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Review Article

Advancements in the Utilization of *Azolla-Anabaena* System in Relation to Sustainable Agricultural Practices

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The nitrogen fixing aquatic pteridophyte *Azolla* has the ability to fix atmospheric nitrogen at cheaper and faster rates due to the presence of a symbiotic cyanobacterium *Anabaena azollae*. Because of this property it has been exploited widely as biofertilizer for rice plants. In addition to this it has several other uses such as food, feed, biogas producer and hyper-accumulator of heavy metals etc. Because of the multifaceted uses the promotion and use of *Azolla-Anabaena* system would be ideal and environment friendly in sustainable agriculture. This article provides a brief account of the importance as well as developments in the utilization of *Azolla-Anabaena* system in agriculture and allied sectors.

Key Words: ***Azolla-Anabaena*; Biofertilizer; Nitrogen; Multifaceted Uses; Sustainable Agriculture**

Introduction

The aquatic pteridophyte *Azolla* is an excellent biofertilizer and green manure having global distribution. Ability of *Azolla-Anabaena* system to fix atmospheric nitrogen at faster rates makes it an outstanding agronomic choice for the cultivation of rice under tropical conditions. Nitrogen fixation potential of the *Azolla-Anabaena* system has been estimated to be 1.1 kg N ha^{-1} day^{-1} and one crop of *Azolla* provided 20-40 kg N ha^{-1} to the rice crop in about 20-25 days (Watanabe *et al*., 1977). The ability of nitrogen fixation is due to the presence of a heterocystous cyanobacterium *Anabaena azollae* which is confined to the dorsal leaf cavity of the fern (Moore, 1969). This cavity is formed by the enfolding of the adaxial epidermis in the dorsal leaf lobe (Peters, 1976). *Azolla* has multifaceted uses and has gained considerable importance in the recent times as biofertilizer, green manure and as poultry feed and cattle fodder (Singh and Subudhi, 1978). Increase in body weight of the chicks upon feeding with *Azolla* was observed. *Azolla* is a rich source of protein and essential amino acids and contains several vitamins such as vitamin-A, vitamin B-12 and beta carotene. It is also rich in minerals such as Calcium, Phosphorous, Potassium, Magnesium, Copper and Zinc etc. The protein composition of *Azolla* is 25-35% on dry weight basis and is easily digested by poultry (Parashuramulu *et al*., 2013). *Azolla* has many uses such as animal feed, human food, medicine, production of biogas, hydrogen fuel, water purifier, weed control, reduction of ammonia volatilization and is aptly referred it as green gold mine (Wagner, 1997). *Azolla* plants in huge numbers sequestered significant quantities of atmospheric CO_2 and converted it directly into biomass of *Azolla* (Speelman *et al*., 2009). According to them the plants died and sank to the bottom of the Arctic Ocean and were deposited as sediments that now form a layer beneath the Arctic Ocean. Thus these plants had a significant impact on

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Earth's climate 50 million years ago (Bujak, 2007). The plant has the ability to multiply fast which is a remarkable aspect in its exploitation as biofertilizer. Agronomic potential of *Azolla* has been demonstrated and successful exploitation of this organism in the agriculture context of Asian countries has also been highlighted (Singh, 1977a; Singh, 1989). Besides this there are several reports on its potential and agronomic significance (Watanabe, 1982). Rice ecosystems are characterized by the pattern of rain fall, depth of flooding and drainage and by the adaptation of rice to these agro-ecological factors and rice is grown in flooded conditions in more than 128 million hectares and is suitable for the cultivation of *Azolla* (IRRI, 1994, Gillar, 2002). Increase in yield due to application of *Azolla* was demonstrated by several studies conducted in the past at several locations in the country. Similar results have been obtained with *Azolla* along with the application of chemical nitrogen fertilizers (Singh *et al*., 1992). Highest grain yield in rice plants is observed when a comparison of *Azolla* application is made with other biofertilizers. Suppression of weeds and reduction in the volatilization of ammonia in rice fields due to the formation of a thick mat in rice fields by *Azolla* is observed (Singh, 2000). Use of *Azolla* is very popular in India and the most widely distributed species of *Azolla* include *A. pinnata*, *A. fuiliculoides*, *A. rubra*, *A. microphylla*, *A. mexicana* and *A. caroliniana*, respectively (Hills and Gopal, 1967). There are also reports on its successful use in crops other than rice. Trials have been conducted using *Azolla* successfully in wheat also as biofertilizer to enhance the yield (Marwaha *et al*., 1992). Mahapatra and Sharma (1989) observed beneficial effects of *Azolla* on subsequent wheat crop with increase in grain yield. Application of 20 tonnes of *Azolla* along with 60 Kg nitrogen recorded highest yield of wheat (Sharma *et al*., 1999). The plant is also used in phytoremediation programs to clean up the polluted and contaminated waters. Phytoremediation is fast emerging as an excellent option to ward off the pollutants from aquatic ecosystems (Arora *et al*., 2003).

However, the full potential of the plant is still underutilized despite its advantages and concerted attempts are required for the popularization of the system. Therefore, the present review takes stock of the developments related to the successful exploitation and application of *Azolla*-*Anabaena* system and its multifaceted uses for sustainable agricultural practices.

Distribution, Habit and Habitat and Morphology

Azolla is found in both temperate and tropical regions. It grows luxuriantly in ditches, fresh water ponds and paddy fields. The *Azolla* plants are delicate, small and triangular or polygonal in shape (Fig. 1). It is free floating and aquatic but can grow on moist soils as long as the moisture persists in the soil. The sporophytic plant has a horizontal rhizome of 0.5 to 7 cm in diameter with branches having densely arranged and overlapping leaves. A leaf consists of a thick dorsal lobe and a thin ventral lobe. The symbiotic Blue Green Alga is confined to the dorsal lobe (Peters and Mayne, 1974). An epidermis covers the surface of the dorsal lobe and the epidermis has vertical rows of single celled stomata and trichomes of one or more cells. The ventral lobe which helps in floating due to its convex surface touching water has a few stomata and trichomes (Eames, 1936).

Growth and Multiplication

Azolla is found in both temperate and tropical regions and it grows luxuriantly in ditches, fresh water ponds and paddy fields. The pH requirement of the soil is 7.2 for an ideal growth coupled with a temperature of 32°C. However, studies conducted elsewhere in

Fig. 1: *Azolla* plants

India have shown that the region specific choice of the species for their use as biofertilizer. A study was conducted at Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi to compare the biomass production and nitrogen fixation potential of different species of *Azolla* (Arora and Singh, 2003). Based on the experiments *A. microphylla* was found to perform better and hence selected for mass multiplication. This strain is maintained round the year as it is able to withstand both high as well as low temperature conditions.

