International Journal of Agricultural Technology 2023 Vol. 19(1):63-82
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Azolla as the multifunctional fern in organic agriculture: Prospects and challenges: A Review Article

Herath, B. M. M. D.[1], Karunarathna, S. C.[2], Ishaq, M.[3], Wariss, H. M.[4] and Yapa, P. N.[1*]

[1]Faculty of Applied Sciences, Rajarata University of Sri Lanka, Mihintale, 50300, Sri Lanka; [2]Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing,Yunnan 655011, P.R. China; [3]Department of Botany, Hazara University, Mansehra, Khyber Pakhtunkhwa, 21120, Pakistan; [4]Department of Botany, University of Sargodha, Sargodha 40100, Pakistan.

Herath, B. M. M. D., Karunarathna, S. C., Ishaq, M., Wariss, H. M. and Yapa, P. N. (2023). Azolla as the multifunctional fern in organic agriculture: Prospects and challenges. International Journal of Agricultural Technology 19(1):63-82.

Abstract *Azolla* species are the world's smallest but most commercially significant, macrophytes, which float on the water surface and are found in freshwater and brackish waters. *Azolla* is one of the fastest growing plants on the globe, and it can double its surface area every 5 to 10 days, making it an extremely valuable resource. *Anabaena azollae*, a cyanobacteria, which is harboured in the leaf lobe of *Azolla*, is capable of fixing atmospheric nitrogen while making it accessible to crop plants. Therefore, the *Azolla-Anabaena* relationship is significant in agronomy. The presence of a symbiotic cyanobacterium, *A. azollae*, which occupies the dorsal lobe of the leaves, contributes to the system's nitrogen-fixing capabilities. As a result of this characteristic, it has been widely used as a biofertilizer for rice plants. Apart from that, it may be used for a variety of other things, such as food and feed, biofuel production, and heavy metal accumulation. Because it has so many uses, promoting and using the *Azolla-Anabaena* system in sustainable agriculture would be helpful and good for the environment.

Keywords: *Azolla*, Biofuel, Biological nitrogen fixation, Food and feed, Organic agriculture, Phytoremediation

Introduction

Azolla is a widely-used biofertilizer and green manure. The *Azolla-Anabaena* system is ideal for tropical rice production due to its mutualistic symbiosis with *Anabaena azollae*, which fixes atmospheric nitrogen more efficiently than other systems. The heterocystous cyanobacterium *A. azollae* in the fern's dorsal leaf cavity fixes nitrogen. *Azolla* has gained popularity as a

[*] **Corresponding Author:** Yapa, P. N.**; Email**: neelamanie@as.rjt.ac.lk

biofertilizer, green manure, poultry feed, and cow fodder. This organism's agronomic potential and usage in Asian agriculture have been established (Dawar and Singh, 2002).

Several global studies have shown that using *Azolla* increases crop yield. Use of *Azolla* with artificial nitrogen fertilizers has also been successful. Compared to other biofertilizers, *Azolla* treatment increased rice grain yield. *Azolla's* thick mat reduces weeds and ammonia volatilization in rice fields (Jha *et al*., 2004). Aquatic ecosystems are becoming increasingly crucial for human life, and experts throughout the world are seeing the growing public awareness of water's importance. Unquestionably, a low-cost, ecologically acceptable method is needed to remove impurities, especially heavy metals, to improve water quality. Phytoremediation is a rising trend (Arora *et al*., 2006).

Azolla can be used to eliminate phenol from industrial effluent and manage weeds. *Azolla* has been used as a biosorbent for metal-bearing effluents (Bennicelli *et al*., 2004). According to studies, *Azolla* may remove dyes and phenolic compounds from aqueous solutions (Vafaei *et al*., 2012). *Azolla* is rich in proteins, amino acids, vitamins (A, B12, beta-carotene), growth promoter intermediates, and minerals (calcium, phosphorus, potassium, iron, copper, and magnesium). *Azolla* is one of the most cost-effective and efficient feed substitutes for cattle due to its high protein and low lignin content. It is also easily digested by animals (Wagner, 1997).

Biodiesel production is diverse. Scientists are focusing on harnessing microalgae to produce green energy. Harvesting is costly and energy-intensive. Macroalgae oil production produces up to 58,700 litres per hectare, more than oily seeds, but *Azolla* may be farmed cheaply. *Azolla* oil's fatty acid concentration makes it a biodiesel ingredient (Gouveia *et al*., 2009). *Azolla* may be able to attain its maximum development rate via revolutionary separation techniques, especially as petroleum fuel costs rise and supply declines, as they have in recent years (Kumar *et al*., 2010).

Biology and physiology of *Azolla*

Azolla belongs to the Division Tracheophyta, Polypodiopsida class, and Salviniales order. It belongs to the Azollaceae family, which has two subgenera and six species. The presence or location of glochidia on tissue may also help identify *Azolla*. Rhizosperma species contain glochidia on the inner surface of *A. pinnata*, but they are missing in *Azolla nilotica*. However, identification of *Azolla* species is usually difficult owing to the lack of sporocarps (Ladha *et al*., 2000).

Azolla is found in tropical, subtropical, and warm-temperate freshwater environments. Formerly widespread in Eastern North America, and the Caribbean, *A. filiculoides* was also found in South America, Western North America and Alaska. Sculthorpe (1967) claims *A. filiculoides* was originally endemic throughout Europe but died out during the Ice Ages. There are four species of *A. microphylla*: *A. mexicana* (from Northern South America to Western North America), *A. nilotica* (from Sudan to Mozambique), and *A. pinnata* (from the remainder of Asia and the tropical African coast) (Singh and Mandal, 2000). *Azolla* macrophytes, or fronds, vary in size from 1 cm to 2.5 cm in *A. pinnata* to 15 cm or more in *A. nilotica*. It has a central rhizome that branches into secondary rhizomes with small leaves alternately positioned. Unbranched adventitious roots drop from rhizome ventral surfaces into water. The roots take nutrients directly from the water, but in shallow water they may contact the soil for nutrients. Each leaf has two lobes: a chlorophyllous dorsal lobe and a colourless, cup-shaped ventral lobe that gives buoyancy. The superior surface of the ventral lobe has vertical rows of stomata. Each dorsal lobe has a leaf cavity containing the symbiotic *A. azollae*. *Azolla* reproduces sexually, asexually, or vegetatively (Serag *et al.*, 2000).

