Progressive Research – An International Journal
Vol. 5 (Special Issue) : 424-433 (2010), ISSN : 0973-6417

Society for Scientific Development in Agriculture and Technology, Meerut (U.P.) INDIA

USE OF AZOLLA FOR SUSTAINABLE AGRICULTURE

Arun Kumar Jha
Deptt. of Soil Science and Agril. Chem., Bihar Agricultural College, Sabour, Bhagalpur, Bihar
Email : jhaak_ss@rediffmail.com

ABSTRACT

Sustainable agriculture is the production of food, fibre, or other plant or animal products using farming techniques that protect the environment, public health, human communities, and animal welfare. To achieve sustainability in agriculture, it is essential to retrieve the soil health and to popularize potential inputs of eco-friendly nature. *Azolla* is a small aquatic fern harbouring symbiotic association with diazotrophic, nitrogen fixing cyanobacteria and having potential of biological N_2 – fixation. Because of this property, it has been exploited widely as bio-fertilizer for rice plants. Owing to fast multiplication rate of biomass, it is also used as green compost. In addition to this it has several other uses such as food, feed, biogas producer and hyperaccumulator of heavy metals etc. Due to multifaceted uses of this fern, *Azolla* would be ideal environmental friendly input for rainbow revolution. Thus, an effort has been made in present article to provide a brief account of the importance as well as developments in the utilization of *Azolla* in agriculture and allied sectors.

Key words : *Azolla, green compost, N_2 – fixer, fertilizer substitution, multifaceted uses, eco-friendly agri-input, sustainable agriculture.*

Sustainable agriculture integrates three main goals-environmental health, economic profitability, and social and economic equity. The key to sustainable agriculture is finding the right balance between the need for food production and the preservation of environmental ecosystem. It is important to point out that for reaching towards the goal of sustainable, more emphasis is required to be given on the use of potential Agri-inputs which are eco-friendly in nature.

The aquatic pteridophyte *Azolla* is a nutritious fern which can be used as a bio-fertilizer, green compost, livestock feed, phytoremediating plants and as an enrichment material for vermicompost. Ability of *Azolla*-Anabaena system to fix atmospheric nitrogen and fair concentration of phosphorus, potassium and micronutrients in its biomass makes this fern outstanding agronomic choice for fertilizer economy during rice cultivation. In fact, a species of *Anabaena* (*A. azzollae*) is associated with the aquatic fern *Azolla* occurring in a ventral pore in the dorsal lobe of each vegetative leaf (Peters, 1976). The endophyte fixes atmospheric nitrogen biologically and resides inside the tissues of the water fern. In North Vietnam, an added advantage is that the plant plant multiplies fast and provides higher yields of green compost (200–300 t/ha/yr) than the conventional green manure plants such as *Sesbania*, *Crotalaria* and *Tephorsia* which are known to yield 30 – 50 t/ha/yr (Subba Rao, 1995). The disadvantages are that the plant is susceptible to parasites and sensitive to fluctuations in temperature, thus biomass production rate varies from place to place. The benefit from *Azolla* growth to the associated rice field has been variously estimated and it ranges from 95 Kg N/ha/yr to 670 Kg N/ha/yr (Subba Rao, 1995). *Azolla* can be used also for rapid multiplication of compost worms and for enrichment of vermicompost.

The importance of *Azolla* as a sustainable feed for livestock and poultry is well established. Fresh biomass of *Azolla* can be used to increase milk and meat yields of livestock. Due to high nutritional content and suitability of *Azolla* for dual cropping with rice–fish culture, it can be used as a feed for fish (Singh and Subudhi, 1978). Chemical composition of *Azolla* has been presented in table-1.

Heavy metals are among the most important sorts of contaminants in the environment. Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and difficult to get optimum results. Currently, phytoremediation through *Azolla* is an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil and water. The technology is environmental friendly and cost effective. *Azolla* can also be used to draw down the carbon from atmosphere and to incorporate into soil (Lal, 2003).

