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REVIEW ARTICLE

Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change

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Abstract This review addresses the perspectives of *Azolla* as a multifaceted aquatic resource to ensure ecosystem sustainability. Nitrogen fixing potential of cyanobacterial symbiont varies between 30 and 60 kg N ha^{-1} which designates *Azolla* as an important biological N source for agriculture and animal industry. *Azolla* exhibits high bioremediation potential for Cd, Cr, Cu, and Zn. *Azolla* mitigates greenhouse gas emission from agriculture. In flooded rice ecosystem, *Azolla* dual cropping decreased CH_4 emission by 40 % than did urea alone and also stimulated CH_4 oxidation. This review highlighted integrated approach using *Azolla* that offers enormous public health, environmental, and cost benefits.

Keywords *Azolla* · Agriculture · Environment · Bioremediation · Greenhouse gasses · Climate change

Introduction

Azolla species are the world's smallest but most economically important macrophytes which float on the water surface. *Azolla* is unique, because it is one of the fastest growing plants on the planet which can double the area it covers in just 5 to 10 days (Brouwer et al. 2015). This aquatic fern is native to Central and South America and western North America and is also widely established in the UK and Ireland and across Europe, Morocco, southern Africa, Australia, New Zealand, Hawaii, and Asia (Sadeghi Pasvisheh et al. 2013). It propagates vegetatively by breaking up to form separate plants and sexually by means of large female spores and tiny male spores. It contains within its leaf cavities a symbiotic cyanobacterium *Anabaena azolae*. Anabaena draws down up to 1000 kg of atmospheric nitrogen per acre per year (Carrapiço 2010). The nitrogen provides a natural fertilizer for *Azolla*'s growth, freeing the plant from its reliance on soil and enabling it to grow free floating on freshwater bodies. Nitrogen is the element that most often limits food production (Mosier et al. 2013). In rice, the amount of nitrogen absorbed to produce grain is nearly constant at 19–21 kg N t^{-1} of whole grain rice (Pittelkow et al. 2012). Therefore, yields can be raised significantly by increasing the amount of nitrogen available to crops. In order for agriculture to be sustainable, nutrients must be replenished. Poor N recovery by rice causes substantial economic loss to farmers and creates negative impacts in the environment. The challenge, therefore, is to develop new management techniques which can curtail the high N losses and improve the poor N use efficiency by rice.

Due to intensive agriculture and industrialization to meet the demands of growing population, humans have caused fragility of ecosystem. The negative environmental impacts of the current agricultural practices include soil degradation, water depletion, contamination, loss of biodiversity, and climate change. The warming of the earth's atmosphere, due to the accumulation of carbon dioxide (CO_2) and methane (CH_4), promises to be the major issue of the next century. Fossil fuel-based industrial development is the major cause of the environmental imbalance; however, agricultural practices are major factors with the capacity of adding to greenhouse gases (the consequence of most modern production technologies) or reducing them by environmentally friendly development schemes. To reduce the impact, there is need of alternative strategies for sustainable agriculture. The sustainable

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agriculture may be defined as any set of agronomic practices that are economically viable, environmentally safe, and socially acceptable.

Sustainability in agriculture can be achieved by (1) increasing production to food grains to meet the demand of the population, (2) minimizing agricultural input to lower the cost of agricultural production, (3) reducing inorganic or chemical fertilizer use to lessen impact on environment and soil biodiversity that are key to the soil function, (4) developing technologies to use waste water resources for agriculture and other useful purposes to meet the risk of problems associated to water scarcity, (5) exploring bioremediation technologies to utilize contaminated lands/waste lands for agriculture and other uses, (6) making use of degraded lands for agriculture through eco-friendly techniques to reduce the relative abundance of waste/degraded land, and (7) identifying strategies to reduce greenhouse gas emission from agriculture. Organic farming has been identified as the best strategy or alternative method to meet the sustainability, which can address the multidimensional problems that is not only to increase the agricultural output but also to maintain soil health and enable biosphere function (Tuomisto et al. 2012). The organic agriculture focuses on "living soil" on optimizing the use of biological processes and on avoiding the use of synthetic chemicals and fertilizers.

Use of *Azolla* including *Azolla pinnata*, *Azolla filiculoides*, and *Azolla Africana* as nutrient source for crops is promising alternative strategies in agriculture. Although a lot of studies has been carried out in the past using *Azolla* for soil and agricultural management, there is lack of a comprehensive review on its application for other global problems such as bioremediation of pollutants and greenhouse gas mitigation. Therefore, this review is outlined to address the sustainability using *Azolla*. This review comprises of three major parts. The first part discusses *Azolla* being used for fertilizer management. The second part focuses its role toward the ecosystem management including bioremediation of toxic trace metals and organic pollutants. The third part identifies its application to minimize greenhouse gas (GHG) emission from agriculture as well as mitigation of atmospheric GHGs.

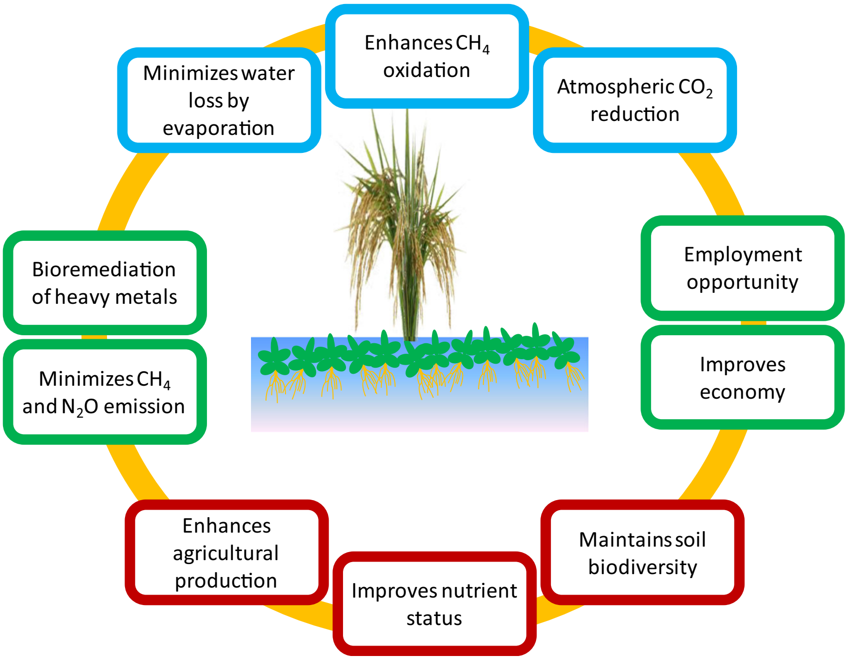
Azolla biofertilizer: perspective to agriculture and ecosystem sustainability

