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A review on the significance of *Azolla* for water and wastewater treatment

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ABSTRACT

Industrialization, urbanization, and population growth release many pollutants into water resources. Conventional mechanical wastewater treatment plants require a lot of electricity, are not sufficient to remove all pollutants, and generate a large amount of sludge. Therefore, it is crucial to develop economically accepted and environmentally friendly methods for wastewater treatment. The main advantages of bioremediation over conventional treatment methods include low cost, high efficiency, and minimizing the generation of chemical or biological sludge. Aquatic macrophytes can improve water quality by bioaccumulation/biosorption of toxic substances, excess nutrients and metals. Therefore, phytoremediation as a natural system presents itself as an environmentally friendly treatment technology for water remediation. This paper presents recent research on *Azolla* fern as a phytoremediation agent. *Azolla* can remove many pollutants from wastewater, including fluoride, ammonium, bisphenol A, dyes, chemical oxygen demand, nitrogen, phosphorus and toxic metals. *Azolla* is a floating water fern and its biomass produced during phytoremediation has various uses, such as animal feed, human food, production of hydrogen fuel, biogas production, medicine, water treatment, and biological fertilizer in rice fields. Therefore, phytoremediation with *Azolla* is recommended as an advanced treatment after conventional wastewater treatment.

Keywords: Azolla; Bioaccumulation; Fern; Macrophytes; Wastewater treatment

1. Introduction

In 2020, about two billion people still lack access to safely managed water, while about 3.6 billion people worldwide have inadequate sanitation. In addition, about 44% of household wastewater was discharged without safe treatment in 2020. By 2030, current progress must be quadrupled to achieve universal access to safely managed water, sanitation, and basic hygiene services [1]. Therefore, it is crucial to develop economically feasible and environmentally friendly methods for wastewater treatment [2]. Aquatic plants growing in or near the water, known as macrophytes, can be used as a suitable polisher when nutrient concentrations are low [3]. Toxic metals enter the aquatic environment from metal refining, mining, smelting, battery manufacturing, tanneries, petroleum refining, paint manufacturing, pesticides, pigment manufacturing, printing and photographic industries, etc. [4]. In addition, other pollutants such as excess nutrients (especially nitrogen and phosphorus) from livestock watering, fertilizer application, animal feed and animal metabolic residues are also released into aquatic ecosystems [5]. Eutrophication occurs when high concentrations of nutrients, especially nitrogen and phosphorus, enter surface waters [6]. Nutrient removal by expensive methods such as electrocoagulation [7], electrochemical precipitation [8], anaerobic/anoxic/aerobic (A^2/O), and biological aerated filter (BAF) has been widely studied [9]. These mechanical methods are expensive and use electricity to accelerate treatment processes that occur naturally [10]. Reducing energy consumption and operating costs of wastewater treatment is a major concern even for small communities in developed countries [11]. Therefore, it is important to develop

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innovative methods to increase treatment efficiency while reducing overall costs. Phytoremediation, as a natural water reclamation system, is an environmentally friendly approach that uses plants as purification agents [12]. Known advantages of using macrophytes for wastewater treatment include simplicity, good biosorption potential, low operating costs, availability, and ease of propagation [4,13]. In addition, biosorption methods offer a good cost-benefit ratio for wastewater treatment and often provide better results than chemical methods [14]. Aquatic macrophytes such as *Azolla* improve water quality due to their great ability to remove organic and inorganic pollutants [15]. Fig. 1 illustrates some of the many possible applications of *Azolla* [16,17].

Based on morphological and reproductive aspects, there are seven obvious species of *Azolla*, which are divided into two subgenera: *Euazolla* and *Rhizosperma*. *Euazolla* includes *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *Azolla microphylla* and *A. rubra*, while *Rhizosperma* includes *A. pinnata* and *A. nilotica* [18]. *A. filiculoides* Lam. which grows in Europe, North America, South Africa, Australia, and New Zealand [19] has advantages such as a high growth rate and the ability to absorb pollutants [20]. Therefore, it is a suitable candidate for phytoremediation [12].

Researchers investigated the ability of *Azolla filiculoides* to remove fluoride [21], ammonium [15], bisphenol A [22], dye [23], chemical oxygen demand (COD) [24], nitrogen, phosphorus, zinc ions and heavy metals (e.g., Pb, Cd, Ni and Cu) from wastewater [6,25–27]. The results of the previous researches as well as the review articles show that the use of *Azolla* can be a suitable and cost-effective option for phytoremediation of polluted water [28]. In this review, most of the capabilities of *Azolla* in water reclamation are presented. In addition, the bioaccumulation and biosorption capacity of *Azolla*, the mechanisms of sorption processes, and the potential of using the biomass produced are discussed.

2. Geographical distribution and characteristics of Azolla

Azolla is a native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and America [29]. This is a small-leaf floating fern (Fig. 2).

