25

Azolla Farming for Sustainable Environmental Remediation

Abin Sebastian[1], Palengara Deepa[2] and Majeti Narasimha Vara Prasad[3]

[1] Department of Botany, St George's College, Aruvithura
[2] Department of Botany, KAHM Unity Women College, Manjeri, India
[3] School of Life Sciences, University of Hyderabad, Central University, P.O., Gachibowli, Hyderabad, Telangana, India

25.1 Introduction

Environment is contaminating with pollutants [1–3]. Industrial effluents, mining activity, application of fertilizer, use of phytosanitory products, and pharmaceutical waste disposal are major source of pollutants in the environment. The usage of industrial waste water for irrigation also resulted hazardous material contamination in arable lands. The release of nitrates and phosphates in excess are a serious environmental concern during farming activities. Accumulation of pollutants such as hydrocarbons and heavy metals are very common in crop plants grow in contaminated areas. Hydrocarbons release into environment via dispersion, evaporation, dissolution, adsorption, and other processes including petroleum combustion [4]. The hydrocarbons can be aliphatic hydrocarbons, aromatic hydrocarbons, and polycyclic aromatic hydrocarbons. Many of the polycyclic hydrocarbons namely, benzo anthracene, benzo pyrilene, and benzopyrene are persistent chemical pollutants in the ecosystem. So these compounds are resistant to environmental degradation through chemical, biological, and photolytic processes respectively [2].

Hydrocarbons and associated chemicals mainly released into crop lands in the form of pesticides. The release of aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene is very common in croplands [5]. These chemicals are applied to control termites, grasshoppers, corn rootworm, and insect pests. But pesticides such as aldrin are also harmful to birds, fish, and humans. The accumulation of aldrin in rice grain reported to kill thousands of shorebirds. The fatal dose of aldrin accumulation in human is about 5 g. The usage of dairy products and animal meats is one of the major sources of aldrin accumulation in humans. Studies show that average daily intake of aldrin and its byproduct dieldrin is about 19 μg per person in India [6]. The food contamination with polychlorinated biphenyls (PCBs) was also a serious health concern [7]. Rice plants accumulated PCBs in grain. The consumption of rice contains PCBs resulted pigmentation of nails, swelling of eyelids, nausea, and vomiting. Fertilizer application had resulted pollution in environment, especially, agriculture fields [8]. The release of nitrate and phosphate is the major environmental concern in the course of fertilization. Phosphate fertilizer is very often contaminated with trace elements such as Cd. It reported that Cd levels in the mono ammonium phosphate fertilizer reach up to 50.9 $mg\,kg^{-1}$ because the parent rock

Handbook of Assisted and Amendment-Enhanced Sustainable Remediation Technology, First Edition.
Edited by Majeti Narasimha Vara Prasad.

material contain Cd more than 188 mg kg^{-1} [9]. Fluoride and radioactive elements contamination are also common during the application of fertilizers in arable lands. The toxic effect of fluoride also disturbs microbial activity in the soil, and hence disturbs soil microbial processes important for nutrient cycling. The presence of U-238 at the level of 1–67 $pCig^{-1}$ detected in phosphate fertilizer [10].

Waste water release from industry and mines contaminated many sites across the globe [11]. The contaminants range from metal ions to various organic compounds such as phenolic acids. Building up of salinity in arable lands is a well known drawback of irrigation. Rice paddies contaminated with heavy metals such as Cd because of irrigation using waste water release from mines [3]. The contaminated water bodies had maximum level of 100 μg Cdl^{-1}. It reported that Cd tainted rice result accumulation of about 20–40 μg Cd per day in humans. Rice also found to accumulate toxic levels of Pb, Cr, Hg, and As that are harmful to human health [12]. The agriculture produces are also contaminating with pharmaceuticals, hormone regulators, and various agrochemicals in the course of irrigation [11]. Geogenic contamination of environment is also very common where ground water use for irrigation. The major geogenic contaminant in arable lands is the metalloid As [13]. The entry of pollutants in environment is harmful to human health because of mutagenic, carcinogenic, immunotoxic, and teratogenic effects. Therefore, low cost and ecofriendly methods are preferred in the remediation of environment.

Phytoremediation use plants to cleanup pollutants from the environment [3]. Plants acquired hypertolerance to pollutants as a result of adaptive evolution to hostile environment. The pollutants inside the plant body very often detoxified with the help of vacuolar sequestration. Thus, the plants concentrate toxic material in the plant body, and the pollutants removed during harvest. Plants that are able to concentrate pollutant more than 1 g kg^{-1} dry weight utilized for phytoremediation [14]. Trace elements such as Cd, Zn, Pb, and Hg are effectively removed from the environment using plants. Also, plants secrete low molecular weight organic acids that chelate metal ions or organic compounds present in the soil. This strategy helps to both immobilize and extract pollutants present in the environment. Phytodegradation is effective in the removal of organic contaminants. The carbon based compounds are degraded with the help of enzymes produce in the plant. The enzymes may secrete into the environment resulting extracellular degradation of pollutant in the rhizosphere zone [14]. The pollutant entered in the plant body is also subjected to various enzymatic detoxification with the help of enzymes such as Cytochrome P450. Thus it is clear that plant based environmental remediation is highly promising for the environmental cleanup.

Aquatic macrophytes grow as emergent, submergent, or floating plants in water bodies [15]. These plants are well known for rapid multiplication and high biomass productivity over a short period. A well adapted root system helps efficient nutrient uptake in aquatic macrophytes. Many aquatic plants live synergetically with microbes such as nitrifying bacteria. This kind of growth adaptations helps aquatic macrophytes to filter water bodies for the removal of pollutants. The cell wall contains functional group such as –OH and –COOH that can be utilized for immobilizing pollutants [3]. Therefore, the biomass of aquatic macophytes are also useful for biosorption and development of cost-effective adsorbents. *Azolla* is one of the aquatic macrophyte having potential for invasive nature [16] (Figure 25.1). This plant grows rapidly with the help of N fixing bacteria namely, *Anabaena*. This high biomass yielding plant offers vast opportunities in the remediation of environmental contaminants. The present chapter discusses the potential of *Azolla* farming for the cleanup of pollutants with a focus to boost bioeconomy.