The *Azolla-Anabaena* symbiosis

In its lifetime, *Azolla* forms symbiotic relationships with cyanobacteria. *Anabaena* belongs to the Phylum-Cyanobacteria, Order- Nostocales, and Family-Nostocaceae members. *A. azollae* was previously known as *Nostoc*. Unbranched trichomes contain three types of cells: vegetative cells, wide and bead-like, highly pigmented; heterocysts, larger than vegetative cells and with thick walls; and akinetes, thick-walled and resting spores. Sanginga and Van Hove (1989) describe the structure of the cyanobacterium *A. azollae*. But reports of a free-living culture of nitrogen-fixing cyanobacteria isolated from various *Azolla* species have been published previously. Although the use of a symbiont-free *Azolla* and a cultured *Anabaena* or *Nostoc* isolate to replicate the link between the related *A. azollae* and these isolates has been hypothesized, it has never been demonstrated. To meet the symbiotic *Anabaena*'s total nitrogen requirement, the nitrogenase enzyme is activated, which breaks down atmospheric nitrogen. Nitrogen comprises 3–6% of the association's dry weight (Zahran *et al*., 2007).

As a result of the *Azolla* application, the soil's microbial health has improved. Small-scale rice farming relies on the mineralization of organic nitrogen to ammonia, which is essential. Among other things, the C:N ratio affects mineral formation rate. In contrast, *Azolla* with a high C:N ratio mineralized in 5 days (van Der Heide *et al*., 2006). The *Azolla-Anabaena* symbiosis is unique in that both the eukaryotic *Azolla* and the bacterial *Anabaena* can fix CO_2. A chemical reaction occurred when $^{14}CO_2$ was introduced into *A. azollae* leaf cavities. No indication of cyanobacterial photosynthesis using ^{14}C-sucrose was discovered. Serag *et al.* (2000) say that *A. azollae* may be able to use either photoheterotrophic or myotropic metabolism, with the sucrose made by the fern acting as a source of less carbon.

Azolla as an input in organic agriculture

Insufficient N may hinder plant growth and development. Nutrients may also improve root volume, area, diameter, total and main root length, and dry mass output and also enhance nutrient uptake, balance, and dry mass production. Soil organic carbon content increased with organic nutrient management, which included *Azolla* inoculation. Compared to chemical fertilizers, organic management increases soil physical features, including soil available water capacity (AWC) and water retention capacity (WRC) (Goyal *et al*., 2005). AWC increased as micro- and microporosity increased. *Azolla-Anabaena* may be used as a biofertilizer for crops such as rice, wheat, and others. Using biofertilizers instead of chemical fertilizers has many benefits.

(A) *Azolla* uses widely available solar energy, nitrogen from the air, and water to enrich the soil. It is quite inexpensive.
(B) Besides delivering nitrogen for crops, it also gives vitamins and growth hormones to animals.
(C) Unlike chemical fertilizers, which are made from petroleum, organic fertilizers are made from sustainable resources. So biofertilizers are non-polluting in nature.

There are three main methods for administering *Azolla* to crops. The crop may be planted as a monocrop during the fallow season, then cultivated and worked into the soil before planting the target crop. Second, *Azolla* may be sown as an intercrop between crops. *Azolla* fonds may be collected from ponds, swamps, or flooded fields and applied to a variety of target crops, either as a soil amendment or as a mulch around the roots of the agricultural plants. Sometimes, a combination of these methods is utilized (Rai (2009)). These fertilizers assist in speeding up microbial activity while also supplying enough nutrition levels to the soil and the plants they feed. While the mixture is still liquid, add more nutrients, cell protectants, and inducers for cell, spore, and cyst formation. Biofertilizer also boosts hormones, vitamins, auxins, and other growth-promoting substances (Setiawati *et al*., 2018).

The utilization of *Azolla* on rice and other crops

Given its ability to grow in flooded rice fields, *Azolla* is widely being used as a biofertilizer for rice fields, where it has been shown to significantly increase nitrogen content within weeks of inclusion. In fact, *Anabaena* and *Azolla* alter the physical, chemical, and biological characteristics of the soil and soil-water interface in rice fields, affecting agronomic productivity (Mischler *et al*., 2014). The reduction of NH_3 volatilization losses and the suppression of weeds under *Azolla* cover are all key impacts that may benefit rice crops (Raja *et al*., 2012). *Azolla* reduces soil pH and water temperature, inhibits NH_3 evolution, and inhibits weed and mosquito growth. One crop of *Azolla* can fix 20-40 kg Nha^{-1} into a rice crop in around 20-25 days, while the *A. azollae* system can fix 1.1 kg N ha^{-1} per day. The usage of *Azolla* with urea has increased urea efficiency (Bhuvaneshwari and Singh, 2015).

Depending on the circumstances, intercropping may be integrated into the mud or left to die naturally due to fungal rot or light deprivation (Bocchi and Malgioglio, 2010). However, a variety of applications is usually advised. Growing *Azolla* as a monocrop, incorporating it into the rice field, and then planting it as an intercrop with one or more additional incorporations after the rice is transplanted can benefit the rice. *Azolla*'s benefits as a green manure in rice farming have been debated recently (Kollah *et al*., 2016). *Azolla* dual cropping has a variety of effects on CH_4 emissions, most of which are due to soil physical-chemical regulation. There has been much research conducted on the influence of *Azolla* on methane emissions in paddy fields, (Bharati *et al*., 2000), double rice (Ying *et al*., 2000), and triple rice (Ying *et al*., 2012). However, there is no agreement on how *Azolla* affects methane emissions in rice fields.

Azolla must be used heavily just before the rice planting season to prepare the fields. This is done in nursery expansions, canals, ponds, and fields. During multiplication, *Azolla* mats must be regularly partitioned to avoid competition for light, space, and nutrients. *Azolla*'s high biomass production rate improves soil physical structure by providing a large amount of organic matter when planted. *Azolla* also solubilizes zinc, iron, and manganese for the rice crop. This process helps rice crop growth by releasing plant hormones and vitamins. It stops water from evaporating, stops illness, stops weeds from growing, helps plants bloom and bear fruit, and helps seedlings and transplants get established quickly and stay alive (Biswas *et al*., 2005). Dual cropping with green *Azolla* at 500 kg/ha increases soil nitrogen by 50 kg/ha and reduces nitrogen requirements by 20–30 kg/ha. Compared to other approaches, *Azolla* increases rice output by 20–30%.

