180^{c}	1.51^{ab}
Pattencheri	39.5^{a}	3.0^{b}	**14.09^{a}**	17.50^{b}	0.184^{c}	1.41^{cd}
Elappully	37.2^{b}	3.3^{a}	11.30^{c}	20.78^{a}	0.216^{ab}	1.41^{cd}
Perumatty	**22.9^{f}**	2.7^{bc}	**8.55^{f}**	17.00^{bc}	0.172^{c}	1.42^{bcd}
Kozhinjampara	32.5^{d}	**2.6^{c}**	12.79^{b}	**15.92^{c}**	**0.156^{d}**	**1.32^{e}**
CD (0.01)	**1.539**	**0.332**	**1.266**	**2.063**	**0.187**	**10.759**
(0.05)	**1.148**	**0.248**	**0.944**	**1.539**	**0.139**	**8.024**

Based on the results given in table 35, ranking was done for the factors which proved significant.For this a scoring methodology was adopted. Individual scoring was done for all the parameters under a particular location. Values with same superscript under each parameter was clubbed and allotted with a score. This was applied for all the parameters under consideration. The sum total of all the scores for all the parameters in a given location was taken as the overall score for further ranking. The location with least score value was considered superior. The details are furnished in table 36

Table 36: Scoring methodology to select ideal location in terms of Azolla quality

Locations	Carbon (%)	Nitrogen (%)	C/N ratio	Crude protein (%)	Phosphorus (%)	Potassium (%)	Sum total of ranks
Vadavannur	5	2	5	2	3	3	20
Muthalamada	3	3	2	3	3	6	20
Nalleppilli	1	1	4	1	1	1	9
Kollengode	3	1	5	1	2	1	13
Pudunagaram	1	3	1	3	3	2	13
Pattencheri	1	2	1	2	3	5	14
Elappully	2	1	3	1	2	5	14
Perumatty	5	3	6	3	3	4	27
Kozhinjampara	4	4	2	4	4	7	25

From the results it could be inferred that Nalleppilli panchayath was most preffered to produce a good quality Azolla .

4.2.24 Location wise details on soil and floodwater quality parameters in relation to Azolla growth.

The results are provided in table 37

Table 37: Location wise details on soil and floodwater quality parameters in relation to Azolla growth.

	SOIL										FLOOD WATER		
Locations	pH	EC	Organic carbon	Avail. nitrogen	Available phosphorus	available potassium	total Fe content	total Mn content	total Zn content	total Cu content	Soluble Fe	Soluble Mn	Soluble Cu
Vadavannur	7.12^{cd}	**0.17^{c}**	**1.64^{a}**	**129.18^{d}**	61.2^{ab}	**194.3^{a}**	**1506.1^{a}**	**228.1^{d}**	21.7^{bc}	18.3^{fg}	**10.2^{e}**	12.3^{cd}	1.25^{e}
Muthalamada	7.23^{abc}	0.27^{ab}	1.56^{a}	144.2^{c}	57.3^{c}	148.1^{de}	1625.6^{bc}	267.4^{b}	23.1^{ab}	**16.9^{g}**	12.1^{bcd}	13.4^{b}	**1.20^{e}**
Nalleppilli	7.34^{abc}	0.30^{a}	1.56^{a}	153.6^{b}	63.3^{a}	191.2^{a}	1646.2^{bc}	234.0^{cd}	23.3^{ab}	24.8^{c}	13.1^{abc}	12.5^{cd}	2.12^{c}
Kollengode	**7.47^{a}**	0.29^{ab}	1.41^{b}	154.3^{ab}	61.4^{ab}	193.3^{a}	1631.6^{bc}	**309.6^{a}**	**24.3^{a}**	20.8^{ef}	12.6^{abcd}	12.0^{d}	1.54^{de}
Pudunagaram	7.4^{abc}	0.30^{a}	1.36^{bc}	153.0^{b}	52.4^{d}	188.9^{ab}	1646.2^{bc}	301.7^{a}	22.4^{abc}	29.7^{b}	13.0^{abc}	**11.1^{e}**	2.55^{b}
Pattencheri	7.42^{abc}	**0.30^{a}**	1.32^{bc}	152.0^{bc}	52.4^{d}	178.7^{bc}	1648.0^{bc}	247.9^{c}	21.5^{bc}	21.8^{de}	13.2^{ab}	13.0^{bc}	1.72^{d}
Elappully	7.44^{b}	0.29^{ab}	1.3^{bc}	**161.8^{a}**	61.3^{ab}	158.9^{d}	1622.4^{b}	231.8^{cd}	20.8^{c}	24.3^{cd}	11.6^{d}	12.2^{cd}	2.36^{bc}
Perumatty	**6.84^{d}**	0.25^{b}	1.28^{bc}	157.1^{ab}	58.6^{bc}	173.2^{c}	**1653.9^{c}**	278.6^{b}	**16.6^{d}**	24.2^{cd}	**13.7^{a}**	**14.7^{a}**	2.37^{bc}
Kozhinjampara	7.13^{bcd}	0.27^{ab}	**1.23^{c}**	159.1^{ab}	**50.2^{d}**	**140.3^{e}**	1623.2^{b}	248.5^{c}	22.4^{abc}	**36.2^{a}**	11.9^{cd}	13.7^{b}	**3.03^{a}**
CD (0.05)	0.317	0.048	0.139	8.024	3.875	11.547	26.68	17.30	2.038	2.889	1.270	0.984	0.380

The soil and floodwater parameters of the locations covered under Azolla growth was non-uniform or the locations differed with respect to composition of soil and floodwater attributes (Table 38). In order to arrive at the ideal location in terms of soil and water quality congenial for Azolla growth, significant values for each parameter under soil and floodwater were considered. Ranking of values was done in accordance with the preference.Values with same superscript under each parameter was clubbed and allotted with a score. This was followed for all the parameters under soil and floodwater quality. The sum total of all the scores for all the parameters in a given location was taken as the overall score for further ranking. From the overall total value the location with least total value was considered excellent.

Table 38: Scoring methodology to select ideal location in terms of soil and flood water characteristics.

Locations	Soil pH	Soil EC	Soil Organic carbon	Soil available nitrogen	Soil available phosphorus	Soil available potassium	Soil total Fe content	Soil total Mn content	Soil total Zn content	Soil total Cu content	Soluble Fe	Soluble Mn	Soluble Cu	Sum total of ranks
Vadavannur	2	1	1	6	2	1	1	5	4	7	6	4	7	47
Muthalamada	4	3	1	4	4	6	3	2	2	8	3	2	7	49
Nalleppilli	4	5	1	2	1	1	3	4	2	3	2	4	4	36
Kollengode	6	4	2	3	2	1	3	1	1	6	2	5	6	42
Pudunagaram	4	5	3	2	5	2	3	1	3	2	2	6	2	40
Pattencheri	4	5	3	5	5	3	3	3	4	5	2	3	5	48
Elappully	5	4	3	1	2	5	2	4	5	4	5	4	3	47
Perumatty	1	2	3	3	3	4	4	2	6	4	1	1	3	37
Kozhinjampara	3	3	4	3	5	7	2	3	3	1	4	2	1	41

Discussion

5. DISCUSSION

The results of the present study are discussed in the light of related and supporting findings on similar line. It is categorized under the following two headings:

5.1 Surveying of areas in Palakkad eastern plains (AEU 23)

5.2 Collection and characterization of soil, flood water and Azolla samples

5.1 SURVEYING OF AREAS IN PALAKKAD EASTERN PLAINS (AEU 23)

The areas surveyed for prevalence of Azolla is represented in the map (Fig1) provided based on GPS data which is represented in map .The typical features of Palakkad eastern plains (AEU 23) are as follows:

The Palakkad Eastern Plain Agro-Ecological Unit is delineated to represent the drier parts of Palakkad plain in the gap region of Western Ghats, having low rainfall, long dry period and fertile soils. The unit comprises 11 panchayats in eastern Palakkad. The climate is tropical dry sub humid monsoon type (mean annual temperature 27.6 °C; rainfall 1340 mm) and dry period around six months.

Probability of moderate annual drought is thrice in ten years. The same probability holds for moderate drought during NE monsoon period as well. In addition, severe drought during NE monsoon period might also occur for two years in a block of ten years. Probability of two consecutive weeks receiving more than 20 mm rainfall is high from 2nd week of June to 2nd week of November. Soil moisture is adequate for crops from 1st week of June to 3rd week of December (Fig. 142). The length of growing period for annual crop is almost 29 weeks while the length of dry period (duration of soil moisture deficit) extends to almost five and half months. There is a high degree of probability of soil moisture deficit from 34th to 40th std. weeks (mid-August to first week of October).

The uplands of the unit have slightly acid or neutral red clay soils well supplied with bases and plant nutrients. Lower parts of the uplands and lowlands have alkaline, swell-shrink clay soils. The fertile soils are rich in bases and plant nutrients. Lowlands have impeded drainage. Coconut, arecanut and mango are the major plantation crops. Annual crops include rice, groundnut, cotton, banana, maize, jowar and sugarcane. The unit covers 47,049 ha (1.21 %) geographical area of the state.

AEU 23 consists of 4 blocks viz Alathur, Chitoor, Kollengode and Nenmara with 8, 7,6 and 7 number of panchayaths respectively under each block. Survey was conducted following the procedure mentioned under section 3 and the important observations are furnished herewith.

1. Among the 28 panchayaths surveyed dominance of Azolla was noticed mostly in rice fields followed by open water bodies, mainly ponds. The microclimate of rice fields provides a suitable environment for cyanobacteria both free living and living symbiotically with the water fern Azolla (Krock., 1988 ; Watanabe and Liu. 1992) .The association of Azolla with other aquatic plants like eichornia, pistia, lemna etc. appear to protect it from turbulence, drifting and high solar radiation as reported by Fiore and Gutbord (1987). Another reason for the presence of Azolla in the stagnant waters of small ponds, rice fields indicated that relatively calm and quite water favours its growth (Douglas *et al.*, 2002, Martin, 2008)
2. The growth and multiplication of Azolla was found to be influenced by solar radiation which was more pronounced in the open water bodies where characteristic browning was noticed towards the center of the water body receiving maximum sunlight. Toward the sides of the pond bordered by coconut palms, Azolla maintained the green sheen. The lower amounts of chlorophyll produced during severe summer and winter may be the reason for the typical browning which is in conformity with the findings of Satapathy and Singh(1992)
3. In all locations of the experimental area the species identified was *Azolla pinnata.* The suitability of tropical conditions, especially temperature, on the growth and multiplication of *Azolla pinnata* and *Azolla rubra*, has been observed by Pereira *et al.* (2001), Evrard and Van Hove (2004) However in the present study only the *A.pinnata* species could be located.

