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Mini Review Article

The effectiveness of Azolla as a Waste Decomposer and Bio-fertilizer

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Abstract:

In modern-day agriculture, we are well aware of the utilization of artificial fertilizers in the soil to supply major soil nutrients for plant growth and development. The most common used nutrients in agroecosystem are nitrogen (N), phosphorus (P), potassium (K), and sulfur (S). These fertilizers cause an increase in crop production, but the excess use of these fertilizers has several health hazards for the plants and animals feeding on them. Presently the consumer's preference has been shifted from synthetic fertilizer-based food to organic food. This paper aims to review the results of the research work done in the past few decays on the environment detoxification and beneficial effects of Azolla (aquatic fern) on plants and soil health. Hopefully, it will help the collaborative research on this "green gold" in the future.Azolla fixes the free-living atmospheric N as they have symbiotic Cyanobacterium Anabaena Azolla. Azolla adds organic matter and provides natural mulch to the soil because of these benefits is known as bio-fertilizer. Azolla remediates industrial and sewage water by accumulating heavy metals in their body and plays an active role in the reclamation of salt-affected soil by accumulating heavy metals and salts and by increasing the soil organic matter.

Keywords: Aquatic fern, Nitrogen fixation, Organic agriculture, Organic mulch, Green manure, Phytoremediation, Biofiltration of toxic elements.

Abbreviations: %DM (Percentage Dry Mass), DO (Dissolved Oxygen), C/N (Carbon and Nitrogen Ratio).

Introduction:

Azolla (Azolla sp.) is a miraculous herb cheap, and rich in a nutrient that grows on water. By using this Farmer can produce the best food in a short time with limited resources for the duck, poultry, goat, fish, and rabbit farming and also for livestock. It widely uses in China, Kuwait, Malaysia, and the Philippine. Azolla can easily grow and exploited in Pakistan as in other countries. Besides 25 to 30 percent protein, Azolla also contains vitamin A, B 12 beta carotene minerals, and a small amount of lignin, because of which animals can easily digest it (Basak, Pramanik, Rahman, Tarafdar, & Roy, 2002). Azolla is used in combination with other animal food. Adding Azolla to the diet 15 to 20 percent increase can be again in milk and animal weight (Rawat, Kumari, Singh, & Gilhare, 2015). Similarly, feeding Azolla to chickens and duck can increase their meat and egg production. According to research, Azolla has an important agricultural history. For centuries Azolla is a viable fern in South China and North Vietnam, used as a bio-fertilizer and green manure for the rice crop because of its N-fixing abilities (Van Hove & Lejeune, 1996). This can increase up to 20% Paddy rice production. Azolla produces huge biomass in a short time. Azolla is rich in nutrients and its compost is used for organic farming and kitchen gardening (Biso, 2019). Azolla act as a waste decomposer as it grows on the sewage, industrial, and agricultural waste water rich in nitrogen (N), phosphorus (P), and other heavy metals that pollute the groundwater, canal water, and the food grown by this water. Azolla grows on this

polluted water and produces bulk biomass by utilizing N, P, and other heavy metals and helps in the reclamation of polluted water by reducing the concentration of these pollutants (Sela, Garty, & TELOR, 1989). As the soil fertility is a key factor in crop production (Rehim et al., 2018a, 2018b), this acts as a nourishing factor for soil health. We can also use Azolla in the field for natural mulch. A thick layer of Azolla in paddy crop prevent other weeds from coming out and larvae of mosquitoes and other insects die because of lack of oxygen. That's why Azolla is also known as mosquito fern (Anjana, Koshy, & Mathew, 2020). Using Azolla has significantly reduced the expense of pesticides and fertilizers in the paddy crop (Cagauan, Branckaert, & Van Hove, 2000)

Figure-1: Azolla covering the stagnant water

We also use Azolla as a biofuel. Azolla produces huge biomass in a short duration of time. There are two different varieties of Azolla, *Azolla filiculoides* and *Azolla pinnata*, use as raw material for the mass production of functional hydrocarbons through hydro-thermal liquefaction, bio-hydrogen, and bio-ethanol (Miranda et al., 2016). This proves that Azolla is really not less than a blessing.