Table 1. Comparative assessment of organic carbon, N, P in soil, yield, and Harvest index percentages from rice field under different treatments of *Azolla*

Azolla Species	Treatment	Organic carbon %	N in soil %	P in soil %	Yield %	Harvest index %	References
A. pinnata	5 tons/ha of compos powder *Azolla*	2.78	2.20	0.24	64.96	NR	(Setiawati *et al*., 2018)
A. pinnata	2.5 ton/ha of compos powder *Azolla*	2.75	2.18	0.25	57.75	NR	(Setiawati *et al*., 2018)
A. pinnata	20 ton/ha of fresh *Azolla*	2.80	2.04	0.26	65.61	NR	(Setiawati *et al*., 2018)
A. pinnata	10 ton/ha of fresh *Azolla*	2.76	2.01	0.22	52.27	NR	(Setiawati *et al*., 2018)
A. filiculoides	30g of dry *Azolla* + 2g of fresh *Azolla*	11.79	5.88	NR	17.38	43.3	(Cheng *et al*., 2015)
***Azolla* sp.**	7.5 ton/ha of *Azolla* in short rain season	1.56	0.13	22.99	10.25	NR	(Oyange *et al*., 2019)
***Azolla* sp.**	15 ton/ha of *Azolla* in short rain season	1.49	0.13	31.14	12.82	NR	(Oyange *et al*., 2019)
***Azolla* sp.**	7.5 ton/ha of *Azolla* in long rain season	1.72	0.15	21.37	11.53	NR	(Oyange *et al*., 2019)
***Azolla* sp.**	15 ton/ha of *Azolla* in long rain season	1.71	0.16	26.89	15.38	NR	(Oyange *et al*., 2019)
***Azolla* sp.**	1 ton/ha of *Azolla*	NR	28.57	NR	16.27	NR	(Bahadur *et al*., 2015)
A. pinnata	*A. pinnata* in wet season	0.76	0.095	1.68	76.21	NR	(Satapathy, 1999)
A. pinnata	*A. pinnata* in dry season	0.80	0.099	20.0	53.6	NR	(Satapathy, 1999)
A. pinnata	2 ton/ha of fresh *Azolla* before transplanting + 0.5 ton/ha of fresh *Azolla* after transplanting	0.76	0.08	12.5	63.38	NR	(Singh, 1988)
A. microphylla	60% of the total biomass of *A. microphylla* was submerged before transplanting and remaining 40% of *A. microphylla* was allow to grow after transplanting	NR	2.07	NR	17.97	47.7	(Ventura *et al*., 1987)

Azolla released nitrogen more slowly than chemical fertilizer, which released 87% of its nitrogen in 10 days. So, using *Azolla* with chemical fertilizers is good for rice farming (Raja *et al*., 2012). Watanabe *et al*. (1988) discovered that inoculating rice fields with phosphorus-enriched Azolla can multiply 5-7 times before becoming phosphorus deficient. This was followed by 3 days of harvesting the Azolla for use as a rice fertilizer. Adding phosphorus to the field once or twice every two weeks for two weeks after inoculation boosted the *Azolla's* biomass production. *A. pinnata* may also be utilized as a biofertilizer on acidic soils in Kerala (Farahpour-Haghani *et al*., 2017). Table 01 shows the comparative assessment of organic carbon, N, and P in soil, yield, and harvest index percentages from rice field under different treatments of *Azolla.*

Phytoremediation and Biosorption of metals and compounds by *Azolla*

Aquatic macrophytes can treat wastewater better than terrestrial plants because they grow faster and produce more biomass. They can also purify water better than terrestrial plants and can take in more pollutants than terrestrial plants. They affect water quality by controlling the amount of oxygen in the water, the cycle of nutrients, and the collection of heavy metals in the water column (Dhote *et al*., 2009).

As an environmental remediation resource, *Azolla* can absorb heavy metals like chromium and nickel, as well as cadmium, copper, and uranium. *Azolla* shows resistance to metal ions and concentration capacity, which have also been studied previously (Hegazy *et al*., 2017). The use of *Azolla* sp. in combination with other aquatic plants to clean wastewater has also been researched earlier. There is very little progress in using *Azolla* biomass to remove heavy metals via passive methods like *Azolla* biofilters or *Azolla* biomatrix. The *Azolla* biomatrix can be used to remove harmful heavy metals and concentrate valuable metals (Sood *et al*., 2012). Awodun (2008) studied the impact of *Azolla* on soil physiochemistry soil pH, organic matter, N, P, K, Ca, Mg, and Na and found higher values with decreasing soil bulk density.

Using *A. pinnata* as a model, Rai (2008) demonstrated that the plant can remove 70–94 percent of heavy metals from ash slurry and chlor-alkali effluent in the Singrauli district of Uttar Pradesh, India and that heavy metal concentrations in *A. pinnata* tissues range between 310 and 740 mg Kg^{-1}, depending on the species. Color removal from textile effluent may be achieved by biological methods such as biosorption, bioaccumulation, and biodegradation. Bacteria, fungi, and algae are important biosorbents for color removal (Aksu and Tezer, 2005). *Azolla* is a metal-collecting aquatic free-floating fern with substantial phytoremediation and biosorption capability. Table 02 shows some heavy metal bioaccumulation by various *Azolla* species.

Table 2. Heavy metal bioaccumulation by various *Azolla* species

Azolla species	Pollutant	Duration	Initial concentration	Removal rate	References
A. pinnata	Cr	28 Days	50 ppm	63 %	Pandharipande and Gadpayle (2016)
	Cu	28 Days	10 ppm	6 %	Pandharipande and Gadpayle (2016)
	Cr (VI)	13 Days	3 ppm	88 %	Rai (2009)
	Hg	13 Days	1 ppm	95 %	Rai (2008)
	Cd	13 Days	1 ppm	91 %	Rai (2008)
	Cr	7 Days	2 ppm	98 %	Mandakini *et al*., (2016)
	Ni	7 Days	2 ppm	57 %	Mandakini *et al*., (2016)
	Cd	7 Days	0.5 ppm	88 %	Mandakini *et al*., (2016)
	Pb	7 Days	8 ppm	86 %	Mandakini *et al*., (2016)
	Hg	21 Days	10 ppb	68 %	Mishra *et al*., (2009)
A. caroliniana	Hg (II)	12 Days	1 ppm	93 %	Banach *et al*., (2012)
	Pb (II)	12 Days	0.5 ppm	90 %	Banach *et al*., (2012)
	Cd (II)	12 Days	1 ppm	22 %	Banach *et al*., (2012)
	Cr (III)	12 Days	0.1 ppm	91 %	Banach *et al*., (2012)
	Cr (IV)	12 Days	0.1 ppm	100 %	Banach *et al*., (2012)
	Pb (II)	4 Days	0.1 ppm	90 %	Stepniewska *et al*., (2005)
	Cd (II)	4 Days	1 ppm	22 %	Stepniewska *et al*., (2005)
A. filiculoides	Cd	15 Days	15 ppm	93 %	Naghipour *et al*., (2018)
	Ni	15 Days	25 ppm	77 %	Naghipour *et al*., (2018)
	Pb	15 Days	15 ppm	97 %	Naghipour *et al*., (2018)
	Ni	18 Hours	9 ppm	40 %	Sela *et al.*, (1989)
	Cd	18 Hours	9 ppm	62 %	Sela *et al.*, (1989)
	Cu	18 Hours	9 ppm	50 %	Sela *et al.*, (1989)
A. microphylla	Ni	15 Days	1.5 ppm	52 %	Biswas *et al*., (2021)
A. imbricata	Cd	9 Days	0.5 ppm	37 %	Diaz *et al*., (2006)

