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Short communication

Distribution of *Azolla* in Palakkad eastern plains (AEU 23)

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Abstract

A study was conducted in the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Vellanikkara during the period 2013 to 2015 to study distribution of *Azolla* in Palakkad eastern plains. The electrochemical properties of soil were studied in the nine locations coming under two blocks *viz.,* Chittoor and Kollengode of AEU23, as described by Kerala State Planning Board. General composition of *Azolla* was analyzed to get information on parameters like moisture, dry matter, carbon, nitrogen, C/N ratio, crude protein, phosphorus and potassium. Correlation was also worked out between the soil parameters and *Azolla* composition. Locational effect was significant on all the soil parameters studied. Significant positive correlation was noticed between the carbon content of *Azolla* and the electrochemical soil properties like pH and EC. The contents 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.

Keywords: Azolla, Composition, Distribution, Soil properties

Cyanobacteria are present abundantly in paddy fields and are important in maintaining fertility of rice fields through nitrogen fixation. Soils of many rice fields contain a high density of cyanobacteria and over 50% are heterocystous filamentous forms. The surface of rice field water provides a suitable environment for cyanobacteria, both free living and living symbiotically with the water fern *Azolla* (Watanabe and Liu., 1992). *Azolla* has a symbiotic association with the nitrogen fixing cyanobacteria *Anabaena azollae*. It can fix 30 to 60 kg N ha^{-1} in 30 days. The fern is also used as an important biological source to improve the nitrogen balance of rice fields. *Azolla*, with its rich protein content, could be remuneratively used as a substrate along with FYM in biogas plants for enhancing gas production and enriching slurry, besides serving as a feed ingredient for livestock, fish and human beings.

The present study is envisaged to gather information on the congenial factors of environment and quantifiable promoting features of soil and water that favour *Azolla* growth. This would help to assess Azolla's contribution towards nitrogen fixation, the nitrogenase activity in soil being more dependent on ecological and climatic conditions. The investigation is expected to hold information on factors which could further be translated as a practical tool for better field management of *Azolla* spp. at this juncture, when organic farming is getting wider acceptance. The main objectives of the present study are surveying of areas in Palakkad Eastern plains (AEU23) to study about the abundance, distribution and utilization of *Azolla* in its natural environment and to study the soil factors favoring the growth of *Azolla.*

An initial survey was carried out to identify the areas

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where *Azolla* was prevailing in Palakkad eastern plains (AEU23, as described by Kerala State Planning Board) with the help of Global Positioning System (GPS). Two stage random sampling procedure was adopted for the survey. 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. Soil samples were collected and analysed in order to study the soil conditions favouring the growth of *Azolla*. The collected soil samples were oven dried and sieved using 0.5 mm and 2 mm sieves and subjected to analysis employing standard procedure. General composition of *Azolla* from different locations was also found out. Before the initiation of the experiment, moisture per cent of *Azolla* and dry matter percentage was determined and then *Azolla* samples were thoroughly washed, put in brown paper covers and oven dried at 50 - 70^{o}C. The dried samples were ground, properly mixed and used for estimating the moisture content. In addition, they were also analyzed for C, N, P, K and crude protein. Crude protein content was calculated by multiplying N with the factor 6.25. 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). After the comprehensive survey in Nenmara, Chittur, Kollengode and Alathur of Palakkad eastern plains, the prevalence of *Azolla* was established only in Chittoor and Kollengode blocks of Palakkad Eastern Plains. Soil samples were analyzed for pH, EC, OC, available N, P, K and total heavy metals like Fe, Mn, Zn, Cu, Cr, Cd, Ni and Pb. The results are given in Tables 1, 2 and 3.

Table 1. Electrochemical properties of soil of experimental site

Areas	pH	EC(dS m^{-1})
Elappully	7.44^{b}	0.29^{ab}
Kollengode	7.47^{a}	0.29^{ab}
Kozhinjampara	7.13^{bcd}	0.27^{ab}
Muthalamada	7.23^{abc}	0.27^{ab}
Naleppilly	7.34^{abc}	0.30^{a}
Pattencheri	7.42^{abc}	0.30^{a}
Perumatty	6.84^{d}	0.25^{b}
Vadavannur	7.4^{abc}	0.17^{c}
CD (0.01)	0.425	0.064
(0.05)	0.317	0.048

Significant differences were noticed between locations with respect to the electrochemical properties of soil. Soil pH ranged from 6.84 (Perumatty) to 7.47 (Kollengode). Electrical conductivity ranged from 0.17 (Vadavannur) to 0.30 (Nalleppilly and Pattencheri) dS m^{-1}.