Azolla is commonly found in stagnant or slow-moving water in ponds, lakes, marshes, swamps, and streams. *Azolla* leaves turn red under stress, such as nutrient deficiency, salinity or high temperatures [31]. Under favorable conditions, *Azolla* rapidly produces biomass (90.0–97.2 kg·dry·weight·ha^{-1}·d^{-1}) with high protein yields (208–176 g·kg^{-1}) without the need for nitrogen fertilization [32]. This unique freshwater fern grows rapidly in N-free medium (relative growth rate greater than 0.5 d^{-1}) [33]. Due to its symbiotic relationship, *Azolla/Anabaena* fixes atmospheric nitrogen and releases it as NH_4^+ in leaf cavities, which is taken up by ferns to produce biomass [34,35]. Colonies of *Anabaena/cyanobacteria* are found in the apical meristem within the megasporangium indusium and in an ovoid cavity on the adaxial side at the base of each dorsal leaf-lobe of the *Azolla* leaf [19]. The studies carried out on *A. filiculoides* have shown that the growth rate in real wastewater and in artificial media with low nitrate concentration or even without nitrogen does not show a significant difference (0.122–0.126 d^{-1}). A high concentration of ammonium ions in a mineral medium has an inhibitory effect on the *Azolla/Anabaena* association [33]. It is noteworthy that the high ammonium concentration in artificial media (34–40 mg·N·L^{-1}) has a negative effect on the growth and nitrogen fixation of *A. filiculoides*, but this effect was not observed in real wastewater [36]. Although *Azolla* has several advantages, it is sometimes considered an invasive plant in wetlands because dense *Azolla* mats shade submerged macrophytes [29,37]. Fig. 3 shows the floating *Azolla* mat on the water surface.

The availability of water is a crucial factor for the growth of *Azolla*. Optimal growth conditions are light intensity (15–18 K Lux), water temperature (18°C–28°C), relative humidity (55%–83%), and the presence of macro- and micronutrients. Wind and turbulent water can fragment and kill *Azolla* [29]. *Azolla* is not able to grow sustainably in a saline

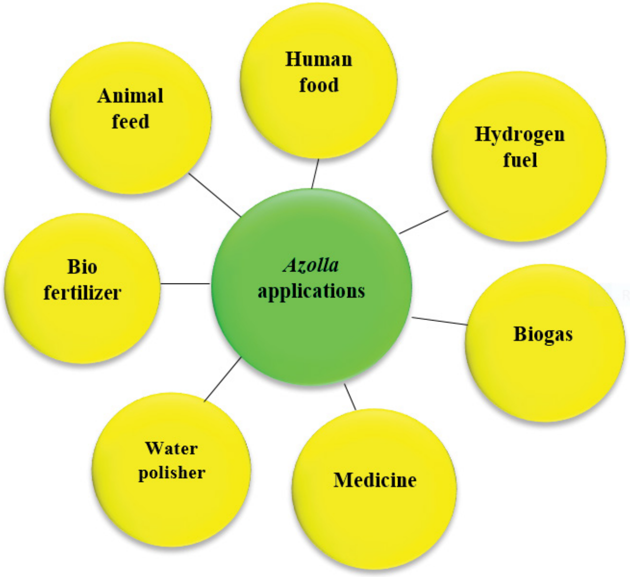

Fig. 1. *Azolla* applications.

Fig. 2. *Azolla* fern in the water [30].

Fig. 3. Floating *Azolla* mat on the surface of water [30].

medium. Salinity has a strong negative influence on photosynthesis of *Azolla*. Moreover, *Azolla*/*Anabaena* symbiosis in nitrogen fixation is also affected by the negative effect of salinity [38]. Six *Azolla* species (*A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. pinnata*, *A. rubra*, and *A. caroliniana*) were studied in saline growth medium by adding 0.32% NaCl under polyhouse conditions. The results showed that *A. microphylla* and *Azolla rubra* were more tolerant to salinity. It was also found that *A. microphylla* performed better than the other species in terms of biomass productivity [39].

3. Mechanisms of Azolla for the degradation of pollutants

Bioremediation is a controlled process that uses microorganisms to degrade hazardous organic pollutants to harmless compounds such as CO_2, CH_4, and H_2O [40]. Phytoremediation is a bioremediation process that uses plants and their associated microorganisms to remove pollutants from water or soil [41]. The four main mechanisms of phytoremediation are shown in Table 1 [42]. *Azolla* can uptake metals, organic and inorganic pollutants from the aquatic ecosystem, either in the form of living biomass (bioaccumulation) or inactive/dead biomass (biosorption) [13,43]. Plants generally use several extracellular and intracellular mechanisms to remove heavy metals. The external mechanism is the exudation of phytosiderophores into the rhizosphere. The exudation mechanism can chelate and dissolve metals bound to the soil. Two ways to enhance metal accumulation in plant tissue are acidification of the rhizosphere and ion exchange sites on cell walls to bind metal ions. Metals are bound by the cell wall, transport systems, and intracellular mechanisms for uptake across the plasma membrane [43].