Table 3. Literature on heavy metal biosorption by *Azolla*

Azolla Species	Type of bio-sorbent	Compound/ Metal	Operating condition			Uptake (mg/ g)	Removal %	References
			Temperature	pH	Time			
A. filiculoides	Native	2,4,6- Trichlorophenol	NR	3	120 min	NR	95 %	Zazouli *et al*., (2013)
A. filiculoides	Native	Acid blue 15	NR	7	12 h	116.28	NR	Padmesh *et al*., (2006)
A. imbricata	Modified (Ai-dp)	U (VI)	303 K	2	1.5 h	3.90	97.61 %	Liu *et al*., (2021)
***Azolla* sp.**	Native	Sr^{+2}	NR	10	1 h	NR	99.9 %	Cohen-Shoel *et al*., (2002)
A. pinnata	Chemically modified (treated with 0.01M $Na_2EDTA.\ 2H_2O$)	Ni	NR	7	1 h	28.22	NR	Badawy *et al*., (2020)
		Al	NR	3	1 h	28.46	NR	
		Cu	NR	5	1 h	39.67	NR	
		Pb	NR	5	1 h	41.27	NR	
		Fe	NR	3	1 h	35.64	NR	
		Cd	NR	5	1 h	21.70	NR	
A. filiculoides	Native	Red 198 (RR198)	NR	7	90 min	12.2	97.3 %	Zazouli *et al*., (2013)
A. filiculoides	Native	Cr (VI)	25 ^{0}C	2	9 h	203	20.3 %	Ahmady- Asbchin *et al*., (2015)
A. filiculoides	Native	Acid red 88 (AR88)	NR	7	12 h	109.0	NR	Padmesh *et al*., (2005)
		Acid green 3 (AG3)	NR	3	12 h	133.5	NR	
		Acid orange 7 (AO7)	NR	3	12 h	109.6	NR	
A. rongpong	Native	Acid red 88 (AR88)	30 ^{0}C	2.5	12 h	81.30	NR	Padmesh *et al*., (2006)
		Acid green 3 (AG3)	30 ^{0}C	2.5	12 h	83.33	NR	
		Acid orange 7 (AO7)	30 ^{0}C	2.5	12 h	76.92	NR	
		Acid blue 15 (AB15)	30 ^{0}C	2.5	12 h	76.34	NR	
A. filiculoides	Native	Au	NR	2	5 h	98	98.2 %	Umali *et al*., (2006)
A. filiculoides	Native	Rhobamine B (RMB)	30 ^{0}C	5	4 h	91.8	NR	Padmesh *et al*., (2008)
		Methylene blue (MB)	30 ^{0}C	5	4 h	116.7	NR	

The experimental conditions (pH, temperature) utilized in various studies make it impossible to directly compare the *Azolla* biosorption potential to other macrophytes. The observed variability while using the same *Azolla* sp. for the same metal may be explained by the use of varied experimental setups. This might be owing to the biomass being processed or chemically altered to boost biosorbent characteristics, or the testing conditions being different (Table 3). Lead removal was maintained at 90% between 10 and 50 ℃, while biomass had no influence on lead removal at any temperature (Sanyahumbi *et al*., 1998). Fogarty *et al*. (1999), investigated the removal of Cu from *A. filiculoides* biomass after processing and immobilization of the biomass.

The researchers say *Azolla* based systems have higher biosorption capabilities than similar biomass systems and are compatible with commercial sorbent exchange values. They discovered that epichlorohydrin-immobilized *Azolla* removed more Cu than milled-sieved *Azolla* and untreated *Azolla*, indicating that this method is more effective. At 60% biomass saturation, the most Ni was taken up, while the most Ni was taken up in batch experiments was 43.3 mg g-1 (Zhao and Duncan, 1998).

***Azolla* as a livestock feed**

Protein, essential amino acids, vitamins (A, B12, Beta- carotene), growth promoter intermediates, trace elements, and minerals are abundant in *Azolla* (Bamidele and Nyamali, 2008). By dry weight, *azolla* contains 25-35 percent protein, 10-15% minerals, and 7-10% amino acids, bioactive compounds, and biopolymers. *Azolla* is low in carbohydrates and fat, making it a nutritious snack. The bio -composition of *Azolla* makes it one of the most cost-effective and efficient cow feed choices. *Azolla* is also readily digestible by animals because of its high protein content and low lignin level (Bhatt *et al*., 2020a).

When cultivated in favorable conditions, all *Azolla* strains contain a balanced combination of essential amino acids and high-quality protein (Table 4). By using its nutrients more efficiently than ruminants, *Azolla* has lower amounts of acid detergent fiber (ADF) and neutral detergent fiber (NDF). *Azolla* is an excellent source of plant proteins, pro-vitamin A, carotenoids, and lutein (Kathirvelan *et al*., 2015). The outcomes of *Azolla* as a fish feed as alternative experiments have also been reported (Lejeune *et al*., 2000) (List in table 5). Rawat *et al*. (2015) found a 11.85% increase in milk output when *Azolla* was supplemented with concentrate in a 1:1 ratio in crossbred cows. Sharma (2012) studied the effects of *Azolla* (*A. microphylla*) supplementation on milk production and milk quality in crossbred bovine animals.

Table 4. Nutrient's composition of different *Azolla* species (Kathirvelan *et al*., 2015, Gupta *et al*., 2018)

Nutritional Content	*A. caroliniana*	*A. microphylla*	*A. pinnata*
Crude protein (%)	23.07	23.69	17.59
Crude fiber (%)	13.19	15.02	16.54
Total Ash (%)	29.17	28.71	25.28
Dry matter %	NR	NR	90.00±0.77
Organic matter %	NR	NR	81.05±0.44 04
Ether extract %	NR	NR	3.25±0.76 05
Calcium (%)	2.07	2.07	1.67
Phosphorus (%)	0.59	0.77	0.46
Iron (%)	0.269	0.249	0.231
Manganese (%)	0.238	0.274	0.205
Sodium (%)	1.240	0.488	0.777
Potassium (%)	2.44	4.93	2.19
Copper (ppm)	16.37	17.55	15.90
Zinc (ppm)	64.51	71.75	46.77
Magnesium (ppm)	0.15	0.173	0.155
Moisture (%)	5	5	5