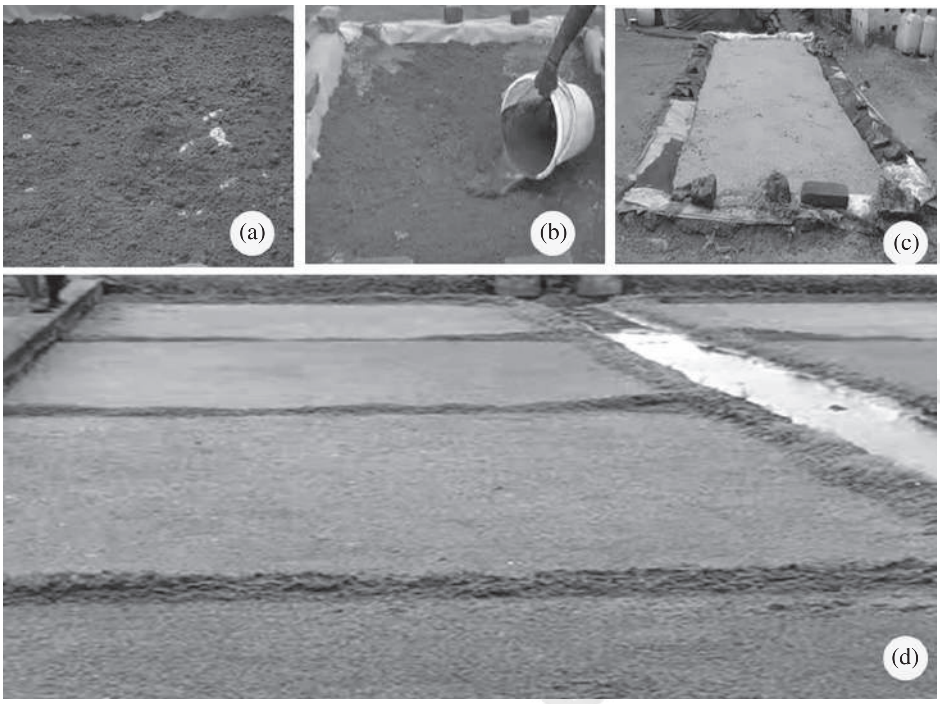

Figure 25.1 *Azolla* as a tool for environmental remediation. The plant had high biomass productivity in nutrient deficient environment and shows invasive nature. The plant can be multiplied in a low cost manner prior to application in the field. (a) Water storing blocks with soil for inoculation of culture. (b) Addition of green manure to boost multiplication rate. (c) *Azolla* culture inoculum. (d) *Azolla* mat in paddy field. *See color inset section for the color representation of this figure.*

25.2 Diversity and Ecological Distribution

The pteridophyte, *Azolla* is commonly known as mosquito fern, duckweed fern, fairy moss, and water fern. The genus consisted of two subgenera, *EuAzolla* and *Rhizosperma*. *EuAzolla* characterized by three megaspore floats with septate glochidia whereas nine megaspore floats with simple glochidia in *Rhizosperma* [17]. *EuAzolla* consists of four species including *Azolla caroliniana* Willd., *Azolla filiculoides* Lam., *Azolla mexicana* C. Presl. and *Azolla microphylla* Kaulf. Two Old World species namely, *Azolla pinnata* R. Br. and *Azolla nilotica* Decne ex Mett. present in *Rhizosperma*. *Azolla* is a dichotomously branched free floating aquatic fern (Figure 25.2). The shape of the Indian species is typically triangular measuring about 1.5–3 cm in length, and 1–2 cm in breadth. The sporophyte of *Azolla* is distinguishable in to rhizome, leaves, and roots [17]. Rhizome is profusely branched with dense leaves on the upper surface. The leaves are alternate, imbricate, and deeply bilobed. The leaves arranged in two rows on rhizome. Each leaf has two lobes namely, upper (dorsal) and lower (ventral) lobes. Green colored upper lobe had cavity called stoma which contain cyanophycean alga namely, *Anabaena azollae*. In contrast, the hyaline lower lobe is thin, and completely submerged in water to provide buoyancy. Under favorable conditions, the leaves and rhizomes turn to red because of the accumulation of anthocyanins. The smaller roots arise from the lower surface of rhizome as singly or cluster.

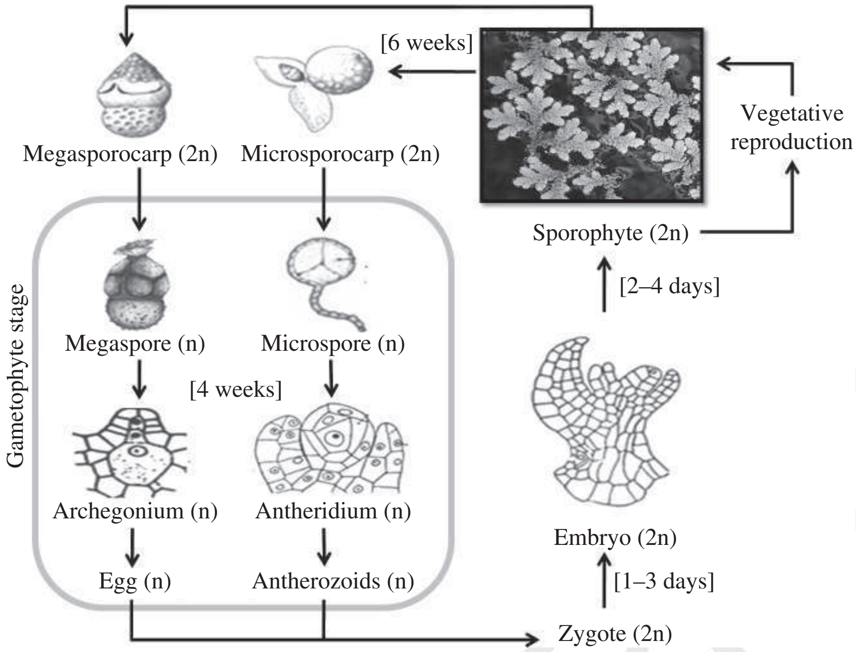

Figure 25.2 Life cycle of *Azolla* showing various developmental stages. The sporophyte had rhizome, leaves, and roots. Rhizome develop dense leaves contain cyanophycean alga namely, *Anabaena azollae* on the upper surface. The relatively short duration of developmental stages allows rapid multiplication of the plant. *See color inset section for the color representation of this figure.*

Azolla shows a permanent symbiotic association with heterocystic nitrogen fixing *Anabaena azollae* which has high potential to fix atmospheric nitrogen. Along with the cyanobacteria, several genera of bacteria namely *Arthrobacter*, *Corynebacterium,* and *Agrobacterium* are also associated with this fern [18]. Cyanobacterial filaments are distributed in peripheral region of the stoma, and embedded in mucilage with center being gaseous [19]. The cavity contains about 2000–5000 cyanobacterial cells. There are trichomes protruding in to the mucilage layer to exchange metabolites with *Anabaena azollae*. The mucilage consisted of different polysaccharides like glucose, galactose, and fucose, while rhamnose present in *A. pinnata*, *A. caroliniana*, *A. filiculoides*, and *A. rubra*. The mucilage cavity bordered with teat cells that mechanically protect the cavity from external ingress, and escapes of the symbionts [20]. The teat cells can also take as taxonomic key character, since teat cell projections are only present in *EuAzolla* and never observed in *Rhizosperma* [20].

The heterocyst frequency is higher in *Azolla* associated *Anabaena azollae* compared to the free living filaments [21]. About 50–90% of fixed N is delivered by *Anabaena* to the fern in the form of ammonium which transfer in to different parts of plant as aminoacids [22]. A considerable decrease in glutamine synthetase (GS) activity in the *Azolla* cyanobiont promotes ammonium transport to the *Azolla*. In the symbiotic association, the cyanobiont growth synchronized with that of the host plant. The growth rates of both partners are highest in the apical parts of the fern. At the zero growth rate of *Azolla*, cyanobiont cells cease to divide. The reproductive structure in *Azolla* is heterosporous sporocarp. Both megasporocarp and microsporocarp can easily distinguish in all species of *Azolla* (Figure 25.2).