Taxonomic Status of *Azolla*

The Genus *Azolla* was established by Lamarck in the year 1783 and placed it in the family Salviniaceae under the order Salviniales. However, *Azolla* is placed in the monotypic family *Azollaceae* and there are seven extant Species of *Azolla* (Hills and Gopal, 1967; Konar and Kapoor, 1972). *Azolla* is categorized into two Sub-Genus viz. EuAzolla and Rhizosperma (Svenson, 1944). The Sub-Genus EuAzolla is characterized by the presence of three floats of megasporocarps and consists of Species such as *A. caroliniana, A. filiculoides, A. mexicana, A. rubra* and *A. microphylla*. In contrast, the Sub-Genus Rhizosperma consists of nine megaspore floats. *A. pinnata* and *A. nilotica* belong to this Sub-Genus. The trichomes are important in the identification of the organism at the Species level (Lumpkin and Plucknett, 1982; Nayak and Singh, 1988). The basic chromosome number in all Species of the section as well as the section *Rhisosperma* is n = 22 except in *A. nilotica* where n = 26. The cytological investigations show that the somatic chromosome numbers for Species of the Section Rhizosperma are *Rhizosperma* are *A. pinnata* R.Br. (India) 2n = 44, *A. pinnata* R.Br. (Africa) 2n = 44, *A. pinnata* R.Br. (Vietnam, Green) 2n = 66 and *A. nilotica* Decne.2n = 52 (Tan *et al*., 1986). The somatic chromosome numbers for Species of the Section Euazolla are *A. Mexicana* Presl. 2n = 48, *Azolla filiculoides* Lam. 2n = 40 with the basic chromosome numbers n = 24 and n = 20, respectively (Nayak and Singh, 1989). The taxonomic assignment of *Azolla* is difficult because many Accessions do not form sporocarps under culture conditions. The normal mode of reproduction is vegetative but sexual reproduction has also been noticed although this has been observed in limited time periods of the year. This has resulted in problems related to precise identification of the Species. Therefore molecular tools have been employed for precise identification of the species. RFLP and isozyme patterns to identify the Sections of *Azolla* are employed (Zimmerman *et al*., 1991a; Zimmerman *et al*., 1991b; Coppenolle *et al*., 1993). Taxonomy of the Family Azollaceae is highly controversial (Reid *et al*., 2006). The uniqueness of *A. nilotica* has been confirmed from the data obtained from the loci of plastid genome (Metzgar *et al*., 2007). Integration of different types of data based on morphology, vegetative characters and molecular biology to provide a firm footing for the taxonomy of *Azolla* has been suggested (Perreira *et al*., 2011). Species specific SCAR primers (sequence characterized amplified region) for the precise identification of different species of *Azolla* is developed recently (Abraham *et al*., 2013).

Sporulation and its Importance in *Azolla*

Mode of reproduction in *Azolla* is mainly vegetative and because of this reason the biomass has to be maintained round the year. The fern is heterosporous and produce both mega and micro sporocarps and their germination is influenced by several factors

Table 1: Sporocarp germination in *Azolla* as influenced by various conditions

Treatment	No. of sporocarps incubated	No. of sporocarps germinated	Germination (%)
Control (distilled water)	188	31	24.4
IRRI medium (solid)	155	44	37.1
IRRI medium (liquid)	181	37	26.7
Soil solution	184	46	28.9
Phosphorous (30 ppm)	126	100	63
Kinetin (100 ppm)	197	132	55.2
Gibberellic acid (100 ppm)	192	106	48

Singh *et al*., (1990)

Table 2: Effect of *Azolla* and various organic treatments on rice (*Pusa Basmati* 1) grain yield during *kharif* (2003-08)

Treatment*	Rice grain yield (t/ha)						
	2003	2004	2005	2006	2007	2008	Mean
Azolla (A)	2.87	2.54	2.43	2.36	2.29	2.06	2.43
BGA (B)	2.70	2.46	2.35	2.29	2.19	1.90	2.32
FYM (F)	2.69	2.24	2.26	2.12	2.17	2.04	2.25
Vermicompost (V)	2.90	2.66	2.53	2.60	2.39	2.18	2.54
A+B	3.35	3.25	3.03	3.16	3.02	2.68	3.08
A+F	3.70	3.42	3.38	3.29	3.18	2.57	3.26
A+V	4.08	3.85	3.67	3.57	3.43	2.64	3.54
B+F	3.33	3.26	3.43	3.48	3.37	2.60	3.25
B+V	3.91	3.50	3.47	3.52	3.48	2.82	3.45
F+V	3.75	3.58	3.64	3.56	3.61	3.02	3.53
A+B+F	4.05	3.66	3.79	3.68	3.82	3.34	3.72
A+F+V	4.08	3.70	3.81	3.89	3.93	3.45	3.81
B+F+V	4.10	3.82	3.88	3.93	3.91	3.64	3.88
A+B+F+V	4.19	4.35	4.38	4.16	4.48	3.68	4.20
$N_{80}P_{40}K_{30}$	4.93	4.68	4.21	4.34	4.61	3.46	4.37
$N_0 P_0K_0$ (Control)	2.02	1.84	1.76	1.78	1.89	1.68	1.82
C.D (at @5 %P)	0.95	0.48	0.31	0.41	0.32	0.26	0.46

*Rate of application/ha: *Azolla* 1.0 t (fresh); BGA 2 kg (dry); FYM 5.0 t; Vermicompost 5.0 t.

(Table 3, Fig. 2). However, production of sporocarps is under the influence of the environment and no uniformity has been observed regarding their time of production by the plants. For example *Azolla microphylla* sporulates throughout the year (Kar *et al.*, 1999). Density of population of the plants especially high density has been found to induce the efficiency of sporocarp production (Watanabe, 1982). Production of sporocarps was generally favoured by relatively short day and cool nights at Cuttack (Singh *et al.*, 1987). Increase in the sporocarp germination is influenced by combined nitrogen sources. The germination of sporocarps in *Azolla caroliniana* varies in response to light, amino acids, sugars and abscissic acid (Singh *et al.*, 1990). Megasporocarps of *Azolla* and their germination in varied paddy soils has also been reported (Nayak *et al.*, 2004). Enhanced sporulation in *Azolla microphylla* and *Azolla pinnata* due to application of Gibberellic acid has been reported (Kar *et al.*, 1999). Similarly high sporocarp yield in relation to exogenous application of certain Auxins in combination with Gibbrellic acid has also been reported (Kar *et al.*, 2002). However, these methods using chemicals besides being uneconomical

Fig. 2: *Azolla microphylla* plants showing sporulation

Table 3: Chemical composition of *Azolla*

Constituents	Dry matter (%)
Crude protein	24-30
Crude fat	3.3-3.6
Nitrogen	4-5
Phosphorous	0.5-0.9
Calcium	0.4-1.0
Potassium	2-.4.5
Magnesium	0.5-0.65
Manganese	0.11-0.16
Iron	0.06-0.26
Soluble sugars	3.5
Crude fire	9.1
Starch	6.54

Source: Singh and Subudhi (1978a)

also lead to environmental contamination. Influence of fertilizer application on the sporocarp production has been observed (Singh *et al*., 1987) and induction of sporulation under phosphorous deficient conditions was recorded (Kannaiyan *et al*., 1988). An increase in the sporulation frequency and sporocarp number without compromising the biomass yield is observed due to the change in schedule of phosphorous application in *Azolla* (Kar *et al*., 2001). The sporocarps germinate after 3-5 months of dormancy period and percent germination is higher during August to October (Singh *et al*., 1984b). Farmers have to carry large quantity of the fresh inoculums to the field for application every time which involves transportation and many a times and this leads to perishing of the culture. This is also one of the reasons responsible for the poor adoption of *Azolla* by the farmers. Hence to make the *Azolla* biofertilizer technology more effective and serious attempts have to be made to successfully propagate the fern through sporocarps.

Production Technology for *Azolla* Biofertilizer and Modes of Application

The propagation of *Azolla* is generally carried out in soil-based nurseries. However, to maintain the germplasm medium based cultures is advisable. Effects of nutrient status of the medium on the productivity and nitrogen fixation in *Azolla* are reported (Kushari and Taheruzzaman, 1990). A comparative study on the growth, pigments, nitrogen fixation and nutrient status of *Azolla* maintained as soil and nutrient based cultures showed that the soil based cultures are as good as those raised on nutrient media (Dawar and Singh, 2002). *Azolla* production can be carried out in nursery plots, ponds, ditches, canals, concrete tanks and polythene lined ditches. The field selected for *Azolla* cultivation needs to be thoroughly prepared and leveled uniformly. Generally 20 m x 2 m size plots are made in the field with suitable bunds and irrigation channels with a water depth of at least 10 cm and in each plot water (20 liters) is added and inoculated with *Azolla* (8-10 Kg). Single super phosphate (100 g) in 2-3 split doses is applied at an interval of 4 days to each plot. Furadon or carbofuran (3 % active granules) can be applied in the plots (100 g plot^{-1}) with or after a week of inoculation. 100-150 Kg fresh *Azolla* can be harvested from each plot after 15 days from each plot. The same method can be used to produce *Azolla* in bigger plots. The quantities of in-puts need to be varied as per requirement. Depending on the availability and need of the inocula, *Azolla* can also be maintained in nursery in trays or earthen or cemented pots of any dimension. If the production is carried out in a pond or canal fertilizers and insecticides are not applied. Cattle slurry and animal dung for the production and utilization of *Azolla* as biofertilizer for rice has been reported (Singh *et al*., 1993). The cattle slurry and animal dung are effective as phosphorous fertilizers and application of *Azolla* is found to enhance the C, N and available P content of the soil.