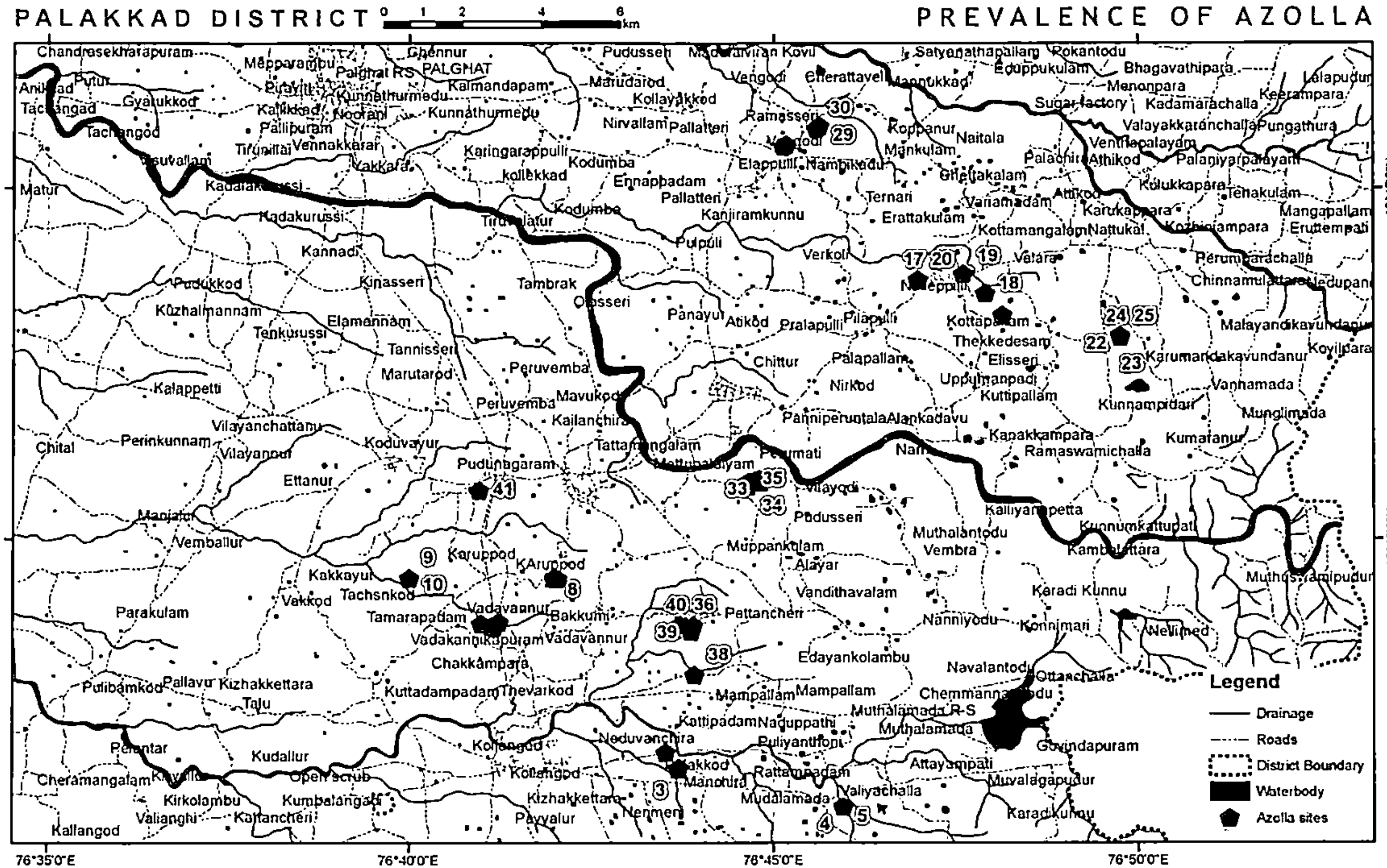


Fig. 1: Map showing the prevalence of Azolla in Palakkad Eastern Plains

5.2 COLLECTION AND CHARACTERIZATION OF SOIL, FLOOD WATER AND AZOLLA SAMPLES

A. Soil sample

5.2.1 pH

The pH of the experimental sites ranged from 6.84 (Perumatty) to 7.47 (Kollengode) whereas the range was 7.17 (Perumatty) to 7.61 (Elappully) in the non- growing locations as shown in Table 5 (Fig. 2). Usually, Azolla prefers a medium near to neutrality or to some extent, acidic conditions. The optimal pH varies from 4.5 to 7.5 (Cary and Weerts, 1992), but Azolla can survive even at pH values ranging from 3.5 to 10, provided all important elements are available (Serag *et al.,* 2000). *A. filiculoides* and *A. pinnata* can, however, grow well at pH values between 5 and 7, while *A. pinnata* can grow relatively well in a pH range between 5 and 8. Results obtained in the present study are in agreement with earlier findings as referred above. The effect of pH on Azolla growth may be due to increased nutrient availability towards neutrality. In general the pH of most acid and alkaline soils changes toward a range of 6-7, a few weeks after flooding (Ponnamperuma, 1972) and this may be the reason for the neutral pH recorded in the present study in rice fields.

5.2.2 Electrical conductivity

Though the EC values were significantly different in the Azolla growing and non-growing locations, it was in the safe limit (Table 6, Fig. 3). The water for Azolla cultivation should not contain more than 0.3 percent salt since higher salt concentrations decreased plant N (Mishra and Singh, 2006; Singh *et al.*, 2008). The maximum value obtained for EC was within the permissible limit especially in the growing locations. The reduced salt content observed in the present study might have helped in overcoming osmotic problems leading to increased chlorophyll content, photosynthesis, respiration and N fixation.

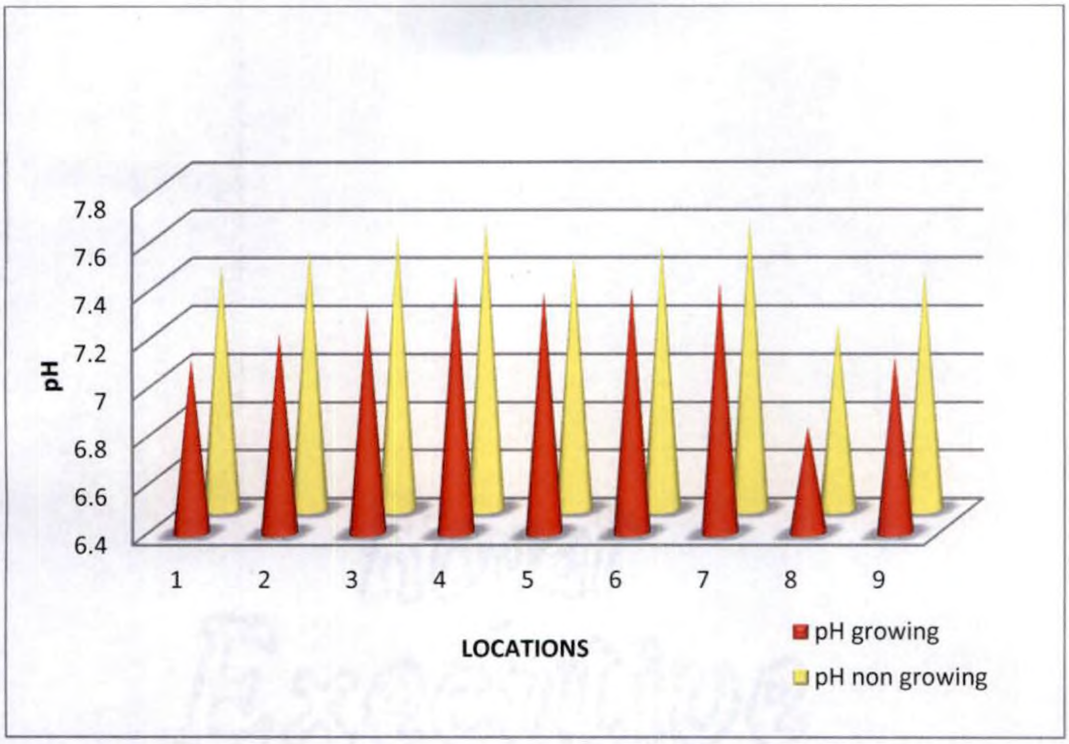


Fig. 2: Soil pH of Azolla growing and non-growing locations

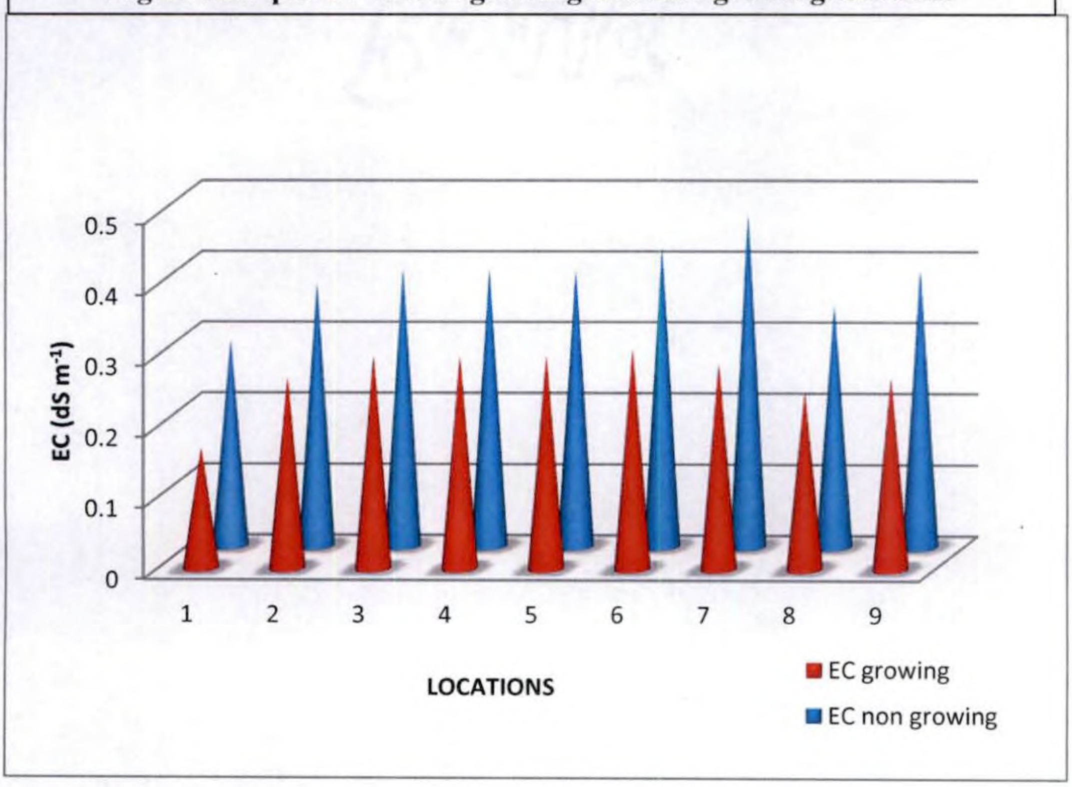


Fig. 3: Soil electrical conductivity (dS m^{-1}) of Azolla growing and non-growing locations

5.2.3 Organic carbon

The benefit of Azolla in paddy fields as an organic green manure is harnessed only if it is incorporated in to the field. In the present study such an effect was not considered hence the increased organic carbon status in the Azolla growing locations (Table7, Fig. 4) may be ascribed to the sloughing –off roots during stress condition and also due to excessive growth (Uheda *et al.*, 1999). Continued application of organic manures in rice fields may be yet another reason for their increased amounts of soil carbon pool (Liu *et al.*, 2005)