Chemical nitrogenous fertilizer is produced by industrial nitrogen fixation, and during this process, for every single unit of nitrogen fertilizer produced, two units of petroleum are required (Hamdi 1982). Depending on the type of nitrogen fertilizer produced and the efficiency of the process, production of a kilogram of N requires 51 to 68 MJ (1 megajoule = 10^6 J) of energy (McLaughlin et al. 2000). This expensive mode of production, combined with the cost of transport, makes the application of fertilizer too expensive for the majority of farmers in developing countries. The utilization of biofertilizers has several advantages over chemical fertilizers. (1) Biofertilizers like *Azolla* are inexpensive, making use of freely available solar energy, atmospheric nitrogen, and water. (2) It utilizes renewable resources, whereas the production of chemical fertilizers depends on petroleum, a diminishing resource. Therefore, biofertilizers are nonpolluting in nature (3). Besides supplying nitrogen to crops, it also supplies other nutrients such as vitamins and growth substances for animals. *Azolla* contains essential amino acids, vitamins (vitamin A, vitamin B12, and beta-carotene), growth promoter intermediaries, and minerals like calcium, phosphorous, potassium, ferrous, copper, and magnesium (Pillai et al. 2005). Dry weight basis contains 25–35 % protein, 10–15 % minerals, and 7–10 % of amino acids, bioactive substances, and biopolymers. The crude protein content of leaf protein concentrate and residual pulp fiber of *A. africana* is 71.3 and 12.6 %, respectively (Brouwer et al. 2015; Fasakin 1999). (4) It also improves the general fertility of the soil by increasing the organic matter in soil, thus improving soil structure. *Azolla* utilization has been identified to be more promising than inorganic fertilizers are in minimizing greenhouse gas mitigation, in helping development of economy, and employment opportunities (Denning 1989; Munasinghe et al. 2010), and in other soil and water management functions Fig. 1.

The utilization of *Azolla* in rice-based cropping system

Azolla anabaena is one of the promising biofertilizers for a variety of crops, including rice (Joshi et al. 2012), wheat (Babu et al. 2015), taro (Petruccelli et al. 2015), and soybean (Sholkamy et al. 2015). *Azolla* is beneficial to wheat when applied in a rotating rice-wheat cropping system (Gaind and Singh 2015). The most suitable crop for the application of *Azolla* is lowland rice, since both plants require a flooded habitat. *Azolla* may be grown either as a monocrop or as an intercrop with rice. Monocropping is done before rice cultivation or in a land to use it as source of green manure. Intercropping is practiced by growing with rice and continuously incorporated or harvested as and when required, providing an additional source of income during the entire period from sowing to harvesting of paddy (Pabby et al. 2003; Ren et al. 2008). As an intercrop, it is usually inoculated into the field just after transplanting the rice and, after a period of growth, it may be incorporated into the mud by manual mixing into soil or allowed to die naturally by fungal rot or light starvation (Aminifar and Ghanbari 2014). However, a combination of applications is usually recommended (Bocchi and Malgioglio 2010). *Azolla* application improves soil fertility by increasing total nitrogen, organic carbon, and available

Fig. 1 Socioeconomic and ecosystem services rendered by *Azolla* when applied to agriculture



phosphorus in the soil. The C/N ratio of the *Azolla* green manure ranges between 9 and 10 and favors release of NH_4^+−N when applied to rice soil. Among the *Azolla* species, *A. pinnata* is efficient to release NH_4^+−N more efficiently than *A. filiculoides* and *A. mexicana.* Indian isolate of *A. pinnata* released a maximum of 88 % NH_4^+−N, whereas Vietnam isolate recorded 77 % at 40 days of flooding. *Azolla* increased soil available P to 26 ppm from 18 ppm of compost (Singh et al. 1981).

The most effective application for increasing soil fertility was first culturing *Azolla* as a monocrop in the fallow period without diminishing the rice growing period, incorporating it before transplanting, and subsequently culturing it as an intercrop with two incorporations (Xie et al. 2010). In the Niger basin, *A. pinnata* was applied as an intercrop during 5 days after transplanting and incorporating it and re-inoculating at 27 days after transplantation. Grain yield of rice increased to 27 % compared to urea when both were applied at 30 kg N ha^{-1}(Kondo et al. 1989). *Azolla* improves soil physical structure when incorporated because of its high productivity, which supplies large quantities of organic matter to soil (Subedi and Shrestha 2015). Use of *Azolla* as green manure is also reported to improve soil porosity (3.7–4.2 %) and decrease the specific gravity of soils (Mandal et al. 1999). In a pot experiment, *Azolla* at different levels applied to soil reduced the bulk density from 1.44 g cm^{-3} in the control to 1.34 g cm^{-3} in 80 g kg^{-1} soil treatment. Similarly, porosity increased 6.52 % over unamended control (Awodun 2008). Another benefit of applying *Azolla* as a biofertilizer is that in low-potassium environments, it has a greater ability to accumulate potassium than rice does. *Azolla* can absorb traces of potassium as low as 0–5 ppm from water, while rice requires at least 8 ppm K for effective absorption. Thus, when the *Azolla* decomposes, it acts indirectly as a potassium fertilizer for soil (Van Hove and Nations 1989).