Methods of Application of *Azolla*

The most common mode of application of *Azolla* in the field is as green manure or as a dual crop along with rice. In case of application as green manure *Azolla* collected directly from ponds/ditches is applied in the field. It may be grown in nurseries as specified earlier and can also be applied in the field. A thick mat of *Azolla* will be formed after application in about 2-3 weeks time and can be incorporated in the soil. Rice can also be transplanted in the field subsequently. Single super phosphate (25-50 kg ha^{-1}) is applied in split doses. After analyzing the soil P-status the dosage of the same can be reduced. Cattle dung or slurry may also be used instead of single super phosphate. In case of pest infestation or attack, pest control measures have to be undertaken. *Azolla* application by this mode contributes around 20-40 kg N ha^{-1}.

In dual cropping, *Azolla* is grown along with rice and each crop of *Azolla* contributes on an average 30 kg N ha^{-1}. After 7-10 days of transplantation fresh inoculums of *Azolla* is applied in the field at the rate of 0.50-1.0 ton ha^{-1}. Single super phosphate is applied at the rate of 20 kg ha^{-1} in split doses. In about 15-20 days time a thick mat of *Azolla* is formed. *Azolla* thus incorporated decomposes in about 8-10 days time and release the fixed nitrogen. Another crop of *Azolla* can be raised in a similar way during the crop cycle of rice. *Azolla* production technology is simple and not very expensive and at the same time it is very efficient in terms biomass accumulation and nitrogen fixation. The rice growing season is also conducive for the growth of *Azolla* plants. The dual application does not have any negative influence on the rice crop.

Biological Nitrogen Fixation and *Azolla* in Relation to Soil Fertility

The ability to fix atmospheric nitrogen at substantially higher rates has led to the exploitation of the organism as biofertilizer. Application of *Azolla* in rice paddy fields has a positive role in improving the soil fertility index. The ability of nitrogen fixation is due to the presence of the symbiotic cyanobacterium *Anabaena* that occurs in the dorsal leaf cavities of the fronds (Peters and Meeks, 1989). The symbiont is able to meet the entire nitrogen requirement of the association. Calvin cycle operates in both the partners and the primary end product of photosynthesis is sucrose (Van Hove, 1989). A strong interaction exists between nitrogen fixation and the photosynthesis and the source of ATP and NADPH is photosynthesis. The capacity of *Azolla* to fix nitrogen in the field has been estimated to be 1.1 kg N ha^{-1} day^{-1} and this fixed nitrogen is sufficient to meet the entire nitrogen requirement of rice crop within a few weeks (Lumpkin and Plucknett, 1980). The rapid and substantially higher rates of nitrogen fixation coupled with production of high biomass have made the organism an outstanding agronomic choice.

Azolla strains have been successfully exploited as an efficient biofertilizer for rice paddy fields because of the nitrogen fixing potential. Dr. P K Singh and coworkers from the Central Rice Research Institute, Cuttack successfully popularized the use of *Azolla* as a promising biofertilizer in the Eastern parts of India. *A. pinnata* fixes 75 mg N g^{-1} dry weight day^{-1} and produces a biomass of 347 ton fresh weight ha^{-1} in a year. This biomass contains 868 kg N which is equivalent to 1900 Kg urea. A wide variability regarding growth and nitrogen fixation among different strains of *Azolla* is observed by a study conducted at Central rice Research Institute, Cuttack (Singh, 1988). Among the several factors that influence the growth and nitrogen fixing potential of *Azolla* are nutrient availability, rate and the time of inoculation etc (Kannaiyan, 1993; Singh and Singh, 1995). In addition to sustaining rice yields, inoculation of *Azolla* has been reported to enhance the soil biological health. It is important to optimize use of organic, inorganic and biological inputs in an integrated manner taking into consideration the ecological and soil conditions to sustain crop productivity. Soil enzyme activity is considered as an index of microbial activity and fertility of the soil. *Azolla* decomposes rapidly in soil and supply nitrogen to the crop plants. It contributes significant amounts of phosphorus, potassium, sulfur, zinc, iron and molybdenum in addition to other micronutrients besides addition of nitrogen. The biological health of the soil due to application of *Azolla* has resulted in improving mineralization and consequent increase in the microbial status of the soil. In low land rice cultivation mineralization of organic nitrogen to ammonia is an important process (Sahrawat, 1983). The rate of mineralization is influenced by factors such as C: N. *Azolla* species with a low C: N mineralized in 2 days while the species with high C:N mineralized in 5 days (Wang *et al*., 1987). The decomposed organic matter plays an active role in the development of microbial population irrespective of the time taken for mineralization. Soil fertility is also influenced by the humic substances formed during the decomposition of *Azolla* (Bhardwaj and Gaur, 1970). For wetland rice cultivation *Azolla* and *Sesbania* are used as green manure (Ventura and Watanabe, 1993). Continuous application increased the organic nitrogen content of the soil significantly. Increased celluloytic and urea hydrolyzing activities in addition to significant increase in the population of heterotrophic bacteria were recorded (Kannaiyan and Subramani, 1992; Kannaiyan and Kalidurai, 1995). Increased soil urease and phosphatase activity has also been observed due to incorporation of *Azolla* (Thanikachalam *et al*., 1984; Thangaraju and Kannaiyan, 1989).

Combined incorporation of nitrogen fixing green manures such as *Sesbania* and *Azolla* shows significant enhancement in the activity of soil enzymes such as dehydrogenase, phosphatase, cellulose and amylase (Kumar and Kannaiyan, 1992). Similar enhancement in the microbial population, total bacterial, cellulolytic, phosphate solubilising and urea hydrolysing bacteria was observed (Gopalaswamy and Kannaiyan, 2000c). *Azolla* helps to sustain soil nitrogen supply by returning N to the soil in quantities roughly equal to those extracted from soil by the rice plants (Cisse and Vlek, 2003). Maximum population of bacteria, fungi and actinomycetes and high urease and dehydrogenase activities due to organic farming using *Azolla* as one of the components was reported (Krishnakumar *et al*., 2005). Field experiment studies have been conducted at the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi. In these studies optimum yield in organic *Basmati* rice was recorded and improvement in grain and soil quality noticed. The inputs used in this study included *Azolla*, blue-green algae, farmyard manure and vermicompost as organic amendments (Singh *et al*., 2007). Experiments conducted at Banaras Hindu University, Varanasi showed beneficial effects of *Azolla* in the cultivation of rice (Bhuvaneshwari, 2012). Application of *Azolla* has been found to significantly improve the physical and chemical properties of the soil especially nitrogen, organic matter and other cations such as Magnesium, Calcium and Sodium released into the soil (Bhuvaneshwari and Kumar, 2013).

