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Azolla as Water Purifier: a Systematic Review

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Abstract: *Water pollution is a critical issue for human health, aquatic plants, and animal biodiversity. While there are several different approaches to resolve this issue, our research investigates one possible solution using aquatic plants as a natural treatment system. The objective of this study is to find evidence supporting Azolla as a water purifier from different data electronic bases. This review-based study was done with the systematic review method, in which only research papers published in the last 10 years were included. The research papers were searched in four data hubs NCBI; SpringerLink; Scopus, and PubMed based on the electronic database. Results of the study show that the Azolla has a symbiotic relationship with the cyanobacteria Nostoc azollae, which live in special cavities inside Azolla's leaves. Finally, Azolla could play as one of the most promising agents for COD removal and treatment of nitrogen-free and rich phosphorus wastewater.*

Keywords: Sewage treatment; *Azolla* purifier; Physicochemical parameters; *Azolla*; water treatment.

I. INTRODUCTION

The aquatic fern Azolla, a small-leaf floating plant, which lives in symbiosis with a nitrogen-fixing cyanobacterium (Anabaena) is an outstanding plant due to its high biomass productivity beside its highest rate per unit area for nitrogen-fixation.

One of the biggest issues facing modern society is water contamination. Water usage has been increasing at a rate of twofold every twenty years (Forni *et al*., 2011). Only one-third of Earth's surface is covered by fresh water, and by 2025, 2.3 billion people will reside in regions with persistent water shortages if serious water preservation measures are not taken (WHO, 2005). A comprehensive classification of the consequences of water contamination includes physiochemical, biological, toxic, and pathogenic effects. Sewage is the official name for the wastewater produced by household, agricultural, and industrial human activities. Approximately 99 percent of sewage is made up of water, while 1 percent of organic solids (Agnihotri, 2011). In our nation, sewage disposal is carried out so carelessly that it seriously pollutes both water and land (Taghilou *et al*., 2011). Most of the time, residential sewage is released into surrounding water pollution problems in its raw, partially-treated, or treated condition (Sood *et al*., 2012).

As a result, wastewater recycling is necessary because it is currently being used in both India and abroad to produce fish (Rai *et al*., 2017; Agnihotri and Tiwari, 2020). Therefore, treating sewage wastewater before disposal is crucial. To make this poisonous water less toxic and use it for aquaculture, agriculture, and other reasons, several treatment techniques were developed (Toledo *et al*., 2011). Degradation is a process that uses microorganisms, plants, and animals to quickly break down potentially harmful organic pollutants in soils, water sludges, and residues to levels that are safe for the environment (Banach *et al*., 2020).

The knowledge of bioabsorption and bioaccumulation by plants and animals involving water plants like water hyacinth, *Azolla*, bivalves, algae, and fungi has recently been highlighted by studies. Numerous papers describe the use of aquatic weeds and plants for the bioaccumulation or bioremediation of sewage wastewater. Using the *Azolla* for other applications, such as using it as a decontaminant plant in affordable wastewater treatment systems, is becoming more popular nowadays (Fačkovcová *et al*., 2020). Wastewater can be recycled for irrigation, and the biomass produced can be used to fertilize paddy fields or for other purposes.

II. METHODOLOGY

This review-based study was done with the systematic review method. In this study, only research papers published in the last 10 years were included. The research papers were searched in four data hubs NCBI; SpringerLink; Scopus, and

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PubMed based on the electronic database. Only peer-reviewed and English-language research articles are included in the study.