Azolla species shows maximum growth in summer season followed by the spring season. It can grow quickly with a doubling time of 2–5 days. About 40–55 kg fresh *Azolla* can be formed from 8 kg inoculums within 15 days in rice plots with 10 cm water column. The plot require nutrient supplements in the form of 10 kg cow dung and 100 g triple super phosphate in three splits at 4 days of interval [16] (Figure 25.2). This aquatic fern multiply via both vegetative and sexual methods. In vegetative method, each branch will develop in to new plants. *Azolla* had heterosporous life cycle which includes the production of two types of genetically different spores such as microspore and megaspore. The spores are produced in the sporangia enclosed in a sporocarp. Microsporocarp is larger in size with many microsporangia whereas the smaller megasporangium enclosed in a megasporocarp with symbiotic cyanobacterium [18]. Germination of spores is influenced by nitrogen, light, amino acids, sugars, and abscissic acid [23]. The microspore encloses eight spermatozoids after metamorphosis (Figure 25.2). Meanwhile, germination of megaspore results in the development of archegonium with an egg cell. After fertilization, subsequent divisions of zygotic cell produces the primary organs namely root, stem, and leaf respectively. When air chambers develop in the first leaf, the embryo will rise to the water surface. Together with the multiplication of *Azolla*, the cyanobiont is transferred from one generation to the next through either leaf lobes or megasporocarps.

The species of *Azolla* is mainly distributed in fresh water ecosystems of tropical, sub-tropical and warm temperate regions of the world. *Azolla* species grows luxuriantly in ditches, fresh water ponds, and paddy fields [18]. As per the species distribution studies, *A. caroliniana* occurs in Eastern North America, Central America, North South America, the Caribbean, Mexica, and West Indies. *A. filiculoides* found in South America, Western North America to Alaska, and Anzali wetland. *A. mexicana* distributed in northern South America to British Columbia, Western America, and eastward to Illinois. *A. microphylla* occurs in South Western America and the West Indies. *A. pinnata* distributed over tropical Africa and Southern Africa, South East Asia, Japan, and Australia. *A. nilotica* spreads in Central Africa, Upper Nile Sudan, Uganda, Tanzania, Congo, and Namibia [16, 18]. Among the species, *A. pinnata, A. microphylla,* and *A. caroliniana* are also reported to found in all over the world [19].

25.3 Growth Conditions for Optimal Biomass Productivity

The consumption of primary and secondary macro nutrients have important role in growth and multiplication of *Azolla* (Table 25.1). In *Azolla*, the atmospheric nitrogen can be fixed through the symbiotic association with the blue green algae, *Anabaena*, that are present in the leaf cavity and sporocarp. The biologically fixed nitrogen transfers to tissues of *Azolla*, and further utilize for metabolic activities. The N deficiency signals promote the symbiotic N fixation in *Azolla* [23]. At the time of *Azolla* cultivation, a mixture of fertile soil and cow dung spread at the bottom of the cultivating area. To maintain the better growth and multiplication of *Azolla*, cow dung and super phosphate should be added in 10 : 1 ratio once in two weeks. *Azolla* is a strong phosphorus accumulator [34]. Supplementation of P gradually enhances the growth rate of this aquatic fern by catalyzing many biochemical reactions. Recent studies reveal the role of optimum concentration of P is about 30 mg l^{-1}. In contrast, the presence of highest number of heterocyst cells observed under lower concentration of N and P [35]. The deficiency of both Ca and P decrease the growth and N content of *Azolla*. But the deficiency of K and Mg has no more effect on growth [31].

Table 25.1 Growth responses of *Azolla* in response to nutrients.

Media/Nutrients in media	Response	References
Hoagland	+	[24]
IRRI2	+	[25]
IRRI1-Fe10x	+	[25]
Waste water with less N	+	[26]
MS	+	[27]
Urea	+	[28]
P	+	[29]
K	+	[30]
Ca	+	[31]
Co	+	[32]
Mo	+	[33]
Fe	+	[33]
Mn	+	[33]
Cu	+	[33]
NH_4^+	–	[28]
Nitrite	–	[29]

+: growth enhancement –: retardation of growth.

Azolla rapidly grows in presence of micronutrients like Fe, Mn, Cu, Co, Zn, B, and Mo [33]. Among these nutrients, Mo and Co enhances the nitrogen fixation capacity of *Azolla* [33]. Also, it reported that the species *A. caroliniana* is able to tolerate excess Mn, and therefore it suggested for phytoremediation of Mn [36]. Over accumulation of Cu, Co, Pb, and Zn induce toxic effect in *Azolla* whereas that of iron and manganese is non-toxic to the plant [37] (Table 25.1). The application of gibberellic acid or indole acetic acid had synergistic effects on the formation of sporocarps. This approach significantly increases the biomass production, irrespective of the presence of metabolic inhibitors viz. dinitrophenol, sodium azide and thiourea [38]. In *A. filiculoides*, cytokinin found to enhance root meristem development [39]. Among the amino acids, tryptophan enhances growth, N fixation, endogenous phytohormone levels, biomass, and doubling time of *A. pinnata* fronds [40]. Similarly, jasmonic and salicylic acid emphatically influenced on the abundance of cyanobiont [41].

Major abiotic factors influencing growth rate of *Azolla* are light intensity, temperature, photoperiod, pH, salinity, and humidity. Insects such as *Lepidoptera*, *Diptera*, and *Cephalapoda* also affect the growth of *Azolla* via grazing on the biomass. The optimal light intensity for growth of *Azolla* is 15–18 klx. The growth and photosynthesis inhibited at higher light intensities [42]. However, *A. pinnata* had maximum growth at 82 klx of sun light. In *A. microphylla*, the exposure to 75 klx sunlight increased the growth of symbiont too [43]. The color of *Azolla* changes in to brownish-red because of exposure to high light in summer season, and it becomes green during return of shade. The optimum photoperiod for the growth of *Azolla* is about 20 hours [16].

Habitually, *Azolla* prefers a medium that is near to neutrality or acidic. For proper growth, the optimal pH of medium varies from 4.5 to 7.5 [44]. The pH of the medium from 5 to 8 encourages the growth and nitrogen fixation in *A. pinnata* [31]. In case of *A. filiculoides*, optimum pH range is 5–7. Salinity gradually inhibited growth of *A. pinnata, A. caroliniana, A. rubra, A. mexicana, A. microphylla,* and *A. filiculoides* [45]. But, the addition of P in saline water can enhance the growth of *Azolla* [46]. *A. pinnata* can tolerate NaCl at a concentration of 60 mM [47]. *A. filiculoides* is more sensitive to high salt concentration than *A. pinnata*. Normally, the salt concentrations above 10 mM NaCl inhibit the growth of *A. filiculoides*. *Azolla* species grow well at relative humidity level of 70–75%. The plantbecomes dry and fragile at a relative humidity of less than 70%. Similarly, the relative humidity higher than 75% negatively influences growth because of low rate of transpiration and decrease in nutrient uptake respectively [26]. *Azolla* grows maximum at an optimum temperature range of 18–28 °C. Compared to other species, *A. pinnata* had highest tolerance to extreme temperature. Species such as *A. filiculoides* also found to survive under frost conditions [48].

In vitro culture of *Azolla* is mainly done using tissue culture media or sterile nutrient liquid media. H40, IRRI1- Fe1x, SH, and IRRI2 are the foremost liquid media for culturing *Azolla* that contain macronutrients, micronutrients, and iron sources for boosting the growth. In *A. filiculoides*, the lowest doubling time, highest relative growth rate, and root formation fostered in IRRI2 medium compared to IRRI1- Fe1x and H40 media [25]. The explants may be rhizome tip, root, or leaf for *in vitro* culture from which the new plants regenerate through direct or indirect organogenesis. Callus induction from leaf explants of *A. pinnata* achieved in modified SH media supplemented with indole-3-acetic acid (IAA) (1 mg l^{-1}), 1-naphthaleneacetic acid (NAA) (0.5 mg l^{-1}), 2,4-dichlorophenoxyacetic acid (2,4-D)(1 mg l^{-1}), and 6-benzylaminopurine, benzyl adenine (BAP) (0.5 mg l^{-1}) [27]. Similarly, the half strength Murashige and Skoog (MS) medium with 2.26 μM 2,4-D and 6.6 μM 6-BAP also induce callus from *Azolla* explants. Transferring onto medium having 6.6 μM 6-benzylaminopurin (BA) or IAA causes indirect regeneration of shoots and roots from *Azolla* callus. Thus, it is clear that the biomass production in *Azolla* can be enhanced with manipulation of nutrients and growth conditions.