Improvement in Crop Productivity Due to Application of *Azolla*

Productivity of rice is related to the availability of nitrogenous fertilizers. Nitrogen can also be supplemented to the rice crops through biofertilizers such as *Azolla* and cyanobacteria. Application of *Azolla* to rice plants is ideal as the growth requirement for both the organisms are similar. The organism is able to multiply fast which is a remarkable aspect in its exploitation as biofertilizer. Successful exploitation of this organism in the Asian context has also been highlighted (Singh, 1989). Field experiments conducted at Central Rice research Institute, Cuttack using high yielding varieties of rice showed that application of 10 tons ha^{-1} of fresh *Azolla* is as efficient as basal application of 30 kg of N (Singh, 1977; Singh, 1978). Their experiments resulted in increase of height, tiller, dry matter, number and weight of panicles, grain and straw yield etc. Increase in yield due to application of *Azolla* was demonstrated by several studies conducted at several locations. A single crop of *Azolla* provides 20-40 kg N ha^{-1} (Singh, 1977b). Similar results have been obtained with integrated application of *Azolla* and chemical nitrogen fertilizers. When a comparison of *Azolla* is made with other biofertilizers highest grain yield in rice is observed (Singh *et al*., 1992). After the decomposition of the organism nitrogen content is made available to the rice plants. It was found that the plant decomposed 8-10 days after incorporation into the soil and rice plants are benefitted after 20-30 days (Singh, 1977d). The release of N by *Azolla* is slow and its availability to the first crop of rice is about 70% to that of ammonium sulphate. Moreover, the release of nitrogen is faster as compared to nitrogen fixing cyanobacteria (Saha *et al*., 1982). Fresh *Azolla* releases its N faster as compared to dried *Azolla* due to rapid mineralization and mineralization of N is faster at room temperatures (Singh, 1979a; Singh, 1979b). A comparative study was conducted at Cuttack on the N release efficiency of chemical N fertilizers, *Azolla* and BGA and other organic manures. It was found that the release of N by *Azolla* was comparatively slower and 87% of N in chemical fertilizer is released within 10 days. Hence the application of *Azolla* in combination with chemical fertilizers offers an excellent choice for rice cultivation (Singh *et al*., 1981). Increase in grain yield with application of *Azolla* alone and chemical nitrogen fertilizer (150 kg N ha^{-1}) is reported (Singh *et al*., 1992). At Central Rice Research Institute, Cuttack, field trials demonstrated that use of *Azolla* enhanced crop yield and crop N uptake significantly as compared to treatments without *Azolla* (Manna and Singh, 1989). Utilization of *Azolla pinnata* as biofertilizer in acidic soils of Kerala has also been reported (Sevichan and Madhusoodanan, 1998). Suppression of weeds and reduction in the volatilization of ammonia in rice fields due to the formation of a thick mat in rice fields by *Azolla* is also observed (Singh *et al*., 1981). Comparison of Phosphorous enriched and un-enriched *A. caroliniana* on the performance of *Azolla* dual cropping with rice showed higher biomass and N yield by applying phosphorous to P-enriched inocula (Singh and Singh, 1995). Potential exploitation of the system as phosphorous biofertilizer to improve the nitrogen and phosphorous balance in polluted environments was attempted (Singh *et al*., 2010).

Azolla as an Input in Organic Agriculture

Recently there is a surge in the interest in organic farming and *Azolla* is used as an important and potential component in the organic farming of rice. Positive effect on the BGA and *Azolla* on rice yield has been reported earlier (Singh and Mandal, 1997; Singh and Mandal, 2000). *Azolla* has been used successfully as a component of organic cultivation in rice based cropping system. Field experiments have been conducted at Indian Agricultural Research Institute, New Delhi during 2003-2009 to find out suitable organic amendments for sustainable productivity of *Basmati* rice-wheat-green gram cropping system (Singh *et al*., 2011). Different treatment combinations comprising of organic amendments such as *Azolla* @1.0 ton ha^{-1}, Blue Green Algae @ 2.0 kg ha^{-1}, vermicompost and farm yard manure @ 5.0 ton ha^{-1} applied alone or in combination were tested. Results revealed significant enhancement in grain yield of rice over absolute control due to the application of organic amendments like *Azolla* applied alone or in combination (Table 2). Optimum yield of Basmati rice (cv. *Pusa Basmati* 1) can be obtained in all the years with the application of four amendments (*Azolla*, BGA, vermicompost and FYM) together. Besides, enhancing and sustaining the productivity of organic rice-wheat system, higher productivity of vegetables like cauliflower, broccoli, cabbage and carrot grown after organic rice under organic nutrition were recorded (Singh *et al*., 2012). There was no serious incidence of any insect pest or disease in organically grown rice crop during these years. Microbial populations (Actinomycetes, Bacteria, Fungi and BGA) were found to enhance over the years due to the application of organic amendments in comparison to total control and recommended fertilizer application that accordingly result in a notable enhancement in dehydrogenase enzyme activity. The treatments have led to changes in the composition of microbial communities due to fertilization treatment and application of organic matter (Irisarri *et al*., 2001; Jha *et al*., 2004; Singh and Dhar, 2011; Singh *et al*., 2012). Rice grain analysis for Iron, Zinc, Manganese and Copper contents showed a significant increase in these essential ion contents in the treatments having two or more organic amendment added altogether over control. Similar results have been obtained by Bhattacharya and Chakraborty (2005). Organic nutrient management including *Azolla* inoculation showed considerable built up in soil organic carbon content. The values of soil physical parameters like available water content (AWC) and water retention capacity (WRC) are higher under organic management compared to INM and chemical fertilization. The increase in AWC was related to the increase in micro-and macro-porosity. Lower bulk density (BD) was observed in organic treatment as compared to INM and chemical fertilizer treatments (Singh *et al*., 2012). Highest net return is also recorded with integrated nutrient management (INM) of rice that included use of *Azolla* in addition of chemical fertilizer and it is followed by organic management with four inoculants including *Azolla*. These results clearly show how *Azolla* could be successfully integrated with other bio-inoculants in rice as well as vegetable based organic cultivation.

Use of *Azolla* as Livestock Feed

The importance of *Azolla* as a sustainable feed for livestock and poultry is reviewed recently (Gouri *et al.*, 2012). According to them *Azolla* can be used as an ideal source of feed for cattle, sheep, goats, pigs, rabbit and fish (Fig. 3). Because of the high nutrient content *Azolla* can be used as fodder for cattle and fish as well as poultry feed. It has Proteins, Vitamins, Calcium, Phosphorus, Iron, Copper, Magnesium, Beta carotene and Amino acids (Table 3). *Azolla* can be maintained throughout the year due to proper planning and cultivation practices and this in turn will ensure round the year availability of the organism for fodder purposes. *Azolla* in dried form is not preferred by the birds and hence the use of fresh biomass is an excellent poultry feed with no side effects (Singh and Subudhi, 1978a). About 20-25% of the commercial feed can be replaced by the incorporation of fresh *Azolla* biomass (Subudhi and Singh, 1978b). Utilization of *Azolla* as animal feed is suggested to be advantageous for the better productivity of livestock which comprise an integral part of the agriculture (Banerjee and Matai, 1990). Studies conducted on *Azolla pinnata* indicate that the level of Amino acids in the leaf protein compare favorably with the standards of FAO reference pattern and chick requirements (Dewanji, 1993). Integration of *Azolla* powder in the fish meal and feeding experiments is found to enhance the weight of the carp *Osteobrama belangeri* and resulted in better feed conversion efficiency and protein efficiency ratio (Basudha and Vishwanath, 1997). Use of *Azolla* protein supplement for the fish *Tilapia mossambica* and observed increase in feeding, absorption and growth rate (Sithara and Kamalaveni, 2008). They further observed that mixing of the *Azolla* biomass with other agricultural byproducts such as wheat and rice bran will lead to improvement in digestibility and protein quality.

