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Azolla: A Boon or Bane

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Abstract

The increasing levels of heavy metals in the environment, their entry into the food chain and the overall health effects on people who consume fish are of the major concern to researchers in the field of food and nutrition, because heavy metal toxicity is a result of long term, low level exposure to pollutants through air, water and numerous consumer food products. Heavy metals are environmental pollutants and their toxicity is a problem in increasing significance of ecological, evolutionary and nutritional reasons. They are ubiquitous in environment and higher concentrations poses serious threat to the aquatic ecosystem.

Keywords: *Azolla* species; Food chain; Pollutants; Environment

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Prologue

The aquatic free-floating fern, *Azolla* - universally called by names, 'mosquito fern', 'fairy moss', 'aquatic fern'. Its diameter is about 1-3 cm (**Figure 1**). The genus belongs to the family *Salvinaceae* which grows in association with the blue-green algae-a symbiotic partner *i.e.*, *Anabaena azollae*. Family *Salviniales* constitute of 2 genera: *a. Salvinia* (approx. 12 species) and *b. Azolla* (approx. 7 species) as shown in **Table 1**.

It is having a capability to double its biomass within 3-5 days. Compared to other aquatic macrophytes, the protein content of *Azolla* is higher with all essential amino acids which are higher to wheat bran, maize, *offals*, etc. One of the interesting facts is that, *Azolla* plant contains probiotics and biopolymers and under some unfavourable conditions, an anthocyanin pigment gives the fern a reddish-brown colour (**Figure 2**).

Azolla as a Fish Nourish

Azolla is very common in rice fields, natural ponds and marshy ditches. It is considered as potential in fish culture, especially in integrated culture of rice cum fish systems. Increased fish production can be camouflaged in integrated rice with fish cultivation. Increased production of fish faeces from with *Azolla* fodder may be directly consumed by bottom dwellers in addition to being used as an organic (nitrogenous) fertilizer to increase overall pond productivity.

Conversely, it should be unspoken that the contribution of *Azolla* to aquaculture is interesting and alone could not ensure high

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productivity. It can be a useful supplement to natural feed in low-input aquaculture. It has attracted attention as a nitrogenous fertilizer and as a source of dietary nitrogen for herbivorous fish and livestock. Therefore, it is an excellent inexpensive feed for fishes. Dried and processed *Azolla* has been experimented as feed ingredient to a number of fish species (e.g. tilapia, carps, ornamental fishes, turtles etc.) for their effect on growth and yield. In the similar manner, fresh *Azolla* as a complete diet for high-density cage culture may not be economically viable. However, *Azolla* may be useful in low density and low input cage culture.

The high rates of decomposition of *Azolla* make it a suitable substrate for enriching the detrital food chain or for microbial processing such as composting, prior to application in ponds. 'Rice-fish-*Azolla*' integration increase the yield of both rice and fish compared to rice-fish culture alone. The probable reasons for the increase in rice yield are improved soil fertility resulting from the augmented production of fish faeces from *Azolla*



Figure 1 Azollaplant.

Table 1. Various species of Azolla.

<i>Salvinia</i> species (12 Nos.)	<i>Azolla</i> species (6 Nos.)
<i>Salvinia auriculata</i>	<i>Azolla intertrappea</i>
<i>Salvinia biloba</i>	
<i>Salvinia cucullata</i>	<i>Azolla berryi</i>
<i>Salvinia hastata</i>	
<i>Salvinia herzogii</i>	<i>Azolla prisca</i>
<i>Salvinia minima</i>	
<i>Salvinia molesta</i>	<i>Azolla tertiaris</i>
<i>Salvinia natans</i>	
<i>Salvinia nymphellula</i>	<i>Azolla primaeva</i>
<i>Salvinia oblongifolia</i>	
<i>Salvinia radula</i>	<i>Azolla boliviensis</i>
<i>Salvinia sprucei</i>	



Tiny up to 3cm long and fern like and non

Sensible roots (Brown/Black colour)

Figure 2 Characters of species.

fodder reduce weed growth and a decreased incidence of insects and pests. Fish yields can also be increased through the direct consumption of *Azolla* (Figures 3 and 4).

World aquaculture production is likely to grow continuously, but at slow rate. *Azolla* has gained its importance in aquaculture due to higher crude protein content (13 to 30%) and Essential Amino Acid (EAA) composition than most green forage crops and other aquatic macrophytes and it contain several vitamins (Vit. A, B-12 and β -carotene) (Figures 5 and 6) (Table 2).