The root surface (rhizosphere) of *Azolla* hosts a microbiome that can support successful phytoremediation of metals. The identification of rhizobacteria in the root zone of *A. filiculoides* L. treated with metals (Pb, Cd, Cr(VI), Ni, Au, Ag) reveals the presence of known metal-tolerant genera (Mucilaginibacter, Pseudomonas, Mycobacterium, Corynebacterium, Stenotrophomonas, Clostridium, Micrococcus, Achromobacter, Geobacter, Flavobacterium, Arthrobacter, and Delftia) [44]. Rhizofiltration is an effective sub-process of phytoremediation that takes place in the roots of plants and can reduce concentration of heavy metals in polluted waters [45]. In phytoremediation, the interaction between plants and their associated microorganisms causes the release of various metabolites (e.g., 1-aminocyclopropane-1-carboxylic acid deaminase, indole-3-acetic acid, siderophores, organic acids, etc.). These compounds with acidifying, chelating and/or reducing effects facilitate phytoremediation through the processes of translocation, transformation, chelation, immobilization, solubilization, precipitation, volatilization and complexation of heavy metals [46]. A comparison of the rhizofiltration capacity of *Azolla*, *Pistia* (water lettuce) and *Eichhornia* (water hyacinth) in reducing the initial concentration of As (5,000 $\mu g\cdot L^{-1}$), Cu (25 $mg\cdot L^{-1}$), Pb (66 $mg\cdot L^{-1}$), Ni (58.16 $mg\cdot L^{-1}$) and fluoride (50 $mg\cdot L^{-1}$) was performed. The results showed that *Eichhornia* had the greatest effect in reducing Pb and As, so that the final concentrations of these two elements were 0.189 ± 0.007 and 12.683 ± 1.002, respectively. *Azolla* had the best performance in absorbing nickel, while *Pistia* had a greater effect in reducing the initial concentration of copper, and the final concentration of these two elements in water was 1.479 ± 0.032 and 0.265 ± 0.036, respectively. These observations were associated with an increase in the content of organic acids (citric acid and malic acid) as root exudates, so that Nickel treated with *Azolla* significantly increased the content of these 2 acids by 1.7 times [47].

Table 1
Phytoremediation processes and mechanisms of contaminant removal [42]

Process	Mechanism	Contaminant
Rhizofiltration	Rhizosphere accumulation	Organics/ inorganics
Phytostabilization	Complexation	Inorganics
Phytoextraction	Hyper-accumulation	Inorganics
Phytovolatilization	Volatilization by leaves	Organics/ inorganics
Phytotransformation	Degradation in plant	Organics

4. Removal of pollutants from aqueous media by Azolla ferns

As there are many kinds of pollutants in the environment and many technologies to deal with them. In this situation, we summarized a comprehensive and instructive review based on the previous published literatures of phytoremediation of various *Azolla* species in aqueous solutions for removing organic and inorganic contaminants (Table 2).

According to Table 2, *Azolla* species, living or non-living biomass, single/multiple pollutant solution, solution type, chemical properties of the pollutant, initial pollutant concentration, and contact time mainly affect the removal efficiency. It was reported that light intensity, water temperature, pH, macro- and micronutrient availability, and water turbulence were the most important factors affecting the growth of *Azolla*. Thus, *Azolla* shows higher removal efficiency in

Table 2
Summarizes the uptake of various pollutants by *Azolla* reported in the literature

Species of *Azolla*	Type of medium	Pollutants	Removal rate	Condition	References
Azolla filiculoides	Aqueous solution	Pb^{2+}, Cd^{2+}, Ni^{2+}, and Zn^{2+}	Inactivated *Azolla* with methanol: Pb^{2+}: 36%, Cd^{2+}: 33%, Ni^{2+}: 34% Zn^{2+}: 24% Inactivated *Azolla* with ethanol: Pb^{2+}: 41%, Cd^{2+}: 36%, Ni^{2+}: 38% and Zn^{2+}: 31%.	100 mL of the heavy metals solution (25 mg/L for each metal ions) were prepared individually. The solutions were incubated with 2.0 g (dry *Azolla*)/L and pH 5.5 ± 0.2 at 22°C ± 2°C with orbital shaking (150 rpm). After 1 h exposure, metals-laden biomass was separated by centrifugation	[14]
Azolla filiculoides	Olive mill wastewater (OMWW)	COD and polyphenols	Traditional extraction systems (TS) of OMWW: COD: 52%, polyphenols: 53% Continuous extraction systems (CS) of OMWW: COD: 95%, polyphenols: 65%	- 5:1 OMWW to *Azolla*-fresh-weight ratio - COD (mg/L) in TS 92,000 ± 2,200 and in CS 44,400 ± 800 - Total polyphenols (mg/L) in TS 7,360 ± 290 and in CS 4,367 ± 130 - Fresh *Azolla* biomass (200 g), the OMWW volumes 1 L, contact time 30 min	[24]
Azolla pinnata	Aqueous solution	Acid Blue 25	36.5 ± 0.5% at the fifth cycle; 41.2 ± 3.6% at the first cycle.	- A 500 mg/L AB 25 stock solution - C_i ranged from 40 to 500 mg/L and temperature (35°C, 45°C, 55°C and 65°C) - Both adsorbents were treated with 100 mg/L AB 25 of adsorbent dosage 2.0 g/L - pH 2.0, and short duration of contact time at 180 min	[48]
Azolla pinnata	Water body (Lake)	Heavy metals	Cu (97.7%), Pb (79.7%), Cr (74%), Cd (85.7%) and Zn (97.1%)	- 10 g of healthy plants, exposed to 4 mg/L concentration of 5 different heavy metals - 10 d experimentation period - During experimental period pH 7.0 ± 0.2, water temperature 25°C ± 2.0°C	[49]
Azolla pinnata R.Br.	Dairy wastewater (DWW)	pH, electrical conductivity, total dissolved solids, total Kjeldahl's nitrogen, and total phosphorus	9.41%, 61.42%, 71.56%, 73.25%, and 65.37%	- Five different concentrations of DWW including, 0%, 25%, 50%, 75%, and 100% were used - The average temperature, humidity, and light intensity of the experimental room were 20°C, 55%–70%, and 2,000 lux, respectively	[50]
Azolla pinnata R.Br.	Integrated industrial effluent (IIE)	TDS, BOD, COD, TKN, Ca, Mg, Na, K, MPN, SPC, P, Cd, Cu, Cr, Fe, Pb, and Zn	25.1, 54.1, 68.8, 71.8, 74.1, 81.9, 45.0, 30.0, 20.9, 41.9, 60.0, 54.1, 60.0, 57.3, 53.9, 58.1, 56.1, 72.4, and 60.0	- Experimental period: 12 h for 14 d - Effluent samples were collected from each concentration of treatment pot at the 0, 7, 14, 21, and 28th days during the phytoremediation experiments	[51]
Azolla filiculoides	Wastewater	Ni and Cu	Maximum uptake capacities for Ni 0.77 and Cu 0.54 mmol/g (dry *Azolla*)	- 0.5 to 1 mm size particles of *Azolla* - Experiments were carried out at room temperature (25°C ± 1°C)	[20]
Azolla filiculoides	Aqueous solution	Acid Green 3 (AG 3) dye	99.1% in optimum conditions	- Optimum conditions: pH = 3, contact time = 90 min, adsorbent dosage = 4 g/L and dye concentration = 10 mg/L	[52]