5.2.4 Available nitrogen

Available N status was more in the Azolla growing region (Table 8, Fig.5). Research findings (Singh, 2000; Macale and Vlek, 2004; Biswas *et al.*, 2005) state the beneficial effect of Azolla in curbing ammonia volatilization thereby decreasing N losses. Azolla can act in a number of ways to curb ammonia volatilization loss from rice fields. Azolla may 1) Form a physical barrier for the escape of ammonia 2) Intercept the incoming light which is necessary for prolific algal growth 3) Absorb a high amount of NH_3 or NH_4^+ temporarily store it for future release 4) Exude protons while absorbing NH_3 etc. These may be the probable reasons for increased N content in soils under Azolla growing locations. Available N in soil has not been reported as a limiting factor for Azolla growth under normal conditions. The ability of Azolla to fix atmospheric N allows this fern to grow successfully in aquatic habitats lacking or having low levels of N. Inorganic N fertilizer along with P and K fertilizers were effective in promoting Azolla growth during summer months (Pabby *et al.*, 2003; Sood *et al.* 2007)

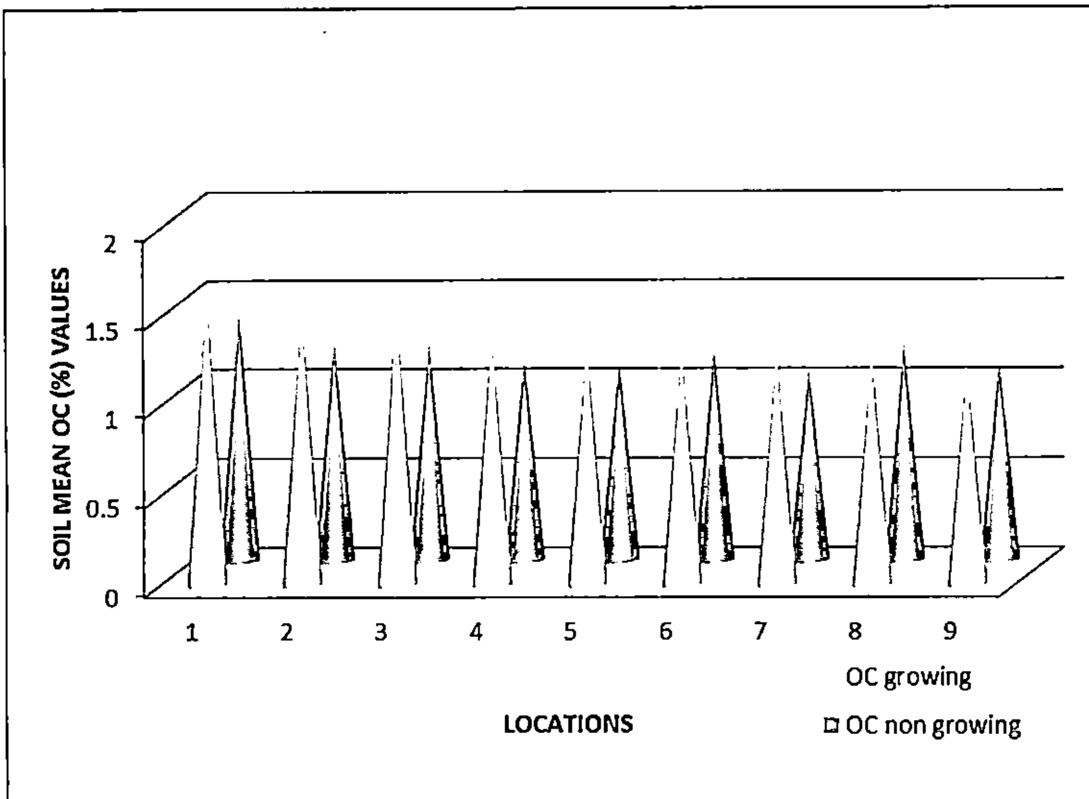


Fig. 4: Soil organic carbon (%) of Azolla growing and non-growing locations

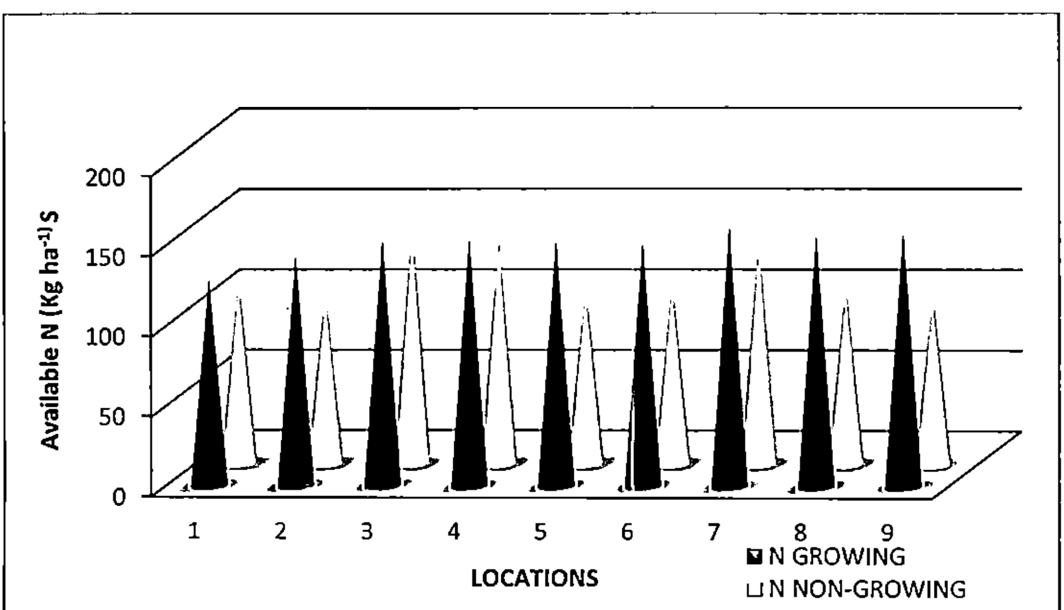


Fig. 5: Soil available N (kg ha^{-1}) of Azolla growing and non-growing locations

5.2.5 Available phosphorus

The data (Table 9, Fig.6) on available P of soil samples collected from Azolla growing and non-growing locations revealed significant difference. P is one of the most important and often limiting nutrients for Azolla growth (Kobayashi *et al.*, 2008). The near neutral pH of the experimental site (Table 5) would have helped in the release of P and better growth of Azolla which is evident from the lower values of phosphorus in the growing locations.

5.2.6 Available potassium

The significant difference (Table10, Fig. 7) in available K in Azolla growing locations, both paddy fields and water body, can be due to the release of K from inorganic and organic fertilizers applied to rice fields. The soil status of K is not a crucial one for growth and multiplication of Azolla. However in general K has been reported to be important for enzyme activation and osmotic regulations (Cerna *et al.*, 2009)

5.2.7 Heavy metals

Heavy metals studied included Fe, Mn, Zn, Cu, Cr, Cd, Ni and Pb (Tables 11 to 14; Fig. 8 to 11). Among these significant difference was noticed with respect to Fe, Mn, Zn and Cu.

Nitrogen fixing ability of Azolla is dependent to a greater extent on soluble Fe followed by Mn. The Fe content in the Azolla growing locations was less as against the non-growing locations which may be due to its uptake by Azolla for N fixation. This finding is well supported by that of (Newton and Herman, 1979; Watanabe and Roger, 1984 and Weisany et al., 2013). The lower values of Mn, Zn and Cu in the Azolla growing locations may be due to its absorption by Azolla. Like any other plant Azolla also requires all micronutrients in the required quantity. The results are in agreement with the findings of Biswas *et al* (2005); and Zahran *et al.* (2007)

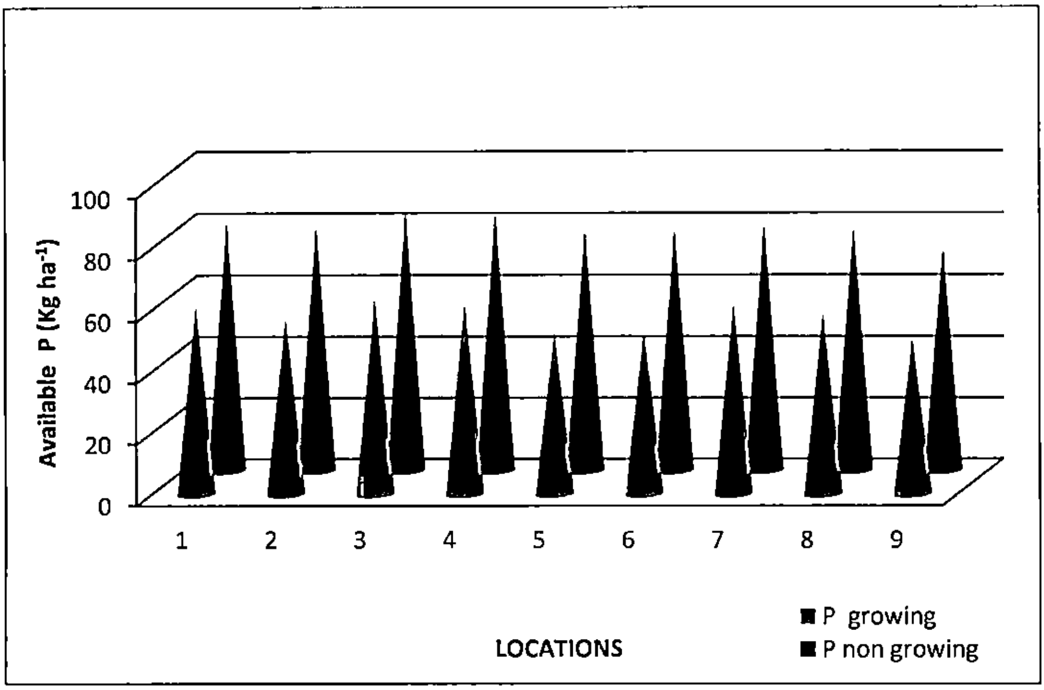


Fig. 6: Soil available P (kg ha^{-1}) of Azolla growing and non-growing locations