Anabaena *Azollae* strains can fix 30–60 kg N ha^{-1} in 30 days (Wagner 2011). The fern is used to a great extent in China (Shao et al. 2011), India (Raja et al. 2012), Bangladesh (Ali et al. 2014), and Vietnam (Phong et al. 2011) as an important biological source to improve the N balance of rice fields. The nitrogen fixed by the cyanobacterial symbiont is either released upon decay of the incorporated *Azolla* (Ortiz-Marquez et al. 2014) or leached into the standing water from the growing *Azolla* (Veluci et al. 2006) and is available for uptake by the rice crop.

Rice is a poor competitor with *Azolla* for available N early in the growing season. *Azolla* effectively competes with young rice plants for applied urea, capturing nearly twice the urea N compared to the rice plants. Using ^{15}N balance study, it was clearly showed that the presence of *Azolla* leads to an early reduction of N losses and an increased recovery of applied N by rice. However, a substantial fraction of the applied N was locked up by *Azolla* leading to a reduction of the *Azolla* biological nitrogen fixation (BNF). The remobilization of this conserved N is of paramount importance for the final recovery and grain yield at maturity. In another experiment, it was established that around 65 % of the N immobilized by *Azolla* was remineralized during the rice growing season, whereby 28.7 and 42 % were taken up by the rice crop following the basal and second split application of urea, respectively. This protective urea immobilization followed by the subsequent re-mineralization contributed to the elimination of NH_3volatilization from applied urea. Together with the input from BNF by *Azolla*, these processes result in a synergism which is expressed in grain yield as well as in nitrogen yield of the standing rice crop (Cissé and Vlek 2003).

This leads to the conservation of urea N in the system, yielding a complete N recovery in the system early in the growing season. It was found that, 6 weeks after fertilization with 100 mg N pot^{-1}, altogether, 50 mg of the applied urea N was immobilized in the *Azolla* biomass (Cissé and Vlek 2003). Due to decomposition of dead *Azolla* biomass, N is released and was available for rice absorption. Around tillering time, the rice plants are developed enough to compete with *Azolla* for applied nitrogen (Cissé and Vlek 2003). In total, the combination of *Azolla* and urea produced yields that were 10 % higher than without cover (de Macale and Vlek 2004). Overall, using *Azolla* as a surface cover in combination with urea can be an alternative management practice worth considering as a means to reduce NH_3 volatilization and improve N use efficiency as well as rice yields (de Macale and Vlek 2004). *Azolla* can act as a physical barrier to trap the liberated NH_3 and absorb the incoming solar radiation. *Azolla* thus conserves N and suppresses algae-induced rise in floodwater pH, and temperature in effect, influencing the chemical and microbiological processes in the floodwater (Waddell and Moore 2008). It significantly suppressed the rise in floodwater pH by 0.9 to 1.4 pH units and maintained pH below 8.0 (Agami and Reddy 1990). Floodwater temperature is another factor in the change of floodwater chemistry (Carpenter et al. 2011). There was a 5 °C difference with *Azolla* cover for the wetland rice of the Philippines (de Macale and Vlek 2004). The temperature effect might be more prominent in the tropics, where air temperature is high. Floodwater temperature has an indirect effect on ammonia volatilization by affecting the partial pressure of NH_3 (Cissé and Vlek 2003). The fine-tuned system should time the *Azolla* inoculation such that an *Azolla* cover is present at the time of fertilizer N application. An initial cover of 50 %, 5–10 days before fertilizer N application is sufficient to assure full coverage (100 % biomass) at the time of N fertilization (Kern et al. 2007).

Evaporation of water is a major concern in flooded water agroecosystem such as rice. By growing *Azolla* along with rice water, evaporation is substantially reduced, a phenomenon which is extremely desirable in many tropical rice farms (Esiobu and Van Hove 1992). Evaporation of water causes salt concentration of soil to increase. It is reported that repeated *Azolla* cultivation in the saline sea water over a 2-year period reduced the salt content from 6 to 43 % (Shang et al. 1987).

Azolla biofertilizer for crops other than rice

Azolla can be beneficial to many crops other than rice. Particularly, any agricultural crop that grows in a flooded soil ecosystem can be a suitable target crop for *Azolla*, like taro (*Colocasia esculenta*). In an experiment with the utilization of *A. mexicana* on taro in the Cook Islands, *Azolla* was incorporated into mud at 20 t ha^{-1} and also applied to soil at 0.5 kg m^{-2} at planting of taro. It was found that the incorporation of *Azolla* into the mud and subsequent intercropping with *Azolla* resulted in 54.6 % greater yields than the control, while taro intercropped with *Azolla* in slowly flowing water gave yields that were 87.3 % greater than the control. Both *Azolla* treatments gave significantly higher yields than did treatments fertilized with chemical nitrogen (40 Kg NH_4SO_4-N ha^{-1}) and phosphorus (10 Kg ha^{-1} P as triple super phosphate) (Tekle-Haimanot and Doku 1995).