Fig. 3: *Azolla* used as cattle feed by farmers

Feeding experiments conducted on buffalo calves suggest that *Azolla* meal is a potential and unconventional source of protein (Indira *et al*., 2012). Although most of these studies advocate the use of fresh biomass, studies show that heat treated *Azolla* meal is partially substituted for fish meal resulted in significant growth of *Labeo rohita* (Maity and Patra, 2008). An experiment was carried out to investigate the effect of dietary inclusion of sun dried and ground *Azolla* (*Azolla pinnata*) on production performance of broiler chicken (Balaji *et al*., 2009). The per cent giblet yield of birds fed with 4.5% *Azolla* is found to be significantly higher than control and other treatments. Based on the experiments it is concluded that dietary inclusion of dried *Azolla* up to 4.5% levels did not have any adverse effect on production performance of broiler chicken. It is observed that aquatic plant species especially *Azolla* offer a great potential as a source of protein for animals due to ease of cultivation, productivity and nutritive value (Prabha and Kumar, 2010). Therefore, keeping in view of the importance of organic aquaculture these days use of *Azolla* is found to be one of the cheapest ways to increase the fish production in organic fish farming. Increase in fish production was observed when *Azolla* is used in organic aquaculture and it is strongly suggested that this practice can be an effective tool to increase the farm income and living standards of low income farmers from the Meghalaya region (Majhi *et al*., 2006). Poultry fed on *Azolla* show significant increase in the body weights and consequently have resulted in an increase in the net return (Rai *et al*., 2012). These studies in general indicate the tremendous possibility of using the *Azolla* biomass effectively in feed and also to bring down the cost involved in feeding. However, there is a need to address the protein quality as well the Amino acid and nutrient composition of different species of these plants. This will help in the selection of promising species with superior protein quality, amino acid and nutrient composition that can be used as efficient dietary supplement.

Bioremediation Potential of *Azolla*

Another interesting and applied aspect of utilization of *Azolla* which is gaining popularity in the recent times is its application in the process of bioremediation. Increase in population and rapid rates of industrialization has resulted in the release of huge quantities of pollutants into water resources. The pollutants released are toxic in nature and pose great concern due to their adverse impact on plant and animal health. Therefore phytoremediation offer an excellent option to ward off the pollutants from aquatic ecosystems. *Azolla* may be used to clean up the polluted and contaminated waters (Table 4). Efficient removal of gold from waste water solutions using the fern *Azolla filiculoides* is reported (Antunes *et al*., 2001). Similarly Elmachliy *et al*., (2010) demonstrated removal of Silver and Lead ions from waste waters using *Azolla filiculoides*. Successful cultivation of *A. microphylla* biomass in secondary treated Municipal waste water of Delhi is attempted by Arora and Saxena (2005). *Azolla* biomass is also employed in the phytoremediation of toxic heavy metals**.** Recently the phytoremediation potential of three different species of *Azolla* such as *A. microphylla*, *A. pinnata* and *A. filiculoides* is studied and it is found that *A. microphylla* accumulated more metal as compared to the other species (Arora *et al*., 2006). The biomass of *Azolla* (both live and dead) has also been used in the bio-sorption of several heavy metals (Umali *et al*., 2006; Mashkani and Ghazvini, 2009). Extensive work has been done using *Azolla* in the removal of heavy metals from aquatic environments (Rai 2008; Rai and Tripathi, 2009; Rai 2010a; Rai, 2010b). However, this is an area where no serious efforts have been attempted despite the potential of the organism for bioremediation. Capability of the organism to reduce these ions in to metallic particles is possible since the plant itself could act as a strong reducing agent.

Table 4: Bioaccumulation potential of heavy metals by various *Azolla* spp.

Azolla spp.	Heavy metal	Concentration of heavy metal accumulated (µg metal g^{-1})
A. pinnata	Cadmium	2759
	Mercury	450
A. caroliniana	Lead	416
	Chromium	964
A. filiculoides	Nickel	28443
	Chromium	12383
A. microphylla	Nickel	21785
	Cadmium	1805

Source: Sood *et al*., (2011)

A hydroponic system is developed to decontaminate the water containing Cadmium and Copper was developed recently (Valderrama *et al*., 2012). Phytoremediation potential of *Azolla* was reviewed recently (Sood *et al*., 2011). All these results show that *Azolla* may be successfully employed in phytoremedation of polluted water bodies. The municipal sewage water waste contains heavy load of Phosphorous and Nitrogen and bioremediation programs involving *Azolla* may be planned to decontaminate them. The used biomass may be used as green manure in case it is not having any heavy metal load in it. However, when used for bioremediation of waste water having heavy metal load the biomass may be dried and extracted for the recovery of the metal or incinerated to prevent the recycling of the heavy metal in the environment.

Research Perspectives for Future Research

In view of the declining soil health and productivity due to increased anthropogenic activities maintaining the sustainability is a challenging task ahead. The *Azolla*-*Anabaena* system is an excellent biofertlizer for rice crop and it also has several other uses. In order to improve its utility in agriculture and allied sectors focused attention is required. Thus, there is an urgent need to address certain key issues in *Azolla* for its exploitation and better utilization. These include the difficulties encountered in identification, observation of sporulation, storage of sporocarps and abiotic stress tolerance etc. These issues are needed to be addressed on priority basis to further enhance the utility of this organism in agriculture and allied sectors. In view of the progresses made using morphological and molecular approach in taxonomical research on *Azolla* an integrative approach is needed to solve the problems related to classification using several accessions as per the list of International Rice Research Institute, Philippines. Therefore, an integrative approach involving morphological and molecular characters must be taken in to consideration to assign species and create molecular finger prints. Contamination is a frequently encountered problem in many germplasm collections. The contamination can lead to the development of hybrids and we do not have any concrete tools to detect such a situation other than the conventional morphological identification. Hence reliable and reproducible fingerprints must be developed for the precise identification of the plants.

The mode of reproduction in *Azolla* is mainly vegetative and because of this reason the biomass has to be maintained round the year. The farmers have to carry large quantity of the fresh biomass to the field for application every time. At times it also involves transportation to long distances leading to perishing of the cultures. This is also one of the reasons responsible for the poor adoption of *Azolla* by farmers. Hence to make *Azolla* biofertilizer technology more effective, attempts must be made to successfully propagate the organism through sporocarps. Hence an important pre-requisite in the sporocarp technology is to have an understanding regarding the time of sporulation in different species of *Azolla* in relation to the factors controlling it in an ecological niche. Once we understand the pattern of sporulation and conditions specific to it, then strategies could be developed for induction of sporulation and its storage.

Screening of the plants against abiotic stress conditions such as salinity, high temperature, pesticide, UV-B and heavy metals is essential. Increasing soil salinity is a major deterrent for growth and development of plants in general. However, works conducted elsewhere showed varied response by *Azolla* to increase in salinity. Thus, the studies conducted on *Azolla* regarding salinity tolerance show the potential of salinity tolerance in different species but no further advancement has been made in this direction using advanced molecular biology tools. Since in the environment the organism is exposed to high and low temperature conditions, it is worth understanding the tolerance mechanisms using advanced techniques. Such an approach will lead to understanding of the common proteins involved in stress tolerance. It is imperative to identify novel proteins, genes and their pattern of expression and functions in stress adaptation in order to improve the stress tolerance. This could help in better understanding the abiotic stress tolerance mechanism in *Azolla*. With the imminent threat of climate change scenario and increasing contamination due to pesticides and heavy metals such approaches could be helpful in deciphering the mechanism of tolerance. In this regard, proteomics and genomics play an important role.

Azolla may be successfully employed in phytoremedation of polluted water bodies. However, this is an area where no serious efforts have been attempted despite the potential of the organism for bioremediation. The municipal sewage water waste contains heavy load of phosphorous and nitrogen and bioremediation programs involving *Azolla* may be planned to decontaminate them. The used biomass may be applied as green manure in case it is not having heavy metal load. However, when used for bioremediation of waste water having heavy metal load the biomass may be dried and extracted for the recovery of the metal or incinerated to prevent the recycling of the heavy metal in the environment.

The incidence of insects and pests is a very common problem encountered in the outdoor mass multiplication tanks of *Azolla* during the humid seasons. These insect pests belong to Lepidopterous and Dipterous orders and can damage the plants in 2-3 days completely (Singh, 1977). At the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi of late the incidence of pests is also observed during the months of October and November which are otherwise months that support good *Azolla* growth (Unpublished observations). Changes in the climate scenario may be responsible for such attacks and may be investigated in detail. At present there are chemical as well as biological controls to check the incidence of pests and insects. Hence species which can repel the insect attack could be screened. Determination of phytochemical composition of different strains is also important especially when *Azolla* is used as an important input in organic rice farming and poultry/ cattle feeding.