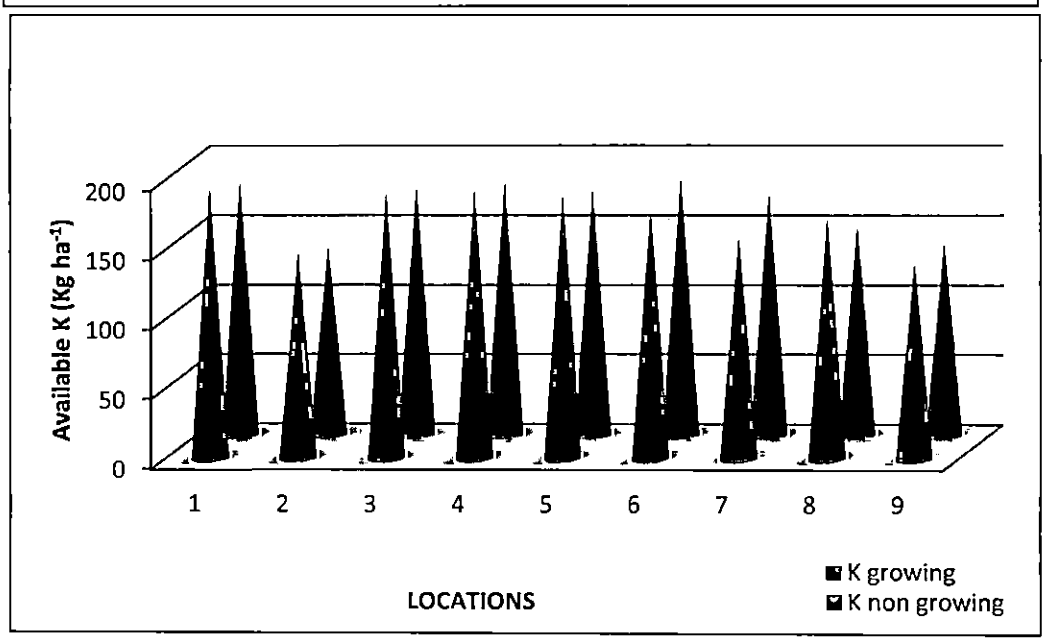


Fig. 7: Soil available K (kg ha^{-1}) of Azolla growing and non-growing locations

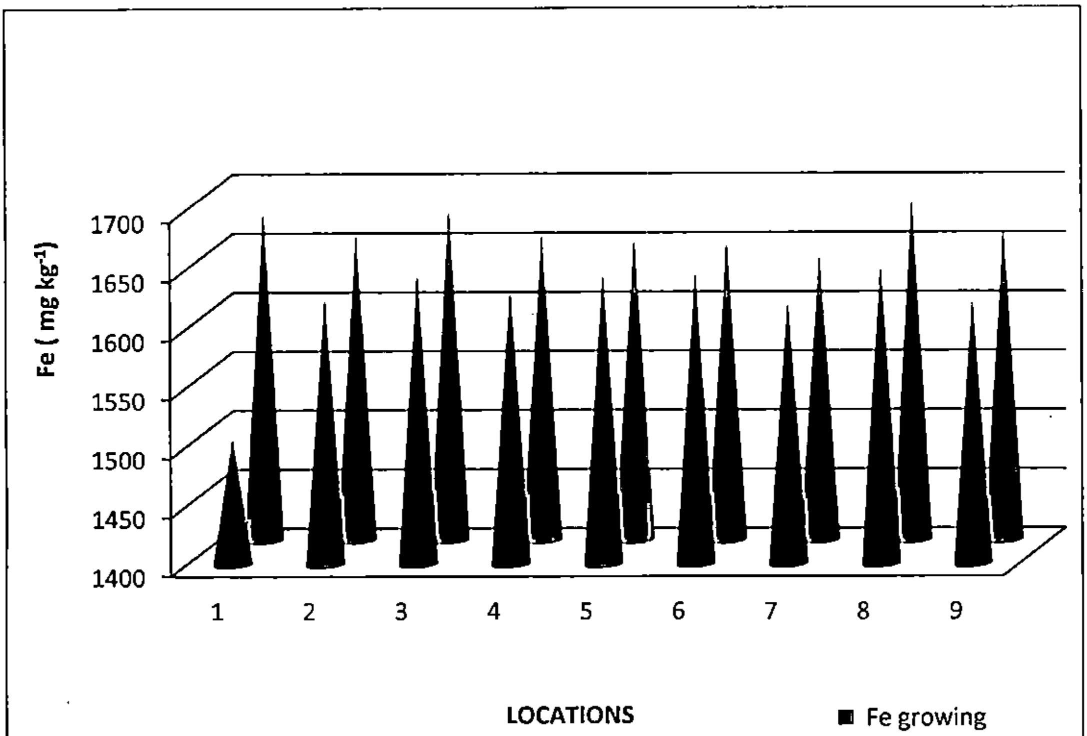


Fig. 9: Soil total Fe content (mg kg^{-1}) of Azolla growing and non-growing locations

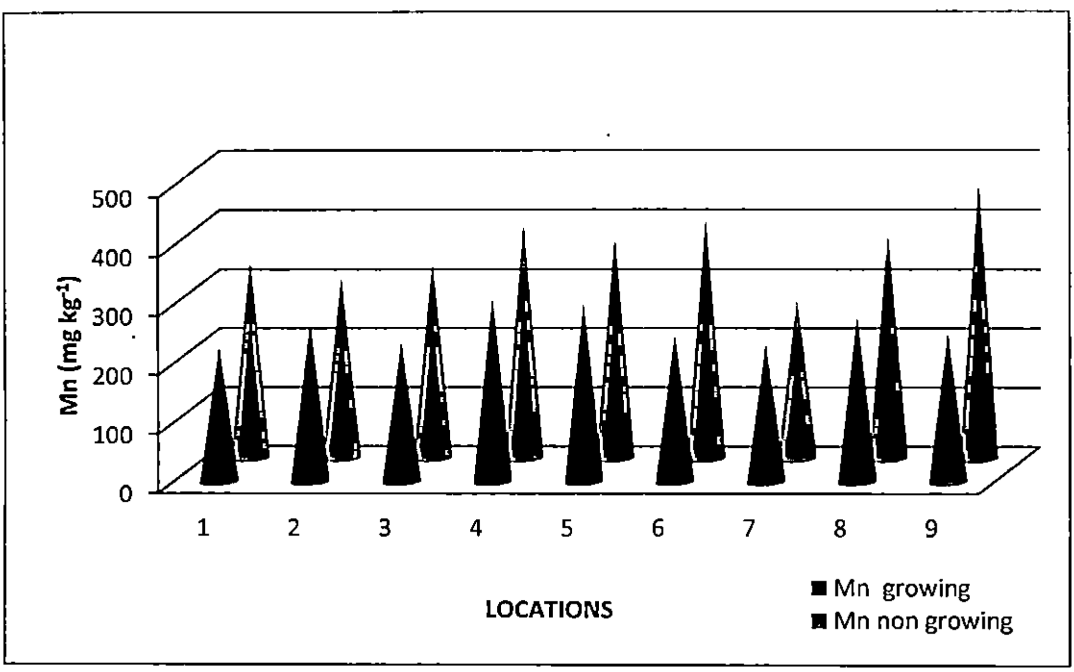


Fig. 9: Soil total Mn content (mg kg^{-1}) of Azolla growing and non-growing locations

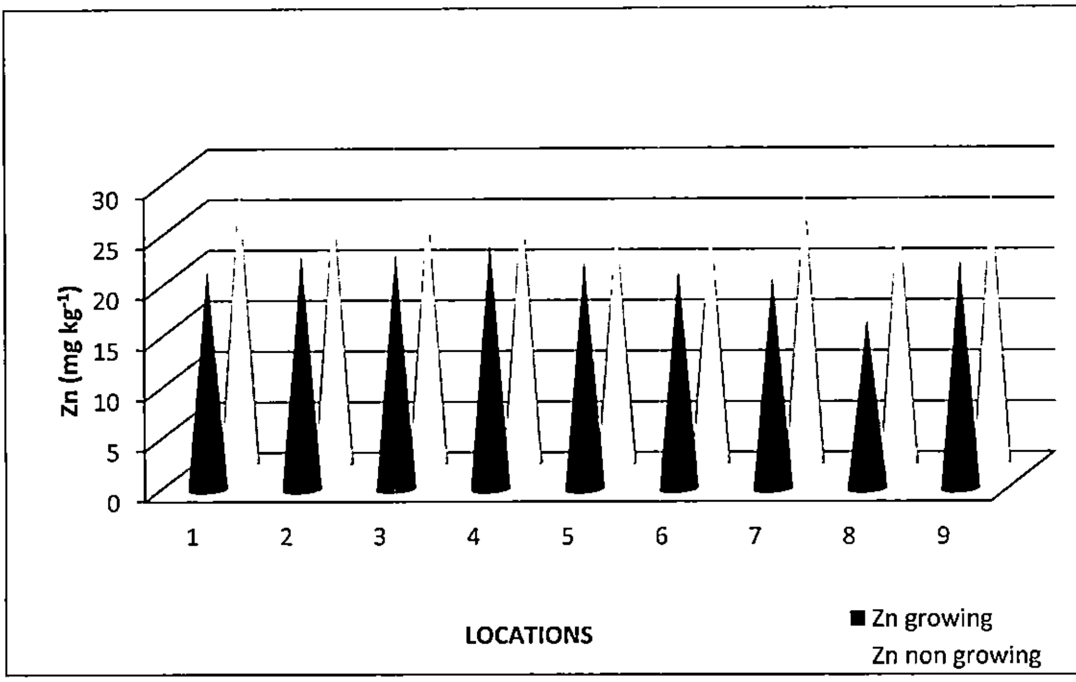


Fig. 10: Soil total Zn content (mg kg^{-1}) of Azolla growing and non-growing locations

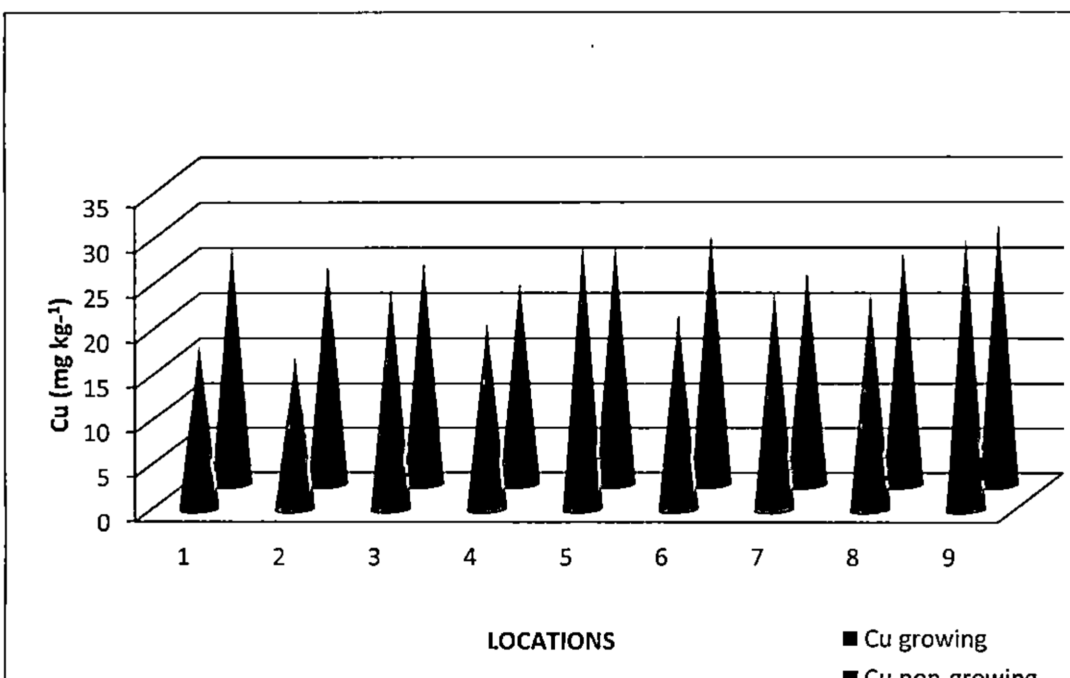


Fig. 11: Soil total Cu content (mg kg^{-1}) of Azolla growing and non-growing locations

The presence of other heavy metals like Cd, Cr, Ni and Pb could not be located either in the Azolla growing locations or in the non-growing locations revealing the pollution free nature of the study area. Azolla, a free-floating, fast growing, and N fixing pteridophyte seems to be an excellent candidate for removal, disposal, and recovery of heavy metals from the polluted aquatic ecosystems (Arora *et al.* 2006; Umali *et al.* 2006)

B. Flood water sample

5.2.8 pH

Azolla growing and non-growing locations recorded a significant difference with respect to pH of flood water sample though the latter recorded comparatively higher values (Table 15, Fig. 12). The lower soil- water interface and floodwater pH of Azolla covered water may be due to respiration of Azolla roots and excretion of hydrogen ions for ion- exchange (Brady 1984)

The Azolla canopy prevents light penetration into the flood water which inhibits the growth of other phototrophs and depresses photo dependent carbon dioxide uptake. Thus, under Azolla canopy the floodwater pH remains lower than in Azolla free conditions (Roger, 1996).Vlek *et al.*, 2002 also justified the lower floodwater pH in the presence of an Azolla cover. The reduction in pH values due to the presence of Azolla cover was to the tune of 0.1-0.3 units.