Distribution, Growth Habit, and Morphology: Azolla is a short, branched, floating stem with roots that hang from the water in the form of duckweed. The diameter of Azolla fern ranges from 1/3 to 1 inch (1-2.5 cm) for small species like *Azolla pinnata* and 6 inches(15cm) or more for *Azolla nilotica* (Ferentinos, Smith, & Valenzuela, 2002). Azolla has a triangular to polygonal appearance and float on the surface of the water individually or in clumps and appears as a greenish-red color exception for *Azolla nilotica (*Watanabe, Ramirez, & Nutrition, 1990*)*. Azolla plants show natural vegetation only in the colder months (November-April) of the year and do not survive in extreme heat. (S Ali & Malik, 1987). The optimum relative humidity for Azolla growth is between 85% and 90%. Azolla will dry out and be sensitive to relative humidity below 60% (Huntington, Hasan, & Paper, 2009; T. A. Lumpkin & Plucknett, 1980). The temperature required for the optimum growth of Azolla is between 18^{o}Cto 28^{o}C (64-82^{o}F).The Azolla can survive in water pH 3.5-10 but the optimum pH range is 4.5 to 7 (Ferentinos et al., 2002). A new heat-tolerant strain of Azolla which can grow at a temperature prevailing in the Punjab province of Pakistan is selected by growing native Azolla of Pakistan in cold water containing

FYM (farmyard manure), shaded from strong direct sunlight during hot days for about 3 years (S Ali & Malik, 1987).

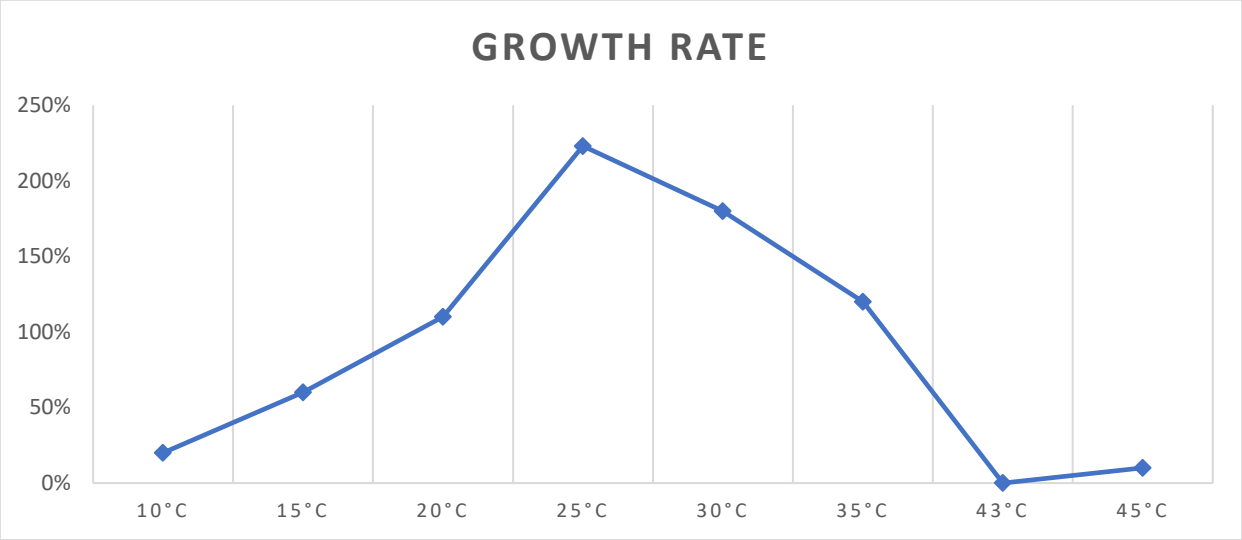

Figure-2: Effect of temperature on the growth rate of Azolla(FAO -Rome, 1979)