Environmental Benefits

Limitation of nitrogen volatilization

When light intensity gets reduced in subsurface water, then *Azolla* will start the algae inhibition which leads to subsequent increase in pH and NH_3 volatilization. Because, up to 50% of nitrogen fertilizer applied to paddy fields is lost in volatilization, *Azolla* could help to reduce the amount of nitrogen fertilizers in rice crops.



Figure 3 *Azolla* as a feed ingredient for ruminants.



Figure 4 Dense mats of *Azolla*.



Figure 5 *Azolla* web diagram.

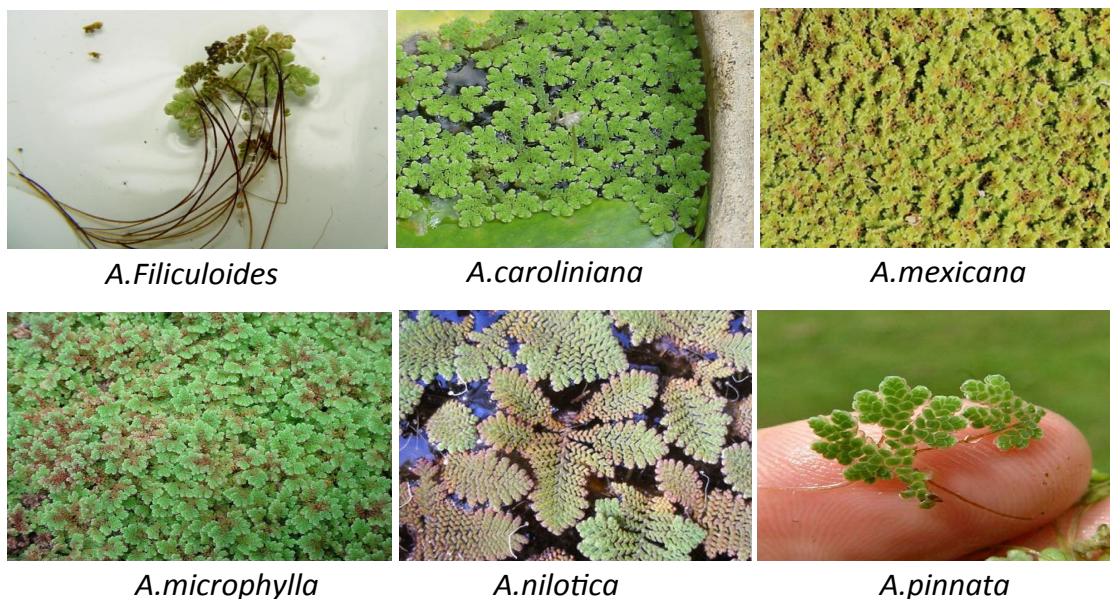


Figure 6 Species of Azolla.

Table 2. Azolla composition.

Sl. No.	Constituents	Dry matter (%)
1	Ash	10
2	Calcium	0.4-1.0
3	Chlorophyll	0.34-0.55
4	Crude fat	3.3-3.6
5	Crude protein	14.0-30.0
6	Iron	0.06-0.26
7	Magnesium	0.5-0.65
8	Nitrogen	4.0-5.0
9	Phosphorus	0.5-0.9
10	Potassium	2.0-4.5
11	Soluble sugars	3.4-3.5
12	Starch	6.5

Weed control and role in waste water treatment

Effluents from aquaculture ponds may add to receiving waters, high levels of Biochemical Oxygen Demand (BOD), inorganic particulate matter, living and dead particulate organic matter, dissolved organic matter, ammonia, nitrite, nitrate, phosphate and other potential contaminants. Therefore, regulatory agencies have developed standards and criteria for the aquaculture industry concerning effluents [1].

Azolla covering water surface reduce light penetration of soil surface, resulting in the depreciation in the germination of weeds (70% of the weed). Thus, growth of *Azolla* reduces aquatic weeds in flooded rice fields. It has been empirically observed and well appreciated by rice farmers that nutrients can be removed completely with the help of tertiary treatment especially, nitrogen (N) and phosphorus (P) which are discharged from wastewater treatment plants that accelerated the eutrophication of lakes and streams.