It is clear from above discussion that *Azolla* has multifaceted uses and has gained considerable importance in the recent times as bio-fertilizer, green manure and as poultry feed and cattle fodder. However, the full potential of the plant is still underutilized despite its advantages and concerted attempts are required for popularization of the system. Therefore, the present review takes stock of the developments related to the successful exploitation and application of *Azolla* and its multifaceted uses for sustainable agricultural practices.

Taxonomy and species recognition in *Azolla* Lam : *Azolla* taxonomy is confused by inadequate recognition and description of species, which results in difficulties in identification. Owing to the facts, there is a variation in the classifications proposed by various workers. Six extant species of *Azolla* and 25 fossil species have been recorded so far. The genus is divided into two subgenera viz., *Euazolla* , a new word *Azolla* (3 floats) and *Rhizosperma* (9 floats), primerly based on the reproductive organs such as megaspores floats and glochidia. The glochidia of the species belonging to *Euzolla* (*A. filiculoides* Lamark, *A. caroliniana* Wild, *A. microphylla* Kaulfus and *A. maxicana* Prest) are septate, while those of the *Rhizosperma* (*A. Pinnata* R.Brown) are simple and absent in A. nilotica. The use of septa in glochidia as a distwingishing characteristic was questioned, because of the presence of the extensive morphological variation within a given species (Kannaiyan and Kumar, 2006).

The location of glochidia on the massulae can also be distwingishing feature and in the sub genus, *Rhizosperma*, the glochidia are replaced by a root like structure emerging from the massulae in the microsporangium. In *A. nilotica* neither the glochidia nor the root like structure is present in the massulae. The species to the *Euazolla* (*A. filiculoide*, *A. caroliniana*, *A. microphylla* and *A. maxicana*) have glochidia positioned on the entire surface of the massulae while those of the *Rhizosperma* are located on the inner surface of *A. pinnata* and are absent in *A. nilitica*. Kannaiyan and Kumar (2006) described taxonomical classification of *Azolla* as under :

Division	:	Pteridophyta
Class	:	Filicopsida
Order	:	Salvinales
Family	:	Azollaceae
Genus	:	Azolla
Sub genera	:	*Azolla* and *Rhizosperma*
Species of *Azolla*	:	A. filiculoides Lamark, *A. caroliniana* Wild, *A. microphylla* Kaulfus, *A. rubra* R. Brown and *A. maxicana* Prest.
Species of *Rhizosperma*	:	A. nilotica Decne. Ex.Mett, *A. imbricate* Roxb. Ex.Griff, *A. pinnata* R. Brown, *A. pinnata* sub sp. pinnata, *A. pinnata* sub sp. Africana and *A. pinnata* sub sp. asiana.

Dunham and Fowler (1987) have proposed tentative taxonomic conclusion based on critical assessment of vegetative and reproductive characters using light microscopy, thin-sectioning, scanning electron microscopy, and transmission electron microscopy. Taxonomic proposal of these scientists has been presented in table-2. They found that apart from leaf trichomes and possible root anatomy, vegetative features provide little assistance in taxonomic separation. The precise identification of *Azolla* is somewhat complex due to similarity in vegetative characters. But, vegetative characters of various members of Rhizosperma group are distinct and due to the fact, it is easier to distinguish. The trichomes have also been identified as an important parameter for identification of the organism at the Species level (Lumpkin and Plucknett, 1982; Nayak and Singh, 1988).

With the hope of resolving the complex taxonomical problems, attention has now been focused on the cytology of genus. The basic chromosome number (n) in all species of the section as well as the section *Rhisosperma* is 22 except in *A. nilotica* where n is equal to 26. The cytological investigations show that the somatic chromosome number (2n) for species of the section *Rhizosperma ,* especially of *A. pinnata* varies from 44 to 66, however, that of *A. nilotica* is 52 (Tan *et al.,* 1986). The somatic chromosome numbers (2n) of the members of *Euazolla* are ranging from 40–48 (*A. mexicana*-48, *Azolla filiculoides*-40) (Nayak and Singh, 1989). The taxonomic assessment of *Azolla* is difficult because sporocarp formation under different agro climatic and culture conditions is not very much assured. Now a day, molecular tools are being 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). It has been concluded by Reid *et al.,* (2006) that taxonomy of the Family Azollaceae is highly controversial. On the basis of the loci of plastid genome, it has been found that there is uniqueness in different strains of *A. nilotica* (Metzgar *et al.,* 2007). Species specific SCAR primers (sequence characterized amplified region) for the precise identification of different species of *Azolla* has been developed most recently which may prove helpful for systematic taxonomical classification of *Azolla*.