25.4 Phytoremediation of Water Bodies

Aquatic macrophytes are well known for rhizofilteration because of rapid growth of roots [3]. This method removes contaminants with the help of absorption and precipitation. The roots of these plants establish stable contact with polluted water and the roots absorb or chemically transform contaminants. The absorbed pollutant may either store in roots or translocated to aerial parts. The plants continuously accumulate pollutants from water until the harvest. This method is successfully utilized to filter pollutants from water bodies including ground water, industrial waste water and solutions containing radio nuclides. The concentration factor is an important concern during rhizofilteration [49]. This factor represents a ratio of pollutant inside the plant body against pollutant present in the solution. Rhizofilteration method having higher pollutant concentration factor is preferred for purification of water bodies. *Azolla* sps had higher metal concentration factor which made them ideal for rhizofiltration of heavy metals (Table 25.2).

The ability to grow in N deficient conditions makes *Azolla* a candidate plant for rhizofilteration. This plant found to effectively remove trace metals from water bodies. *A. pinnata* removed more than 90% of Hg and Cd from industrial effluents [56]. The plant body contained about 7.4 g Cd kg^{-1} dry weight. This study indicated that *A. pinnata* is a suitable to remove heavy metals from ash slurry and chlor-alkali effluent. Studies with various strain of *Azolla* indicated potential of this plant in the removal of As [60]. The highest level of As found in the fronds of *A. caroliniana*. This plant accumulated more than 0.25 g As kg^{-1} dry biomass. The lowest level of As found in the fronds of *A. filiculoides* where the concentration of As was less than 0.06 g As kg^{-1} dry weight.

Table 25.2 Phytoremediation capability of *Azolla*.

Pollutant	Accumulation	References
Pyrocatechol	5 mg/0.9 g fwt	[50]
Rhodamine B	10 mg/0.4 g fwt	[51]
Atrazin	18 μg/1 g fwt	[52]
Ethyl-benzenes	5 mg/0.1 g fwt	[53]
CH_4	58 kg/ha land	[54]
N	60 kg/ha land	[55]
Cd	75 mg/Kg dwt	[56]
Zn	31 mg/Kg dwt	[57]
Ni	41 mg/Kg dwt	[57]
Pb	9 mg/Kg dwt	[58]
As	28 mg/Kg dwt	[59]
Hg	70 mg/Kg dwt	[56]

fwt - fresh weight, dwt - dry weight ha-hectare.

But *A. filiculoides* found to accumulate higher levels of Cd, Cr, Cu, and Zn ranging from a concentration of 1000–10 000 ppm [57]. This species also found to accumulate more than 1.8% Pb in biomass. Studies show that species of *Azolla* such as *A. microphylla*, *A. filiculoides*, *A. pinnata*, and *A. microphylla*, had bioconcentration factor greater than 90% for Pb^{2+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} respectively [61]. Mercury is a well known pollutant which undergoes phytovolatilization [62]. Therefore, chemical transformation of Hg is essential for efficient phytoremediation. Generally, Hg transformed to methyl mercury which later transform to ionic (Hg^{2+}) and elemental (Hg^0) forms. The accumulation of Hg in *A. caroliniana* reported to be greater than 800 mg kg^{-1} dry matter [63]. This species also found to accumulate significant amount of Cr, Cd, and Pb. Therefore, *A. caroliniana* can be utilized for cleaning municipal wastewater. The metal accumulation capacity of *Azolla* species found to be influenced by physiochemical properties of the solution such as the presence of diverse metal ions. For example the accumulation of Pb found to decrease in *A. pinnata* when there was excess Zn ion in the solution [37].

Several studies compared metal accumulation capacity of *Azolla* plants with other aquatic macrophytes. It reported that *Azolla* sps efficiently removed Hg from aquatic habitats compared with *Vallisneria* plants [64]. But the ability to accumulate Hg was lower compared to *Pistia* and *Eichhornia*. However, a direct comparison between aquatic macrophytes for metal accumulation is not feasible because of difference in both initial metal ion concentrations in the solution and amount of biomass used to start the culture. The metal accumulation capacity of *Azolla* sps also found to vary with metal ions. *Azolla* plants had high affinity to accumulate Ni. It reported that *A. filiculoides* accumulate Ni more than 28 mg kg^{-1} [65]. Also, order of metal accumulation in *A. pinnata* reported as Ni> Zn> Co = Cd > Cu > Pb> Cr [65]. The higher Ni accumulation capacity could be the outcome of relatively low toxicity of Ni unlike other heavy metals such as Cd and Pb.

Azolla found to effectively mitigate CO_2 from the atmosphere as well as water bodies. It reported that *Azolla* can fix 1.86 t CO_2 ha^{-1} within 1 year period [55]. The release of NH_3 is very common in fresh water lakes and rivers. The nitrogen rich fertilizer, livestock waste, household cleaning products, atmospheric deposition, and sewage treatment plants contribute formation NH_3 in water.

Ammonia found in water as NH^{4+} ion which is a weak base. An elevated level of NH_3 causes toxicity in fish via disturbing pH balance and gas exchange. The lethal concentration of NH_3 in water is 0.2–2 mg l^{-1}. But *Azolla* plants found to effectively absorb NH_3 from water bodies [55]. It estimated that *Azolla* can fix about 30–60 kg N ha^{-1}. Studies pointed that *A. pinnata* remove NH_3 from solution at a faster rate than *Eichhornia crassipes*. It found that growth of *Azolla* remove more than 5 mg l^{-1} NH_3 from lake water [55]. This study also revealed that combination of *Azolla* and *Eichhornia* effectively remove phosphate which is a potential agent of eutrophication in fresh water. The emission of CH_4 which is a greenhouse gas in flooded rice paddy system decreased more than 40% after cropping with *Azolla* [54]. It also found that the growth of *Azolla* stimulate oxidation of CH_4 in rice paddies because of creation of oxygen rich conditions in standing water. The presence of cyanobacteria had a critical role in the release of oxygen which build oxidation environment in the water. However, it must be noted that the presence N and C content in the soil is the key regulatory factor for CH_4 efflux during co cultivation of *Azolla* in rice paddies. The formation of N_2O which is also a greenhouse gas occurs in rice paddies because of the microbial transformation of nitrogen present in soils and manures [66]. The efficient N utilization capacity of *Azolla* also found useful to decrease N_2O emission from wet arable lands [66].

Hydrocarbon polluting sites are often N limited. The presence of *Azolla* not only increase N but also enhance microbial growth. The bioremediation capacity of *Azolla* on petroleum products found to depend on both bioaugmentation – enhanced degradation of pollutants with help of microbes and biostimulation – the improvement of physico-chemical conditions of the plot. A study conducted on diesel contaminated site revealed that *Azolla* significantly decrease the contaminant in 16 weeks with the help of microbes [67]. The amount of xylenes and ethyl-benzenes lowered about 100 times in the presence of *Azolla* culture. The growth of bacteria consortium with genes of *A. pinnata* decreased aromatic compounds up to 50% during growth on 4% diesel containing mineral medium [53].