Azolla can be beneficial to wheat under rice-wheat cropping system (Rana et al. 2015). *Azolla* grown as a monocrop between the wheat and rice crops or applied as an intercrop with rice has a significant beneficial effect on subsequent wheat crops in rice-wheat cropping system. Application of *Azolla* with *Sesbania* as green manure had beneficial residual effects on subsequent wheat crops, raising grain yield by 56–69 % over controls (Mahapatra and Sharma 1989). *Azolla* (especially fresh fronds) increased grain yield of wheat, though straw yield and the number of tillers per plant were largely unaffected. The method is also practiced in Senegal (Van Hove and Nations 1989), where a succession of vegetable crops is planted on the banks of *Azolla* ponds. In the case of bananas, *Azolla* is applied as mulch on the soil surface around the bases of the plants. When there is an overproduction of *Azolla*, it can be mixed with rice straw to form compost. Incorporation of 6–24 t ha^{-1} of fresh *Azolla* into the soil significantly increased its water-holding capacity, organic carbon, ammonium-N, nitrate-N, and its available phosphorus, potassium, calcium, and magnesium, while it decreased pH and bulk density. *Azolla* used as a green manure significantly raised the yield of mungbeans (Nuraisyah 2002).

Other usages of *Azolla* in agriculture

Azolla is used as a food supplement fresh dried or ensiled for a variety of animals, including pigs, rabbits, chickens, ducks, and fish (Devendra and Leng 2011). *Azolla* fed to broilers resulted in growth and body weight values similar to those resulting from the use of mustard oil cake meal (Ashraf et al. 2015). In an experiment in Bangladesh, *A. pinnata* was tested as a feed ingredient for broiler ration at 5–15 %. The composition of *Azolla* meal contained 25.78 % crude protein, 15.71 % crude fiber, 3.47 % ether extract, 15.76 % ash, and 30.08 % nitrogen free extract on the air-dry basis. Live weight, production number, and protein efficiency were ($P < 0.01$) significantly improved at the level of 5 % *Azolla* meal in broiler ration (Basak et al. 2002). In another experiment, effect of *Azolla* (*A. pinnata*) meal in broilers was studied at Assam, India. Conventional feed was replaced with 0, 5, 10, and 15 % *Azolla* meal. Broilers fed with 5–10 % *Azolla* meal showed significant body weight gain, feed consumption, and fed efficiency to that of control group (Saikia et al. 2014). *A. filiculoides* fed as partial replacement (0, 15, and 30 %) of the protein in a soybean-based supplement to the pig in a commercial farm in the Valle del Cauca in Colombia. Results were at par with the expensive soybean-based meal. Therefore, replacing pig feed with *Azolla* was important in an economic standpoint (Cherryl et al. 2013). Studies have been conducted on the use of *Azolla* as feed to tilapia. Up to 30 % of fish meal-based diet fed to Nile tilapia could be successfully replaced with dried *Azolla* meal (Youssouf et al. 2012). Growth and survival of *Labeo fimbriatus* fry fed with feed containing varied levels of dried *Azolla* (*A. pinnata*) was evaluated. The control feed constituted 45 % groundnut oilcake, 45 % rice bran, and 10 % finger millet flour as pelleting binder. Dried *Azolla* powder was added into the control feed at 10–40 % levels replacing the groundnut cake and rice bran proportionately. Feed with 40 % *Azolla* reduced the cost of feed by 25 % without affecting the yield (Gangadhar et al. 2015).

Isonitrogenous fish feed (crude protein 27.75–28.26 %) replaced with 40 % *Azolla* increased specific growth rate of *Labeo rohita* by 0.67 than control (Panigrahi et al. 2014). A rice-*Azolla*-fish culture system has proven to be quite successful in Fujian, China (Lu and Li 2006). Rice-*Azolla*-fish culture is of great significance in increasing freshwater fish yield. The yield of fish has in general reached 225–750 kg ha^{-1} in China. This system can result in eradicating weeds and harmful insects, loosening soils, increasing dissolved oxygen, and improving the fertility of paddy fields, so rice production may be increased at between 8 and 47.3 %. This practice has the characteristics of low cost, quick effectiveness, and better economic returns and has been recognized as an additional source of food and/or income in rural areas (Kangmin 1988). The nitrogen-fixation role of this system increased the content of organic matter, total nitrogen, and total phosphorus in the soil by 15.6–38.5 % (Lu and Li 2006).

Azolla appears to be fit for human consumption. A few researchers have experimented with the preparation of *Azolla* in soup or "*Azolla-meat*" balls as food for man. However, such recipes are as yet unpublished (Van Hove and Nations 1989). A book in China in the 16th century described the medicinal properties of *Azolla* (Shi and Hall 1988). In Tanzania, *Azolla* has been reported to be used effectively as a traditional cough medicine (Wagner 1997).

Environment management using *Azolla*

Whenever good quality water is scarce, water of marginal quality or even waste water will have to be considered for use in agriculture. Use of wastewater in agriculture could be an important consideration when its disposal is being planned in arid and semiarid regions. From the viewpoint of irrigation, use of marginal quality or waste water requires more complex management practices and more stringent monitoring procedures than when good quality water is used. Waste water is characterized with high concentration of pollutants like heavy metals, organic solvents, oil, xenobiotics, and other industrial wastes. The source of waste waters are generally sewage, industrial effluents, agricultural runoff, and oil-spilled natural water bodies.

Azolla can be used to manage such wastewaters by bringing contaminant concentration to a lower level for further use in agriculture (Rai 2007). A study conducted on growing *Azolla microphylla* on municipal wastewaters revealed that *Azolla* can act as a biofilter to remove pollutants. The biomass produced can be used for fertilizing paddy fields or for other applications, and polished wastewaters can be recycled for irrigation purposes (Arora and Saxena 2005). *Azolla* exhibits a remarkable ability to concentrate trace metals, petroleum compounds, pesticides, pharmaceutical antibiotics, and dyes. *Azolla* live biomass acts as potential bioaccumulator for toxic pollutants, while the dead biomass regulates pollutant concentration through biosorption. Hyperaccumulation of heavy metal involves several steps, such as transport of trace metal across plasma membrane, translocation of heavy metal, detoxification, and sequestration at cellular and whole plant level (Rascio and Navari-Izzo 2011; Shah and Nongkynrih 2007). In most aquatic plants, bioaccumulation is carried out by metal chelators which include phytochelatins, metallothioneins, organic acids, and amino acids. Metallothioneins has been characterized in *A. filiculoides* grown under heavy metal stress. These metallothioneins have low molecular weight (4–10 k Da) and are cysteine-rich and metal-binding proteins that bind metals via the thiol groups of cysteine residues (Schor-Fumbarov et al. 2005). *Azolla* species tested for their potential to bioremediate pollutants are listed in Table 1.