Azolla prefers a medium near to neutrality or to some extent, acidic conditions. The optimal growth 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). 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.

According to Chinese researchers the water for *Azolla* cultivation should contain no more than 0.3% salt since higher salt concentrations decreased plant nitrogen (Mishra and Singh, 2006; Singh et al., 2008). The reduced salt content observed in the present study might have helped in overcoming osmotic problems, leading to increased chlorophyll content, photosynthesis, respiration and nitrogen fixation.

Pronounced locational effect existed with respect to available nutrient status of soil. The organic carbon status was high as per soil rating. The increased organic carbon status in the *Azolla* growing regions (Table 2) may be ascribed to the sloughing –off of roots during stress condition and also due to excessive growth (Uheda et al., 1999). Available nitrogen in soil has not been reported as

Table 2. Available nutrient status of soil

Areas	OC (%)	Avail. N(kg ha^{-1})	Avail. P(kg ha^{-1})	Avail. K(kg ha^{-1})
Elappully	1.3^{bc}	161.82^{a}	61.3^{ab}	158.9^{d}
Kollengode	1.41^{b}	154.32^{ab}	61.39^{ab}	193.3^{a}
Kozhinjampara	1.23^{c}	159.12^{ab}	50.2^{d}	140.3^{e}
Muthalamada	1.56^{a}	144.22^{c}	57.3^{c}	148.1^{de}
Naleppilly	1.56^{a}	153.60^{b}	63.32^{a}	191.2^{a}
Pattencheri	1.32^{bc}	152.00^{bc}	52.41^{d}	178.7^{bc}
Perumatty	1.28^{bc}	157.14^{ab}	58.56^{bc}	173.2^{c}
Vadavannur	1.64^{a}	129.18^{d}	61.19^{ab}	194.3^{a}
CD (0.01)	0.187	10.759	5.195	11.557
(0.05)	0.139	8.024	3.875	8.620

Table 3. Total soil micronutrient status

Areas	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
Elappully	1622.4^{b}	231.78^{cd}	20.82^{c}	24.26^{cd}
Kollengode	1631.6^{bc}	309.58^{a}	24.28^{a}	20.76^{ef}
Kozhinjampara	1623.2^{b}	248.50^{c}	22.44^{abc}	36.22^{a}
Muthalamada	1625.6^{bc}	267.42^{b}	23.10^{ab}	16.98^{g}
Naleppilly	1646.2^{bc}	234.04^{cd}	23.32^{ab}	24.76^{c}
Pattencheri	1648.0^{bc}	247.94^{c}	21.54^{bc}	21.84^{de}
Perumatty	1653.9^{c}	278.64^{b}	16.58^{d}	24.20^{cd}
Vadavannur	1506.1^{a}	228.14^{d}	21.68^{bc}	29.66^{b}
CD (0.01)	35.77	23.20	2.733	3.873
(0.05)	26.68	17.30	2.038	2.889

a limiting factor for *Azolla* growth under normal conditions due to its ability of *Azolla* to fix atmospheric nitrogen. Phosphorus 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 1) would have helped in the release of phosphorus and better growth of *Azolla.* The significant difference in available potassium in *Azolla* growing regions (Table 2) can be due to the release of potassium from inorganic and organic fertilizers applied to rice fields.

Nitrogen fixing ability of *Azolla* is dependent to a greater extent on soluble Fe followed by Mn (Newton and Herman, 1979). Zinc and copper are also required for the growth of *Azolla* in tracer quantities. So like any other plant *Azolla* also requires all micronutrients in the required quantities (Table 3).

There was no locational effect regarding the moisture and dry matter percentage of *Azolla.* 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% which is in agreement with the findings of Huggins (2007), and Bocchi and Malgioglio, (2010). Dry matter percentage, a measurement of the mass of *Azolla* when completely dried (Table 4), registered values between 5 to 6%. Since a major share of the composition is occupied by moisture, the values on dry matter obtained tallies with that of similar research works in *Azolla* (Singh and Subidhi, 1978; Herzalla et al., 2001).