Azolla also exhibited a notable ability to remove herbicides, pesticides, pharmaceutical waste products, and dyes. *A. caroliniana* grow in 10 ppm atrazine contaminated solution had accumulated about 18 μg of this compound per g fresh biomass [52]. Studies show that *A. microphylla* tolerate exposure to organochlorine insecticide – Endosulfan, and organophosphate insecticide – Monocrotophos. Tolerance to these pesticides achieved with the help of antioxidant enzymes and accumulation of proline, respectively [68]. Pyrocatechol use as a precursor chemical in pharmaceutical and perfume industry. Growth of *A. filiculoides* helped to remove more than 90% of this compound from 5 ppm pyrocatechol solution [50]. *A. pinnata* accumulated more than 75% of rhodamine B from aqueous solution contain 10 ppm of dye [51]. The pH of the solution found to influence accumulation of rhodamine B where maximum phytoremediation capacity observed at pH 3. Also, *A. pinnata* reported to effectively remove malachite green and methyl violet 2B dyes from aqueous solutions [69, 70]. The efficacy of removal was dependent on dosage where concentration of 15 ppm malachite green and 20 ppm methyl violet 2B found to be ideal for phytoremediation. Thus, it is clear that *Azolla* is a highly promising plant for the phytoremediation of water bodies.

25.5 Prospects in Sustainable Remediation and Bioeconomy

Sustainable remediation practices maximize the utility of a system for human health and environment [71]. This approach assesses impact of remediation on society, environment, and the economy. Apart from reduction of harmful impact on environment, the safety of community is focused in sustainable practices. The concept tries to minimize usage of petrochemical based energy

Figure 25.3 Sustainability of *Azolla* farming. The cultivation of *Azolla* bring socio-economical development in rural area because of potential to generate value added products such as compost and job opportunities. (a) *Azolla* growth ponds. (b) Facile seeding of *Azolla* in fields. (c) *Azolla* intercropped in rice paddies. (d) Washing of *Azolla* biomass for composting. (e) Inoculation of *Azolla* biomass in compost pits. (f). *Azolla* vermicompost. *See color inset section for the color representation of this figure.*

whereas maximize that of renewable energy [71]. There is higher demand for reuse, and recycle materials and waste in the course of sustainable remediation. *Azolla* plants widely applied for fertilizer management in rice paddies [3] (Figure 25.3). The ability to concentrate nitrogen in nitrogen deficient condition made this plant as an alternative of N fertilizer [19]. The lowland rice reported as ideal for application of *Azolla* because both the plants require flooded habitat. *Azolla* can be grown as monocrop or intercrop with rice. Monocrop method practices prior to rice culture. In this method, the biomass acts as a source of fertilizer in the field. But additional economy comes to attention when *Azolla* grow as an intercrop. So it is clear that sustainable remediation using *Azolla* can be performed both field and biomass based approaches.

Economy of handling *Azolla* culture is an important aspect when in use for environmental remediation. Large scale cultivation of *Azolla* practice using green manure such as cowdung [72]. Mineral salts are used when *Azolla* grown for biomass which can be converted to fodder and compost. Magnesium sulfate, murate of potash, and superphosphate are common salts used in large scale *Azolla* farming [72]. All the expenditure including labor for the production of 1 kg *Azolla* biomass is less than 1 US $in developing countries such as India (Table 25.3). Therefore, the utilization of *Azolla* biomass in sustainable remediation programs had tremendous scope. However, the drawback is that co culturing of *Azolla* with crop plants can only be practical under submerged conditions. Secondly, the short life span and dissolution of metal ions from dead colonies restrict usage of *Azolla* in phytoremediation.

Table 25.3 Bioeconomics of *Azolla* farming.

Manure/fertilizer expenditure	Expenditure (INR)
90 kg cow dung (Rs 1 for kg^{-1})	90
250 g Magnesium sulphate (Rs 20 kg^{-1})	5
100 g Murate of potash (Rs 50 kg^{-1})	5
2 kg Super phospahte (Rs 10 kg^{-1})	20
2 kg Mineral mixture (Rs 30 kg^{-1})	60
4.45 sq.m silpalin sheet	800
Bricks 30 nos	300
Labor 12 per year @ Rs 600 per day	7200
Total expenditure per year	8480
Azolla production	**Cost (INR)**
Compost 100 kg/tank for 2 kg per day / tank (350 days 700 kg)	500
Expenditure for 700 kg of *Azolla* f.wt.	8000
Azolla production cost per kg	11

Azolla very often intercropped with rice plants where the introduction of the plant helped to decrease the usage of nitrogen fertilizer [73]. Crop plants utilizes the ammonium either leaching from living *Azolla* plants or release during decomposition of biomass [74]. Also, *Azolla* intercropping help to increase the utilization of N in the field via trapping excess NH_3 release from the fertilizer. Secondly, intercropping with *Azolla* prevents growth of algae in the field because it acts as a physical barrier of sunlight. Thus growth of *Azolla* in paddy field prevents increase of soil pH and microbial process due to algal growth. The presence of *Azolla* also helps to decrease the temperature of the field up to 5% which is a critical factor affecting crop yield in tropics [75]. It estimated that two unit of petroleum required for the production of one unit of nitrogen fertilizer. This is because the production of 1 kg nitrogen fertilizer requires minimum energy of 50 MJ [76]. But *Azolla* plants can fix 30–60 kg N ha^{-1} in 30 days [77]. So the usage of *Azolla* in farming system helps to boost economy via decreasing the usage of synthetic nitrogen fertilizer. Synthetic fertilizer also had expenses during synthetic and transportation processes. Dry biomass of *Azolla* contains 25–35% protein, 10–15% minerals, and 7–10% amino acids, bioactive substances, and biopolymers. So besides nitrogen, *Azolla* co culturing helps to provide minerals such as phosphorus, amino acids, and vitamins essential for crop growth. Also, the growth of *Azolla* oxygenates rhizosphere of crop plants under submerged conditions.

Biomass conversion to green manure, fodder, biodiesel, and biosorbing agents ensure sustainable environmental applications and bioeconomy with *Azolla* farming (Figure 25.4). Soil physicochemical properties such as porosity and specific gravity of soil improved after mixing with *Azolla* manure [78]. It also reported that the addition of fresh biomass of *Azolla* at the rate of 20 kg ha^{-1} increased water holding capacity, organic carbon, ammonium-N, nitrate-N, and its available P, K, Ca, and Mg in the banana field [54]. Dry biomass of *Azolla* contains 3.32% N, 1.64% Ca, 2.71% K, and 0.34% P respectively. So the usage of *Azolla* biomass as green manure helps to replenish macronutrient deficiency in agriculture fields. Study pointed that *Azolla* manure treatment increases grain yield in rice more than 25% compared with urea [79]. *Azolla* manure also reported to increase

Figure 25.4 Bioeconomic potential of *Azolla* farming. The plant biomass can be used for the generation of value added products such as compost, fodder, biosorbents, and biofuels. The proteinaceous nature of *Azolla* biomass makes it as an ideal fodder. (a) Maintenance of *Azolla* culture in nursery. (b). Manual separation of *Azolla* harvested from field. (c,d) *Azolla* feeding in pigs. (e,f) *Azolla* use as a fodder for cows. *See color inset section for the color representation of this figure.*

yield of wheat, mungbeans, banana, and colocasia [54]. *Azolla* can use as mulch and compost. The preparation of compost can be done after mixing with rice straw. It is noteworthy that the usages of *Azolla* not only increase fertilizer use efficiency in the field but also prevent water pollution.