5.2.9 Electrical conductivity

The efficacy of Azolla mat in absorbing various kinds of salts from water than other phytoplankton is a proven fact. Covering open water bodies with Azolla in a way to ensure 25, 50 and 75% shade revealed that the 75% shading helps to decrease electrical conductivity from 1.51 to 0.93 mS cm^{-1}(Tawwab, 2008). In the present study, EC values of the soil samples was within the safe range which in turn would have reduced the salt concentration of floodwater (Table16, Fig.13)

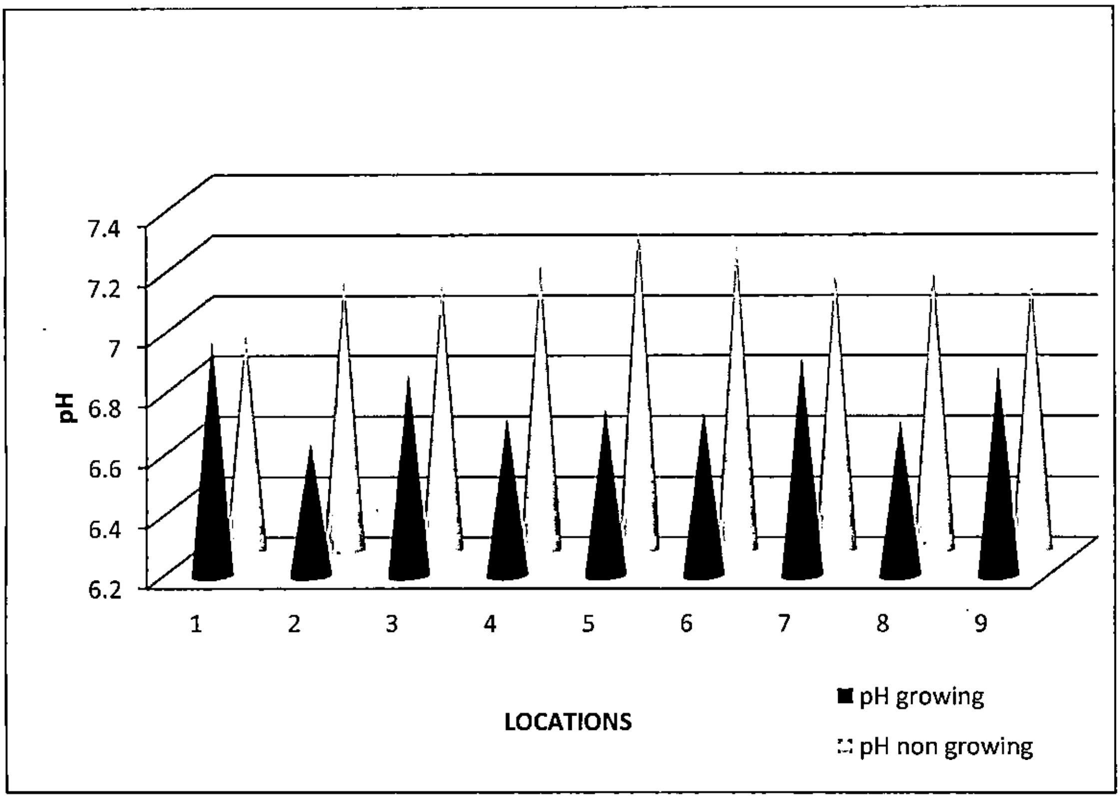


Fig. 12: Floodwater pH of Azolla growing and non-growing locations

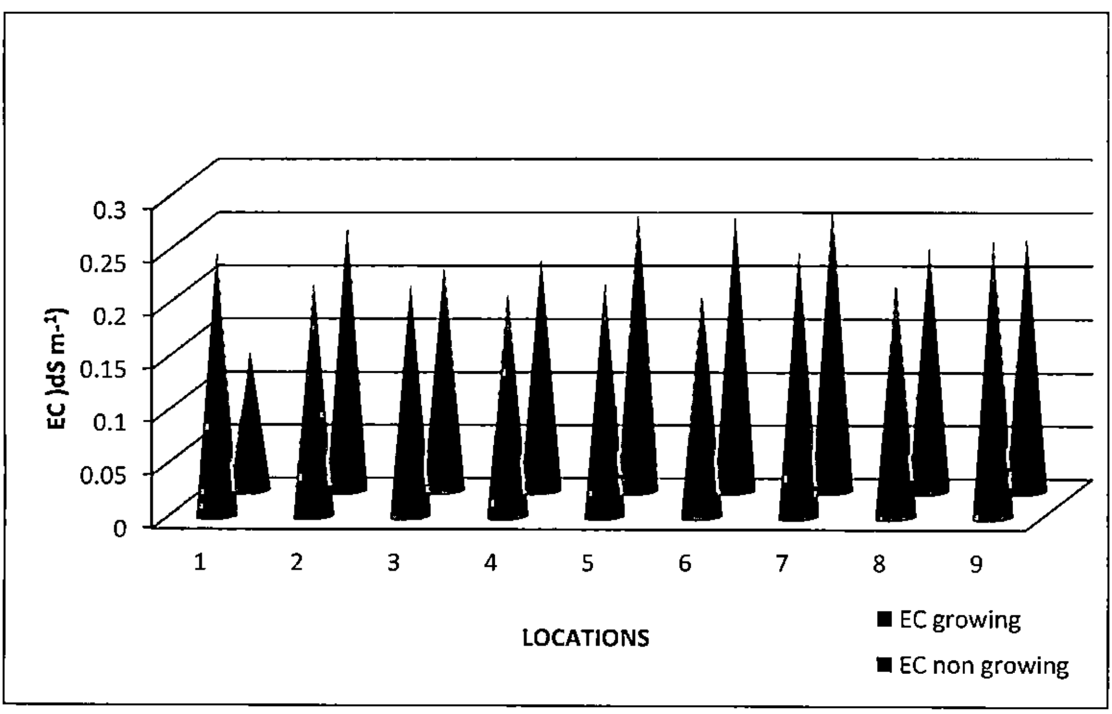


Fig. 13: Floodwater electrical conductivity (dS m^{-1}) of Azolla growing and non-growing locations

5.2.10 Flood water temperature

The floodwater temperatures of the Azolla-covered plots were significantly lower ($P < 0.05$) than that in the Azolla-free plots (Table17, Fig. 14). On an average the difference in temperature was to the extent of 1 to 2 ^{0}C. In the presence of an Azolla cover, the rapid heating of the floodwater from morning until midday was prevented. An Azolla cover resulted in a mean floodwater temperature reduction of 0.6 to 2.6 ^{0}C and the maximum floodwater temperature difference between Azolla-covered and Azolla-free plots was 5^{0}C as disclosed by Macale and Paul (2004) from a work undertaken to study the role of Azolla cover in improving the nitrogen use efficiency of lowland rice. The optimum temperature required for Azolla growth was reported as 20- 35^{0}C (Vitousek *et al.*, 2002; Douglas, 2002) which matches very well with that of present experiment. Temperature is probably the most important limiting environmental factor in Azolla cultivation which is also very difficult to manipulate. Its direct effects are not as serious as its indirect influences. The most serious problem with temperature is its stimulating effect on Azolla pests, e.g., insects, pathogenic fungi and free-living algae. At higher temperatures, the generation time for insects and the growth rate of fungi greatly increase; also it will adversely affect the Nitrogenase activity (Sadeghi *et al.*, 2013).

5.2.11 Dissolved oxygen

The data on dissolved oxygen (Table 18, Fig. 15) revealed that Azolla growing locations had a positive effect in increasing dissolved oxygen content. Dissolved oxygen is probably the single most important water quality factor. The leaf cavity in the dorsal lobe of Azolla is surrounded by photosynthetic mesophyll that evolves O_2 in the presence of light, thereby increasing the O_2 concentration in the environment (Mutuskin and Kolesnikov 1991). Therefore, Azolla plants may play an important role in regulating the levels of dissolved O_2 of freshwater environment.

Combination of Azolla and cyanobacterial systems recorded a dissolved oxygen content of 7.4 ppm. The floating masses of Azolla act as aerators in paddy field ecosystem. The oxygen released during the photosynthetic activity got liberated as minute air bubbles thereby aerating the water impounded in paddy field resulting in increased dissolved oxygen content (Selvi and Sivakumar, 2011).