Bioremediation of hazardous pollutants

There has been considerable interest in the area of metal accumulation from aqueous solution by microbes and plants (Elifantz and Tel-Or 2002). Trace metals cannot be degraded; therefore, it must instead be extracted from contaminated sites (Sahoo et al. 1992). Potential of many aquatic macrophytes for trace metal removal has been investigated extensively (Cheng 2003; Dhir et al. 2009; Marques et al. 2009; Rai 2007). Aquatic plants vary widely with respect to the amount of trace metal accumulation indicating that phytoremediation potential of aquatic plants is dependent upon the tolerance level and toxicity of the plant genera or species employed in a particular study. In addition, within a particular plant genus and/or species, there is variation in accumulation potential for the same heavy metal. Aquatic plants such as *Ceratophyllum demersum*

Table 1 Bioremediation of environmental pollutants by *Azolla* sp

Pollutants	Components	*Azolla* sp. tested for bioremediation	Reference
Trace metals	Cu, Cd, Pb, Ni, Cr, Hg, As, Au, Zn	*Azolla filiculoides*, *Azolla microphylla*, *Azolla pinnata*, *Azolla caroliniana*	Jafari et al. (2010); Kanoun-Boulé et al. (2009); Mufarrege et al. (2010)
Petroleum	Diesel hydrocarbon, BTEX, Crude oil	*Azolla pinnata, Azolla africana*	Cohen et al. (2002); Edema et al. (2010)
Antimicrobial pharmaceuticals	Sulfadimethoxine	*Azolla filiculoides*	Forni et al. (2006)
Dyes	Acid red 88 (AR88)	*Azolla microphylla*	Padmesh et al. (2005)

accumulate Zn and Cd (Aravind et al. 2009). *Lemna minor* has been found to tolerate Cu (Kanoun-Boulé et al. 2009). In a study, it was observed that *Eichhornia crassipes* was more efficient in removal of trace metals (Fe, Zn, Cu, Cr, and Cd) followed by *Pistia stratiotes* and *Spirodela polyrrhiza* (Mufarrege et al. 2010).

Azolla carries out bioremediation of trace metals through accumulation and biosorption. Heavy metals present in wastewater can be managed by *Azolla* through bioremediation and are listed in Table 1. After bioaccumulation, its biomass is easy to harvest as it desiccates quickly (Sachdeva and Sharma 2012). Bioaccumulation properties of *Azolla* make it a perfect candidate for bioremediation systems (Cohen 2006), which can be used to treat polluted waters or sewage water for agricultural use. *Azolla* species vary in their potential to bioremediate trace metals. *A. pinnata* removed 70–94 % of trace metals (Hg and Cd) from ash slurry and chlor-alkali effluent in Singrauli (India), and the concentration of these trace metals ranged between 310 and 740 mg kg^{-1} dry mass in tissues of *Azolla* (Rai 2008). In a hydroponic experiment, it was found that *Azolla* grew under As concentration ranging from 29 to 397 mg kg^{-1} dry mass. *Azolla caroliniana* accumulated highest As (284 mg kg^{-1} dry weight), while *A. filiculoides* accumulated 54 mg kg^{-1} dry weight. *A. filiculoides* accumulated trace metals such as Cd, Cr, Cu, and Zn at 10,000, 1990, 9000, and 6500 ppm respectively (Sela et al. 1989). Different *Azolla* species vary in their absorption potential to the trace metals. It was observed that highest bioconcentration potential of Pb^{2+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} was 94 % in *A. microphylla*, 96 % in *A. filiculoides*, 71 % in *A. pinnata*, and 98 % in *A. microphylla*, respectively (Jafari et al. 2010). *Azolla* biomass produced after phytoremediation can be used as source for bioenergy production or bio-ore for recovery of marketable amount of precious trace metal. The biomass left after the extraction of heavy metals can be a good source of protein-rich feed for animals or can be used as green manure. Much research is still needed on metal transporters, biosorption, and their regulatory genes. This will provide effective strategies to utilize *Azolla* for treatment of multielement-contaminated wastewater.

Bioremediation of petroleum products

Often oil spilled in land and water bodies are challenging to clean up. The biodegradation of hydrocarbon pollutants in open systems is limited by the availability of a utilizable nitrogen source. *Azolla* can be used to resolve the hydrocarbon pollution by indirectly stimulating microbes that have potential to degrade hydrocarbons (Edema et al. 2010). In an experiment, *A. pinnata* as well as *P. stratiotes* and *Salvinia molesta* were applied to plots containing soil that had been surface-contaminated with diesel fuel (2.4 l m^{-2}) and flooded with water. All plants quickly died and bacterial flocs developed around the dead *A. pinnata* fronds. After 16 weeks, diesel concentrations in the plant-added plots were less than half those of the control plot, and concentrations of xylenes and ethylbenzene were 50–100 times lower. In previous study conducted in laboratory experiments, a consortium composed of *A. pinnata*-derived bacteria displayed dense growth in a 4 % diesel-containing mineral salts medium and was found to lower the concentration of aromatic compounds by approximately 50 % after 19 days (Cohen et al. 2002). It is concluded that the observed enhancement of diesel degradation in the plant-added plots was due to the release of bacteria (bioaugmentation) and physiochemical improvement of the plot conditions (biostimulation) (Cohen et al. 2002).