Growth and Multiplication: Azolla emerges from stagnant or slow-moving water in ponds, ditches, lakes, and rivers. The easiest way of growing Azolla is to dig a pit of 20 to 25 cm deep, 3 feet wide, and 7 to 10 feet long in a shaded place (Divya, Kanimozhi, Poornima, & Tamilarasu, 2020). Cover it with a plastic sheet to prevent the water from leaching down. Add 10 to 15 kg soil, super-phosphate, or 4 to 5 days old animal dung as a source of P and fill the pit up to 10 to 30 cm with water. Then leave it for two to three days so that the soil settles down. Add 2 to 3 kg Azolla. After 2 weeks you can get up to one to three kilograms of Azolla daily and use it as fodder for animals. And remember to change 20 to 30% water every five days and again add animal dung or super-phosphate. Clean it after every six months to avoid stinking. Sprinkling Azolla can also grow anywhere in standing water or in the rice field as inter-crop. Duck and fish farming in the rice field inter-cropped with Azolla have many benefits. Ducks and fishes eat Azolla and insects and in return provide the rice field with their waste which is highly rich in nutrients like P (Cagauan et al., 2000).

Harmful Impact of Artificial Fertilizers: Fertilizers improve the crop yield, but their continued use causes organic matter degradation, soil distortion, decrease soil aggregation & diminish fertilizer efficiency. Besides, essential nutrient inorganic fertilizer also contains certain compounds and salts. The plant cannot absorb (compounds & salts), and it remains in the soil and changes its chemistry resulting in loss of soil productivity. Nitrogen Application in excess badly affects the soil PH. Soil becomes more acidic or alkaline depending on nitrogen. As nitrogen is absorbed too quickly, it will dehydrate and dry up soil. Alkaline fertilizers reduce soil fertility and make it barren. Excess fertilizers may hurt the beneficial microorganism by salt accumulation (Sood, Uniyal, Prasanna, & Ahluwalia, 2012). Many salts like chloride, Sodium, and Boron are poisonous to the plant because it deteriorates the enzyme system or blocks the biochemical pathway. Though fertilizer makes plants grow faster, plants do not have enough time to develop a mature and strong root system, strong stem, nutritious fruits, and vegetables. Overdose mainly cause defoliation, wilting, deceased growth, leaf Scorching if leaves are wet, burn crops, destroy soil organism, reduce

the colonization of plant roots with Mycorrhizae. Sometimes plant death also occurs. It also causes root burn as it does not allows plants to take enough water. So, their excessive use may be fetal. Organic fertilizers are the only right solution for getting healthy food (Sood et al., 2012).

Nitrogen Fixation: Azolla has an important agricultural history. For centuries Azolla is a viable fern in South China and North Vietnam, used as a bio-fertilizer and green manure for the rice crop because of its N-fixing abilities (Van Hove & Lejeune, 1996).There exists a symbiotic relationship between the Azolla (*Anabaena azollae)* and nitrogen-fixing blue-green algae (Cyanobacterium). Azolla supplies the anabaena colonies with the nutrients and protective cavity in each leaf and the Anabaena in return fixes the N for Azolla and also provides other growth-promoting substances (T. A. Lumpkin & Plucknett, 1980). The fixed N is ultimately available to the crops. The use of a certain amount of P (0.5 to 1.0 kg P / ha/week) helps in the successful growth of Azolla but does not mean that an increase in the amount of P fertilizer is essential for rice production (T. Lumpkin & Plucknett, 1985). Total 30 Kg per acre P_2O_5 is recommended for the rice crop. This phosphorus is applied in splits to minimize the loss of phosphorus. Azolla takes excessive phosphorus and releases it back slowly when their colonies die. In this way, the phosphorus's fixation into the soil as calcium and aluminum phosphate does not occur.

Green Manure: Organic amendments applied in agriculture have vast positivity impact on agricultural production (Rehim et al., 2020). Azolla is particularly used as green manure in the paddy field. Azolla is used as a green manure by many farmers in China for rice and other crops. The compost is used as a bio-fertilizer in the wheat crop at the rate of 30,000 Kg/ha same as for maize and rape. An increase in crop yield has been found by 800 Kg/ha for wheat, 710 kg for beans, and 945 Kg/ha for maize (FAO -Rome, 1979).The practice of using Azolla is site and time specific. In the temperate regions, the field is flooded with water up to the depth of 3 to 4 cm, and the mother culture of Azolla is applied to this muddy water at the rate of 7,500 Kg/ha which should be collected from fresh Azolla nurseries (FAO -Rome, 1979). After fifteen to twenty days the field will be covered with a 6 to 8mm thick (about 20 ton/ha) layer of Azolla. After the drainage of water, Azolla is incorporated into the soil. Rice crop is transplanted after two to three days of plowing the Azolla into the soil(Sikander Ali, Hamid, Khan, & Malik, 1998)