2.1 Result

Table 1: Included article in the study

Sl.	Title of article	Conclusion	Author & Year
1.	Potential of water fern (*Azolla pinnata* R.Br.) in phyto-remediation of integrated industrial effluent of SIIDCUL, Haridwar, India: removal of physicochemical and heavy metal pollutants	Findings of this study showed that *A. pinnata* was useful for the eco-friendly treatment of Integrated industrial effluent and could minimize potential wastewater management issues.	Kumar *et al*., 2020
2.	*Azolla filiculoides* L. as a source of metal tolerant microorganisms	The study evidenced that *A. filiculoides* possess a microbiome whose representatives belong to metal-resistant species which makes the fern the source of biotechnologically useful microorganisms for remediation processes.	Banach *et al*., 2020
3.	Uptake of Trace Elements in the Water Fern *Azolla filiculoides* after Short-Term Application of Chestnut Wood Distillate (Pyroligneous Acid)	The results suggested that, under the experimental conditions, element uptake is positively influenced by time and negatively by increasing concentrations of wood distillate, likely due to the acidification of the medium. On the whole, the element concentrations measured in *A. filiculoides* were low and did not pose any toxicological concern.	Fačkovcová *et al*., 2020
4.	Aquatic Plants, *Landoltia punctata*, and *Azolla filiculoides* as Bio-converters of wastewater to Biofuel	*A. filiculoides* and *L. punctata* can be used as a new generation of feedstock, which can treat different types of wastewater and represent renewable and sustainable feedstock for bioenergy production	Miranda *et al*., 2020
5.	Improving the efficiency of wastewater treatment plants: Bio-removal of heavy metals and pharmaceuticals by *Azolla filiculoides* and *Lemna minuta*	The higher removal capacity of A. filiculoides compared to L. minuta can be attributed to its superior tolerance of the contaminants, probably in turn related to the presence of the nitrogen-fixing microorganism in its fronds.	Bianchi *et al*., 2020
6.	*Azolla pinnata, Aspergillus terreus*, and *Eisenia fetida* for enhancing the agronomic value of paddy straw	The results show that *Azolla* reduces dependence on cattle dung for recycling the carbon-rich rice straw and enhances its agronomic value.	Arora & Kaur, 2019
7.	*Azolla* along a phosphorus gradient: biphasic growth response linked to diazotroph traits and phosphorus-induced	Due to luxury consumption, P sequestration rates increased to 50 µmol P l^{-1}. At higher P concentrations (≥50 µmol	Temmink *et al*., 2018

	iron chlorosis	l^{-1}), however, chlorosis occurred that seems to be caused by iron- (Fe-), and not by N-deficiency.	
8.	Isolation and chemical characterization of a novel immuno-stimulating galactofucan from freshwater *Azolla filiculoides*	Water-soluble polysaccharides were isolated from *Azolla filiculoides* to determine their chemical and structural characteristics as well as anticancer and immuno-stimulatory activities.	Shemami *et al.*, 2018
9.	Wastewater management through biomass of *Azolla pinnata*: an eco-sustainable approach. *Ambio*,	Only electrical conductivity and pH were reduced by plants compared to the control without plants. Thus, A. caroliniana and S. auriculata show low potential for improving effluent quality.	Rai, 2017
10.	Nano modification of NZVI with an aquatic plant *Azolla* filiculoides to remove Pb(II) and Hg(II) from water: Aging time and mechanism study	However, coated NZVI with *Azolla filiculoides* as a green and environmentally friendly support suppressed rapid oxidation and aggregation of the immobilized NZVI, therefore vastly enhancing the probability of environmental transport and reducing the sedimentation and potential for toxicity.	Arshadi et al., 2017
11.	Plant growth promotion using microbial IAA producers in conjunction with *Azolla*: a novel approach.	It was found that plots 3 (only culture) and 4 (culture with *Azolla*) showed better plant growth as compared to others. Based on the IAA-producing ability of G2, pot experiments, and field studies, it was concluded that isolate G2 can be used alone or in conjunction with *Azolla* as a biofertilizer to get better plant growth.	Raut *et al.*, 2017
12.	Is there foul play in the leaf pocket? The metagenome of floating fern *Azolla* reveals endophytes that do not fix N_2 but may denitrify	The persistent but less abundant heterotrophic Rhizobiales bacteria possibly contributed to lowering O_2 levels in leaf pockets but did not release detectable amounts of the strong greenhouse gas N_2O.	Dijkhuizen *et al.*, 2017
13.	The aquatic fern *Azolla* is a natural plant factory for ammonia removal from fish-breeding fresh wastewater.	The ammonia removal rate (A RR) was both light and temperature dependent and the highest rate (6.394 h(-1)) was attained at a light intensity of 140 μmol m(-2) s(-1) and a temperature of 28 °C; the lowest (0.947 h(-1)) was achieved at 20 μmol m(-2) s(-1) and 15 °C. The depth of the fish-wastewater pool also affected the A RR with the relation between A RR and	Carlozzi & Padovani, 2016