5.2.12 Heavy metals

The content of Fe, Mn, Zn and Cu in the water sample of Azolla growing locations was slightly less (Tables 19 to 22; Fig. 16 to 19). The nutrition of Azolla is mainly from the dissolved nutrients in the impounded water. The soil status of Fe, Mn, Zn and Cu was lower in the Azolla growing locations thus reducing the quantity of respective water soluble nutrients. Another reason for the decreased levels of Fe, Mn and Cu may be the near neutral pH recorded in the Azolla growing tracts which agreed fully with the findings of Lumpkin & Plucknett, 1980; Watanabe, 1982. The increase of H ion concentration affects the mobilization intensity of heavy metals. In highly acidic soils, the mobility of metallic elements is much higher than in soils with neutral and alkaline reaction (Matos *et al.*, 2001)

Other heavy metals like Cd, Cr, Ni and Pb was below detectable limits in the water sample much similar to that of soil. The rhizo-filtration effect of Azolla would have alleviated the chances of heavy metal pollution (Jafari *et al.*, 2010; Rai, 2010)

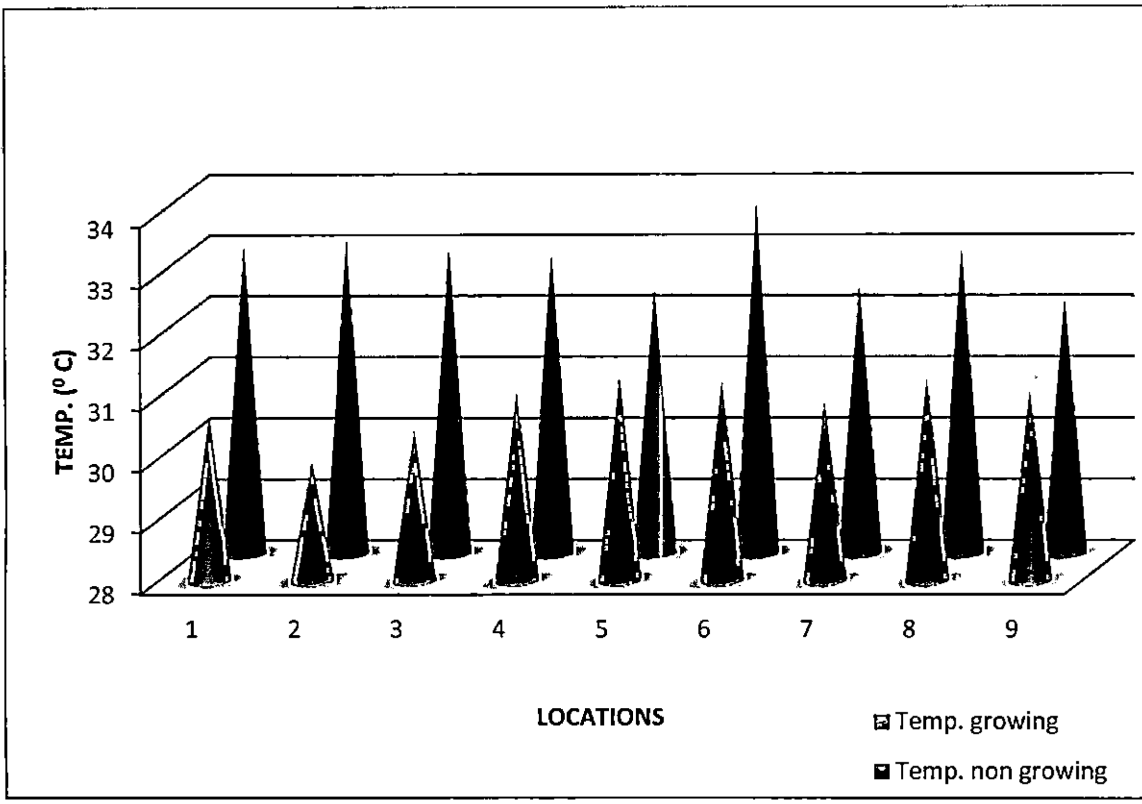


Fig. 14: Floodwater temperature (^{0}C) from Azolla growing and non-growing locations

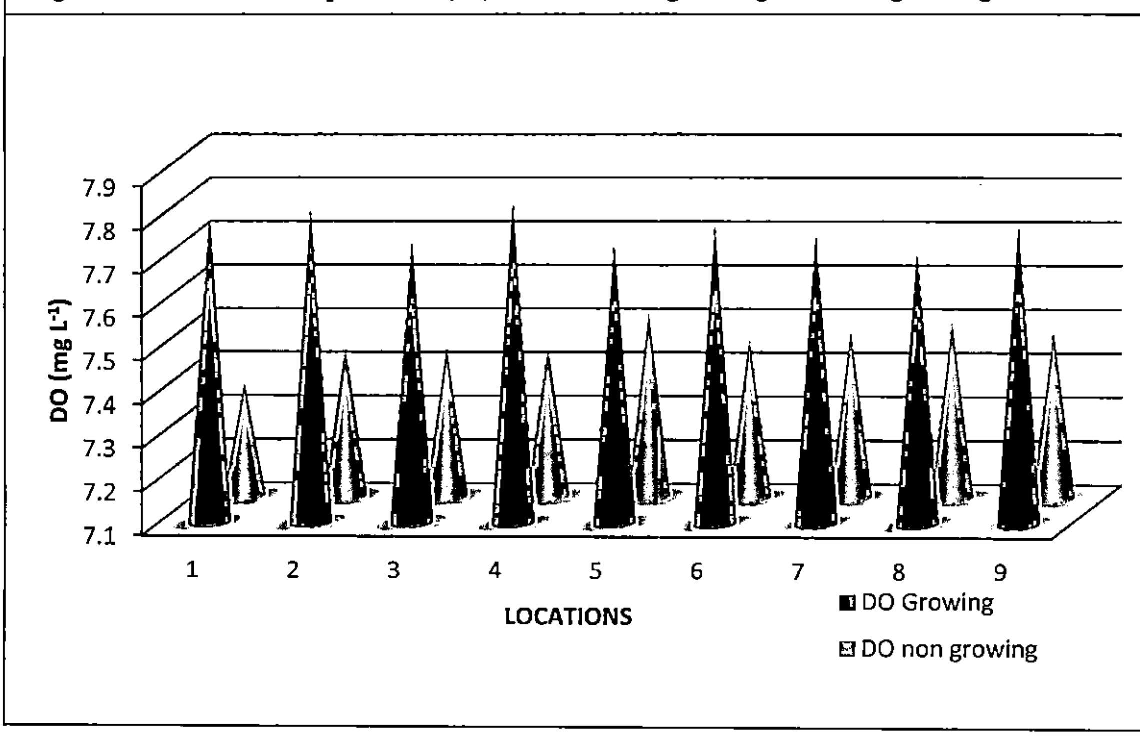


Fig. 15: Floodwater dissolved oxygen (DO) (mg L^{-1}) in Azolla growing and non-growing locations

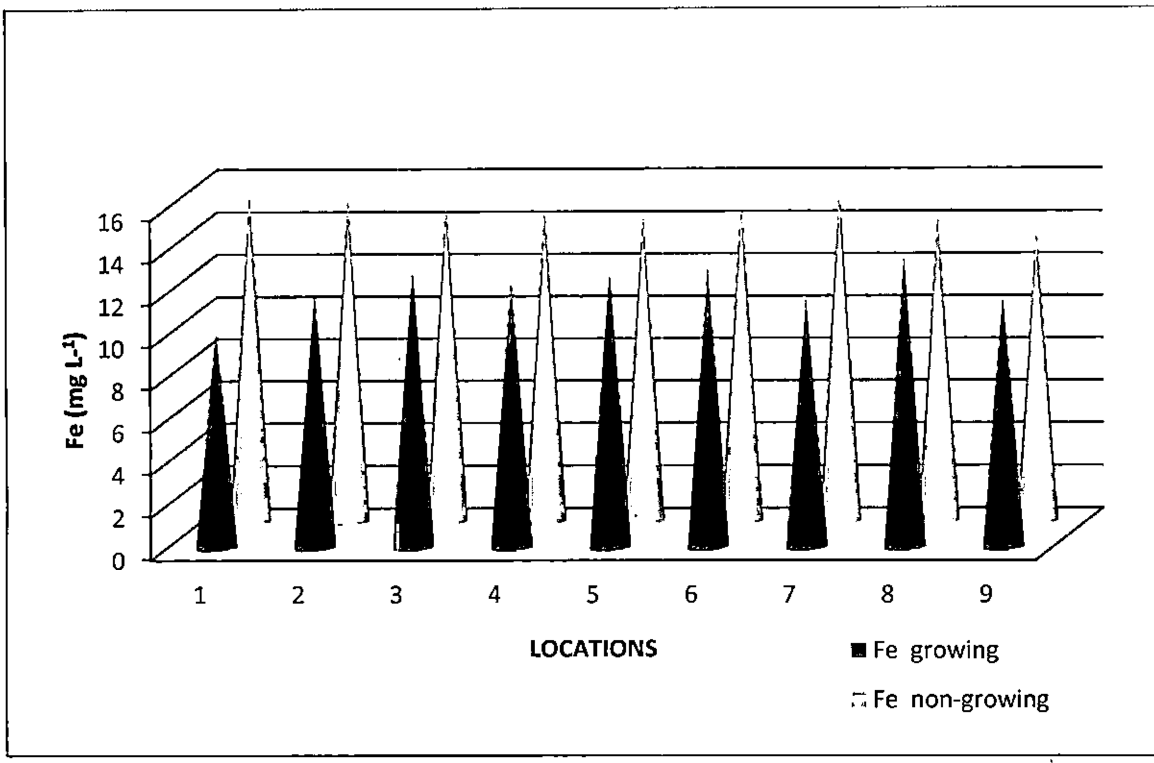


Fig. 16: Floodwater soluble Fe (mg L^{-1}) of Azolla growing and non-growing locations

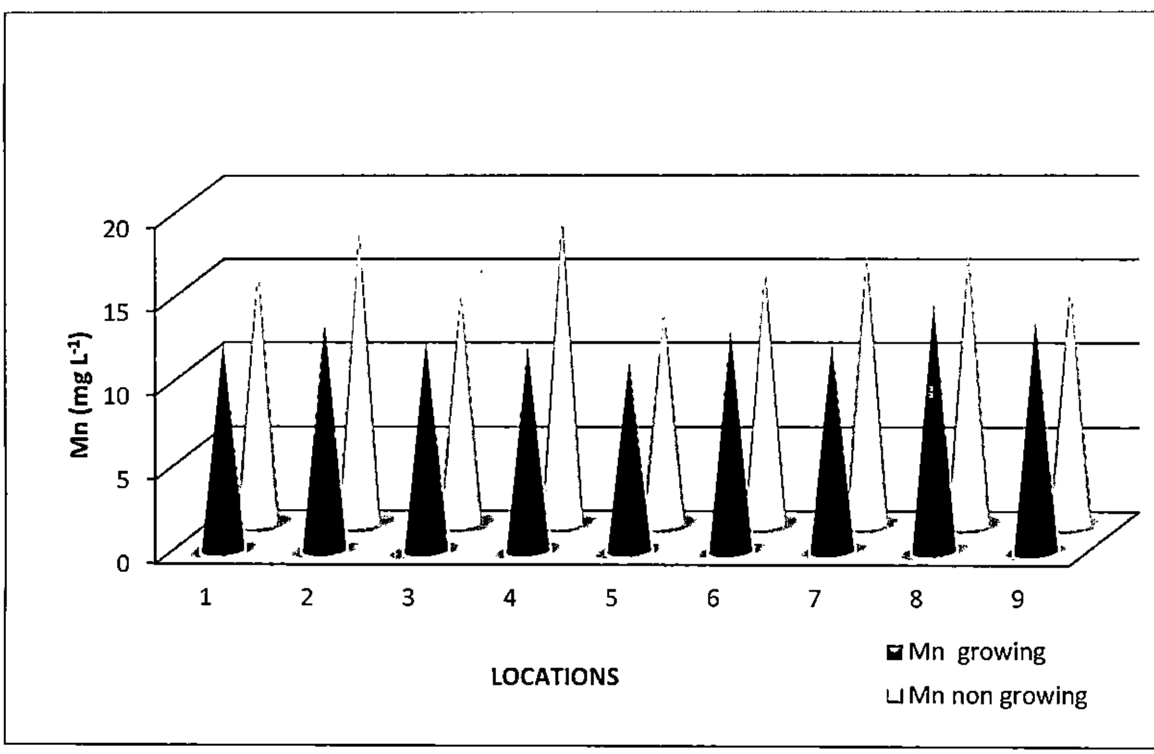


Fig. 17: Floodwater soluble Mn (mg L^{-1}) of Azolla growing and non-growing locations