Apart from being a bio-fertilizer, *Azolla,* being inherently rich in carbon, adds to the soil carbon pool immensely. The sample analysis revealed that *Azolla* contains 20 - 40% carbon (Table 4). The carbon richness of *Azolla* has also been reported by

Table 4. Composition of *Azolla*

Areas	Moisture (%)	Dry matter (%)	Carbon (%)	Nitrogen (%)	C/N	Crude protein(%)	Phosphorus (%)	Potassium (%)
Elappully	94.6	5.94	27.6^{e}	2.9^{b}	9.52^{e}	18.12^{b}	0.210^{b}	1.48^{abc}
Kollengode	94.56	5.46	32.5^{d}	2.6^{c}	12.58^{b}	16.25^{c}	0.174^{c}	1.39^{de}
Kozhinjampara	93.81	6.18	38.6^{a}	3.6^{a}	10.80^{cd}	22.31^{a}	0.224^{a}	1.55^{a}
Muthalamada	94.23	5.81	34.5^{c}	3.4^{a}	10.15^{de}	21.25^{a}	0.218^{ab}	1.54^{a}
Naleppilly	94.45	5.60	38.6^{a}	3.6^{a}	14.08^{a}	22.5^{a}	0.180^{c}	1.51^{ab}
Pattencheri	94.03	5.94	39.5^{a}	3.0^{b}	14.09^{a}	18.75^{b}	0.156^{d}	1.32^{e}
Perumatty	94.15	5.62	37.2^{b}	3.3^{a}	11.30^{c}	20.70^{a}	0.216^{ab}	1.41^{cd}
Vadavannur	93.47	5.88	22.9^{f}	2.7^{bc}	8.50^{f}	17.00^{bc}	0.172^{c}	1.42^{bcd}
CD (0.01)	NS	NS	1.539	0.332	1.266	2.063	0.018	0.108
(0.05)			1.148	0.248	0.944	1.539	0.013	0.081

Herzalla et al. (2001, and Bocchi and Malgioglis (2010). The locational effect was also significant in deciding the carbon content which can be attributed to the soil and environmental factors. Total nitrogen content of *Azolla* varied between 2 to 4% (Table 4). The high nitrogen content is due to its ability to fix nitrogen in the form of ammonium (Costa et al., 2009; Sadeghi et al., 2012). The amount of nitrogen was higher than that of phosphorus and potassium. Kimball et al. (2002) 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 nitrogen fixed by the symbiotic cyanobacteria *Anabaena.*

In the present study C/N ratio of *Azolla* species collected from diverse locations varied from 9- 14 (Table 4) thereby emphasizing locational impact. Similar ratios were also reported by Lumpkin and Plucknett (1982). The crude protein content of *Azolla* collected from various locations ranged from 15 to 22% (Table 4). Many experiments with *Azolla* have yielded a crude protein content of 19 - 30% (Singh and Subidhi, 1978) which is higher than green forage crops, which are supposed to be protein rich sources, and aquatic macrophytes. Complementing to this crude protein status is the favourable amino acid composition 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).

Phosphorus is one of the most essential elements for the growth of *Azolla* and its nitrogen fixing ability. The tissue content of phosphorus ranged

Table 5. Correlation between composition of Azolla and soil parameters

Azolla composition	Soil/water parameters			
	pH	EC	P soil	K soil
Moisture	-	-	-	-
Dry matter	-	-	-	-
Carbon	0.90**	0.75*	-	-
Nitrogen	-	-	0.99*	-
Crude protein	-	-	0.79*	-
C/N ratio	-	-	-0.75*	-
Phosphorus	-	-	0.84**	-
Potassium	-	-	-	0.89**

*Significance at 5% level

** Significance at 1% level

between 0.2 and 0.3% (Table 4). The role of potassium has paramount importance in deciding growth of *Azolla* whenever the temperature is low (Costa et al., 1999). The tissue content of Azolla ranged from 1.3- 1.5 % (Table 4).

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 (Table 5). 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, so with increase in availability of soil phosphorus, the nitrogen fixation and nitrogen content in *Azolla* will be more, thus reducing the C/N ratio which paves way for easy mineralization. The potassium content in *Azolla* showed a significant positive correlation with the potassium content of soil.

Electrochemical properties and available nutrient status in soil immensely influence the growth of *Azolla* in its natural habitat. Locational effect is prominent with respect to soil conditions and the composition of *Azolla.*

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