		the depth being a hyperbolic function.	
14.	Response of nitrogen-fixing water fern *Azolla* biofertilization to rice crop	The use of *Azolla* also increased the quality of water.	Bhuvaneshwari & Singh, 2015
15.	Dual application of duckweed and Azolla plants for wastewater treatment and renewable fuels and petrochemicals production	Batch, continuous, and continuous with the slow stirring systems were used. Live *Azolla* was an efficient, inexpensive, and affordable bio-adsorbent for dairy wastewater treatment.	Muradov *et al*., 2014
16.	Phytoremediation potential of aquatic macrophyte, *Azolla*	The impact of the uptake of heavy metals on the morphology and metabolic processes of *Azolla* has also been discussed for a better understanding and utilization of this symbiotic association in the field of phytoremediation.	Sood *et al*., 2012
17.	Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms	The internal nitrogen to phosphorus ratio decreased from 23:1 to 11:1 when increasing the loading rate. This combination of findings demonstrates that microalgal biofilms can be used for removing both nitrogen and phosphorus from municipal wastewater effluent.	Boelee *et al*., 2011
18.	Evaluation of the fern *Azolla* for growth, nitrogen, and phosphorus removal from wastewater	PO(4)3- concentration was very low in Water and sewage waters and decreased in the Hoagland medium. The results obtained confirm the ability of the fern to grow in sewage water.	Forni *et al*., 2011
19.	Performance of *Azolla caroliniana* Willd. and *Salvinia auriculata* Aubl. on fish farming effluent	However, S. auriculata grew slightly better in fish farming effluent (0.030 d-1) than in Hoagland ½ medium (0.025 d-1). The species contributed to reducing nitrate and phosphate concentration in Hoagland ½ medium.	Toledo *et al*., 2011

The ability of different species of *Azolla* plants to purify water by removing nitrogen and phosphorus, two substances that contribute to water eutrophication, has recently been investigated by researchers. Additionally, it may get rid of radioactive elements like U as well as sulfa medications and metals like Sr, Cu, Cd, Zn, Cr, Ni, Pb, Au, and Pt.

Numerous studies are being conducted in nations like the USA to see if aquatic vascular plants can remove nutrients from sewage wastewater and natural water (Temmink *et al*., 2018). The free-floating aquatic fern *Azolla* microphylla has distinct advantages over other plants because of its high biomass productivity, high rate of nitrogen fixation, ability to grow in a variety of environments, and numerous uses as a biomonitor, biofertilizer, animal feed, biofilter, and weedicide. It also can concentrate heavy metals and nutrients from flood waters. Aquatic water fern *Azolla* is effective at bio-filtering a variety of hazardous metals (Arshadi *et al*., 2017; Carlozzi *et al*., 2019).

According to Agnihotri & Tiwari (2019), these floating plants were chosen because they are common floating plants inhabiting India and research suggests water hyacinth, *Azolla*, and duckweed affect quality. While that research provided a deep analysis of each of the plants, there are no comparisons of those plants in India. Therefore, the comparison between the three different types of floating plants will be the key to this research (Semami *et al*., 2018)

The study done by Shemami *et al*., 2018 showed that the six measurements in this experiment-conductivity, pH, turbidity, dissolved oxygen, color, and the number of bacteria colonies-showed that water hyacinth, *Azolla*, and duckweed did not always affect water quality. For instance, there were subtle differences between the experimental groups and the control group for conductivity, pH, and dissolved oxygen (Muradov *et al*., 2014). On the other hand, turbidity and the number of bacteria in water changed drastically when the plants were added into the water; experimental groups were more effective at water purification compared to the control group since they had a larger decrease in turbidity and fewer bacteria growing on the culture media (Dijkhuizen *et al*., 2018).

However, despite the limitations this research contains, we expect that the application of a natural treatment system using floating plants will bring a positive impact on water quality, providing better habitat for aquatic life as well as increasing the usefulness of the water. For instance, duckweed can be added to a pond in which wildlife cannot propagate due to contamination from wastewater. This can reduce the peril of wastewater with less cost than physical or chemical treatments (Rout *et al*., 2017).

The government can even adopt constructed wetlands, where microorganisms and aquatic plants act as a filter as water slowly enters the wetland; nutrient and pollutant in water naturally breaks down and is taken up by plants and bacteria (Bianchi *et al*., 2020). This way, the wastewater from agricultural runoff or human, and industrial waste can be treated at low cost and energy consumption, protecting the environment (Bhuvaneshwar *et al*., 2015).

However, there are some limitations to using duckweed. Duckweed has a high rate of reproduction, however, the excess quantity of duckweed can contaminate water quality when it covers the water surface and blocks the sunlight needed for microorganisms that decompose contaminants (Semami *et al*., 2018; Agnihotri, 2019; Agnihotri & Tiwari, 2020). Some countries had to undergo the disposal of duckweed in sewage systems as duckweed propagated more than they expected. Hence, future studies could focus on the relationship between the number of duckweed covering the water surface and the water purification level.

III. CONCLUSION

The ability of different species of *Azolla* plants to purify water by removing nitrogen and phosphorus, two substances that contribute to water eutrophication, has recently been investigated by researchers. Conclusion: The current study demonstrated the viability of implementing a "sustainable" and environmentally friendly method of sewage wastewater treatment that emphasizes degradation and recycling/reuse of sewage water for aquaculture and biocomposite production. Further large-scale research should be done in the future with a particular focus on resource use and phytoremediation, as this is simply a laboratory-scale baseline study.

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