Conclusions

The fundamental to sustainable intensification of agriculture is effective soil health management and *Azolla* has been known to influence the dynamics of the total soil and microbial population of nitrogen fixing bacteria. The soil fertility index will improve due to the accumulation soil enzymes. Therefore we need to have efficient strains of *Azolla* to maintain the soil fertility. Efficient strains of *Azolla* in terms of nitrogen fixation from different agro-ecological zones are to be screened and their performance under various abiotic stress conditions such as salinity, heavy metals and UV-B need to be evaluated. Strain improvement must be attempted using innovations in molecular biology. Soil fertility index studies using soil enzymes need to be conducted employing *Azolla* alone or as a consortium with other micro-organisms. Sound extension strategies are also the need of the hour to promote the use of this biofertilizer with other benefits. The organism has tremendous potential and its real potential to enrich soil organic matter, soil enzymes and the soil microbial population has not yet been fully exploited. Concerted efforts are required from the part of policy makers, scientists and farmers to promote *Azolla* as a viable bio-inoculant for sustainable crop production and development.

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References

Abraham G, Pandey N, Mishra V, Chaudhary A A, Ahmad A, Singh R and Singh P K (2013) Development of SCAR based molecular markers for the identification of different species of *Azolla Ind J Biotechnol* **12** 489-492

Antunes A P M, Watkins G M and Duncan J R (2001) Batch studies on the removal of gold (III) from aqueous solution by *Azolla filiculoides Biotechnol Lett* **23** 249-251

Arora A, Pabby A and Singh P K (2003) Heavy metal accumulation by *Azolla* in contaminated soils *In*: (Eds: Calabrese E J, Kostecki P T and Dragum J) (Proc. 18th Ann. Intl. Conf. on Contaminated Soil Sediments and Water), Vol 8 Amherst Scientific Publishers, *Amherst Scientific Publishers Amherst*, USA **8** 39-48

Arora A and Saxena S (2005) Cultivation of *Azolla* microphylla biomass on secondary-treated Delhi municipal ef?uents *Biomass and Bioene* **29** 60-64

Arora A, Saxena S and Sharma D K (2006) Tolerance and phytoaccumulation of chromium by three *Azolla* species *World J Microbiol & Biotechnol* **22** 97-100

Arora A and Singh P K (2003) Comparison of biomass productivity and nitrogen fixing potential of *Azolla* spp *Biomass and Bioenergy* **24** 175-178

Balaji K, Jalaludeen A, Churchil R R, Peethambaran P A and Senthilkumar S (2009) Effect of dietary inclusion of *Azolla* (*Azolla pinnata*) on production performance of broiler chicken *Ind J Poultry Sci* **44(2)** 195-198

Banerjee A and Matai S (1990) Composition of Indian aquatic plants in relation to utilization as animal forage *J Aquat Plant Mange* **28** 69-73

Basudha C and Vishwanath W (1997) Formulated feed based on aquatic weed *Azolla* and fish meal for rearing medium carp *Osteobrama belangeri* (Valenciennes) *J Aqua Cult Trop* **12(3)** 155-164

Bhardwaj K K R and Gaur A C (1970) Effect of humic and fulvic acid on growth and efficiency of nitrogen fixation by *Azotobacter chroococcum Folia Microbial* **15** 364-367

Bhattacharya P, Chakraborty G (2005) Current Status of Organic Farming in India and other countries *Ind J Fert* **1(9)** 111-123

Bhuvaneshwari K (2012) Beneficial effects of blue green algae and *Azolla* in rice culture Environment Conservation **13(1&2)** 1-5

Bhuvaneshwari K and Ajay Kumar (2013) Agronomic potential of the association *Azolla-Anabaena Sci Res Reporter* **3(1)** 78-82

Bujak J P (2007) The *Azolla* Story: climate change and Arctic hydrocarbons GeoExPro Magazine, September, pp 66-72

Cisse M and Vlek P L G (2003) Influence of urea on biological N_2 fixation and N transfer from *Azolla* intercropped with rice *Plant Soil* **250** 105-112

Coppenolle B V, Watanabe I, Van, Hove, C, Second G, Huang N and Mc Couch S R (1993) Genetic diversity and phylogeny analysis of *Azolla* based on DNA amplification by arbitrary primers *Genome* **36(4)** 686-693

Dawar S and Singh P K (2002) Comparison of soil- and nutrient-based medium for maintenance of *Azolla* cultures *J Plant Nutr* **25** 2719-2729

Dewanji A (1993) Amino acid composition of leaf proteins extracted from some aquatic plants *J Agric Food Chem* **41** 1232-1236

Eames A J (1936) Morphology of Vascular Plants. McGraw Hill Book Co, New York

Elmachliy S, Chefetz B, Tel-Or E, Vidal L, Canals A and Gedanken A (2010) Removal of silver and lead ions from water wastes using *Azolla filiculoides*, an aquatic plant, which adsorbs and reduces the ions into the corresponding metallic nanoparticles under microwave radiation in 5 min *Water Air Soil Pollut* DOI 10.1007/s11270-010-0650-3

Gillar E (2002) Nitrogen fixation in tropical cropping systems *CABI Publishers UK*

Gopalaswamy G, Kannaiyan S (2000c) Influence of phosphorus on the growth of *Azolla* hybrids. Proceedings of National Seminar on Agricultural Scenario Challenges and opportunities. College of Agriculture, Gwalior, Madhya Pradesh, pp 55-56

Gouri M D, Jagadeesh S, Gopinath C R and Kalibavi C M (2012) Importance of *Azolla* as a sustainable feed for livestock and poultry *Agric Rev* **33(2)** 93-103

Hills L V and Gopal B (1967) *Azolla primaeva* and its phylogenetic significance *Can J Bot* **45** 1179-1191

Indira D, Sarjan R K, Suresh J, Venugopal N K and Ravi A (2012) *Azolla* (*Azolla pinnata*) as feed supplement in buffalo calves on growth performance *Ind J Anim Nutr* **26(4)** 345-348.

Irisarri P, Gonnet S, Monza J (2001) Cyanobacteria in Uruguayan rice fields diversity nitrogen fixing ability and tolerance to herbicides and combined nitrogen *J Biotechnol* **91** 95–103.

IRRI (1994) *Rice facts*, International Rice Research Institute Philippines.

Jha M N, Prasad A N and Misra S K (2004) Influence of source of organics and soil organic matter content on cyanobacterial nitrogen fixation and distributional pattern under different water regimes *World J Microbiol and Biotechnol* **20** 673-677

Kannaiyan S (1993) Nitrogen contribution by *Azolla* to rice crop *Proc Ind Natl Sci Acad Part B Biol Sci* **59** 309-314

Kannaiyan S, Goyal S K, Goyal S S and Rains D W (1988) Some factors influencing the growth and sporulation in *Azolla Phykos* **27** 197-205

Kannaiyan S and Kalidurai M (1995) Nitrogen fixing potential of stem nodulating *Sesbania rostrata* and its effect on rice yield, Rice Management Biotechnology, (Ed: Kannaiyan S), pp. 173–195, Associated Publishing Company, New Delhi, India

Kannaiyan S and Subramani S (1992) Use of *Azolla* as biofertilizer for rice crop, Cyanobacterial Nitrogen Fixation, (Ed: Kaushik B D), pp. 281-289, Indian Agricultural Research Institute, New Delhi

Kar P P, Mishra S and Singh D P (1999) Influence of gibberellic acid on the sporulation of *Azolla caroliniana Azolla microphylla* and *Azolla pinnata Biol Fert Soils* **29** 424-429

Kar P P, Mishra S and Singh D P (2001) Influence of different phosphorus management strategies on the sporulation and the growth of *Azolla Expl Agric* **37** 111-114

Kar P P, Mishra S and Singh D P (2002) *Azolla* sporulation in response to application of some selected auxins and their combination with gibberellic acid *Biol Fertil Soils* **35** 314-319

Konar R N and Kapoor R K (1972) Anatomical studies on *Azolla pinnata Phytomorphology* **22** 211-223

Krishnakumar S, Saravanan A, Natarajan S K, Veerbadaran V and Mani S (2005) Microbial population and enzymatic activity as influenced by organic farming *Res J Agric & Biol Sci* **1** 85

Kumar K and Kannaiyan S (1992) Changes in the activity of soil enzymes during decomposition of N fixing green manures.