Table 2 (Continued)

Table 2

Species of *Azolla*	Type of medium	Pollutants	Removal rate	Condition	References
Azolla/ Anabaena	Freshwater fish breeding areas	Ammonia	Highest removal rate was 6.394/h at light intensity of 140 μmol/m^2·s and at a temperature of 28°C; the lowest was 0.947/h at 20 μmol/m^2·s and 15°C	- Under artificial light of 20, 70, and 140 μmol/m^2·s - Water temperatures for the growing *Azolla* (15°C, 22°C, and 28°C)	[53]
Azolla filiculoides	Industrial wastewater	Cr(VI)	83.3% at pH of 2	30 mL of different chromium concentration solutions, 1 h of contact time, with 0.1 g of *Azolla filiculoides* biomass	[54]
Azolla filiculoides	Aqueous solution	Bisphenol A	More than 90% when BPA concentration was 5 ppm and biomass was 0.9 g	- Containers with the capacity of 200 mL - Desired concentration of BPA including 5, 10, 25, and 50 ppm - *Azolla* mass was used 0.3, 0.6, 0.9 and 1.2 g - Contact time 2, 8, 12, 16, and 20 d	[22]
Azolla microphylla with papaya stem	Pond wastewater	Nitrate, ammonia, and phosphate	57%, 62%, and 71%, Respectively	100 g of *Azolla* with 100 g of papaya stem, contact time 24 d	[55]
Azolla filiculoides	Petroleum refinery wastewater	N and P	In separate medium: 36% and 44%, respectively In mixed solution of these two compounds: 33% and 40.5%, respectively	- Volume of wastewater: 1 L - Mass of *Azolla*: 24 g - Contact time 28 d	[34]
Azolla microphylla	Municipal effluents	Total organic C, total Kjeldahl's N, and total P	42%, 39% and 17%, respectively	0.5 g of *Azolla*, 200 mL of Espinase and Watanabe medium, contact time 7 d	[56]
Azolla pinnata	Aqueous solution	Cu, Pb, Cr, Cd and Zn	44%, 3.94%, 2.85%, 6% and 34%, respectively	*Azolla* were exposed to 4 mg/L concentration of different heavy metals, contact time 10 d, 5 L trays	[57]
Azolla pinnata	Industrial effluents	Hg^{2+} and Cd^{2+}	70%–94%	Contact time 13 d, volume of aquariums: 40 L, 58.5 g *Azolla* added to each aquarium	[58]
Azolla pinnata	Reservoir water	Hg	80%–94%	Contact time 6 d, volume of glass aquariums: 40 L, volume of *Azolla*: 210 g/aquarium	[59]
Azolla pinnata	Aqueous medium	Cr(III)	70%–88%	Contact time 13 d, aquariums capacity: 40 L, 58.5 g *Azolla* added to each aquarium	[60]
Azolla filiculoides	Aqueous solutions	Acid Blue (AB) 15 dye	98%	200 mg/L initial AB 15 concentration, contact time 10–180 min, 100 mL solution, optimum condition (pH 3, contact time 90 min, adsorbent dose 10 g/L and AB 15 concentration 10 ppm	[61]
Azolla filiculoides	Industrial wastewater	Reactive Red 198	97.3%	- Initial dye concentration was 10, 25, 50, 100, and 200 ppm - Absorbent dosage: 0.2–1.4 g - Contact time: 10, 20, 30, 45, 60, 90, 120, 180 and 240 min - pH: 3, 5, 7, 9 and 11	[62]