Sporulation and sexual propagation of *Azolla* : Mode of reproduction in *Azolla* is mainly vegetative mainly due to problems associated with sporocarp formation and germination of seeded sporocarps. Sporulation does not takes place at everywhere in all the species of *Azolla* and secondly, a significant number of seeded sporocarps refuge to convert into *Azolla* plants. Viability of *Azolla* sporocarps also reduces with increase in period of storage. sporocarps of *Azolla microphylla* have been found superior over that of the *Azolla pinnata* regarding their germination. Storage of sporocarps under refrigerated condition after mixing in soil in the ratio of 1:15 increases the percentage of sporocarp germination, but, after two months of sporocarp storage, sexual propagation of *Azolla* becomes very difficult. Singh *et al* (1996) has reported that germination percentage of sporocarps also depends on quality of light. Blue coloured light is superior to green, yellow and red colour light to increase germination of sporocarps (table-3). It was also concluded by singh *et al*. (1996) that amino acids increases the percentage of sporocarp germination and out of numerous amino acids, Glutamine had been found the most suitable to promote percentage of sporocarp germination in his lab experiment (table-4). Success of sexual propagation of *Azolla* also depends on conditions provided for sporocarp germination (Singh *et al*., 1990). Phosphorus solution having 30 ppm concentration proves beneficial for sporocarp germination under laboratory conditions (table-5). Due to lack of standard technology of sexual propagation to produce *Azolla* round the year, mode of reproduction in *Azolla* is mainly vegetative. Package of practices of *Azolla* cultivation to use in rice field can be explained through following flow diagram :

Construct 30-45 cm deep pits of small dimensions

Use polythene sheet in the pit to check water loss

Prepare 7.5-10 cm thick layer of fertile soil over polythene sheet (in pit)

Allow 10 cm high level of water to stand over the soil

Apply 4-5 days old cow dung slurry @ 1Kg m^{-2} (Cow dung: Water:: 1:2-4)

Remove the scum using sieve

Apply *Azolla* @ 2 Kg m^{-2}(Preferably, protect the pit with 60% agro net shed)

Maintain 5-10 cm water level and replace one third water with fresh water fortnightly. Apply single super phosphate @ 10 g m^{-2}at weekly interval. Change the soil after 3 months

Prepare plots of 10m X 2m in field and fill up the water (10 cm high)

Introduce 8-10 Kg *Azolla* in each plot after spray of slurry prepared from 10-12 Kg cow dung

Apply 100g single super phosphate in 3 splits at 7 days interval and allow *Azolla* to grow for three weeks

For green manuring of *Azolla*, disperse the biomass in whole field @ 6-10t ha^{-1} and transplant rice at the time of puddling. However, for dual cropping, *Azolla* is applied after one week of rice transplanting. *Azolla* is allowed to grow for three weeks and then managed for its decomposition.

To use *Azolla* biomass for livestock management, 30–45 cm deep pits are dug within or near the livestock farm, especially near the water source and *Azolla* is multiplied in pits. For the purpose, polythene sheet is spread in pit and applying soil and water, *Azolla* biomass is introduced @ 250–500 g/m^2. For nutritional enrichment to the *Azolla*, nitrogen @ 1Kg/ha, muriate of potash@ 0.5 Kg/ha, Magnassium sulphate @ 1.5 Kg/ha and single super phosphate @ 10-20 Kg/ha should also be applied. Collecting the biomass from pit it should be fed to cattle @ 2 Kg/day/animal in divided dose (morning–1 Kg, evening–1Kg) after proper washing with fresh water.