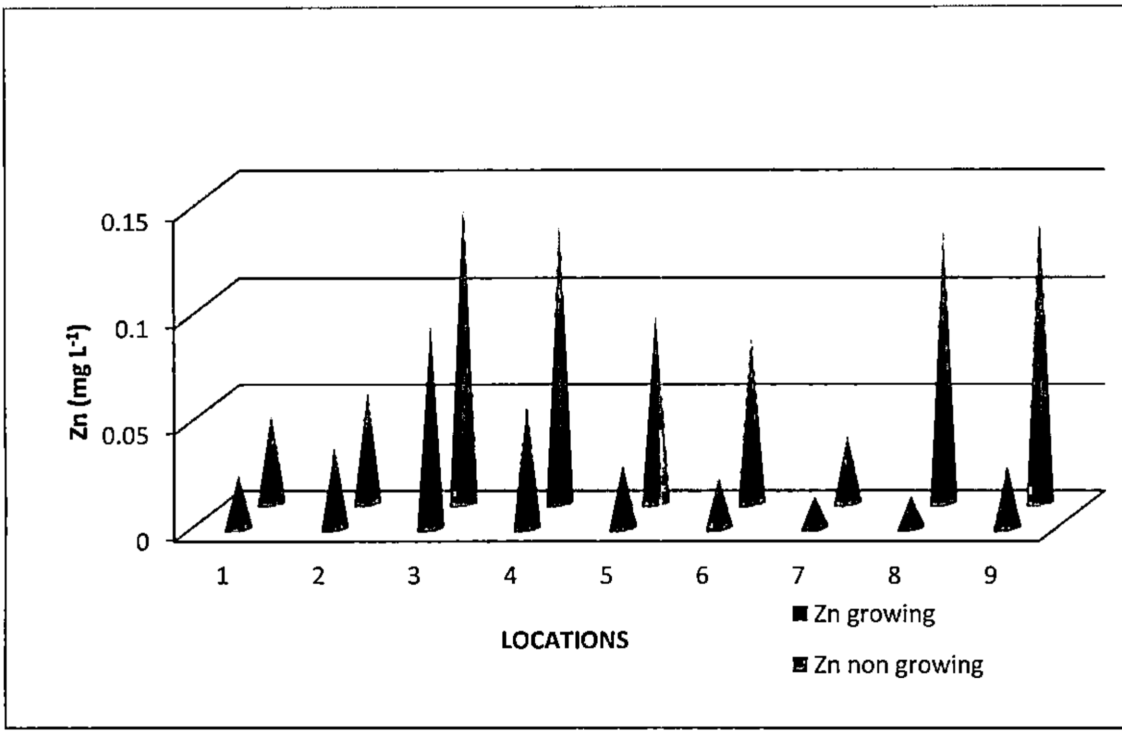


Fig. 18: Floodwater soluble Zn (mg L^{-1}) of Azolla growing and non-growing locations

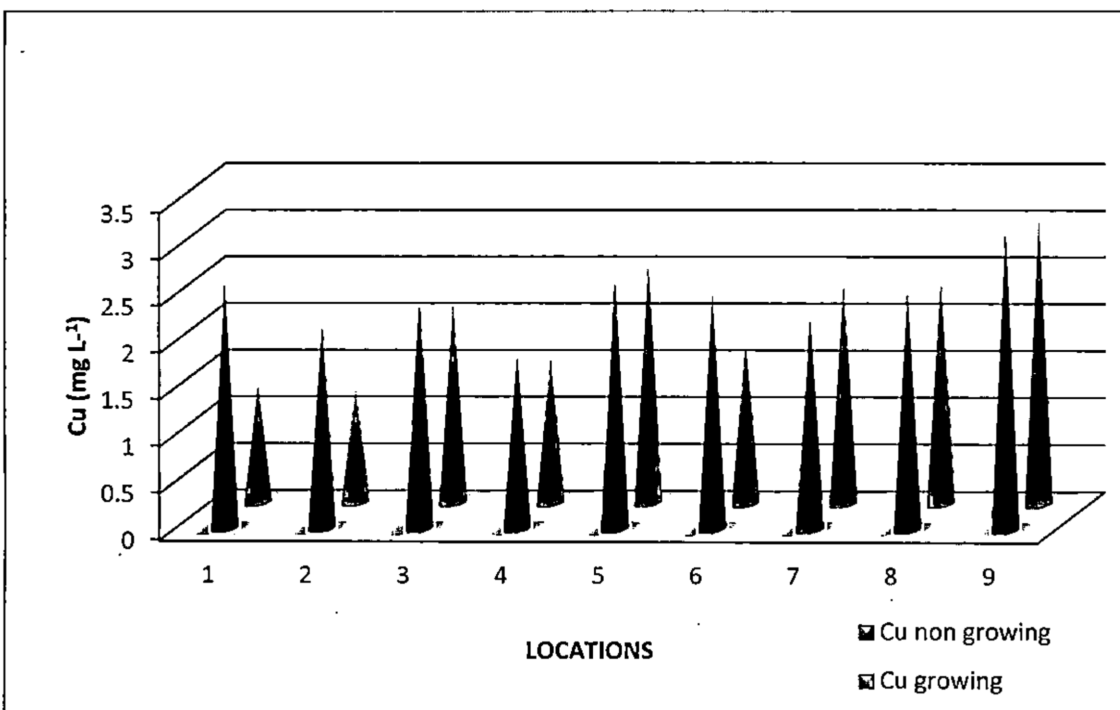


Fig. 19: Floodwater soluble Cu (mg L^{-1}) of Azolla growing and non-growing locations

C. Azolla

5.2.13 Moisture

Moisture content in Azolla depends mostly on factors like water availability, temperature and relative humidity (Biswas *et al.*, 2005). In this study, the moisture content of Azolla ranged from 92 to 95 percent which is in agreement with the findings of Huggins (2007), Bocchi and Malgioglio, (2010). Although the temperature recorded for the growth of Azolla was in the optimum range (25- 30 ^{0}C) in all the locations, the moisture content was found to be the highest for the samples collected from Muthalamada panchayath where the water temperature was relatively lower (Table 23), this may be due to the reduced rate of evaporation from paddy fields in the maximum tillering stage.

5.2.14 Dry matter

The results of dry matter of Azolla (Table 24) registered the values between 5 to 6%. Since a major share of the composition is occupied by moisture the values on dry matter agrees with that of similar reports on Azolla (Singh and Subidhi, 1978; Herzalla *et al.*, 2001)

5.2.15 Carbon

Azolla, inherently being rich in carbon add to the soil carbon pool immensely. The sample analysis revealed that contains 20-40 percent carbon (Table 25). The carbon richness of Azolla has also been reported by Herzalla *et al.* (2001); Bocchi and Malgioglis (2010). The locational effect also was significant in deciding the carbon content which can be attributed to the soil and environmental factors.

5.2.16 Nitrogen

Total N content of Azolla varied between 2 to 4 % (Table 26). The high N content is due to its ability to fix N in the form of ammonium (Costa *et al.*, 2009; Sadeghi *et al.*, 2012). The amount of nitrogen was higher than that of P and K.

Kimball *et al.*(2012), assigned the two probable sources of nitrogen in Azolla to the mineral nitrogen absorbed from the flooding water which reaches originally from soil mineralization and to the atmospheric N fixed by the symbiotic cyanobacteria Anabaena

5.2.17 C/N ratio

The C/N ratio is adjudged as one of the most important factor that controls the rate of mineralization. The suitability of Azolla to rice crop is purely due to its quick decomposition in soil, once incorporated, and efficient release of nitrogen for crop use. Researchers have arrived at differential rates of mineralization based on C/N ratio which varied from two days in Azolla species with low C/N ratio to five days in those with high C/N ratio (Wang *et al.*, 1987, Kannaiyan 1993)

In the present study C/N ratio of Azolla species collected from diverse locations varied from 9- 14 (Table 27) thereby emphasizing locational impact. Similar ratios were also reported by Lumpkin and Plucknett (1982)

5.2.18 Crude protein

The crude protein content of Azolla collected from various locations ranged from 15 to 22 percent (Table 28). Many experiments with Azolla have yielded a crude protein content of 19-30 percent (Singh and Subidhi, 1978) which is higher than green forage crops, so Azolla is supposed to be a protein rich source among aquatic macrophytes. Complementing to this, crude protein status is favourable for animal nutrition. These merits have given an alternate role to Azolla as a feed for livestock, poultry and fish farmers (Herzella *et al.*, 2001)

5.2.19 Phosphorus

Phosphorus is one of the most essential element for the growth of Azolla and its nitrogen fixing ability. Soil P status was low in Azolla growing compared to that of non-growing (Table 9) which indicates the better uptake of P by Azolla The tissue content of P ranged between 0.2 to 0.3 percent (Table 29) which is comparable with a concentration of 0.4-0.5 percent P on dry weight basis (Diara *et al.*, 1987) which was arrived at after preloading Azolla with phosphorus up to its maximum luxury uptake level and thus attaining 1.0-1.6 percent P on fresh weight basis

5.2.20 Potassium

The role of K has paramount importance in deciding growth of Azolla whenever the temperature is low (Costa *et. al.*, 1999) .The tissue content of K in Azolla ranges from 1.3 to 1.6 percent (Table 30) which agrees with the findings of Herzalla *et al.*, 2001

The available status of K in the Azolla growing locations was lower than that of the non-growing sites which may be due to the higher accumulation of K during their effective growing period. Potassium is important for photosynthesis and in the formation of amino acid and proteins from ammonium ions, its deficiency lead to premature death of leaves and an increased sensitivity to stress condition (Costa *et al.*, 1999). The availability of K was found to be in the sufficient range among the Azolla growing tracts thereby overruling the chances of it getting deficient.