Figure-3: Azolla is providing an organic cover

The second method that can be used is to apply the mother culture of Azolla after transplanting the rice into the field. In this technique, Azolla is incorporated very carefully into the soil by hand or this may be plowed easily after the harvesting of rice. The Azolla decomposes within two to three weeks after being incorporated into the soil as the Azolla contains a very low carbon (C) to N ratio (about 10)(Prayoga et al., 2020). Micro-

organisms can easily decompose the organic material with a low C/N ratio. After the decomposition of Azolla many types of nutrients especially organic N, P, and adequate calcium (Ca) are released which positively enhances the growth of plants (Yadav, Abraham, Singh, & Singh, 2014).

Chemical Composition: Chemical analysis of sun-dried Azolla showed that the dry matter comprises of 25 to 26% crude protein, 14 to 17 percent crude fifer, 35 to 40 percent N Free Extract (NFE is the small part of the dry matter of any substance which contains sugar, carbohydrate, and starch, etc.), 3 to 4 percent ether, 17 to 21 percent ash, 1.5 to 3 percent Ca, 4 percent N, 1 to 1.6 percent P, 2 to 3 percent potassium (K), 0.06 to 0.26 percent iron (Fe), 0.5 to 0.65 percent magnesium (Mg), 0.11 to 0.16 percent manganese (Mn), and 74.5 percent organic matter (Joysowal et al., 2018) (Yadav, Abraham et al. 2014)

Role of Azolla in Environmental Detoxification: Intensive agriculture and industrialization for fulfilling a growing population's needs are affecting the environment by warming the earth's atmosphere with carbon dioxide and methane. Fossil fuel burning is causing environmental imbalance, and modern agricultural techniques are disturbing environmental sustainability. Organic agriculture farming is the only way to reduce toxicity. Using Azolla is a promising technique or strategy for remediation. It has the potential to remove toxic elements from the environment (Kollah, Patra, Mohanty, & Research, 2016).

Heavy Metals Accumulation by Azolla: Heavy metals are dangerous due to their constant, determined, and bioaccumulative nature. It (Hg, Cd, Cr, As, Pb) cause serious environmental and health issue even at very low concentration, Sood and Uniyal found an easy and eco-friendly and less costly method for its removal (Sood et al., 2012). Azolla is considered an eco-friendly and strong agent for removing, disposal and recovering heavy metals. Azolla is beneficial for filtering the polluted ecosystem. The fast growing and free-floating nature of Azolla facilitate easy harvesting and disposal of heavy metals from the effluents. Azolla fronds contain 90-94% water content, so after drying, its volume reduces drastically and provides easy disposal. There are three different types of Azolla; *Azolla pinnata*, *Azolla filiculoides*, and *Azolla caroliniana*. *Azolla pinnata* eradicates 70-90% of heavy metals (Hg & Cd). *Azolla filiculoides* also accumulate heavy metals like Cr (1990ppm), Cd (10,000ppm), Cu (9000ppm), and Zn (6500ppm). *Azolla caroliniana* also can accumulate Hg and Cr. It can take up 75-100% heavy metals (Sood et al., 2012).

Role of Azolla in Water Eutrophication: Urbanization, industrialization, and irrigational activity lead to over-exploitation and deterioration of groundwater resources. The proper availability of clean water is at risk when agricultural activities demand more water for irrigation. There is various type of physical, chemical, and biological processes for purification, recycling, or improving water quality, but these are all unaffordable to common people (Soman, Anitha, & Arora, 2018). Bioremediation is the only suitable, less expensive method to ward off the pollutant. Azolla grew in the least time to remove toxic nutrients from polluted water and prevent water Eutrophication. It removes 54.8 % ammonia, 33.3 % organic matter, 68.65 % phosphorous, 71.4 % nitrate, and 50 % organic carbon from polluted water and prevents Eutrophication in water bodies (Soman et al., 2018). Using other macrophytic plants, phosphorous will remain even after nitrogen removal, but Azolla can remove phosphorous as it possesses nitrogen-fixing capacity.