Table 2 (Continued)

Table 2

Species of *Azolla*	Type of medium	Pollutants	Removal rate	Condition	References
Azolla filiculoides	Wastewater	Au (gold)	98.2% from a gold Plating factory containing 5 mg·gold/L in solution, 100% of gold from diluted wastewater, 98% of gold from undiluted wastewater containing 41 mg·Au/L	- Stock gold solution 1,000 mg/L - Providing contact times of 60 or 20 min, respectively.	[63]
Azolla filiculoides	Aqueous solution	Phenol	up to 95% at 30°C, more than 97% when phenol concentration was 5 ppm and biomass was 0.9 g	Capacity of each container 200 mL, concentrations of phenol solution were 5, 10, 25, and 50 ppm, *Azolla* was used in certain weights including 0.3, 0.6, 0.9 and 1.2 g, measured on days 4, 8, 10, 12, 16, and 14	[64]
Azolla filiculoides	Aqueous solution	Fluoride	98%	Optimum pH was 5, contact time up to 75 min, fluoride concentration of 10 mg/L	[65]
Azolla filiculoides	Aqueous solution	COD, TP, and TN	77.5%, 66.8%, and 78.1%	- Synthetic wastewater prepared in the typical range of municipal wastewater (TP 10.8–84.6 mg/L, TN 20–99 mg/L and COD 66.26–415 mg/L) - 2 g fresh *Azolla* floated in 500 CC prepared wastewater - Contact time 21 d	[66]
Azolla	Wastewater	COD, Cd, Pb, Ni, total phosphorous, and total nitrogen	29.68%–98.82%, 92.84%, 97.12%, 76.82%, 46.45%, and 25.57%	- Number of days are between 0 to 30 d - Contact time and biomass of *Azolla* for heavy metal analysis are 15 d and 0.8 g - Initial concentration for COD, Cd, Pb, and Ni are 48–4,326, 5, 10, and 25 mg/L	[67]
Azolla pinnata	Industrial effluents	Hg and Cd	70%–94%	- Duration of investigation was 13 d and plant biomass was 58.5 g - Initial doses of heavy metals were 0.5, 1, 3 mg/L	[68]
Azolla filiculoides	Aqueous solutions	Bisphenol A (BPA)	60%–90%	- Biomass level: 0.3, 0.6, 0.9, and 1.2 g - Initial concentration of BPA: 5, 10, 25 and 50 ppm - Samples were collected every 2 d	[69]
Azolla pinnata	Palm oil mill effluent	BOD, COD, TS, TSS, oil and grease, NH_3–H, and phosphate	63.67%, 80.02%, 85.89%, 80.58%, 81.50%, 98.44%, 92.92% and 0.90 %	- Quantity of *A. pinnata*: 20–30 g - Concentration of palm oil mill effluent: 10%–60% - Retention time: 1–5 d	[70]

a solution with one pollutant than in a solution with multiple pollutants. However, in a real water body, there is a mixture of pollutants.

4.1. Removal of inorganic pollutants

The extent of bioaccumulation of pollutants in macrophytes varies greatly. The phytoremediation capacity of duckweed (*Lemna minor*, *Lemna trisulca*, *Salvinia natans*) and water fern (*A. filiculoides*) was compared. The results show that duckweed species uptake more K, Mg and Na than water fern. *A. filiculoides* showed lower capacity to bioaccumulate metals than *S. natans* and *L. trisulca*. The content of Cd, Cr, Cu, Fe, Ni, and Pb in *A. filiculoides* was lower than in *L. trisulca* but higher than in *L. minor* [71]. Furthermore, the bioaccumulation capacity in plants is metal dependent. In another study, the phytoremediation capacity of *L. minor* L., *A. filiculoides* Lam. and *Pistia stratiotes* L. in contact with Cr, Cd, Pb and Zn was investigated. It was reported that *L. minor* and P. stratiotes had the highest absorption of Pb, while *A. filiculoides* absorbed more Cr. In general, the removal efficiency of the aquatic plants studied was in this order: *P. stratiotes* > *L. minor* > *A. filiculoides* [72]. The bio-concentration factor (plant tissue concentration/water concentration) and translocation ability (root concentration/shoot concentration) of Cd, Cu, Pb, and Ni in three groups of hydrophytes (free-floating, attached, and submerged plants) were studied. It was found that the free-floating plants had better bio-concentration factor and translocation ability than the attached hydrophytes. This is due to the greater surface area to volume ratio of the free-floating plants [73]. The results of a study conducted to investigate the ability of *Azolla pinnata* to reduce iron in mine wastewater showed that the average value of iron translocation was 1.02. This value shows that the iron concentration is higher in the roots than in the canopy. Iron is taken up by the epidermal cells of the root and enters the xylem vessels of the roots through the apoplast or symplast pathway [74]. Plants synthesized phytochelatins (PCs) upon exposure to heavy metal ions. Phytochelatins are small cysteine-rich peptides that can bind metals via SH groups and play a key role in homeostasis and detoxification of metals and metalloids in plants. Phytochelatins complexes with different toxic metals have different structures, and they are also stable and less toxic than free metal ions [75]. The study of the phytoremediation potential of different *Azolla* species upon bioaccumulation of Cd, Cu, Zn and Ni showed that the level of metallothionein and phytochelatin synthase encoding genes expression depends on the type of metal, the duration of exposure and the metal concentration in the medium. Since the bioaccumulation of heavy metals and gene expression differ among *Azolla* species, it can be concluded that an appropriate species should be used for the elimination of the specific metal [76]. The presence of metals in water affects the growth of *Azolla*. It appears that bioaccumulation with living *Azolla* biomass cannot continue at high concentrations of toxic metals. Therefore, in highly toxic media, biosorption with inactive/dead biomass seems to be a more sustainable option [77].