Bioremediation of other pollutants

There are only a limited number of studies available that examined biological treatment of pollutants such as antimicrobial agents and dyes. Antimicrobial drugs are widely used in intensive farming (including aquaculture) both as feed additives and in mass therapy. They are administered as medicated feed, slowly absorbed and eliminated with feces ending up into the environment. After elimination from the body, these drugs (and their metabolites) can maintain significant residual activity and toxicity. In an experiment, three different floating macrophytes such as water velvet, duckweed, and water lettuce (*A. filiculoides*, *L. minor*. and *P. stratiotes*) challenged against antimicrobial drug, five sulfadimethoxine (S) concentrations (0, 50, 150, 300, and 450 mg l^{-1}). Tests with and without plants under the same environmental conditions showed that in S-treated batches, a higher proportion of drug was removed if the fern was present (from 56 % at S 50 to 88 % at S 450). Sulfadimethoxine's accumulation capability followed the trend as *Azolla*>*Lemna*>*Pistia* (Forni et al. 2006) (Table 1). Biosorption potential of *A. microphylla* for acid red 88 from aqueous solution was investigated. The biomass exhibited the highest dye sorption capacity at optimum conditions of pH 3 and temperature 30 °C.

A. pinnata have shown potential usefulness in the treatment of eutrophicated water system (Sutton and Ornes 1975). Floating macrophytes take up inorganic nutrients mainly by the roots, as well as through the leaves. *Azolla rongpong* is used to clean water bodies for its ability to remove acid dyes such as acid red 88, acid green 3, acid orange 7, and acid blue 15 from the contaminated sites (Padmesh et al. 2005). Biosorption potential of *Azolla* to various pollutants is regulated by pectin content. Pectin, constituting 8–10.5 % (*w*/*w*) of the *Azolla* cell wall, is shown to bind a major portion of ionic pollutants (Sr^{2+} ions). Treatment with pectinase reduced the binding capacity of *Azolla* to Sr^{2+}. Methylation of *Azolla* biomass, known to block the carboxyl groups of pectin by esterification, markedly reduced the Sr^{2+} binding capacity (Cohen-Shoel et al. 2002). A study on the biosorption of basic orange (BO) dye from aqueous solution onto the dried *A. filiculoides* found that the maximum biosorption capacity for BO was 833.33 mg g^{-1} at a temperature of 303 K, a solution pH of 7.0, a biosorbent dosage of 5 g l^{-1} and a contact time of 4 h. Fourier transform infrared spectroscopy revealed that the amino, carboxyl, and hydroxyl groups may be responsible for the biosorption of BO on the biomass (Tan et al. 2010).

GHG mitigation by *Azolla*

Agriculture contributes three major atmospheric greenhouse gases i.e., carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Cole et al. 1997; Houghton 2001). CO_2 is released mainly from microbial decay processes or burning of plant litter and soil organic matter decay (Janzen 2004; Smith et al. 2008). CH_4 is produced during organic material decomposition which usually happens under oxygen-limited environments, notably from fermentative digestion by ruminant livestock, from stored manures, and from rice grown under flooded conditions (Mosier et al. 1998). N_2O is generated by the microbial transformation of nitrogen in soils and manures and is generally stimulated where available nitrogen (N) exceeds plant requirements, especially under wet conditions (Smith et al. 2008).

Role of *Azolla* in CH_4 flux from rice fields

Methane, the most abundant gaseous hydrocarbon in the atmosphere, is an important greenhouse gas that may account for approximately 15–20 % of the total current increase in global warming (Bharati et al. 2000). Flooded soils planted to rice are conductive to the production and emission of CH_4 (Bharati et al. 2000). With the intensification of rice cultivation, CH_4 emission from this important ecosystem is likely to increase. Studies have been carried to verify the effect of *Azolla* on CH_4 efflux from rice fields (Table 2). In a field trial at Cuttack, India, *Azolla* in different forms were applied with urea N to provide a total of 60 kg N ha^{-1} (urea 30 kg N, *Azolla* 30 kg N). *Azolla* was either incorporated as green manure at the beginning of experiment or grown as dual crop in the standing water along with rice crop. Cumulative CH_4 flux followed the order of urea > *Azolla* (incorporated) + urea > *Azolla* (incorporated + dual crop) no N control > urea + *Azolla* (dual crop). Urea alone increased CH_4 emission possibly due to increased plant growth and microbial metabolic activity in soil resulting to higher CH_4 emission (Ying et al. 2000; Zhao et al. 2015). Higher CH_4 flux was observed in *Azolla* incorporated plots compared to no N control until 60 days. This difference was attributed to C supplied by root lysis or exudates after flowering (Schutz et al. 1991). Dual cropping of *Azolla* along with urea N reduced CH_4 flux by 40 % when compared to urea N alone (Table 2). The decrease in CH_4 efflux in plots with dual crop of *Azolla* could be related to the release of oxygen in the standing water by the growing *Azolla* leading to less reduced conditions in the soil (Bharati et al. 2000). The role of *Azolla* and cyanobacteria on CH_4 production and oxidation in a laboratory simulation experiments carried out using soil samples from rice fields (Prasanna et al. 2002). Moist soil core samples (0–5 cm depth) collected from rice fields that had been treated with urea in combination with a cyanobacterial mixture plus *Azolla microphylla* affected distinctly more rapid decrease in head space CH_4 added at 200 μL than did the soil cores from plots treated with urea alone (30, 60, 90, 120 kg N ha^{-1}) irrespective of the rate of chemical nitrogen applied for rice fields. Higher CH_4 oxidation was due to aeration by oxygen released by the cyanobacterium. With their increasing role in CH_4 oxidation, cyanobacteria and *Azolla* can play a major role in mitigating CH_4 emission.