Azolla can serve as fodder for animal husbandry. The plant is enriched with 17–28% protein, and contains amino acids, vitamins, and trace minerals. Dry *Azolla* flakes are used as poultry feed whereas green biomass utilize as feed in pisciculture. *Azolla* can grow in situ in the fish rearing ponds [19]. Simply, a part of the pond is earmarked for inoculums, and a barrier is made using rope made of floating objects such as straw. *Azolla* slowly release to pond by lifting the rope after the formation of mats. *Azolla* plots help farmers to cater fodder when there is scarcity of grass especially during summer seasons. *Azolla* silage is preferred as fodder for pigs and cows (Figure 25.4). The fermentation and the storage processes help to lower moisture content as well as increase nutritional value of silage. *Azolla* silage is usually supplemented with regular feed of the animal.

Biosorption helps to remove toxic material from aqueous media. The biomass of *Azolla* found to efficiently biosorb heavy metals such as Pb, Cd, Cu, and Zn [80]. The optimum uptake range of metal ions was 50–228 $mg\,g^{-1}$ dry weight. Chemically modified form of *A. filiculoides* namely, ferrocyanide *Azolla* sorbents type 1 found to effectively remove Cs and Sr from solution [81]. This study points pretreatments help to increase pollutant binding capacity of cell wall of *Azolla*. The biomass of *Azolla* was very effective to remove chemical oxygen demand and polyphenols from olive mill waste water [82]. The biomass packed in to Imhoff cones and columns to remove the pollutants. The biofilter was able to remove up to 4000 ppm polyphenol in the waste water.

The higher biomass productivity made *Azolla* as a potent plant for biodiesel production. This plant found to have sufficient levels of cellulose/hemicellulose, starch, and lipids, resembling combinations of terrestrial bioenergy crops and microalgae used for biodiesel production [83]. Among various species, *A. filiculoides* and *A. pinnata* reported as ideal bioenergy crop. Hydrothermal liquefaction allows maximum production of 20.2 t ha^{-1} of bio-oil and 48 t ha^{-1} of biochar from *Azolla* in an year [84]. The ethanol production from *A. filiculoides*, 11.7×103 l ha^{-1} in an year, found to be close to that from corn stover (13.3×103 l ha^{-1} in an year), but higher than from miscanthus (2.3×103 l ha^{-1} in an year) and woody plants, such as poplar (1.3×103 l ha^{-1} in an year) [84]. Fermentation of *Azolla* biomass generates 2.2 mol mol^{-1} glucose or xylose of hydrogen with a high C/N ratio. Therefore, *Azolla* is a competitive feedstock for hydrogen production compared to biodiesel producing crops such as *Jatropha*. So it is clear that *Azolla* farming is highly promising with regard to minimizing greenhouse gas mitigation, development of economy, and employment opportunities, respectively.

25.6 Outlook

Azolla farming plays an important role in environmental remediation. The species diversity and adaptability to grow under low nitrogen environment are key characteristics that make *Azolla* ideal for application in environmental remediation. The plant is able to produce large amount of biomass in a short interval of time. Aquatic habitat allows usage of this plant in the cleanup of waste water. The plant accumulate significant amount of heavy metals and carbon compounds that are toxic. The fresh as well as dry biomass of the plant is very useful for making biosorbents, compost, fodder, and biofuel. There is tremendous scope for biomass of this plant for usage in phytoremediation which in turn boost bioeconomy. The optimal culture conditions of *Azolla* against various pollutants need to be standardized. The development of value added products from *Azolla* farming that are useful for sustainable environmental remediation must be in focus. The integrated *Azolla* farming based remediation strategies not only boost bioeconomy but also ensure sustainable environmental remediation.

References

1 Oke, S.A. (2004). On the environmental pollution problem: a review. *J. Environ. Eng. Landsc. Manag.* 12: 108–113.
2 Scheringer, M., Strempel, S., Hukari, S. et al. (2012). How many persistent organic pollutants should we expect? *Atmos. Pollut. Res.* 3: 383–391.
3 Sebastian, A. and Prasad, M.N.V. (2014). Cadmium minimization in rice. A review. *Agron. Sustain. Dev.* 34: 155–173.
4 Freije, A.M. (2015). Heavy metal, trace element and petroleum hydrocarbon pollution in the Arabian gulf: review. *J. Assoc. Arab Univ. Basic Appl. Sci.* 17: 90–100.
5 Sun, S., Sidhu, V., Rong, Y. et al. (2018). Pesticide pollution in agricultural soils and sustainable remediation methods: a review. *Curr. Pollut. Rep.* 4: 240–250.
6 Mitra, A. (2018). Pesticide level in lower Gangetic Delta. In: *Estuarine Pollution in the Lower Gangetic Delta: Threats and Management* (ed. A. Mtra), 183–190. New York: Springer.
7 Aken, V., Correa, B., and Schnoor, J. (2010). Phytoremediation of polychlorinated biphenyls: new trends and promises. *Environ. Sci. Technol.* 44: 2767–2776.

8 Carpenter, S.R. (2008). Phosphorus control is critical to mitigating eutrophication. *Proc. Natl. Acad. Sci. U.S.A.* 105 (32): 11039–11040.

9 Roberts, T.L. (2014). Cadmium and phosphorous fertilizers: the issues and the science. *Proc. Eng.* 83: 52–59.

10 Khater, A.E. (2012). Uranium and trace elements in phosphate fertilizers-Saudi Arabia. *Health Phys.* 102 (1): 63–70.

11 Ma, L., Liu, Y., Zhang, J. et al. (2018). Impacts of irrigation water sources and geochemical conditions on vertical distribution of pharmaceutical and personal care products (PPCPs) in the vadose zone soils. *Sci. Total Environ.* 626: 1148–1156.

12 Kong, X., Liu, T., Yu, Z. et al. (2018). Heavy metal bioaccumulation in rice from a high geological background area in Guizhou Province, China. *Int. J. Environ. Res. Public Health* 15 (10): 2281.

13 Mazumder, D.N., Ghosh, A., Majumdar, K.K. et al. (2010). Arsenic contamination of ground water and its health impact on population of district of Nadia, West Bengal, India. *Indian J. Commun. Med.* 35 (2): 331–338.

14 Peuke, A.D. and Rennenberg, H. (2005). Phytoremediation. *EMBO Rep.* 6: 497–501.

15 Chambers, P.A., Lacoul, P., Murphy, K.J. et al. (2008). Global diversity of aquatic macrophytes in freshwater. *Hydrobiologia* 595: 9–26.

16 Kannaiyan, S. and Kumar, K. (2006). Biodiversity of *Azolla* and its algal symbiont, *Anabaena azollae*. NBA Scientific Bulletin Number-2, National Biodiversity Authority, Chennai. pp. 1–31.

17 Lumpkin, T.A. and Plucknett, D.L. (1980). *Azolla*: botany, physiology and use as a green manure. *Econ. Bot.* 34: 111–153.

18 Carrapico, F. (2017). The *Azolla*–anabaena–bacteria association: a case of symbiotic abduction? In: *Algal and Cyanobacteria Symbioses* (eds. M. Grube, J. Seckbach and L. Muggia), 329–345. Singapore: World Scientific.

19 Roy, D.C., Pakhira, M.C., and Bera, S. (2016). A review on biology, cultivation and utilization of *Azolla*. *Adv. Life Sci.* 5: 11–15.