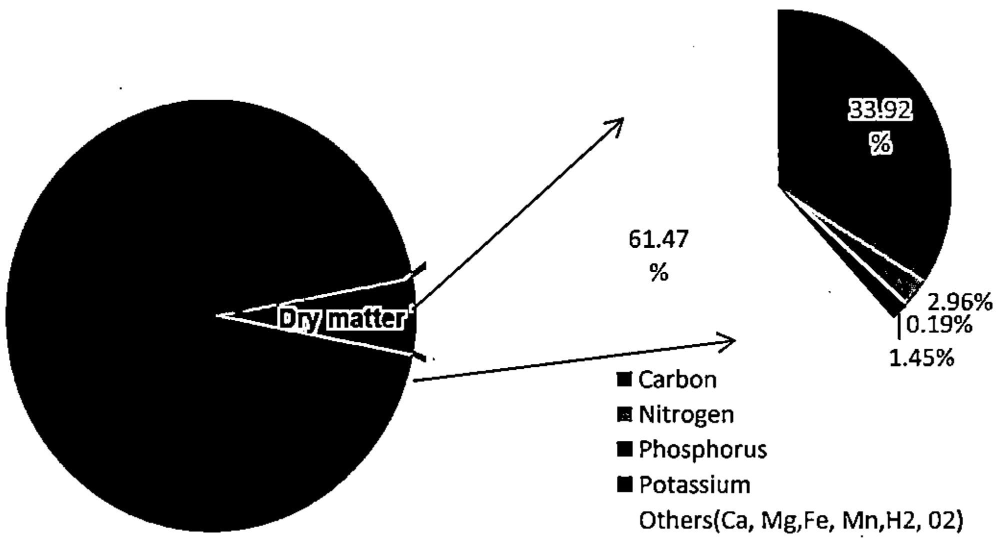


Fig. 20: General composition of Azolla

5.2.21 Nitrogenase activity

Nitrogenase enzyme activity was measured by Acetylene Reduction Assay (ARA) and the results are given in Table 31.The values ranged between 180 to 240 nmoles ethylene g^{-1} h^{-1} which is in conformity with the results of Arora and Singh (2003). The Nitrogenase activity and N fixation of the *Azolla- Anabaena* symbiosis was found to be influenced to a large extent by species and various environmental factors such as temperature, light, pH, salinity and the nutrients like Fe and P (Belnap, 2000; Kathiresan 2007)

Arora and Singh (2003) analyzed biomass accumulation and N fixing potential of six different Azolla species namely *A. filliculoides*, *A. Mexicana*, *A. microphylla*, *A. pinnata*, *A.rubra* and *A. caroliana* of these low biomass production , relative growth rate and lower Nitrogenase activity were reported in *A.pinnata*

Hechler and Dawson (1995) proved that nitrogenase activity was strongly dependent on temperature. Less activity was observed at 0^0C and only a very little amount could be detected at 5^0C. Activity rose sharply above 15^0C and reached its maximum at 25^0C. Nitrogenase activity declined precipitously from 35^0C to 40 ^0C and could not be detected at 45 ^0C. In the present study the temperature was in the range of 30-32^0C (Table 17) thereby favouring the growth of Azolla and the efficiency of nitrogenase enzyme.

High salinity was reported adverse for the nitrogenase activity. Alexander *et al.*, 1978 substantiated that low salinity favoured the presence of heterocystous cyanobacteria while very high salinity mainly supported the growth of non-heterocystous genera. In the present study salinity was congenial (Table 6, 16) for growth of Azolla.

Effect of pH on nitrogenase activity was explained by many researchers (Herrero *et al.,* 2001, Choudhury and Kennedy, 2004) mainly based on availability of nutrients especially P and Fe. A near neutral pH was observed to be optimum for the growth of Azolla (Table 5, 15) since the availability of P availability was more in the near neutral pH.

The general reaction for Nitrogen fixation with the help of Nitrogenase enzyme is as follows:

$$N_2 + 12\ ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + 12\ ADP + 12\ Pi$$

This reaction requires 12 ATPs to provide sufficient energy to break the strong triple bond between the two N atoms of N_2 gas. This energy is obtained through photosynthesis and the nitrogen fixing process proceeds only if P is available in the sufficient range, it being essential for the activity of ATPase enzymes.

Nitrogenase (the enzyme complex) consists of a dimeric Fe- protein (the de nitrogenase reductase) functioning as an electron carrier to the tetrameric Mo Fe-protein (the de nitrogenase). Presence of Fe^{2+} in the soil is a good index for the degree of reduction which normally changes in accordance with pH and E_h; the data furnished in Tables 5, 9, 15 and 19 suggested that the degree of sequential reduction in the soil was one of the most important controlling factors for the N fixing activity.

Effort was made to relate the N content of Azolla with its Nitrogenase enzyme activity. It was noticed that it was difficult to arrive at such a relation since the quantity of acetylene getting reduced to ethylene which was only momentarily influenced by the N content of Azolla or rather the N_2 -fixing activity of Azolla determined at any point of a day could not be referred to as Nitrogenase activity for the whole day, nor could the actual amount of N_2 fixation calculated from the N_2 -fixing activity during the time of analysis.

Though the efficacy of acetylene reduction technique for measuring N fixing activity has been reported by many scientists (Ashton and Walmsky, 1976; Bai and Shi, 1979; Hill, 1977; Peters and Mayne, 1977; Peters *et al.*, 1978; Wang and Tang, 1978) its inadequacy towards precision has always been a part and parcel of all the works. For example, the value of N_2 -fixing activity of Azolla obtained from destructive measurement or from a one-time measurement differed greatly from the actual value in the natural environment. Reports are also available that throw light on regular variations of N_2-fixing activity of Azolla and the actual amount of N_2 fixation in the natural environment(Mao *et al.*, 1982). The conclusion is that the Azolla growing in a natural environment fixed atmospheric nitrogen throughout the day and its Nitrogenase activity varied with environment.

5.2.22 Species identification

Identification of the species (Table 32), according to the standard keys outlined by Lumpkin and Plucknett, 1982; Watanabe *et al.*, 1992, revealed that the species prevalent in the experimental region was *A. pinnata*

Summary

SUMMARY

In order to achieve the objectives of identifying soil and water quality parameters congenial for the growth and Nitrogenase content of Azolla *spp.* in the rice growing tracts of Palakkad eastern plains, a comprehensive survey was conducted. Out of the four blocks viz Alathur, Chitoor, Kollengode and Nenmara the prevalence of Azolla could be located only in two blocks namely Chitoor and Kollengode. Soil, water and Azolla samples were collected from Azolla growing locations under different panchayaths of the selected blocks whereas from non-growing tracts the collection was confined to soil and water samples.

The salient findings on properties of soil and water of the Azolla growing and non-growing locations and on the composition of Azolla are detailed below:

A. Soil

- Significant difference between growing and non-growing locations was noted w.r.t. pH, EC, organic carbon, available N , available P , total Fe , Mn and Zn
- A neutral pH (6.8- 7.5) and low electrical conductivity (0.2-0.3 dSm^{1}) was preffered by Azolla for its growth
- Organic carbon and available N increased with the growth of Azolla
- P, Fe, Mn, Zn and Cu were less in Azolla growing locations
- The heavy metals viz., Cr, Cd, Ni and Pb were below detectable limits in both Azolla growing and non-growing locations.

B. Water

- Significant difference existed between Azolla growing and non-growing locations w.r.t. pH, temperature, dissolved oxygen, Fe, Mn, Zn and Cu

- pH and temperature was low under Azolla growing locations whereas dissolved oxygen was more
- Heavy metals like Fe, Mn, Zn and Cu was low in Azolla growing locations.
- In both the locations heavy metals like Cr, Cd, Ni and Pb were below detectable limits.

C. Azolla

- The moisture content varied from 94 to 95 per cent
- Carbon content was between 22.9 and 39.45 per cent.
- The N content ranged between 2 to 4 per cent and the C/N ratio was in the range 9 to 14
- Crude protein content of Azolla registered values between 16 and 22 per cent
- The P content varied from 0.20 to 0.23 per cent.
- K content recorded values from 1.2 to 1.5 per cent.
- The amount of ethylene produced extended from 192 to 236 nmole ethylene g^{-1} h^{-1}
- Locational effect was significant effect on the quantity of ethylene produced.
- A significant positive correlation was obtained between soil P and the content of nitrogen, crude protein and P of Azolla
- The C/N ratio showed a negative correlation with soil P.
- Soil pH and EC showed a positive correlation with carbon content of Azolla.
- Nitrogenase activity recorded a significant negative correlation with pH and EC.

Conclusion

Among the nine locations studied Nalleppilli panchayath with soil characters (pH 7.34; EC 0.3 dS m^{-1}; OC 1.56%; Available N,P,K- 153.6, 63.3 and 191.2 Kg ha^{-1} respectively; Total Fe 1646.2 mg kg^{-1}; Mn 234 mg kg^{-1};Zn 23.3 mg kg^{-1} and Cu 24.8 mg kg^{-1}) . Flood water parameters (13.1, 12.5 and 2.12 mg L^{-1} of soluble Fe, Mn and Cu). Quality parameters of Azolla from the same location were (C 38.6%; N 2.7%; C/N ratio 12.58; Crude protein 16.95%; P 0.174 % and K 1.39%) was identified to be the suitable for the growth and multiplication of Azolla

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Appendices

Appendix I

CHARACTERISATION OF SOIL AND WATER OF PALAKKAD EASTERN PLAINS IN RELATION TO GROWTH AND NITROGENASE CONTENT OF *AZOLLA* SPP.

By

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ABSTRACT OF THE THESIS

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Abstract

A study intended for "Characterization of soil and water of Palakkad eastern plains in relation to growth and nitrogenase content of *Azolla* spp." was undertaken in the Department of Soil Science &Agricultural Chemistry, College of Horticulture, KAU, Vellanikkara during 2013-2015. The objectives of the study were to conduct a survey of *Azolla* spp. in the rice growing tracts of Palakkad eastern plains and to identify soil and water quality parameters congenial for the growth and nitrogenase content of *Azolla* spp.

The preliminary survey conducted in the four block panchayaths of Palakkad eastern plains to identify the prevalence of Azolla revealed two blocks viz. Chittoor and Kollengode to be positive with respect to Azolla and hence, further study was restricted to the grama panchayaths of these blocks.

Characterization of soil and water was done in both Azolla growing and non-growing regions. Composition of Azolla collected from different locations was also determined. In order to ascertain the nitrogenase enzyme activity, samples of Azolla collected from five different locations were subjected to Acetylene Reduction Assay (ARA).

Among the soil parameters, significant difference was noticed between Azolla growing and non-growing locations with respect to pH , EC, organic carbon, available N, P , total Fe, Mn and Zn in contrast to potassium and copper which did not show any pronounced variation. Soil pH, EC, available P, total Fe, Mn and Zn were comparatively lower in Azolla growing regions. However, available nitrogen in soil was more in Azolla growing locations. Heavy metals like Cd, Cr, Ni and Pb were below the detectable limits in soil.

Analysis on flood water quality showed significant effect in the Azolla growing regions on parameters like pH, temperature and dissolved oxygen, Fe, Mn,

Zn and Cu. Soil analysis data revealed the pH, EC, soluble Fe, Mn, Zn and Cu to be lower under Azolla growing conditions. Heavy metals like Cd, Cr, Ni and Pb were below the detectable limits. Presence of Azolla decreased the flood water temperature but increased the dissolved oxygen content.

On comparison, the composition of Azolla revealed a prominent and significant correlation with location on its content of carbon, nitrogen, phosphorus, potassium and also the C/N ratio. Moisture content varied from 94 to 95 per cent, variation in carbon and nitrogen content was from 22.9 to 39.5 per cent and 2 to 4 per cent respectively among the locations studied. The C/N ratio ranged from 9 to 14. Crude protein content of Azolla registered values between 16 and 22 per cent. The content of phosphorus ranged between 0.20 and 0.23 per cent whereas that of potassium was in the range of 1.2 to 1.5 per cent. Correlation worked out between soil parameters and composition of Azolla disclosed a significant positive effect of soil P on the nitrogen, crude protein and phosphorus content of Azolla. However, soil P was negatively correlated with the C/N ratio of Azolla.

Nitrogenase enzyme activity quantified was profoundly affected by the locations. The amount of ethylene produced extended from 192 to 236 nmole ethylene g^{-1} h^{-1}. It showed a significant negative correlation with soil pH and EC.

The study has given valuable information on the influence of soil and flood water quality parameters and locations on Azolla which could be used for further investigations on its nutrient dynamics.