The effect of different organic amendments on CH_4 emission in rice field soil was investigated (Adhya et al. 2000). Organic sources were applied to fields through incorporation to provide 20 kg N ha^{-1}. The treatments included urea N, Sesbania + urea N, farm yard manure (FYM) + urea N, *Azolla* + urea N. Methane efflux per grain yield were 14, 37.70, 21.96, and 18.55 kg $CH_4\,t^{-1}$ grain yield respectively. Although *Azolla* incorporation increased CH_4 flux over that of urea N alone, the effect was compensated with higher yield. Organic matter (*Azolla* or Sesbania), when amended to

Table 2 Comparative assessment of greenhouse gas (CH_4) efflux from rice field soils under different amendment practice of *Azolla*

Azolla incorporation methods	Amendments	Total soil C (g kg^{-1})	Total soil N (g kg^{-1})	Greenhouse gas (CH_4) flux/production (kg ha^{-1})	References
Control	No N or *Azolla*	7	0.8	95	Bharati et al. (2000)
Urea (60 Kg N)	Control—No *Azolla*			155	
Azolla incorporation (30 Kg N) + Urea (30 Kg N)	Incorporated as Green manure			149	
Azolla dual cropping (30 Kg N) + urea (30 Kg N)	Grown along with crop			89	
Azolla incorporation (30 Kg N) + *Azolla* dual cropping (30 Kg N)	Applied both as green manure and grown along with crop			105	
Urea (170 Kg N)	Control—No *Azolla*	16	0.8	140	Ying et al. (2000)
Urea (170 Kg N) + *Azolla* dual cropping	*Azolla* applied at 156 g m^{-2}, grown for 1 year			230	
Urea (170 Kg N) + *Azolla* dual cropping	*Azolla* applied at 156 g m^{-2}, grown for 5 years			235	
Urea (60 Kg N)	No compost	7	0.8	14	Adhya et al. (2000))
Urea (40 Kg N) + Sesbania	*Sesbania aculeata* (incorporated) 20 kg N			37.70	
Urea (40 Kg N) + Compost	FYM 20 Kg N			21.96	
Urea (40 Kg N) + *Azolla* (20 Kg N)	*Azolla* (incorporated) 20 Kg N			18.55	
Urea (30 Kg N Ha^{-1})	No *Azolla*	3	0.13	378	Prasanna et al. (2002)
Urea (60 Kg N Ha^{-1})				272	
Urea (90 Kg N Ha^{-1})				326	
Urea (120 Kg N Ha^{-1})				316	
Urea (30 Kg N Ha^{-1}) + *Azolla*	*Azolla* incorporated at 1 t ha^{-1}			210	
Urea (60 Kg N Ha^{-1}) + *Azolla*				202	
Urea (90 Kg N Ha^{-1}) + *Azolla*				210	
Urea (120 Kg N Ha^{-1}) + *Azolla*				198	

flooded soil, decomposes into readily mineralizable C that acts as substrates for CH_4. Data suggested that *Azolla* incorporation can reduce CH_4 efflux from rice field significantly compared to other organic amendments. Few studies from China have reported increase in CH_4 efflux from rice field with *Azolla* dual cropping. In these studies, at field and laboratory conditions showed that growing *Azolla* as a dual crop could enhance CH_4 emission from rice fields (Ying et al. 2000) (Table 2). Interestingly, it was found that the CH_4 efflux from Indian soil was lower than from soils originated from China. Soil properties like C and N content can be one of the regulatory factors for the differential response of soils to the CH_4 efflux. Several studies have revealed the role of soil C content on the increasing CH_4 flux (Gollany et al. 2015). However, in the present context, the reason of high CH_4 flux in presence of *Azolla* particularly in high C containing soils is less understood.

Atmospheric CO_2 reduction by *Azolla*

To determine carbon fixing potential, *Azolla* was cultivated in a tank and found that it fixed 0.33 t nitrogen and 1.86 t CO_2 in 1 ha land within a 1-year period. It was estimated that by enhancing *Azolla* cultivation in wet land paddy field of Sri Lanka, 509,422 t of CO_2 can be reduced from atmosphere and can get 8934 t of nitrogen fertilizers every year (Surenthiran and Loganathan 2012). Similarly, a study from UK indicated that *A. filiculoides* sequestered 32.54 metric t CO_2 ha^{-1} $year^{-1}$ which was higher than grassland, forest, and algae (Ben-Amotz 2007; Dawson and Smith 2007). To investigate the influence of elevated atmospheric CO_2 concentrations on *Azolla* growth rates, pot experiments were carried out with growing *Azolla* under higher CO_2 (380 ppm and 680 ppm) concentrations. Distinct increases in biomass production ($p < 0.01$) in response to elevated carbon dioxide concentrations were evidenced. Biomass of *Azolla* increased to 417 mg pot^{-1} over that of ambient (348 mg pot^{-1}) in 16 days (Cheng et al. 2010). *Azolla* is an interesting opportunity for harvesting atmospheric CO_2 (Brinkhuis and Bijl 2014). The amount of biomass produced was corresponding to the CO_2 concentration. Therefore, it is expected that growing *Azolla* in agricultural practices will substantially reduce global CO_2 budget (Table 3). The production and subsequent burial of significant amounts of organic carbon might have affected atmospheric CO_2 concentrations and, as a consequence, global climatic conditions. Estimations reveal a mean sea surface

Table 3 Regulation of climate changing factors by *Azolla*

Climate change factors	Remarks	References
Energy saving	Reduces energy use by minimizing inorganic fertilizer production. Energy use is related to greater C foot print	McLaughlin et al. (2000)
N loss	Minimizes N loss through NH_3 volatilization	Cissé and Vlek (2003); Phong et al. (2011)
Greenhouse gas mitigation	CH_4, CO_2, N_2O decrease	Bharati et al. (2000); Janzen (2004); Prasanna et al. (2002)
Global temperature	Reduces Global temperature by CO_2 fixation	Waddell and Moore (2008)
Carbon sequestration	Incorporation of *Azolla* biomass in soil help C sequestration	Surenthiran and Loganathan (2012)
Carbon credits	Cultivation of rice with *Azolla* is a potential approach for incorporating carbon credit markets reducing global carbon foot print.	Brinkhuis and Bijl (2014)

temperature of 13 °C before and after the *Azolla* interval and a mean temperature of 10 °C during the *Azolla* interval (Brinkhuis et al. 2006).