Bioremediation of a solution contaminated with hexavalent uranium (U(VI)) followed by real liquid organic radioactive waste was performed with macrophytes (*Limnobium laevigatum* and *Azolla* sp.). In the solution containing a range of U(VI) (0.25–36 mmol·L^{-1}), *Azolla* showed a higher sorption capacity (0.474 mmol·g^{-1}) compared to *L. laevigatum* (0.026 mmol·g^{-1}). When *Azolla* was contact with real liquid organic radioactive waste, the sorption capacity decreased to 0.010 mmol·g^{-1}. Therefore, it was recommended to use both macrophytes simultaneously [78]. Although *Azolla* is able to hyperaccumulate heavy metals, some heavy metals can suppress the growth of *Azolla*. The extent of the metal effect on growth reduction depends on the type of metal and the *Azolla* species. *Azolla caroliniana* was cultivated in a nutrient solution containing metals (Hg^{2+}, Cr^{3+} and CrO_4^{2-}). The results show that the presence of these ions inhibits the growth of *A. caroliniana* by 20%–31% [79]. Different pollutants have different potential to be taken up by roots. For example, the concentration of various heavy metals (Cr, Pb, Hg, Cu, Zn, Cd, Ag and Ti) in the water body and in *A. filiculoides* was investigated. It was reported that the highest and lowest amounts of heavy metals in *Azolla* samples were Zn and Cd, respectively. This is because Zn is a vital element for the growth of *A. filiculoides* [80].

According to previous studies, *Azolla* has been shown to remove heavy metals such as Pb, Cd, Ni, Zn, Cr, Cu, and Hg from aqueous solutions at rates of 4%–41%, 6%–94%, 34%–38%, 24%–34%, 3%–83%, 44%–88%, and 70%–94%, respectively. In the case of nutrients such as nitrogen (total forms) and phosphate, the removal efficiency reached 36%–62% and 17%–71%, respectively. In addition, *Azolla* could remove gold and fluoride up to 98% from aqueous media.

4.2. Removal of organic pollutants

Macrophytes have different potential for phytoremediation of organic pollutants. The removal of organic pollutants was investigated using water hyacinth. The water hyacinth studied can uptake mesotrione and fomesafen (96.7%–98.2%), naphthalene (100%), formaldehyde (93%), sulphadiazine (83.5%) and oxytetracycline hydrochloride, chlortetracycline hydrochloride and tetracycline hydrochloride (>80) [81]. In other study, under optimal conditions (6.3 d contact time, 11.9 g biomass and 14.7 mg/L tetracycline concentration), *Azolla* removes more tetracycline (100%) compared to chloramphenicol (70%). The mean bio-concentration factors (BCFs) of tetracycline and chloramphenicol were 4.9% and 37.8%, respectively, in *Azolla* [82]. *A. filiculoides* was used as a carbon source for the production of activated carbon (ACAF) and for the biosorption of ampicillin from water. The results confirm the promising abilities of *Azolla*. Under optimal conditions (ACAF dose = 0.8 g/L, pH = 7, ampicillin concentration = 100 mg/L, contact time = 60 min and temperature = 45°C), a removal efficiency of 96.84% (114.3 mg/g) was obtained. The experiments showed that the presence of different concentrations of $NaNO_3$ competitors did not significantly affect the reduction of *Azolla* adsorption capacity [83]. In a laboratory-scale phytoremediation study, *Azolla* was shown to degrade antibiotics in three sequential steps. The first step is the uptake of antibiotics and the release of reactive oxygen species (ROS). In the second step, the effects of ROS are neutralized and minimized by conjugation with enzymes such as glutathione transferase or metabolites such as glutathione. The last step ends with the storage of the assimilated compounds in the vacuoles, the apoplast and the cell wall [84].