Factors affecting growth of *Azolla* : *Azolla* is found in fresh water ecosystem of temperate and tropical regions throughout the world. An analysis of the distribution of *Azolla* shows that four species of the section Euazolla are originally found in North and South America, however, the members of Rhizosperma are found in Tropical Africa, South Africa, Central Africa, South East Asia, Japan, Australia, upper Nile Sudan, Uganda, Tanzania, Congo and Namibia (Kanniyan and Kumar, 2006). But, despite of its global distribution and quality for multifaceted use, *Azolla* is not in common use of farming community probably due to its high sensitivity against climatic fluctuations.

Water is the fundamental requirement for the occurrence of *Azolla*. The plant body consists of 90–95% water, which is required for the maintenance of structural integrity and physiological activity. It has been reported by Hechler and Downson (1995) that the nitrogenise activity of *A. caroliniana* was maximum when the tissue moisture content was 88–95 percent and the activity decreased to less than one fifth of the maximum when tissue moisture content dropped to 80percent. The optimum relative humidity for growth of

Table-1 : Chemical composition of *Azolla.*

Constituent	Content (%) on dry weight basis
Crude protein	24.0 -30.0
Crude fat	3.3 – 3.6
Nitrogen	4.0 – 5.0
Phosphorus	0.5 – 0.9
Potassium	2.0 – 4.5
Calcium	0.4 – 1.0
Magnesium	0.5 - .65
Manganese	0.11 – 0.16
Iron	0.06 - 0.26
Soluble sugar	3.5
Crude fibre	9.1
Starch	6.54

Source : Singh and Subudhi (1978).

Azolla is 85–90 percent (Watenable, 1982). Though, *Azolla* can survive on the wet soil surface or wet peat litters, but the recommended water depth for its cultivation is 5–10 cm (Singh, 1989). The higher water depth does not have any adverse effect on its growth until the availability of nutrients becomes limiting. The lower water depth that allows the plant roots to touch the soil surface adversely affects the fragmentation and dispersal but corrects the nutrient deficiencies.

One of the most important environmental variables governing the growth of *Azolla* is temperature. The optimum temperature for most *Azolla* species is between 20^{0}C and 30^{0}C. Li *et al.* (1982) reported maximum growth of *A.caroliniana* and *A. maxicana* at 25 – 30^{0}C, *A. filiculoide* at 20^{0}C and *A. pinnata* at 25^{0}C. Details about the temperature response of different *Azolla* species have been presented in table 7. The tolerance of of Azolla species to high temperature is in the order of *A. filiculoide <A. caroliniana < A. maxicana <A.pinnata* (Laurinavinchene et.al., 1990). *A. microphylla* is also known to tolerate high temperature (Lin *et.al.*, 1989).

Azolla derives energy for its photosynthesis from light. The quality, intensity and duration of light significantly affect the fern growth. *Azolla* is considered a shade loving plant and maximum biomass is produced with 50 percent shaded light. *A. microphylla* is more adapted to full sunlight, followed by *A. caroliniana* then *A. pinnata*–which perform better under 50 percent sunlight (De Carbalho and Lapes, 1994). But, when *Azolla* is grown with rice as a intercrop, its growth decreases due to shading by rice canopy and the biomass and fixed N are less than that in fallow field (Singh and Singh, 1987).

Azolla can survive wide range of pH from 5 to 10, but the optimum pH range for its maximum growth is 5–7, however, the highest biomass production by *A. filiculoide* and *A. pinnata* have been reported at pH 10 (El- Hadded *et al.,* 1988). The decreasing or increasing pH beyond this range significantly decreased the growth and biological N_2–fixation.