20 Veys, P., Lejeune, A., and Van Hove, C. (2000). The pore of the leaf cavity of *Azolla*: interspecific morphological differences and continuity between the cavity envelopes. *Symbiosis* 29: 33–47.

21 Hill, D.J. (1975). The pattern of development of anabaena in the *Azolla*-anabaena symbiosis. *Planta* 122 (2): 179–184.

22 Ray, T.B., Peters, G.A., Toia, R.E., and Mayne, B.C. (1978). *Azolla*-anabaena relationships: VII. Distribution of ammonia-assimilating enzymes, protein, and chlorophyll between host and symbiont. *Plant Physiol.* 62 (3): 463–467.

23 Singh, P.K., Bisoyi, R.N., and Singh, R.P. (1990). Collection and germination of sporocarps of *Azolla caroliniana*. *Ann. Bot.* 66: 51–56.

24 Yusuf, A., Aparna, M.B., and Heimer, Y.M. (2018). Regulation of glutamine synthetase (GS) activity and root morphological plasticity in the nitrogen fixing *Azolla*-anabaena symbiont under nitrogen limiting conditions. *J. Plant Physiol. Pathol.* 6: 5.

25 Pereira, A.L. and Carrapiço, F. (2009). Culture of *Azolla filiculoides* in artificial conditions. *Plant Biosyst.* 143 (3): 431–434.

26 Costa, M.L., Santos, M.C.R., Carrapiço, F., and Pereira, A.L. (2009). *Azolla*–Anabaena's behaviour in urban wastewater and artificial media–influence of combined nitrogen. *Water Res.* 43 (15): 3743–3750.

27 Sarma, B.K. and Deka, P.C. (1987). Induction of callus from leaf explants of *Azolla pinnata*. *IRRN* 12: 4.

28 Kitoh, S. and Shiomi, N. (1991). Effect of mineral nutrients and combined nitrogen sources in the medium on growth and nitrogen fixation of the *Azolla*-anabaena association. *Soil Sci. Plant Nutr.* 37 (3): 419–426.

29 Kitoh, S., Shiomi, N., and Uheda, E. (1993). The growth and nitrogen fixation of *Azolla filiculoides* Lam. in polluted water. *Aquat. Bot.* 46 (2): 129–139.

30 Guochang, L.C.W.W.Z. and Yiqing, W.B.Z. (1982). Study on the potassium enriching physiology of *Azolla*. *Sci. Agric. Sin.* 4: 82–87.

31 Subudhi, B.P.R. and Singh, P.K. (1979). Effect of macronutrients and pH on the growth, nitrogen fixation and soluble sugar content of water fern *Azolla pinnata*. *Biol. Plant.* 21 (1): 66–70.

32 Johnson, G.V., Mayeux, P.A., and Evans, H.J. (1966). A cobalt requirement for symbiotic growth of *Azolla filiculoides* in the absence of combined nitrogen. *Plant Physiol.* 41 (5): 852–855.

33 Zahran, H.H., Abo-Ellil, A.H., and Al-Sherif, E.A. (2007). Propagation, taxonomy and ecophysiological characteristics of the *Azolla*-anabaena symbiosis in freshwater habitats of Beni-Suef Governorate (Egypt). *Egypt. J. Biol.* 9: 1–12.

34 Temmink, R.J., Harpenslager, S.F., Smolders, A.J. et al. (2018). *Azolla* along a phosphorus gradient: biphasic growth response linked to diazotroph traits and phosphorus-induced iron chlorosis. *Sci. Rep.* 8 (1): 1–8.

35 Handajani, H. (2012). Optimation of nitrogen and phosphorus in *Azolla* growth as biofertilizer. *Makara J. Technol.* 15 (2): 142–146.

36 Lizieri, C., Aguiar, R., and Kuki, K.N. (2011). Manganese accumulation and its effects on three tropical aquatic macrophytes: *Azolla caroliniana*, *Salvinia minima* and *Spirodela polyrhiza*. *Rodriguesia* 62 (4): 909–917.

37 Jain, S.K., Vasudevan, P., and Jha, N.K. (1990). *Azolla pinnata* R. Br. and *Lemna minor* L. for removal of lead and zinc from polluted water. *Water Res.* 24: 177–183.

38 Kar, P.P. and Singh, D.P. (2002). Effect of some metabolic inhibitors and growth hormones on the sporulation and growth of *Azolla* microphylla. *Exp. Agric.* 38 (4): 433–444.

39 deVries, J., Fischer, A.M., Roettger, M. et al. (2016). Cytokinin-induced promotion of root meristem size in the fern *Azolla* supports a shoot-like origin of euphyllophyte roots. *New Phytol.* 209 (2): 705–720.

40 El-Araby, M.M.I., El-Berashi, M.N., El-Shahate, R.M., and Eweda, E. (2010). Enhancement of growth and endogenous phytohormones of *Azolla pinnata* in response to tryptophan. *J. Appl. Sci. Res.* 6 (12): 6598–6604.

41 deVries, S., de Vries, J., Teschke, H. et al. (2018). Jasmonic and salicylic acid response in the fern *Azolla filiculoides* and its cyanobiont. *PlantCell Environ.* 41 (11): 2530–2548.

42 Liu, X., Min, C., Xia-shi, L., and Chungchu, L. (2008). Research on some functions of *Azolla* in CELSS system. *Acta Astronaut.* 63: 1061–1066.

43 Effendi, I. and Pranata, A. (2019). The effect of sun light intensity on the growth of *Azolla* microphylla and its symbiont *Anabeana Azollae* in brackish water. *J. Phys. Conf. Ser.* 1351 (1): 012096.

44 Cary, P.R. and Weerts, P.G.J. (1992). Growth and nutrient composition of *A. pinnata* R. Brown and *A. filiculoides* Lam. as affected by water temperature, nitrogen and phosphorus supply, light intensity and pH. *Aquat. Bot.* 43: 163–180.

45 Masood, A., Shah, N.A., Zeeshan, M., and Abraham, G. (2006). Differential response of antioxidant enzymes to salinity stress in two varieties of *Azolla* (*A. pinnata* and *A. filiculoides*). *Environ. Exp. Bot.* 58: 216–222.

46 Watanabe, I., Berja, N.S., and Del Rosario, D.C. (1980). Growth of *Azolla* in paddy field as affected by phosphorus fertilizer. *Soil Sci. Plant Nutr.* 26 (2): 301–307.

47 Rai, V. and Rai, A.K. (1999). Growth behaviour of *Azolla pinnata* at various salinity levels and induction of high salt tolerance. *Plant Soil* 206 (1): 79–84.

48 Talley, S.N. and Rains, D.W. (1980). *Azolla filiculoides* Lam. as a fallow-season green manure for rice in a temperate climate. *Agron. J.* 72 (1): 11–18.

49 Verma, P., George, K.V., Singh, H.V. et al. (2006). Modeling rhizofiltration: heavy-metal uptake by plant roots. *Environ. Model. Assess.* 11: 387–394.

50 Zazouli, M.A., Balarak, D., and Mahdavi, Y. (2013). Pyrocatechol removal from aqueous solutions by using *Azolla Filiculoides*. *Health Scope* 2 (1): 25–30.

51 Kooh, M.R.R., Dahri, M.K., Lim, L.B.L. et al. (2019). Phytoextraction capability of *Azolla pinnata* in the removal of rhodamine B from aqueous solution: artificial neural network and random forests approaches. *Appl. Water Sci.* 9: 80.

52 Guimarães, F.P., Aguiar, R., Karam, D. et al. (2011). Potential of macrophytes for removing atrazine from aqueous solution. *Planta Daninha* 29: 1137–1147.