33rd Annual Conference of Association of Microbiologists of India, Goa University, India

Kushari D P and Taheruzzaman Q (1990) Multiplication of *Azolla pinnata* in primary production units. National Symposium on Cyanobacterial Nitrogen Fixation, (Ed: Kaushik B D), pp. 353-358, NFBGAC, IARI, New Delhi, India

Lumpkin T A and Plucknett D L (1980) *Azolla:* Botany, Physiology and use as a green manure *Econ Bot* **34** 111-153

Lumpkin T A and Plucknett D L (1982) *Azolla* as a green manure; use and management in crop production. Series No15, pp230, Westview Press; Boulder, Colorado, USA *Westview Press Boulder Colorado* USA **15 (**1982) 230

Mahapatra B S and Sharma G L (1989 Integrated management of *Sesbania*, *Azolla* and urea nitrogen in low land rice under a rice-wheat cropping system *J Agric Sci Camb* **113** 203-26

Maity J and Patra B C (2008) Effect of replacement of fishmeal by *Azolla* leaf meal on growth, food utilization, pancreatic protease activity and RNA/DNA ratio in the fingerlings of *Labeo rohita* (Ham) *Can J Pure Appl Sci* **2(2)** 323-333

Majhi S K, Das A and Mandal B K (2006) Growth Performance and Production of Organically Cultured Grass Carp *Ctenopharyngodon idella* (Val.) Under Mid-Hill Conditions of Meghalaya; North Eastern India *Turkish J Fish Aquat Sci* **6** 105-108

Manna A B and Singh P K (1989) Growth and acetylene reduction activity of *Azolla caroliniana* Willd. As influenced by split applications of urea fertilizer and their response on rice yield *Fert Res* **18** 189-199

Marwaha T S, Singh B V and Goyal S K (1992) Effect of incorporation of *Azolla* on wheat (*Triticum aestivum var HD-2329*) *Acta Bot Indica* **20** 218-220

Mashkani S G and Ghazvini P T M (2009) Biotechnological potential of *Azolla filiculoides* for biosorption of Cs and Sr: Application of micro-PIXE for measurement of Biosorption *Bioresource Technol* **100** 1915-1921

Metzgar J S, Schneider H and Pryer K M (2007) Phylogeny and divergence of time estimates for the fern genus *Azolla* (Salviniaceae) *Int J Pl Sci* **168** 1045-1053

Moore A W (1969) *Azolla*: Biology and agronomic significance *Bot Rev* **35** 17-35

Nayak S K and Singh P K (1988) Some variation in dermal appendages of *Azolla Ind J Fern* **05** 170-175

Nayak S K and Singh P K (1989) Cytological studies in the genus *Azolla Cytologia* **54** 275-286

Nayak S K and Singh P K (2004) Megasporocarps of *Azolla* and their germination in varied paddy soils, Proceedings of 4th International Crop Science Congress held at Brisbane, Australia, 26th Sept. -1st Oct. 2004

Parashuramulu S, Swain P S and Nagalakshmi D (2013) Protein fractionation and *in vitro* digestibility of *Azolla* in ruminants *J Ani Feed Res* **3(3)** 129-132

Perreira A L, Martins M, Oliviera M M and Carrapico F (2011) Morphological and genetic diversity of the family Azollaceae inferred from vegetative characters and RAPD markers *Pl Syst Evol* **297** 213-226

Peters G A and Mayne B C (1974) The *Azolla-Anabaena azollae* relationship I. Initial characterization of the association *Pl Physiol* **53** 813-819

Peters G A and Meeks J C (1989) The *Azolla-Anabaena* symbiosis: basic biology. *Ann Rev Plant Physiol Plant Mol Biol* **40** 193-210

Peters G A (1976) Studies on the *Azolla-Anabaena* symbiosis, Proceedings of the International symposium on nitrogen fixation. (Eds: Newton W E and Nyman C J) pp 592-610, Washington State University Press

Prabha B J and Kumar K (2010) Dried *Azolla* as a nutritionally rich cost effective and immuno-modulatory feed supplement for broilers *Asian J Animal Sci* **5(1)** 20-22

Rai P K (2008) Phytoremediation of Hg and Cd from industrial effluent using an aquatic free floating macrophyte *Azolla pinnata Int J Phytoremed* **10** 430-439

Rai P K (2010a) Heavy metal pollution in lentic ecosystem of subtropical industrial region and its phytoremediation *Int J Phytoremed* **12** 226-242

Rai P K (2010b) Microcosom investigation of phytoremediation of Cr using *Azolla pinnata Int J Phytoremed* **12**: 96-104

Rai P K and Tripathi B D (2009) Comparative assessment of *Azolla pinnata* and *Vallisneria spiralis* in Hg removal from G.B. Pant Sagar of Singrauli Industrial region India *Environ Monitor and Assessment* **148** 75-84

Rai R B, Dhama K, Damodaran T, Hamid A, Rai S, Singh B and Bhatt P (2012) Evaluation of *Azolla* (*Azolla pinnata*) as a poultry feed and its role in poverty alleviation among landless people in northern plains of India *Vet Practitioner* **13(2)** 250-254

Reid J D, Plunkett G M and Peters G A (2006) Phylogenetic relationships in the heterosporous fern genus *Azolla* (Azollaceae) based on DNA sequence data from non-coding regions *Int J Pl Sci* **167** 529-538

Saha K C, Panigrahi B C and Singh P K (1982) Blue green algae and *Azolla* additions on the nitrogen and phosphorus availability and redox potential of flooded rice soil *Soil Biol Biochem* **14** 23-26

Sahrawat K L (1983) Mineralization of soil organic nitrogen under waterlogged conditions in relation to other properties of rice soil *Plant Soil* **42** 305-308

Sevichan P J and Madhusoodanan P V (1998) Utilization of *Azolla* in the acidic paddy fields of Kuttanad, Kerala. *Cyanobacteria and Biofertilizer for Rice Crops* (Ed: Kannaiyan S) pp 200-203, TNAU, Coimbatore

Singh D P and Singh P K (1995) Response of *Azolla caroliniana* and rice to phosphorus enrichment of the *Azolla* inoculum and phosphorus fertilization during intercropping *Exp Agric* **31** 21-26

Singh P K (1977) Multiplication and utilization of fern *Azolla* containing nitrogen algal symbiont as green manure in rice cultivation *Riso* **26** 125-136

Singh P K (1977a) Multiplication and utilization of fern *Azolla* Containing nitrogen fixing algal symbiont as a green manure in rice cultivation *Riso* **46** 642-644

Singh P K (1977d) *Azolla* fern plant-rice fertilizer and chicken feed *Kerala Karshakan* **26** 5-6

Singh P K (1978) Nitrogen economy of rice soils in relation to nitrogen-fixation by blue green algae and *Azolla*. *In*: National Symposium on increasing rice yield in Kharif, CRRI Cuttack

Singh P K (1979 b) Symbiotic algal nitrogen fixation and crop productivity *In*: Annual Review Plant Sciences Vol I, (Ed: C P Malik) pp 37-60 Kaliyani Publishers, New Delhi

Singh P K (1979a) Use of *Azolla* in rice production in India *In*: Nitrogen and rice pp 407-418, International Rice Research Institute, Philippines

Singh P K (1988) Biofertilization of rice crop, Biofertizers: Potential and Problems. (Eds: S P Sen and P Palit), pp 109-114, Plant Physiology Forum, Calcutta

Singh P K (1989) Use of *Azolla* in Asian Agriculture *Appl Agric Res* **4(3)** 149-161