Like other plants, *Azolla* also requires mineral nutrition except nitrogen for its normal growth. Essential plant nutrients must be present in available form in balanced proportion especially in water where this fern is to be grown. Phosphorus is the most important growth limiting elements for *Azolla*. The threshold level level of P in the plant tissue is 0.2 – 0.3% on dry weight basis and 0.06 ppm of P in the medium is adequate for growth of A. pinnata in continuous culture (Subudhi and Wetnable, 1981). Optimum P concentration for N*2*-fixation at 5ppm for *A.pinnata*, 15 ppm for *A. filiculoides* and 20ppm for *A. microphylla*. Phosphorus requirement also depends upon soil characterastics, including phosphate absorbing capacity. The symptoms of P deficiency in *Azolla* include reduced growth, decreased frond size, fragile fronds, root elongation and reddening or browning of leaves. But, reddening of leaves also appears in case of unfavourable climatic conditions. Phosphorus deficiency decreases the nitrogen content of *Azolla* up to 50 percent. Under the limited P conditions, *A. pinnata* grows better than other species. Deficiency of any of the essential plant nutrients imposes adverse effect on growth and biological N_2-fixation by *Azolla*.

Pest problem in *Azolla* : One of the main problems for cultivation of *Azolla* in tropics is the damage caused by insects particularly during summer season. The insects belonging to family Pyralalidae and Chironomiddae and many species of snail are the major insect pests of *Azolla*. Beside snail, species of Chironomos, Pyralis and Nymphula are the important insect of *Azolla* and *A. pinnata* is highly susceptible to insect pests (Singh, 1989). Application of furadan (3% a.i. as carbafuran) @ 2.5 – 3.0 Kg/ha is the practical solution of this problem. But, to control insects in the *Azolla* which is to be utilized for livestock management, some neem based organic product should be tried, though this is a matter of further research.

Fungal and bacterial infections on *Azolla* are observed when it is not harvested for longer period. So, harvesting should be done at right time. In case plant pathogenic problems, spray of 2.5 ppm Benomyl can be practiced because, in case of delayed harvesting

Table-2 : Taxonomy of *Azolla* and proposal of Dunham and Fowler (1987).

Original Name and author	Mettenius, 1847	Mettenius, 1867	Sevenson,1944	Proposal of Dunham and Fowler, 1987
A. portoricensis Spreng. 1827	*A. protoricensis*	-	*A. microphylla*	*A. sp*
A. microphylla Kaulf. 1824	*A. microphylla*	-	-	-
A. bonariensis Bertol. 1860	*A. bonarieensis*	-	-	-
A. mexicana Presl. 1845	*A. mexicana*	-	*A. mexicana*	*A. mexicana*
A. caroliniana Willd. 1810	*A. caroliniana*	*A. microphylla*	-	-
A. densa Desvx. 1827	*A. densa*	*A. caroliniana*	-	-
		A.cristata	*A. caroliniana*	-
A. cristata Kaulf. 1824	*A. cristata*	-	-	-
A. arbuscula Desvx. 1827	*A. arubuscula*	-	-	*A. caroliniana*
A. magellanica Willd. 1810	*A.megellanica*	*A. filiculoides*	*A. filiculoides*	*A. filiculoides*
A. filiculoides Lam. 1783	*A. filliculoides*	*A. rubra*	-	*A. rubra*
A. rubra R. Br. 1810	*A. rubra*	-	-	*A. sp*

Table-3 : Effect of light quality on germination of *A. caroliniana*.

Treatment	No. of sporocarp incubated	No. of sporocarp germinated	Germination in angular value
No light	106	0	0.0
Normal light	119	57	43.8 (47.9)
Yellow light	91	54	50.4 (59.3)
Red light	120	79	54.2 (65.8)
Green light	113	72	53.0 (63.7)
Blue light	112	82	58.8 (73.3)
C.D. (P=0.05)			5.0
C.D. (P=0.01)			7.1

Source : Singh et al. (1996).

growth of *Rhizoctonia* and *Sceratium* may cause economical loss.

Use of *Azolla* for crop production : The ability of *Azolla* to fix atmospheric nitrogen at higher rates has led to the exploitation of the organism as bio-fertilizer. Application of *Azolla* in rice paddy fields has a positive role in improving the soil fertility. The ability of nitrogen fixation is due to the presence of the symbiotic blue green algae (Peters and Meeks, 1989). The microsymbiont, BGA 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/day and this fixed nitrogen is sufficient to meet the entire nitrogen requirement of rice crop within a few weeks (Lumpkin and Plucknett, 1980).