Green House Gases: Agriculture contributes to three types of greenhouse gases (CO_2, CH_4, and N_2O). Carbon dioxide gas is released by burning plant materials and the decay of soil organic matter—methane gas by decomposing matter, from rice field under

flood condition and stored manure (Kollah et al., 2016). NO_2 is generated by microbial transformation of nitrogen in soil and manure, especially under wet conditions. Azolla plays an important role in minimizing greenhouse gas emissions from agriculture and mitigation of atmospheric Greenhouse gases. The study highlighted the role of Azolla in the Eocene period when there was a shift from the greenhouse to the ice age due to plants' uptake of CO_2 (Kollah et al., 2016).

Phytoremediation: The world's population is increasing day by day, with the increase in population demand for food, drink, and shelter is also rising. To fulfill the demands of human being many industries are working day and night to provides the human with food, drink, clothes, and shelter. Industries are producing different fertilizers like Di-ammonium Phosphate, urea, single super-phosphate, and sulfate of potash, etc. for the agriculture sector, and other chemicals like sulfuric acid for textile, beverage, and other industries. Industries are also producing a lot of waste contaminated water containing heavy metals that discharged out into the canals, lakes, and rivers with no proper treatment which is affecting the life directly or indirectly. Domestic sewage water also contains heavy metals, soluble salts, micro, and macro nutrients that pollute the ground and canal's water (Ahmad, Aziz, Zia-ur-Rehman, Sabir, &Khalid, 2016). Farmers use this water with no treatment to irrigate their crops. When plants use this contaminated water, then these pollutants accumulate in their bodies. These pollutants affect the organism when these organisms feed on these contaminated crops (Intawongse, Dean, & contaminants, 2006). Phytoremediation is a biological technique in which plants are used to purify the contaminated water or soil(Mandakini, Bandara, Gunawardana, & Environment, 2016). Many types of aquatic plants are used for the remediation of contaminated water *Azolla pinnata* is one of them. *Azolla pinnata* is an aquatic fern that has the potential to accumulate pollutants such as pesticides, dyes, radio-isotopes, and heavy metals such as Hg (II), Cr (III), Cr (VI) from municipal waste water (Bennicelli, Stępniewska, Banach, Szajnocha, & Ostrowski, 2004; Sood, Uniyal, Prasanna, & Ahluwalia, 2012). *Azolla pinnata* is has grown at a place where wastewater is standing. Azolla explodes and produces biomass in a bulk quantity in a short duration of time and accumulate heavy metals in their body (Muradov et al., 2014).This biomass undergoes the pyrolysis process and produces high-quality bio-oil. Azolla reduces the water Eutrophication by consuming the P from the water and purify the water which was full of P (Shiomi & Kitoh, 1987).

Reclamation of Salt Affected Soil: Azolla can restore saline-sodic soil and produce biogas and bio-energy (Raja, Rathaur, John, & Ramteke, 2012). Azolla can fix the nitrogen and produces organic matter in bulk quantity. The organic matter adds by the Azolla to the soil improves the soil's physical properties like soil structure, soil water holding capacity, permeability, soil organic carbon, and improve the solubility of soil calcium and magnesium, which ultimately helps in the reclamation of saline-sodic soils (Ram, Raja, & Naidu, 1994). Azolla acts as a cover crop (in the flooded area) and organic mulch, which reduces the water evaporation from the soil surface and conserves soil moisture (Small & Darbyshire, 2011). By reducing the soil water transpiration, soil salts' accumulation on the soil surface reduces, and by doing this, soil can be protected from being salted (Kimani, Kanno, Tawaraya, & Cheng, 2020)