Singh P K (2000) Biology of *Azolla* and blue green algae *In*: Biofertilizer-Blue Green Algae and *Azolla* (Eds: Singh PK, Dhar DW, Pabby S, Prasanna R, Arora A) pp 1-23, Venus Printers and Publishers, New Delhi

Singh P K, Bisoyi R N and Singh R P (1990) Collection and germination of sporocarps of *Azolla caroliniana Ann Bot* **66** 51-56

Singh P K, Panigrahi B C and Satpathy K B (1981) Comparative efficiency of *Azolla*, blue green algae and other organic manures in relation to N and P availability in a flooded rice soil *Pl Soil* **62** 35-44

Singh P K, Patra R N and Nayak S K (1984b) Sporocarp germination, cytology and mineral nutrition of *Azolla* sp. Practical application of *Azolla* for rice production. (Eds: Silver W S and Schroder E C), pp 72-75, Martinus Nijhoff/ Dr. W. Junk Pub., The Netherlands

Singh P K, Singh D P and Pandey K D (1987) The Influence of fertilizers on sporocarp formation in *Azolla Pinnata Proc Ind Acad Sci (Pl Sci)* **97** 223-226

Singh P K, Singh D P and Satapathy K B (1993) Use of cattle slurry and other organic manures for *Azolla* production and its utilization as biofertilizer for rice. *In*: Utilization of biogas slurry. Consortium on Rural Technology, New Delhi, pp 135-142

Singh P K, Singh D P and Singh R P (1992) Growth, acetylene reduction activity. nitrate uptake and nitrate reductase activity of *Azolla caroliniana* and *Azolla Pinnata* at varying nitrate levels *Biochem physiol Pflanzen* **118** 121-127

Singh P K and Subudhi B P R (1978a) Utilization of *Azolla* in poultry feed *Ind Farming* **27** 37-39

Singh S S, Mishra A K and Upadhyay R S (2010) Potentiality of *Azolla* as a suitable biofertilizer under salinity through acid phosphatase activity *Ecol Engg* **36** 1076-1082

Singh S, Prasad R, Goyal S K, Singh B V, Marwaha T S and Sharma S N (1992) Effect of *Azolla*, blue-green algae and fertilizer nitrogen on wetland rice (*Oryza sativa*) *Ind J Agron* **37** 569-571

Singh Y V and Saxena A K (2012) Influence of organic nutrient management in aromatic rice on productivity, nutrient concentration and economics. Oral presentation at International conference on Organic Rice Farming and Production Systems held at Montpellier France 27-30 August, 2012

Singh Y V and Dhar D W (2011) Changes in soil organic carbon and microbial population under organically managed rice (*Oryza sativa*)-wheat (*Triticum aestivum* L emend Fiori & Paol)-green gram cropping system *Ind J Agric Sci* **81(4)** 55-58

Singh Y V, Dhar D W and Agarwal B (2011) Influence of organic nutrient management on Basmati rice (*Oryza sativa*)-wheat *(Triticum aestivum*)-greengram (*Vigna radiata*) cropping system *Ind J Agron* **56(3)** 169-175

Singh Y V, Lata, Prasanna R, Pradhan S, Gaind S and Saxena A K (2012) Influence of organic nutrient management in aromatic rice based system on soil carbon dynamics, physical parameters and global warming potential. Oral presentation at International conference on Organic Rice Farming and Production Systems held at Montpellier, France 27-30 August, 2012

Singh Y V and Mandal B K (1997) Nutrition of rice (*Oryza sativa*) through *Azolla*, organic materials and urea *Ind J Agron* **42(4)** 626-633

Singh Y V and Mandal B K (2000) Rate of mineralization of *Azolla* other organic materials and urea in water logged soils *Tropical Agriculture* (Trinidad) **77(1)** 119-122

Singh Y V, Singh B V, Pabbi S and Singh P K (2007) Impact of organic farming on yield and quality of basmati rice and soil properties, http://orgprints.org

Singh P K and Singh D P (2001) Prospect of *Azolla* biofertilizer for rice in India. *In*: Recent advances in Biofertilizer Technology, (Eds: Yadav A K, Raychaudhari S, Motsara M R) pp 257-271, Publication of Society for Promotion and Utilization of Resources and Technology, New Delhi

Sithara K and Kamalaveni K (2008) Formulation of low-cost feed using *Azolla* as a protein supplement and its influence on feed utilization in fishes *Current Biotica* **2(2)** 212-219

Sood A, Uniyal P L, Prasanna R and Ahluwalia A S (2011) Phytoremediation potential of Aquatic Macrophyte, *Azolla Ambio*, DOI 10.1007/s13280-011-0159-z

Speelman E N, Van Kempen M M, Barke J, Brinkhuis H, Reichart G J, Smolders A J, Roelofs J G, Sangiori F, de Leeuw J W, Lotter A F and Sinninghe Damste J S (2009) The Eocene Arctic *Azolla* bloom: environmental conditions, productivity and carbon drawdown *Geobiology* **7(2)** 155-170. doi: 10.1111/j.1472-4669.2009.00195

Subudhi B P R and Singh P K (1978b) Nutritive Value of Water Fern *Azolla* for Chicks *Poultry J* **57** 378-380

Svenson H K (1944) The new world species of *Azolla Amer J Fern* **61** 1-13

Tan B C, Payawal, P, Watanabe I, Laedan N and Ramirez C (1986) Modern taxonomy of *Azolla*: A review. Philipp. *Agric* **69** 491-512

Thangaraju M and Kannaiyan S (1989) Studies on the urease activity and fertility status of soil by incorporating certain *Azolla* cultures. 29th Ann Conf Assoc Microbiologists of India, HAU, Hissar p 87

Thanikachalam A, Rajakannu K and Kannaiyan S (1984) Effect of neem cake, carbofuran and *Azolla* application on phosphatase activity in soil. 25th Annual Conference of Association of Microbiologists of India, GB Pant University of Agriculture and Technology, Pant Nagar, India

Umali L J, Duncan J R and Burgess J E (2006) Performance of dead *Azolla filiculoides* biomass in biosorption of Au from wastewater *Biotechnol Lett* **28** 45-49

Valderrama A, Tapia J, Peñailillo P and Carvajal D E (2012) Water phytoremediation of cadmium and copper using *Azolla filiculoides* Lam. in a hydroponic system *Water and Enviro J* doi:10.1111/wej.12015

Van Hove C (1989) *Azolla* and its multiple uses with emphasis on Africa, Food and Agricultural Organization, Rome

Ventura W and Watanabe I (1993) Green manure production of *Azolla microphylla* and *Sesbania rostrata* and their long-term effects on rice yields and soil fertility *Biol Fertil Soils* **15** 241-248

Wagner G M (1997) *Azolla*: a review of its biology and utilization *Bot Rev* **63** 1-21

Wang De-Xian, Zhao Miao-Zheng and Chen-De-Fu (1987) Studies on the of the mineralization rate and nutrient releasing dynamics, *Azolla* utilization, Proceedings of Workshop on *Azolla* use, pp 275, Fuzhou,Fujian, China, International Rice Research Institute, LosBanos, Philippines

Watanabe I (1982) *Azolla-Anabaena* symbiosis-it's Physiology and use in tropical agriculture. *In*: Microbiology of tropical Soils and Plant Productivity (Eds: Dommergues YR, Diem HG) pp 169-185, Martinus Nijhoff/W. Junk, The Hague

Watanabe I, Espianas C R, Berja N S and Alimagno B V (1977) Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice *IRRI Res Paper Ser* **11** 1-15

Zimmerman W J, Watanabe I and Lumpkin T A (1991a) The *Anabaena-Azolla* symbiosis: diversity and relatedness of neotropical host taxa *Pl Soil* **167** 167-170

Zimmerman W J, Watanabe I, Ventura T, Payawal P and Lumpkin T A (1991b) Aspects of the genetic and botanical status of neotropical *Azolla* species *New Phytol* **119** 561-566.