Azolla is a well established bio-fertilizer for rice. A wide variability regarding growth and nitrogen fixation among different strains of *Azolla* has been 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 (Sahrawat, 1983). The biological health of the soil due to application of *Azolla* has resulted in

Table-4 : Effect of different amino acids on germination of *A. caroliniana* sporocarps.

Treatment	No. of sporocarp incubated	No. of sporocarp germinated	Germination in angular value
Control	83	33	39.1(39.7)
Methionine	78	58	59.5(74.3)
Cystine	90	61	55.4(57.8)
Glutamine	74	58	62.3(78.4)
Adenine	85	56	54.3(65.9)
Alanine	86	56	53.8(65.1)
Proline	80	53	54.5(66.2)
Valine	96	63	54.1(65.6)
Histidene	75	48	53.1(64.0)
Tryptophan	86	60	56.7(69.8)
C.D. (P=0.05)			3.4
C.D. (P=0.01)			4.7

Source : Singh et al. (1996).

Table-5 : Sporocarp germination in *Azolla* as influenced by various conditions.

Treatment	No. of sporocarp incubated	No. of sporocarp germinated	Germination (%)
Control	188	31	24.4
IARI medium (solid)	155	44	37.1
IARI medium (liquid)	181	37	26.7
Soil solution	184	46	28.9
Kinetin (100 ppm)	126	100	63.0
Gibberellic acid (100 ppm)	197	132	55.2

Source : Singh et al. (1990)19210648.0

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). Increased soil urease and phosphatase activity has also been observed due to incorporation of *Azolla* (Thanikachalam *et al.,* 1984; Thangaraju and Kannaiyan, 1993).

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, 2000). *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). Significant improvement in physical and chemical properties of the soil especially nitrogen, organic matter and other cations such as Magnesium and Calcium have been observed due to *Azolla* application in soil.

Utilization of *Azolla* for organic Farming: *Azolla* can be 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). 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). Rice grain analysis for Iron, Zinc, Manganese and Copper

Table-6 : Responses of *Azolla* species to temperature.

Temperature response	Azolla species	Temperature (°C)		
		Optimum	Maximum	Minimum
Cold tolerant and heat sensitive	*A. Filiculoide, A. rubra*	20	38 – 40	-5 to -8
Heat tolerant and cold sensitive	*A. microphylla, A. maxicana*	25 – 30	45	5 to 8
Cold and heat tolerant	*A. caroliniana, A. pinnata var. imbricata*	25 – 30	45	-3 to -5
Not heat and cold tolerant	*A. pinnata var. pinnata, A. nilotica*	25	38	3 to 8

Source : Li et.al. (1982)

Table-7 : Bioaccumulation of heavy metal by *Azolla pinnata* and *Azolla filiculoides.*

Azolla **species**	**Heavy metal**	**Initial concentration (ppm)**	**Duration of experiment (days)**	**Heavy metal accumulated (microgram/g dry weight)**
A. pinnata	Hg	3.0	13	667
	Hg	10.0	21	450
	Hg	3.0	6	940
	Cd	3.0	13	740
	Cd	10.0	7	2759
	Cr (III)	3.0	13	1095
	Cr(VI)	20.0	14	9125
A. filiculoides	As	80.0	7	60
	Cr(VI)	20.0	14	12,383
	Cr(III)	9.0	4	1904
	Cd	9.0	4	10441
	Cd	10.0	7	2608
	Ni	9.0	4	8814
	Cu	9.0	4	9224
	Zn	9.0	4	6408

Source : Elmachliy *et al.,* (2010)

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.

Utilization of *Azolla* in livestock management: *Azolla* is a sustainable feed for livestock and poultry. *Azolla* can be used as an ideal source of feed for cattle, sheep, goats, pigs, rabbit and fish. Because of the high nutrient content *Azolla* it can be used as fodder for cattle and fish as well as poultry feed. It contains Proteins, Vitamins, Calcium, Phosphorus, Iron, Copper, Magnesium, Beta carotene and Amino acids (Singh and Subudhi, 1978a). *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 favourably 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 carpOsteobrama belangeriand 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).