Owing to high increasing treatment costs, the use of various aquatic plants can remove N and P, in view of the low energy consumption to promote the preservation of the environmental conditions. The plants such as *Eichhornia*, *Lemna*, *Spirodella*, *Ipomea* and various higher plants can be used but these plants have disadvantages such as incomplete removal of Phosphorus [2-6]. Eventually, due to the symbiotic N₂ fixation, *Azolla* can grow even after N₂ is depleted in the effluents, resulting in adequate removal of P [7].

Biogas production

Anaerobic fermentation of *Azolla* or a mixture of *Azolla* and rice straw produces methane gas which can be utilized as fuel. The remaining effluent can be used as a fertilizer as it contains all of the nutrients originally incorporated in the plant tissues except for a small amount of nitrogen lost as ammonia [8]. Nevertheless, there has been little systematic research into *Azolla* potentiality as a biogas source. Das et al. mixed cow dung and *Azolla pinnata* residues and found that the best ratio was 1:0.4, which gave a gas

production 1.4 times that of cow dung alone [9]. This indicates *Azolla's* potential to produce biogas on an industrial scale.

Hydrogen source

There has been some research undertaken into *Azolla* to produce hydrogen, a non-polluting, high-energy fuel. When *Azolla-Anabaena* are grown in a nitrogen-free atmosphere or a water medium containing nitrate, the nitrogenase in the symbiont, instead of fixing nitrogen that evolves hydrogen using water as the source [10]. Newton recorded hydrogen production at rates of 760 nmol (nanomole) of H₂ per gram fresh weight of *Azolla* per h [11].

As per the demonstrations by Hall et al. the rate of hydrogen production can be increased by [12]:

- Exposing *Azolla* to a micro-aerobic environment.
- Exposing *Azolla* to a partial vacuum.
- Exposing an argon-enriched or carbon dioxide-enriched atmosphere.
- The immobilization of *Anabaena* cells isolated from the *Azolla*.

Cells may be immobilized by entrapment in transparent or translucent gels or polymers in order to increase the functional life time of the cells. Using a 'trickling-medium' column bioreactor, Park et al. obtained a production rate of 83 ml H₂ per gram per day [13].

Removal of gold from wastewater

Gilding generates large volumes of wastewater with residual gold, posing environmental and financial problems necessitating metal recovery. Following earlier studies on *Azolla* by Khosravi et al. and Umali et al. Found that *Azolla filiculoides* removed more than 98% of gold from waste water in a gold plating factory and developed a continuous flow column system that removed 100% of the gold from waste water [14,15].

Green manure and N-fixation

In agriculture, *Azolla* has been traditionally used as a green manure and an animal feed in Vietnam and China [16,17]. In rainy season, *Azolla* can easily fix enough nitrogen for a 4 to 6 tonnes/ha rice crop within 25 to 35 days whereas, during the summer season, 5 to 8 tonnes/ha crop under irrigation. Soil fertility contributed by the *Azolla*, providing nutrient-rich humus through its decomposition. One of the main reasons for the popularity of *Azolla* among agriculturists is its ability to fix nitrogen, valuable in paddy fields under waterlogged or flooded conditions where, nitrogen fixating legumes cannot grow. It is also a source of green manure for upland rice growing on the most fertile soils that farmers are reluctant to use for legume crops.

Bioremediation

Bioremediation, an internal part of Environmental Biotechnology Programs, explores the use of biological mechanisms to destroy, transform or immobilize environmental contaminants to protect potential sensitive receptors. The use of living organisms (primarily microorganisms and plants) is emerging as one of the most alternative technologies for removing contaminants from the environment, restoring contaminants sites and preventing further pollution. The natural biological processes explored to remediate nutrient-rich water by converting nutrients into forms that could be more easily removed. As the aquaculture industry develops, efficient, cost-effective and environmentally friendly, preventive and bioremediation methods of improving effluent water quality prior to discharge into receiving waters of sensitive areas becomes necessary [18]. Currently, aqua culturist has to follow the discharge guidelines as stipulated by the Environmental Protection Agencies where, Bioremediation or Bio-augmentation can be a great aid. *Azolla* can accumulate excessive amounts of pollutants such as heavy metals, radio-nuclides, dyes, pesticides, etc. Hence, it has been extensively studied and tested since the 2000's as a candidate for the bioremediation of waste waters and effluents [19] (Table 3).

Table 3. Composition of amino acids.