53 Cohen, M.F., Williams, J., and Yamasaki, H. (2002). Biodegradation of diesel fuel by an *Azolla*-derived bacterial consortium. *J.Environ. Sci. Health A* 37: 1593–1606.

54 Kollah, B., Patra, A.K., and Mohanty, S.R. (2016). Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change. *Environ. Sci. Pollut. Res.* 23: 4358–4369.

55 Acero L. (2019). Phytoremediation of phosphorous and ammonia with *eichhornia crassipes* and *Azolla pinnata* in *waste waters from Estero de San Miguel Mendiola Manila Philippines*. E3S Web of Conferences 93. 02004.

56 Rai, P.K. (2008). Phytoremediation of hg and cd from industrial effluent using an aquatic free floating macrophyte *Azolla pinnata*. *Int. J. Phytorem.* 10: 430–439.

57 Sela, M., Garty, J., and Tel-Or, E. (1989). The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*. *New Phytol.* 112 (1): 7–12.

58 Khosravi, M., Ganji, M.T., and Rakhshaee, R. (2005). Toxic effect of Pb, Cd, Ni and Zn on *Azolla filiculoides* in the international Anzali wetland. *Int. J. Environ. Sci. Technol.* 2 (1): 35–40.

59 Sanchez-Viveros, G., Ferrera-Cerrato, R., and Alarcon, A. (2011). Short-term effects of arsenate-induced toxicity on growth, chlorophyll and carotenoid contents, and total content of phenolic compounds of *Azolla filiculoides*. *Water Air Soil Pollut.* 217 (1–4): 455–462.

60 Zhang, X., Lin, A.J., Zhao, F.J. et al. (2008). Arsenic accumulation by aquatic fern *Azolla*: comparison of arsenate uptake, speciation and efflux by *A. caroliniana* and *A. filiculoides*. *Environ. Pollut.* 156: 1149–1155.

61 Jafari, N., Senobari, Z., and Pathak, R.K. (2010). Biotechnological potential of *Azolla* filiculoides, *Azolla* microphylla and *Azolla* pinnata for biosorption of Pb(II), Mn(II), Cu (II) and Zn(II). *Ecol. Environ. Conserv.* 16: 443–449.

62 Hussein, H.S., Ruiz, O.N., Terry, N., and Daniell, H. (2007). Phytoremediation of mercury and organomercurials in chloroplast transgenic plants: enhanced root uptake, translocation to shoots, and volatilization. *Environ. Sci. Technol.* 41 (24): 8439–8446.

63 Bennicelli, R., Stezpniewska, Z., Banach, A. et al. (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. *Chem. Tech.* 55: 141–146.

64 Rai, P.K. and Tripathi, B.D. (2009). Comparative assessment of *Azolla pinnata* and *Vallisneria spiralis* in hg removal from G.B. pant Sagar of Singrauli industrial region, India. *Environ. Monit. Assess.* 148: 75–84.

65 Arora, A., Sood, A., and Singh, P.K. (2004). Hyperaccumulation of cadmium and nickel by *Azolla* species. *Indian J. Plant Physiol.* 3: 302–304.

66 Smith, P., Martino, D., Cai, Z. et al. (2008). Greenhouse gas mitigation in agriculture. *Philos. Trans. Royal Soc. B Biol. Sci.* 363: 789–813.

67 Edema, N.E., Okoloko, E.G., and Agbogidi, O.M. (2010). Physical and ionic characteristics in water soluble fraction (WSF) of Olomoro well-head crude oil before and after exposure to *Azolla Africana* Desv. *Afr. J. Biotechnol.* 7: 3540.

68 Raja, W., Rathaur, P., and Ashiq, R. (2013). Differential sensitivity of nitrogen-fixing, *Azolla Microphylla* to organochlorine and organophosphate insecticide. *J. Appl. Sci. Environ. Manag.* 17 (1): 61–73.

69 Kooh, M.R.R., Lim, L.B.L., Lim, L.H., and Khairud, M. (2016). Phytoremediation capability of *Azolla pinnata* for the removal of malachite green from aqueous solution. *J. Environ. Biotechnol. Res.* 5: 10–17.

70 Kooh, M.R.R., Lim, L.B.L., and Lim, L.H. (2018). Phytoextraction potential of water fern (*Azolla* pinnata) in the removal of a hazardous dye, methyl violet 2B: artificial neural network modeling. *Int. J. Phytorem.* 20: 424–431.

71 Warner, S.D. and Hadley, P.W. (2012). Sustainable remediation: integrating risk, science, and sustainability principles. In: *Encyclopedia of Sustainability Science and Technology* (ed. R.A. Meyers). New York, NY: Springer. https://doi.org/10.1007/978-1-4419-0851-3_63.

72 Aziz, A. (2012). New method of large-scale production of *Azolla pinnata* var. pinnata R: Brown: a multipurpose crop. *Int. J. Appl. Agric. Res.* 7: 1–9.

73 Raja, W., Rathaur, P., John, S.A., and Ramteke, P.W. (2012). *Azolla*: an aquatic pteridophyte with great potential. *Int. J. Sci. Res. Biol. Sci.* 2: 68–72.

74 Veluci, R.M., Neher, D.A., and Weicht, T.R. (2006). Nitrogen fixation and leaching of biological soil crust communities in mesic temperate soils. *Microb. Ecol.* 51: 189–196.

75 De Macale, M.A. and Vlek, P.L. (2004). The roles of *Azolla* cover in improving the nitrogen use efficiency of lowland rice. *Plant Soil* 263: 311–321.

76 McLaughlin, N.B., Hiba, A., Wall, G.J., and King, D.J. (2000). Comparison of energy inputs for inorganic fertilizer and manure based corn production. *Can. Agric. Eng.* 42: 9–18.

77 Wagner, S.C. (2011). Biological nitrogen fixation. *Nat. Educ. Knowl.* 2: 14.

78 Awodun, M.A. (2008). Effect of *Azolla* (*Azolla* species) on physiochemical properties of the soil. *World J. Agric. Sci.* 4: 157–160.

79 Kondo, M., Kobayashi, M., and Takahashi, E. (1989). Effect of phosphorus on *Azolla* and its utilization in rice culture in Niger. *Plant Soil* 120: 165–170.

80 Taghi Ganji, M., Khosravi, M., and Rakhshaee, R. (2005). Biosorption of Pb, Cd, Cu and Zn from the wastewater by treated *Azolla filiculoides* with $H_2O_2/MgCl_2$. *Int. J. Environ. Sci. Technol.* 1: 265–271.

81 Mashkani, S.G., Tajer, P., and Ghazvinia, M. (2009). Biotechnological potential of *Azolla* filiculoides for biosorption of Cs and Sr: application of micro-PIXE for measurement of biosorption. *Bioresour. Technol.* 100 (6): 1915–1921.

82 Ena, A., Carlozzi, P., Pushparaj, B. et al. (2007). Ability of the aquatic fern *Azolla* to remove chemical oxygen demand and polyphenols from olive mill wastewater. *Grasas Y Aceites* 58 (1): 34–39.

83 Gupta, K.S., Chandra, R., Dey, D. et al. (2018). Study of chemical composition and mineral content of sun dried *Azolla pinnata*. *J. Pharm. Phytochem.* 7 (6): 1214–1216.

84 Miranda, A.F., Biswas, B., Ramkumar, N. et al. (2016). Aquatic plant *Azolla* as the universal feedstock for biofuel production. *Biotechnol. Biofuels* 9: 221.