Use of *Azolla* for Phytoremediation of heavy metals from soil and water : 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-11). 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). 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.

CONCLUSIONS

Multifaceted use of aquatic fern *Azolla* is well known. There is a need of intensive research on production technology and uses of *Azolla* for agricultural purposes and phytoremediation of heavy metals and pollutants from soil and water bodies through the use of *Azolla* to popularize this fern for eco-friendly agriculture and livestock management. Breeders and biotechnologist will have to make the effort to develop some temperature insensitive *Azolla*. Food technologist and medical scientists will have to make effort to explore its use for human consumption to overcome the problem of mal nutrition. Possibilities of efficient use of *Azolla* in crops, other than rice are also to be examined. Thus, there is a need of policy decision to fetch maximum benefits from this natural resource.

REFERENCES

1. Arora, A. and Saxena, S. (2005) Cultivation of *Azolla microphylla* biomass on secondary-treated Delhi municipal effluents. *Biomass and Bioene, 29:* 60-64.
2. 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.
3. 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.
4. 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.
5. Bhattacharya, P., Chakraborty, G. (2005) Current Status of Organic Farming in India and other countries. *Ind J Fert, 1(9) :*111-123
6. Cisse, M. and Vlek, P. L. G. (2003) Influence of urea on biological N2 fixation and N transfer from *Azolla* intercropped with rice. *Plant Soil, 250:* 105-112.
7. 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.
8. De Carvalho, E.F. and Lapes, N.F. (1994) Growth, pigmentation and nitrogen fixation in *Azolla* sp. cultivated under four light flux densities. *Pesquisia Agropequaria Brrasilaria, 29:* 221 – 236.
9. Dewanji, A. (1993) Amino acid composition of leaf proteins extracted from some aquatic plants. *J Agric Food Chem, 41:* 1232-1236.
10. Dunham, D.G. and Fowler, K.(1987) Taxonomy and species recognition in *Azolla* Lam. In: *Azolla Utilization*, *Int. Rice Res. Inst.,* Philippines: 7 -16.
11. El-Hadded, M.E., Mahmoud, S.A.Z., Shalan, S.N. and El-Shahat, R.M. (1988) Factors affecting *Azolla* growth in Egypt.1. Hydrogen ion and sodium chloride concentration. Proc. Of second confrance of the Agricultural Development Research, Cairo, 17 – 19 Dec. *In Food Sci. Microbiol:* 184-190.
12. 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.
13. Gopalaswamy, G., Kannaiyan, S. (2000) 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: 55-56.
14. Hechler, W.D. and Dowson, J.O. (1995) Factors affecting nitrogen fixation in *Azolla* caroliana, *Transaction of the Illinois state Academy of Sci., 88:* 97 – 107.
15. 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.
16. 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.
17. Kannaiyan, S. (1993) Nitrogen contribution by *Azolla* to rice crop. *Proc Ind Natl Sci Acad Part B Biol Sci 59:* 309-314
18. Kannaiyan, S. and Kumar, K. (2006) Biodiversity of *Azolla* and its algal symbiont *Anabaena azollae*. National Biodiversity Authority Bulletin,Frontline Ofset Printers, Chennai: 10 - 16.
19. 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
20. 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