Moreover, the chemical properties of water play a crucial role in achieving higher efficiency in the phytoremediation process. For example, one researcher pointed out that the phytoremediation efficiency of water hyacinth significantly depends on the N content [85]. A study on treatability of grey water with water hyacinth showed significant reduction in COD (51.61% ± 13.56%), ammonium-nitrogen (62.15% ± 9.08%), phosphate-phosphorus (58.13% ± 15.23%), turbidity (90.68% ± 7.01%) and total dissolved solids (9.21% ± 2.65%) [86]. The presence of metals can limit or enhance the growth of macrophytes. Some organic substances can also have a toxic effect on *Azolla*. The study of the toxic effects of bisphenol A (BPA) on *A. filiculoides* showed that BPA (≥20 $mg\cdot L^{-1}$) has a negative effect on the growth indices of the *Azolla* (number of branches, leaf area, growth rate of the plant with severe leaf damage, membrane peroxidation, and electrolyte loss) [87]. As an aquatic fern, *A. filiculoides* has a wide range of removal efficiency for COD (52%–95%) and polyphenols (53%–65%). It has been reported that *A. filiculoides* can remove 60%–97% of phenols from an aqueous solution. The reason for the difference in removal efficiency can be explained by the different initial concentrations of phenol or initial *Azolla* mass [24]. In another case, the researchers showed that the removal of phenol was significantly improved by increasing the temperature, decreasing the initial concentration of the pollutant, and increasing the initial *Azolla* mass [64]. In a successive sorption-regeneration study, *Azolla* was shown to remove 36%–99.1% of the Reactive Red dye 198. Removal efficiency depends on the number of regeneration cycles, pH, contact time, plant mass, and initial dye concentration. There is also evidence that 42% of the total organic carbon was removed by *Azolla* [62]. Evaluation of the phytoremediation ability of *A. filiculoides* Lam. in nitrogen-free Hoagland culture medium containing 25, 50, and 75 $mg\cdot L^{-1}$ naphthalene showed that 94% of *Azolla* was absorbed, accumulated, and/or biodegraded after 10 d [88]. Studies have shown that *Azolla* has the ability to remediate water contaminated with phenanthrene at concentrations less than 10 mg/L [89].

Comparison of the use of *A. filiculoides* and *Lemna minuta* in the treatment of wastewater at concentrations of 5 $mg\cdot L^{-1}$ for each metal (Fe(III), Cr(VI), Al(III)) and 1 $\mu g\cdot L^{-1}$ for the pharmaceuticals (diclofenac and levofloxacin) shows the superiority of *Azolla* in removal efficiency and tolerance to pollutants. The removal efficiencies of levofloxacin and diclofenac were 60% and 10%, respectively. Although Cr, Fe and diclofenac had a minor toxic effect on *Lemna*, the presence of Al had a growth-promoting effect on both plants [90]. *A. pinnata* and *L. minor* were used for phytoremediation of palm oil mill effluent (POME) at different dilutions (2.5%, 5%, 10% and 15%). The effluent from this industry is non-toxic in nature and contains a large amount of nutrients (198 $mg\cdot L^{-1}$ COD, 4.3 $mg\cdot L^{-1}$ nitrate, pH 9.53, 4 $mg\cdot L^{-1}$ phosphate, 2.98 $mg\cdot L^{-1}$ ammonia). Compared to *Lemna*, *Azolla* has shown higher efficiency in removing all pollutants within 10 d. *Azolla* and *Lemna* have shown significant COD removal efficiencies of 78% and 66%, respectively at a dilution of 15%. The maximum growth of *Azolla* (296%) was observed at a dilution of 2.5%, but the maximum growth of *Lemna* (535%) was observed at a dilution of 5%. Both plants studied have the ability to absorb nutrients from wastewater and convert them into carbohydrates and proteins for their growth. In *Azolla*, the amount of carbohydrates in 5% dilution was equivalent to 2 $mg\cdot L^{-1}$. The carbohydrate content of *Azolla* increased by 43.71% from the second day to the 10th day. The protein content of *Azolla* at 2.5% dilution was 26%. Although diluted POME is a suitable medium for the growth of these two plants, increasing the proportion had a decreasing effect on the concentration of carbohydrates and proteins. The results of this study show the ability of *Azolla* to treat POME with non-toxic and nutritious substances and to produce qualified biomass for animal feedstock [91].