Table 5. *Azolla* feeding regime for different animals

Animal	Weight gain	Feed intake	References
Nera brown pullets	91.4-101.7 g/bird/week	653-708 g/bird/week	Alalade *et al*., (2007)
Hariana heifers	643.52 g /bird/day	4.20 kg/day	Roy *et al*., (2016)
Boiler ration	1394.33-1637.00 g/bird/week	896.67-981.33 g/bird/week	Basak *et al*., (2002)
Sheep/Goat	300-500 g/animal	NR	Chander *et al*., (2011)
Rabbit	100 g/animal	NR	Chander *et al*., (2011)
Buffalo calves	240-294 g/animal	3.21-2.91 kg/100 kg	Indira *et al*., (2009)
Cattle	8-10 % of animal meat	NR	Kumar and Chander *et al*., (2017)
Goat	10-15 % of milk production	NR	Kumar and Chander *et al*., (2017)
Poultry	10-15 % of egg laying capacity	NR	Kumar and Chander *et al*., (2017)
Pig	0.704-0.784 kg/animal	10.5-12.6 kg/animal	Leterme *et al*., (2009)
Fish	5-10 % of fish	NR	Shiomi and kitoh (2001)

The *Azolla* supplementation increased milk and FCM yields by 11.2% and 12.5%, respectively. He further reported that supplementing *Azolla* 2k/day enhanced feed conversion efficiency (kg DMI / kg FCM yield) in crossbred animals. At the same time, *Azolla* feeding improved hair coating, eye brightness, nose moisture, and kept the animal active. Bhatt *et al*. (2020b) tested Sahiwal female calves divided into three groups (T0, T1 and T2) for 90 days. This group was fed as per ICAR 2013 feeding standards, whereas groups T1 and T2 were fed by replacing 15% and 30% total protein of concentrate with *A. pinnata* on DM basis. The average daily live-weight gain (ADG) was greater in T2 (0.456 ± 0.01kg/day) than in T1 (0.431 ±0.01 g/day) and least in T0 (0.411 ± 0.02) and the difference was significant statistical (P<0.05).

Naghshi *et al*. (2014) examined the effects of *A. pinnata* meal on broiler chick performance and carcass parameters. Compared to other diets, chickens given 5% *Azolla* powder showed considerably higher daily weight gain and feed conversion percentage in all raising stages. Thus, diets with 5% *Azolla* had the lowest feed intake, maximum weight gain, and lowest feed conversion ratio. Mandal *et al*. (2012), discovered that digested *A. pinnata* may be utilized as a fertilizer in fish ponds to improve phytoplankton levels. The use of standard fish feed in a 4:1 ratio with digested *Azolla* mixture promotes the fastest development. *A. pinnata* supplementation in the diets of fingerling and adult Ile tilapia, *Oreochromis niloticus* L., inhibited growth. With good results, *Azolla* has also been used to replace soybean meal in the diets of juvenile black tiger shrimp (*Penaeus monodon*) (Sudaryono, 2006). Abou-Zeid *et al*. (2001) observed that adding 25% sun-dried *Azolla* protein to soybean meal protein maintained feed conversion, weaning litter size, and female weight, but decreased conception rate, birth litter size, and milk output in female mating rabbits.

***Azolla* as the feedstock for biofuel production**

Due to their unique chemical composition, *Azolla* species are intriguing as feedstock for various biofuels. With the use of *A. filiculoides* grown in wastewater as a bio-oil source, up to 33% of biodiesel may be produced without the need for glycerin (Muradov *et al*., 2014). The organic content of the bio-oils was determined by GC-MS. (Table 6) shows that *A. filiculoides* bio-oils included a complex mixture of aromatic and unsaturated hydrocarbons, alkanes, alkenes, phenolic compounds, and alcohols (Huggins, 2007). Golzary *et al*., (2021) found *Azolla* contains 11.7 percent lipids, including palmic acid and unsaturated fatty acids including linoleic acid (ω 6), linolenic acid (ω 3), and oleic acid. These fatty acids make up 27.11, 14.23, 5.58, and 32.8 percent of the total fatty acids in *Azolla*.

Table 6. Main compounds detected in the bio-oils of *A. filiculoides* (Pourkarimi *et al*., 2021)

Formula	Component	Wt. %
C_5H_5N	Pyridine	1.6
C_7H_8	Toluene	3.1
$C_5H_6O_2$	2-furan methanol	2.3
C_6H_7N	3-methyl pyridine	1.6
C_8H_{10}	Ethyl benzene	2.0
C_6H_{10}	3-methyl-2-cycloPentene	0.8
C_8H_7N	Indole	7.6
C_9H_{12}	1-Ethyl-3-methylbenzene	1.75
C_9H_{12}	1,2,3-trimethylbenzene	1.2
$C_6H_8O_2$	3-methyl, 1,2-cyclopentanedione	1.5
C_7H_{12}	2,3-Dimethyl-3-cycloPentene	0.7
C_6H_6O	Phenol	15.5
C_7H_8O	2-Methyl phenol	4.4
C_7H_8O	p-Cresol	11.2
$C_7H_8O_2$	2-Methoxyphenol	0.7
C_5H_5NO	3-Pyridinol	2.6
$C_8H_{10}O$	2-Ethyl phenol	1.00
$C_8H_{10}O$	2,4-Dimethyl phenol	2.7
$C_8H_{10}O$	2,5-Dimethyl phenol	1.6
$C_8H_{10}O$	4-Ethyl phenol	3.1
$C_8H_{10}O$	3,5-Dimethyl phenol	2.0
$C_6H_6O_2$	Catechol	9.2
$C_9H_{10}N_2$	2-Ethyl benzimidazole	1.7
$C_{11}H_{10}$	2- Methyl naphthalene	1.3
$C_7H_8O_2$	4-Methyl-1,2-benzenediol	4.42
$C_{13}H_{26}$	1-Tridecene	1.1
$C_{14}H_{28}$	2-Tetradecene	1.6
$C_{16}H_{34}$	Hexadecane	1.6
$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	1.0
$C_{19}H_{40}$	Nanodecane	1.5

The water-rich *A. pinnata* was pyrolyzed in a glass reactor with a nitrogen carrier gas flow rate of 50 mL/min and a heating rate of 25 per minute by Biswas *et al*. (2017). At 400 ℃, they got 38.5 percent bio-oil production. An estimate of 9.3 t/ha of potential ethanol production from *A. filiculoides* is lower than that from sugarcane but equivalent to that from maize stover and greater than that from miscanthus. For *A. filiculoides*, Pourkarimi *et al*. (2021) reported the highest bio-oil yields of 30.83% (at 461 ℃, 0.5 L/min nitrogen flow rate, and 20 ℃ per minute heating rate) and 34.29% (at 500 ℃, 0.2 L/min nitrogen flow rate, and 10 ℃ per minute heating rate).

Conclusion

The world's environment may be conserved or enhanced by using *Azolla* as a biofertilizer and reducing or eliminating commercial fertilizers. These materials may have additional human uses. This unique natural resource needs further research. The *A. azollae* symbiosis increases nitrogen input in rice growing, which boosts yield. Nitrogen-fixing microorganisms boost rice yields. Better extension is needed to encourage the use of biofertilizers, which offer benefits. Scientists and farmers must promote *Azolla* as a rice biofertilizer.