21. Lal, R. (2003). Global potential of soil C sequestration to mitigate the greenhouse effect. *Critical Reviews in Plant Science 22:* 151-84.
22. Laurinavinchene, T.V., Yakunin, A.F. and Gogotov, I.N (1990) Effect of temperature and photoperiod on growth and nitrogen fixation of *Azolla*. *Soviet Plat Pathology, 37:* 344 – 347.
23. Li, Z.X., Zhu, S.X., Mao, M.F. and Lumkin, T.A. (1982) Study on the utilization of 8 Azolla species in agriculture. *Zhungguo, Nongue Kexur,1:* 19 – 27.
24. Lin, C., Liu, Z.Z., Zengh, D.Y., Tang, L.F. and watanable, I (1989) Re-establishment of symbiosis to Anabaena free Azolla. *Sci., China, 32:* 551 – 559.
25. 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.
26. Lumpkin, T. A. and Plucknett, D. L. (1980) *Azolla*: Botany, Physiology and use as a green manure. *Econ Bot, 34:* 111-153.
27. 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.
28. Nayak, S. K. and Singh, P. K. (1988) Some variation in dermal appendages of *Azolla. Ind J Fern, 05:* 170-175.
29. 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.
30. Peters, G. A. and Mayne, B. C. (1974) The *Azolla-Anabaena azollae* relationship I. Initial characterization of the association. *Pl. Physiol, 53:* 813-819.
31. Peters, G. A. and Meeks, J. C. (1989) The *Azolla-Anabaena* symbiosis: basic biology. *Ann Rev Plant Physiol Plant Mol Biol, 40:* 193-210.
32. 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 noncoding regions. *Int J Pl Sci, 167:* 529-538.
33. 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.
34. Singh, P. K. and Subudhi, B. P. R. (1978b) Utilization of *Azolla* in poultry feed. *Ind Farming, 27:* 37-39.
35. 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 ntercropping, *Exp Agric, 31:* 21-26.
36. Singh, P. K. (1977) Multiplication and utilization of fern *Azolla* containing nitrogen algal symbiont as green manure in rice cultivation. *Riso, 26:* 125-136.
37. 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.
38. Singh, P. K. (1977b) *Azolla* fern plant-rice fertilizer and chicken feed Kerala. *Karshakan, 26:* 5-6.
39. 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.
40. 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.
41. Singh, P. K. (1979a) Use of *Azolla* in rice production in India In: Nitrogen and rice, pp 407-418, *International Rice Research Institute*, Philippines
42. 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.
43. Singh, P. K. (1989) Use of *Azolla* in Asian Agriculture. *Appl Agric Res, 4(3):* 149-161.
44. 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.
45. Singh P K, Bisoyi R N and Singh R P (1990) Collection and germination of sporocarps of Azolla caroliniana *Ann Bot 66:* 51-56
46. 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.
47. 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. (EdsSilver W S and Schroder E C), pp 72-75, Martinus Nijhoff/ Dr. W. Junk Pub., The Netherlands.
48. 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.
49. 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
50. 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.
51. Singh, P. K. and Subudhi, B. P. R. (1978a) Utilization of Azolla in poultry feed. *Ind Farming, 27:* 37-39.
52. 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.

53. 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.
54. 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.
55. 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.
56. 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
57. 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
58. 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.
59. Singh, P.K. and Singh, R.P. (1987) Comparative studies on growth and nitrogenise activities of water fern Azolla germplasm collection. Curr sci.56: 790-794.
60. Singh, P.K., Singh, D.P. and Singh, R.P. (1996) Germination of water fern *Azolla caroliniana* at various light, amino acids and sugars and scorbic acids. *Proc. Indian national Science Academy,B 62, No. 5:*353-358.
61. Subba Rao, N.S. (1995) Nitrogen fixation by symbiotic Blue Green Algae. *Soil Microorganism and Plant Growth*, 3rd Ed., *Oxford & IBH Publishing* Co. Pvt. Ltd., New Delhi: 129 – 137.
62. Subudhi, B.P.R. and Watenable, I. (1981) Differential phosphorus requirement of *Azolla* sp. and strain in phosphorus limited continuous culture. *Soil Sci. Plant Nutr., 27:* 237-247.
63. Tan, B. C., Payawal, P., Watanabe, I., Laedan, N. and Ramirez, C. (1986) Modern taxonomy of *Azolla*: A review. *Philipp. Agric, 69 :*491-512.
64. 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: 87.
65. 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
66. Van Hove, C. (1989) *Azolla* and its multiple uses with emphasis on Africa, *Food and Agricultural Organization,* Rome.
67. 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
68. Watanable, I (1982) Azolla – Anabaena symbiosis – its physiology and use in tropical agriculture. In Microbiology of Tropical Soil and Plat Productivity. Dommergues, Y.R. and Diem, H.S. (eds), Martinus NIjhoff Publ, The Hauge, Nethrlands : 169 – 185.
69. 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.
70. 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.