5. Possibilities of using Azolla biomass

In recent decades, increasing concern about the environmental impact of fossil fuels has focused attention on the use of biofuels. On the other hand, the raw materials for biofuels are many plants such as corn and soybeans, which can be used directly for human consumption or indirectly as animal feed [92]. Ethical and economic criticism of biofuel production mostly focuses on the demand for land, water and other resources, which can threaten human food security, increase food prices [93] and even lead to more greenhouse gas emissions [94]. These challenges have drawn attention to the further development of biofuel production technologies and the use of microalgae as biomass [95]. After phytoremediation, the harvested *Azolla* biomass can be converted into bioenergy in the form of bio-diesel, bio-hydrogen, bio-methane, bio-ethanol and bio-oil as a renewable energy source [96]. A theoretical study to find natural solutions to reduce the risks of fossil fuel consumption has shown that *Azolla* has the ability to sequester 21,266 kg/ha of CO_2 (C) per year from the air and convert it into new biomass [97]. The oil content of *Azolla* is not as high as that of microalgae, but the problems in separating *Azolla* biomass from water are much less than with microalgae. The oil extracted from *Azolla* for biodiesel production is acceptable compared to other feedstock's, and the cost of propagating *Azolla* biomass for biodiesel production is lower compared to growing the plants [28]. In one study, *Azolla* was used to treat tannery effluent. Subsequently, *Azolla* oil was extracted and used to produce biodiesel. The results showed that transesterification of *Azolla* oil to biodiesel in an optimized process (catalyst loading 2%, molar ratio 1:9, temperature between 60°C and 65°C) is cost effective and reliable [98]. The biomass of *A. filiculoides* obtained during phytoremediation can be used as a raw material for biofuel production. The ability of *A. filiculoides* (11.7×10^3 L/ha-y) to produce ethanol has been shown to be equivalent to that of corn stover (13.3×10^3 L/ha-y). *A. filiculoides*, with an efficient C/N ratio of 15 (41% total carbohydrates and 20% proteins), is capable of producing biohydrogen at the rate of 2.2 mol H_2/mol reducing sugars [99]. Biofuel is one of the best options for securing future energy supplies from an agriculture-based economy. Because of the high oil content of *Azolla*, its biomass can be used to produce biogas and biodiesel [100]. *Azolla* can proliferate in wastewater and produce large amounts of biomass [99]. *A. pinnata* has been studied as a potential source for biodiesel production. The calorific value (38.2 MJ/Kg) and dynamic viscosity (4.3 cP) of the synthesized biodiesel were similar to those of normal diesel (44 MJ/Kg and 3.06 cP) [101]. Studies on bio-methane production with *Azolla* biomass grown in water with three different metal concentrations (1, 4 and 8 mg/L) showed that these metals can have different effects. Iron and manganese had no effect on bio-methane production from anaerobic fermentation, but copper, cobalt, lead, and zinc were toxic. At low concentrations, cadmium and nickel have a positive effect on biogas production and methane content, but at high concentrations (8 mg/L) methane production decreases [102]. Review of research on the effect of heavy metals (chromium, cadmium and nickel) in the processes of anaerobic digestion and biogas production from biomass has led to the conclusion that the intensity of inhibition of heavy metals depends on the type of metal and its concentration in dissolved and ionic form in the digester. in addition, the toxic effects of heavy metals on biogas production have been attributed to the disruption of enzyme function and structure by the binding of metal ions to thiol and other groups on protein molecules or by the replacement of naturally occurring metals in the prosthetic groups [103].

Azolla biomass can also be used as animal feed. The dry matter of *Azolla* species contains proteins (21%–26%), fatty acids (41%–66%), and a wide range of active compounds such as phenols, caffeoylquinic acid derivatives, tannins, and carotenes. The predominant fatty acids in *Azolla* are palmitic, linolenic, and lignoceric acids [87, 104], so it is logical that *Azolla* is considered a sustainable source of animal feed in livestock, poultry, and fish diets. On the other hand, *Azolla* can accumulate heavy metals such as Cr, Pb, Zn, Hg, Cu, Cd, Ag, and Ti [80]. It has been demonstrated that feeding *Azolla* to *Cyprinus carpio* growing on water fertilized with sheep manure containing heavy metals (28.5 mg/kg Pb and 139.3 mg/kg Zn) causes histopathological changes and tissue damage in fish [105]. In addition, *Azolla* is a medicinal plant for sore throat and cough in New Zealand and Tanzania. *Azolla* may be effective in curing gram-positive bacterial infections [106].

6. Conclusion and recommendations

This review shows that the study of *Azolla* as an aquatic fern in phytoremediation processes has attracted the attention of scientists. Various research articles show that *Azolla* can be used to remove various pollutants in wastewater, such as excess nutrients, heavy metals, dyes, COD, and fluoride. It is a low-cost natural sorbent available in many regions of the world. In addition, after phytoremediation, *Azolla* can be converted into a nutrient-rich organic fertilizer, making phytoremediation with *Azolla* an environmentally friendly approach to removing pollutants.

Consequently, phytoremediation with *Azolla* is recommended as an advanced treatment after conventional wastewater treatment due to its high efficiency, low cost and environmental friendliness in water remediation. However, further studies are needed to assess the use of *Azolla* as a phytoremediation agent and its impact on real water bodies. In addition, the potential of *Azolla* for phytoremediation needs to be improved to successfully remove new pollutants. The rapid growth rate and high protein and fatty acid content make *Azolla* used as animal feed. In phytoremediation with *Azolla*, the proliferating excess biomass, which may contain a high concentration of pollutants, must be properly disposed of. However, when *Azolla* is grown in polluted water or wastewater, its use as feed is of concern due to bioaccumulation of pollutants. Therefore, bioenergy production using *Azolla* biomass grown in wastewater containing toxic metals or organic compounds harmful to human and animal health appears to be a safer option. Most of the research has been conducted on a laboratory scale, which may not correspond to the expected results under real conditions. It is therefore recommended to conduct studies under real conditions.

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Authors contributions

Samaneh Taghilou: Data curation, Methodology, Writing – original draft;

Mazyar Peyda: Project administration, investigation, Supervision, Writing – review & editing;

Mohammadreza Mehrasbi: investigation, Writing – review & editing.

Availability of data and materials

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Conflict of interest

The authors of this article declare that they have no conflict of interests.

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