Rapid growth, large biomass output, broad root system, easy harvesting, and resistance to heavy metals make it excellent for phytoremediation. *Azolla* is metal-resistant. Using *Azolla* biomass as a bioenergy source or bio-ore for heavy metal recovery may be an integrated method. Findings suggest dried *Azolla* may absorb dye-containing effluents.

Protein-rich *Azolla* is animal feed. *Azolla* helps animals and birds gain weight and produce milk and eggs. *Azolla's* biomass can be utilized as animal feed, compost for organic farming and kitchen gardening, or bioethanol. *Azolla* species are the most attractive, sustainable, and universal feedstock for a wide range of renewable biofuels due to their high productivity, ability to grow on wastewater, and unique chemical makeup. Macroalgae are a cheap and abundant raw material for biodiesel production.

Acknowledgments

Financial assistance given by World Bank Group through the project, Accelerating Higher Education Expansion and Development Operation (AHEAD), DOR, Grant No 79, Rajarata University of Sri Lanka, is highly appreciated.

References

Abou-Zeid, A., Mohamed, F. and Radwan, M. (2001). Assessment of the nutritive value of dried *Azolla* hay as a possible feed ingredient for growing NZW rabbits. Egyptian Journal of Rabbit Science, 11:1-21.

Ahmady-Asbchin, S., Safari, M. and Varposhti, M. (2015). Biosorption optimization of Cr (VI) using response surface methodology and thermodynamics modeling onto *Azolla filiculoides*. Separation Science and Technology, 50:554-563.

Aksu, Z. and Tezer, S. (2005). Biosorption of reactive dyes on the green alga *Chlorella vulgaris*. Process Biochemistry, 40:1347-1361.

Alalade, O. and Iyayi, E. (2006). Chemical composition and the feeding value of *Azolla* (*Azolla pinnata*) meal for egg-type chicks. International Journal of Poultry Science, 5:137-141.

Arora, A., Saxena, S. and Sharma, D. (2006). Tolerance and phytoaccumulation of chromium by three *Azolla* species. World Journal of Microbiology and Biotechnology, 22:97-100.

Awodun, M. (2008). Effect of *Azolla* (*Azolla* species) on physiochemical properties of the soil.World Journal of Agricultural Sciences, 4:157-160.

Badawy, R. K., Shehata, S. M., Aboulsoud, Y. I. (2020) Assessment of phyto-filtration and biosorption treatment on the removal of contaminant form wastewater. Natural Sciences, 18:16-26.

Bahadur, S., Verma, S., Prasad, S., Madane, A., Maurya, S., Gaurav, V. V. and Sihag, S. (2015). Eco-friendly weed management for sustainable crop production-A review. Journal of Crop Weed, 11:181-189.

Bamidele, J. and Nyamali, B (2008). Ecological studies of the Ossiomo river with reference to the macrophytic vegetation. Research Journal of Botany, 3:29-34.

Banach, A. M., Banach, K. and Stepniewska, Z. (2012). Phytoremediation as a promising technology for water and soil purification: *Azolla caroliniana w*illd. as a case study. Acta Agrophysica, 19.

Basak, B., Pramanik, M. A. H., Rahman, M. S., Tarafdar, S. U. and Roy, B. C. (2002). *Azolla* (*Azolla pinnata*) as a feed ingredient in broiler ration. International Journal of Poultry Science, 1:29-34.

Bennicelli, R., Stępniewska, Z., Banach, A., Szajnocha, K. and Ostrowski, J. (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg (II), Cr (III), Cr (VI)) from municipal waste water. Chemosphere, 55:141-146.

Bharati, K., Mohanty, S., Singh, D., Rao, V. and Adhya, T. (2000). Influence of incorporation or dual cropping of *Azolla* on methane emission from a flooded alluvial soil planted to rice in Eastern India. Agriculture, Ecosystems and Environment, 79:73-83.

Bhatt, N., Chandra, R., Kumar, S., Singh, K. and Pratap, N. (2020a). Nutritive analysis of *Azolla pinnata* and its cultivation during winter season. International Journal of Current Microbiology Applied Sciences, 9:2012-2018.

Bhatt, N., Tyagi, N., Chandra, R., Meena, D. C. and Prasad, C. (2020b). Growth performance and nutrient digestibility of *Azolla pinnata* feeding in sahiwal calves (*Bos indicus*) by replacing protein content of concentrate with *Azolla pinnata* during winter season. Indian Journal of Animal Research, 55:663-668.

Bhuvaneshwari, K. and Singh, P. K. (2015). Response of nitrogen-fixing water fern *Azolla* biofertilization to rice crop. 3 Biotech, 5:523-529.

Biswas, B., Kumar, A. A., Bisht, Y., Krishna, B. B., Kumar, J. and Bhaskar, T. (2021). Role of temperatures and solvents on hydrothermal liquefaction of *Azolla filiculoides*. Energy, 217:119330.

Biswas, M., Parveen, S., Shimozawa, H. and Nakagoshi, N (2005). Effects of *Azolla* species on weed emergence in a rice (*Oryza sativa*) paddy ecosystem. Weed Biology and Management, 5:176-183.

Biswas, B., Singh, R., Krishna, B. B., Kumar, J. and Bhaskar, T. (2017). Pyrolysis of *Azolla*, *Sargassum tenerrimum* and *Water hyacinth* for production of bio-oil. Bioresource Technology, 242:139-145.

Bocchi, S. and Malgioglio, A. (2010). *Azolla-Anabaena* as a biofertilizer for rice paddy fields in the Po Valley, a temperate rice area in Northern Italy. International Journal of Agronomy.

Chander, M., Bodapati, S., Mukherjee, R. and Kumar, S. (2011). Organic livestock production: an emerging opportunity with new challenges for producers in tropical countries. Revue scientifique et technique (International Office of Epizootics), 30:569-583.

Cheng, W., Okamoto, Y., Takei, M., Tawaraya, K. and Yasuda, H. (2015). Combined use of *Azolla* and loach suppressed weed Monochoria vaginalis and increased rice yield without agrochemicals. Organic Agriculture, 5:1-10.

Cohen-Shoel, N., Barkay, Z., Ilzycer, D., Gilath, I. and Tel-Or, E. (2002). Biofiltration of toxic elements by *Azolla* biomass. Water, Air, and Soil Pollution, 135:93-104.

Dawar, S. and Singh, P. (2002). Comparison of soil-and nutrient-based medium for maintenance of *Azolla* cultures.Journal of plant nutrition, 25:2719-2729.

Dhote, S. and Dixit, S. (2009). Water quality improvement through macrophytes—a review. Environmental Monitoring And Assessment, 152:149-153.

Diaz, C., Saliba-Colombani, V., Loudet, O., Belluomo, P., Moreau, L., Daniel-Vedele, F., Morot-Gaudry, J.-F. and Masclaux-Daubresse, C. (2006). Leaf yellowing and anthocyanin accumulation are two genetically independent strategies in response to nitrogen limitation in *Arabidopsis thaliana*. Plant and Cell Physiology, 47:74-83.