Sl. No.	Amino acids	Percentage
1.	Alanine	5.3-7.4
2.	Arginine	5.1-6.6
3.	Aspartic acid	8.2-10.3
4.	Cystine	0.7-2.3
5.	Glutamic acid	11.6-13.5
6.	Glycine	4.4-6.6
7.	Histidine	1.6-2.4
8.	Isoleucine	3.7-5.4
9.	Leucine	7-9.2
10.	Lysine	3.5-6.5
11.	Methionine	1.2-1.9
12.	Phenylalanine	5.2-5.6
13.	Proline	3.5-6.8
14.	Serine	3.9-5.6
15.	Threonine	4-5.3
16.	Tryptophan	1.5-2
17.	Tyrosine	3.2-4.1
18.	Valine	3.8-6.8

Removal of heavy metal pollutants from water

Azolla can be used to purify water polluted by metals. Jain et al. found that *Azolla pinnata* and *Lemna minor* (duckweed) removed the heavy metals-iron and copper from polluted water when the metals are present at low concentrations [20]. They concluded that effluent containing metal pollutants in low concentrations could be treated by passing it through ponds containing one or both of these water plants. Saxena found that a mixed culture of *Lemna* and *Azolla* in the ratio of 2:1 was able to purify highly polluted effluent from factories sufficiently for it to be used for agricultural purposes [21].

Azolla's potential to purify water has impending applications for both industrial and mining operations as well as space exploration. Fishes are the bio-indicator of metal pollution and are particularly sensitive to wide variety of toxicants. As fish fauna serves as a food source, it is essential to know the impact of water pollution on these organisms. Reports available in the recent years suggest the contamination of freshwater bodies by heavy metals coming through industrial wastes leading to biological magnification resulting into severe alteration in physiological and biochemical parameters of fish.

Phytoremediation is considered one such remedy to solve the problem of heavy metal pollution in water by using plants. The ability of aquatic plants to accumulate toxic metals from water can be removed by this process.

On phyto-accumulation potential of macrophytes with emphasis on utilization of *Azolla* as a promising candidate for phytoremediation has been dealt. The impact of uptake of heavy metals on morphology and metabolic processes of *Azolla* has been argued for a better understanding and utilization of symbiotic association in the field of phytoremediation [19]. Phytoremediation processes are having significant power to remove Chemical Oxygen Demand (COD) increasing contaminants from polluted water. In addition to this, it is also been proved that water hyacinth and *Ceratophyllum* combinative treatment is more convenient and useful to reduce COD level [22].

Contamination of fish in wastewater ponds

Aquaculture generates considerable amount of wastes, consisting of metabolic byproducts, residual food, faecal matter, residues of biocides and biostats, fertilizer derived wastes, wastes produced during moulting and collapsing algal blooms and residual of prophylactic and therapeutic inputs, leading to the deterioration of water quality and disease outbreaks. As a result, aquaculture is now considered as a potential polluter of aquatic environment and a cause of degradation of wetland areas (b).

No public health problems been reported so far, even though the controversial hypothesis of influenza pandemics from pig-duck-fish-zoonosis in Chinese integrated farming, the possibility of hazards continues to be raised, mainly because of successful experimental infections [23]. Ponds fertilized with untreated sewage or other wastes can contain human pathogenic organisms. Laboratory experiments with tilapia, common carp and silver carp have shown that when bacteria is present in high concentrations in experimental ponds for long periods, could be

recovered from all organs and muscles of fish, although normally they are found in the digestive tract and not in muscle tissues.

Guidelines issued by the World Health Organization (WHO) for the use of wastewater suggest that there is a little accumulation of enteric organisms and pathogens on or penetrating into edible fish tissue when faecal Coliform concentration in fish pond waters is below 103 per 100ml [24]. Greater the concentrations to an extent of 104 and 105 per 100ml, the potential for invasion of fish muscle by bacteria increases with the duration of exposure of the fish to the contaminated water.

Conclusion

Utilizing aquatic plants/microorganisms to detoxify effluents, soils, etc., is getting such wide acceptance that 'bioremediation' and 'phytoremediation' has now become a common buzzword in waste water management. When development and intensification of aquaculture sector is inevitable for food security and social well-being, it should be within the frame work of sustainability and environmental friendly approach. It seems likely that the use of bioremediations will gradually increase and the success of aquaculture in future may be synonymous with the success of bioremediation that, if validated through rigorous scientific investigation and used wisely, may prove to be a boon for the aquaculture industry.

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