Regulation of N_2O emission and NH_3 volatilization from agroecosystem by *Azolla*

N_2O is produced in soil by two dissimilar energy-producing microbial processes, nitrification and denitrification, and very limited chemical process to contribute N_2O emission in soil (Li et al. 2015). Continuously flooded condition establishes anaerobic environment which does not favor N_2O emission. Hence, flooded rice paddies are usually not considered to be an important source of atmospheric N_2O because N_2O would be rapidly reduced to N_2 under such intensive anaerobic conditions (Reay et al. 2012). But, we presume that it may indirectly reduce N_2O flux by reducing doses of inorganic N sources. Inorganic N sources are directly responsible for N loss and N_2O emission (Table 2). *Azolla* as N source acts as slow release nutrient for low N loss as well as N_2O release to atmosphere (Jeyapandiyan and Lakshmanan 2014). The most important factor for NH_3 volatilization or gaseous N (N_2O) loss is high available NH_4 concentration and high pH of flood water. Urease inhibitors and slow release products have been used to decreases NH_4 concentration in the flood water. The influence of an *Azolla* cover in urea-amended plots applied at the rates of 0, 40, 80, 120, and 160 kg N ha^{-1} as compared to plots with urea only was assessed with respect to floodwater chemistry, NH_3 volatilization. Findings revealed that a full *Azolla* cover on the floodwater surface at the time of urea application prevented the rapid and large increase in floodwater pH associated with urea hydrolysis and the photosynthetic activities of the algae. In the presence of an *Azolla* cover, the mean floodwater pH was reduced by as much as 1.9 pH units, and the maximum pH value was kept below 8.3. In contrast, in the absence of a cover, floodwater pH rose above 8.5 and reached a maximum of 10.1. The floodwater temperature was lowered by as much as 5 °C. As a consequence, the partial pressure of NH_3, which is an indicator of potential NH_3 volatilization, was significantly depressed. The total N recovery varied between 77 and 99 %. The N loss accounted for in the *Azolla*-rice-soil system ranged from 0.01 to 23 %. Whereas, in the absence of an *Azolla* cover, N losses ranged from 21 to 49 % (Cissé and Vlek 2003; de Macale and Vlek 2004).

Socio economical evaluation of *Azolla*

The economic benefits of *Azolla* have been mostly studied evaluating its use in different agricultural systems. Previous study from rice fish *Azolla* system in India revealed that the *Azolla* not only increased the yield of both rice and fish but also increased benefit cost ratio. Adaptation of *Azolla* increased benefit cost ratio to 1.88 from 1.57 to 1.77 in rice alone and rice fish, respectively (Shanmugasundaram and Balusamy 1994). The biological potential of *Azolla* as a green manure in rice production is great. Under favorable experimental conditions, a layer of *Azolla* covering a 1-ha rice field releases 20–30 kg organic N. The economic return from *Azolla* adoption including cost savings in chemical fertilizer and weed control is more than 10 % of the total non land cost for rice production in areas where environmental conditions favor *Azolla* growth (McConnachie et al. 2003). *Azolla* produces abundant biomass and has 5–7 % protein respiration abilities, offer cost-effective solutions for fodder security (more than 30–40 % cost saving), and significantly reduces costs toward chemical farming. Experiment was conducted to examine the prospects of supplementing commercial feed with raw *Azolla* (*A. pinnata*), on the production performance of Nicobari fowl. *Azolla* was fed at 200 g per chick per day, in addition to 120 g of basal diet, from 45 to 60 weeks. There was 30.73 % reduction in feed consumption in *Azolla*-supplemented group that culminated in 0.01$ (0.76 Indian Rs) savings on feed cost per egg per day over the control. The study tends to conclude that *Azolla* is a good feed additive for sustainable egg production in Nicobari fowl and proved to be profitable due to savings on feed cost (Sujatha et al. 2013).

Concluding remarks

Azolla is a multi faceted biofertilizer in solving major problems that are of global concern. *Azolla* utilization deciphers positive increase in the area of socioeconomic development, agriculture productivity, bioremediation of toxic pollutants, and climate change (Table 3). Utilization of *Azolla* in agriculture improves social economic status by reducing the agricultural input cost, generating employment opportunities for small-scale industry involved in its propagation. *Azolla* can reduce the energy demand required for fertilizer production, hence, the environmental impact due to energy production. *Azolla*'s leaf structure has evolved to provide a microenvironment for the heterocyst-forming nitrogen-fixing filamentous bacterium *A. Azollae*. This is the key to *Azolla*'s ability to sequester enormous amounts of atmospheric CO_2, an attribute for carbon credit. Greenhouse gases like CH_4, N_2O emission can be regulated from agriculture through dual cropping. *Azolla* technology will ultimately benefit the rice farmers in a positive and self-sustaining way. However, its application for sustainability is still limited because of (1) social and political constraints which lack initiative programs to extend subsidies or credits adapting this technology in most developing countries, (2) less awareness programs for the end users like farmers, and extensive extension program is warranted, and (3) scientific research programs to develop efficient strains designed for specific purpose. Apart from these, its propagation is restricted to tropics if strains to grow at low temperature found it can be used in temperate climate. The review suggests further collaborative efforts in research to make the best use of this important natural resource for sustainable agriculture.

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Compliance with ethical standards

Conflict of interest Authors do not have any conflict of interest in the order of authorship.

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