Farahpour-Haghani, A., Hassanpour, M., Alinia, F., Nouri-Ganbalani, G., Razmjou, J. and Agassiz, D. (2017). Water ferns *Azolla* spp.(Azollaceae) as new host plants for the small China-mark moth, *Cataclysta lemnata* (Linnaeus, 1758)(Lepidoptera, Crambidae, Acentropinae). Nota Lepidopterologica, 40:1-13.

Fogarty, R. V., Dostalek, P., Patzak, M., Votruba, J., Tel-Or, E. and Tobin, J. M. (1999). Metal removal by immobilised and non-immobilised *Azolla filiculoides*. Biotechnology Techniques, 13:533-538.

Golzary, A., Hosseini, A. and Saber, M. (2021). *Azolla filiculoides* as a feedstock for biofuel production: cultivation condition optimization. International Journal of Energy and Water Resources, 5:85-94.

Gouveia, L. and Oliveira, A(2009). Microalgae as a raw material for biofuels production. Journal of IndustrialMicrobiology and Biotechnology, 36:269-274.

Goyal, S., Dhull, S. and Kapoor, K. (2005). Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. Bioresource Technology, 96:1584-1591.

Gupta, S. K., Chandra, R., Dey, D., Mondal, G. and Shinde, K. P. (2018). Study of chemical composition and mineral content of sun-dried *Azolla pinnata*. Journal of Pharmacognosy and Phytochemistry, 7:1214-1216.

Hegazy, A. K., Emam, M., Lovett-Doust, L., Azab, E. and El-Khatib, A. (2017). Response of duckweed to lead exposure: Phytomining, bioindicators and bioremediation. Desalin Water Treatment, 70:227-234.

Huggins, D. (2007). Evaluation of *Azolla* plant as an alternative stock feed source. Goulburn Broken Catchment Authority, Australia.

Indira, D., Rao, K. S., Suresh, J., Naidu, K. V. and Ravi, A. (2009). *Azolla* (*Azolla pinnata*) as feed supplement in buffalo calves on growth performance. Indian Journal of Animal Nutrition, 26:345-348.

Jha, M., Prasad, A. and Misra, S. (2004). Influence of source of organics and soil organic matter content on cyanobacterial nitrogen fixation and distributional pattern under different water regimes. World Journal Of Microbiology Biotechnology Advances, 20:673-677.

Kathirvelan, C., Banupriya, S. and Purushothaman, M. (2015). *Azolla*-an alternate and sustainable feed for livestock.International Journal of Science, Environment and Technology, 4:1153-1157.

Kollah, B., Patra, A. K. and Mohanty, S. (2016). Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change. Environmental Science and Pollution Research, 23:4358-4369.

Kumar, G. and Chander, H. (2017). Study on the Potential of *Azolla pinnata* as livestock Feed Supplement for climate Change adaptation and Mitigation. Asian Journal Advanced Basic Sciences, 5:65-68.

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F. X. and Van Langenhove, H. (2010). Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. Trends in Biotechnology, 28:371-380.

Ladha, J., Dawe, D., Ventura, T., Singh, U., Ventura, W. and Watanabe, I. (2000). Long-term effects of urea and green manure on rice yields and nitrogen balance. Soil Science Society of America Journal, 64:1993-2001.

Lejeune, A., Peng, J., Le Boulengé, E., Larondelle, Y. and Van Hove, C. (2000). Carotene content of *Azolla* and its variations during drying and storage treatments. Animal Feed Science and Technology, 84:295-301.

Leterme, P., Londono, A. M., Munoz, J. E., Súarez, J., Bedoya, C. A., Souffrant, W. B. and Buldgen, A. (2009). Nutritional value of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta Mitchell*) in pigs. Animal Feed Science and Technology, 149:135-148.

Liu, C., Guo, B., Li, H., Fu, Q., Li, N., Lin, Y. and Xu, G. (2021). *Azolla* incorporation under flooding reduces grain cadmium accumulation by decreasing soil redox potential. Scientific Reports, 11:1-10.

Mandakini, L., Bandara, N. and Gunawardana, D. (2016). Analysis of compost quality with time after mixing with different weight percentages of dried sludge from wastewater treatment plants.21 st International Forestry and Environment Symposium 2016. University of Sri Jayawardanapura.

Mandal, R., Pandey, B., Chattopadhyay, D. and Mukhopadhyay, P. (2012). *Azolla*–an aquatic fern of significance to small-scale aquaculture. Aquaculture Asia, 17:11-15.

Mischler, J. A., Taylor, P. G. and Townsend, A. (2014). Nitrogen limitation of pond ecosystems on the plains of Eastern Colorado. PLoS One, 9:e95757.

Mishra, V. K., Tripathi, B. and Kim, K. H. (2009). Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. Journal of Hazardous Materials, 172:749-754.

Muradov, N., Taha, M., Miranda, A. F., Kadali, K., Gujar, A., Rochfort, S., Stevenson, T., Ball, A. S. and Mouradov, A. (2014). Dual application of duckweed and *azolla* plants for wastewater treatment and renewable fuels and petrochemicals production. Biotechnology of Biofuels, 7:1.

Naghipour, D., Ashrafi, S. D., Gholamzadeh, M., Taghavi, K. and Naimi-Joubani, M. (2018). Phytoremediation of heavy metals (Ni, Cd, Pb) by *Azolla filiculoides* from aqueous solution: a dataset. Data In Brief, 21**:**1409-1414.

Naghshi, H., Khojasteh, S. and Jafari, M. (2014). Investigation the effect of different levels of *Azolla* (*Azolla pinnata*) on performance and carcass characteristics of cobb broiler chicks.International Journal of Farming, 3:45-49.

Oyange, W. A., Chemining'wa, G. N., Kanya, J. I. and Njiruh, P. (2019). Effects of *Azolla* biomass growth on flood water temperature and pH, tillering and yield of paddy rice. Journal of Tropical Subtropical Agroecosystems, 22.

Padmesh, T. V. N., Vijayaraghavan, K., Sekaran, G. and Velan, M. (2005). Batch and column studies on biosorption of acid dyes on fresh water macro alga *Azolla filiculoides*. Journal of Hazardous Materials, 125:121-129.

Padmesh, T. V. N., Vijayaraghavan, K., Sekaran, G. and Velan, M. (2006). Application of *Azolla rongpong* on biosorption of acid red 88, acid green 3, acid orange 7 and acid blue 15 from synthetic solutions. Chemical Engineering, 122:55-63.

Padmesh, T., Vijayaraghavan, K., Anand, K. and Velan, M. (2008). Biosorption of basic dyes onto *Azolla filiculoides*: equilibrium and kinetic modeling. Asia-Pacific Journal of Chemical Engineering, 3:368-373.