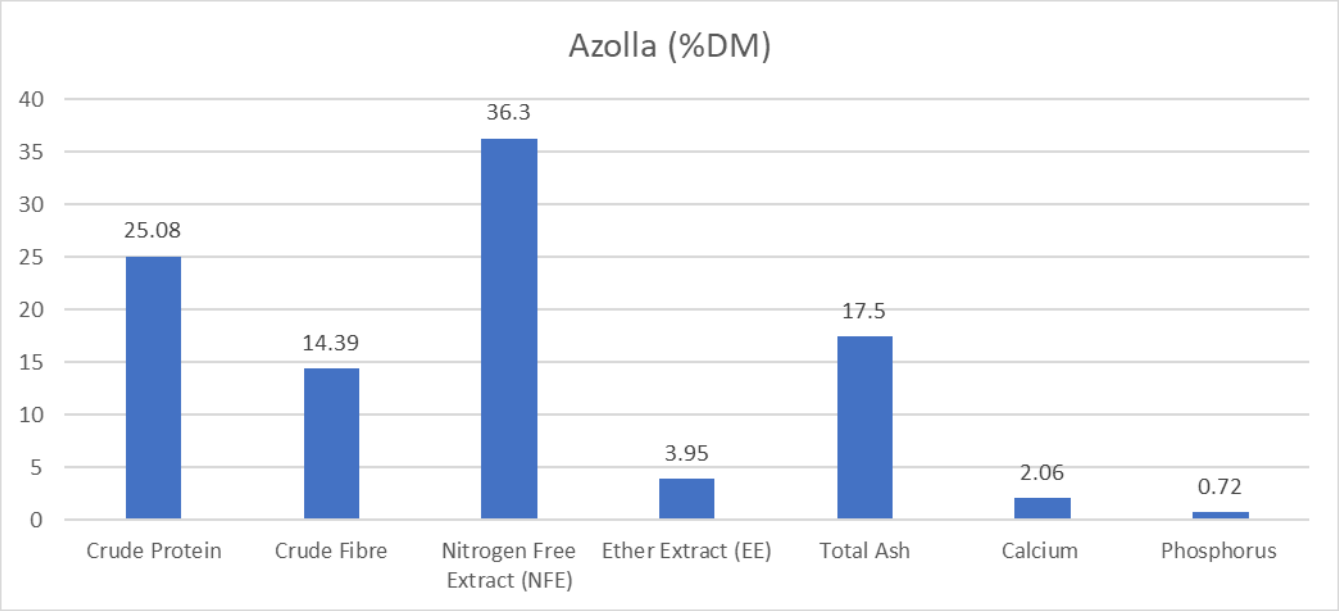

Figure-4: Chemical analysis of Azolla(Joysowal et al., 2018)

Conclusion:

By reviewing these papers, we have concluded that the Azolla is not less than a blessing. Azolla is an animal feed full of protein and other essential nutrients. Animals and birds that feed on Azolla show an excellent increase in body weight, milk, and egg production. Azolla causes a prominent increase in rice production by 30% to 40%. By fixing the atmospheric N, controlling the emergence of weeds, and preventing insects like a mosquito to lay eggs the Azolla reduce the cost of N fertilizers, herbicides, and insecticides. Azolla help in water conservation by providing a cover to the stagnant water in rice field and water ponds, etc. Azolla produces huge biomass in a short time (doubling time 2.5 days almost) which can be used as an animal feed as well as it can be converted into compost that can be used in organic farming and kitchen gardening or it can be converted into bio-fuel like bio-ethanol. Azolla is used for the phytoremediation of sewage and industrial water rich in heavy metals, and P. Azolla protects the P rich water from Eutrophication and improves the water quality by increasing its DO (Dissolved oxygen). Azolla help in the reclamation of salt-affected water and soil by up taking soluble salts and improving the physical conditions of the soil.

Recommendations: Pakistani farmers can easily grow Azolla by inoculating the rice field or any pre-built water pond with its seed that can be easily taken from a pond where Azolla is already grown. Azolla is green gold for the farmers as it has many benefits (already discussed above).

Research Gaps:

- Azolla is rich in nutrients. Therefore, we can use it as human food by growing it in a clean and controlled environment. It can help in improving global food security and minimizing the world's starvation.
- Soil microbiologist can also work on the roots of Azolla for the identification of sulfur (S), and zinc (Zn) solubilizing bacteria.
- Integrated cropping of Azolla and duckweed in salt-affected soil can give satisfactory results.

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