Pandharipande, S. and Gadpayle, P. (2016). Phytoremediation studies for removal of copper & chromium using *Azolla pinnata* and water hyacinth. International Journal of Innovative Research in Science, Engineering Technology, 5:7078-7083.

Pourkarimi, S., Hallajisani, A., Alizadehdakhel, A. and Nouralishahi, A. (2021). Bio-oil production by pyrolysis of *Azolla filiculoides* and Ulva fasciata macroalgae. Global Journal of Environmental Science Management, 7:331-346.

Rai, P. K. (2009). Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. Journal of Critical Reviews in Environmental Science Technology, 39:697-753.

Rai, P. K. J. C. R. I. E. S. (2008). Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*. International Journal of Phytoremediation, 10:430-439.

Raja, W., Rathaur, P., John, S. A. and Ramteke, P. (2012). *Azolla*: An aquatic pteridophyte with great potential. International Journal of Resources and Biological Sciences, 2:68-72.

Rawat, N., Kumari, K., Singh, F. and Gilhare, V. J. A. B. R. (2015). Effect of *Azolla*-supplemented feeding on milk production of cattle and production performance of broilers. Journal of Applied Biological Research, 17:214-218.

Roy, D. C., Pakhira, M. C. and Roy, M. (2016). Estimation of amino acids, minerals and other chemical compositions of *Azolla*. Advances in Life Sciences, 5:2692-2696.

Sanginga, N. and Van Hove, C. (1989). Aminoacidic composition of *azolla* as affected by strain and population density. Journal Plant and Soil, 117:263-267.

Sanyahumbi, D., Duncan, J. R., Zhao, M. and Van Hille, R. (1998). Removal of lead from solution by the non-viable biomass of the water fern Az*olla filiculoides*. Biotechnology Letters, 20:745-747.

Satapathy, K. (1999). Comparative efficiency of blue green algae, *Azolla* and other biofertilizers on growth of rice.Indian Journal of Plant Physiology, 4:100-104.

Sculthorpe, C. D. (1967). Biology of aquatic vascular plants, 610.

Sela, M., Garty, J. and Tel-Or, E. (1989). The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*. New Phytologist, 112:7-12.

Serag, M. S., El-Hakeem, A., Badway, M. and Mousa, M. (2000). On the ecology of *Azolla filiculoides* Lam. in Damietta district, Egypt. Limnologica-Ecology Management of Inland Waters, 30:73-81.

Setiawati, M. R., Damayani, M., Herdiyantoro, D., Suryatmana, P., Anggraini, D. and Khumairah, F. H. (2018).The application dosage of *Azolla pinnata* in fresh and powder form as organic fertilizer on soil chemical properties, growth and yield of rice plant. AIP conference proceedings,. AIP Publishing LLC, 030017.

Sharma (2012). Comparative nutritional evaluation & Effect of supplementation of *Azolla microphylla* on milk yield and composition in crossbred cattle. (MVSc. thesis). NDRI, Kalyani, West Bengal, India.

Shiomi, N. and Kitoh, S. (2001). Culture of *Azolla* in a pond, nutrient composition, and use as fish feed. Soil science and Plant Nutrition, 47:27-34.

Singh, P. K. (1988). Biofertilization of rice crop. In Sen SP and Palit P (eds), Biofertilizers: Potentials and problems, Plant Physiology Forum, Calcutta, India, 109.

Singh, Y. V. and Mandal, B. K. (2000). Rate of mineralization of *Azolla*, other organic materials, and urea in waterlogged soils. Tropical agriculture, 77:119-122.

Sood, A., Uniyal, P. L., Prasanna, R. and Ahluwalia, A. S. (2012). Phytoremediation potential of aquatic macrophyte, *Azolla*. Ambio, 41:122-137.

Stepniewska, Z., Bennicelli, R. P., Balakhnina, T. I., Szajnocha, K., Banach, A. and Wolin˜ska, A. (2005). Potential of *Azolla caroliniana* for the removal of Pb and Cd from wastewaters. International Agrophysics, 19:251-255.

Sudaryono, A. (2006). Use of *Azolla* (*Azolla pinnata*) meal as a substitute for defatted soybean meal in diets of juvenile black tiger shrimp (Penaeus monodon). Journal of Coastal Development, 9:145-154.

Umali, L., Duncan, J. and Burgess, J. (2006). Performance of dead *Azolla filiculoides* biomass in biosorption of Au from wastewater. Biotechnology Letters, 28:45-50.

Vafaei, F., Khataee, A., Movafeghi, A., Lisar, S. S. and Zarei, M. (2012). Bioremoval of an azo dye by *Azolla filiculoides*: Study of growth, photosynthetic pigments and antioxidant enzymes status. International biodeterioration biodegradation, 75:194-200.

Van der Heide, T., Roijackers, R. M., Van Nes, E. H. and Peeters, E. T. (2006). A simple equation for describing the temperature dependent growth of free-floating macrophytes. Aquatic Botany, 84:171-175.

Ventura, W., Mascariña, G. B., Furoc, R. E. and Watanabe, I. (1987). *Azolla* and *Sesbania* as biofertilizers for lowland rice. Philippines Journal of Crop Science, 12:61-69.

Wagner, G. (1997). *Azolla*: a review of its biology and utilization. The Botanical Review, 63:1-26.

Watanabe, I., Lapis, M. T., Oliveros, R. and Ventura, W. (1988). Improvement of phosphate fertilizer application to *Azolla*. Soil Science and Plant Nutrition, 34:557-569.

Ying, J.-Z., Shan, J.-X., Gao, J.-P., Zhu, M.-Z., Shi, M. and Lin, H.-X (2012). Identification of quantitative trait loci for lipid metabolism in rice seeds. Molecular Plant, 5:865-875.

Ying, Z., Boeckx, P., Chen, G. and Van Cleemput, O (2000). Influence of *Azolla* on CH 4 emission from rice fields. Methane emissions from major rice ecosystems in Asia. Springer.

Zahran, H., Abo–Ellil, A. and Al Sherif, E. (2007). Propagation, taxonomy and ecophysiological characteristics of the *Azolla-Anabaena* symbiosis in freshwater habitats of Beni-Suef Governorate (Egypt). Egyptian Journal of Biology, 9.

Zazouli, M. A., Balarak, D. and Mahdavi, Y. J. I. J. O. H. S. (2013). Application of *Azolla* for 2-chlorophenol and 4-chrorophenol removal from aqueous solutions. Iranian Journal of Health Sciences, 1:43-55.

Zhao, M. and Duncan, (1998). Bed-Depth-service-time analysis on column removal of Zn^{+2} using *Azolla filiculoides*. Biotechnology Letter, 20:37-39.

(Received: 21 February 2022, accepted: 30 July 2022)