UDC 581.6:582.37:631.5 https://doi.org/10.15407/biotech15.05.005

WATER FERNS OF *Salviniaceae* FAMILY IN PHYTOREMEDIATION AND PHYTOINDICATION OF CONTAMINATED WATER

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Received 29.07.2022
Revised 27.09.2022
Accepted 31.10.2022

Background. Aquatic ecosystems are subjected to significant stress loads and depletion due to the influx of pollutants of inorganic and organic origin, that pose a serious threat to human health. The United Nations Environment Program has defined phytoremediation as an effective eco-technology for the removal, detoxification and immobilization of pollutants using plants. Water ferns of the *Salviniaceae* family belong to promising phytoremediants. They are characterized by high growth rates, resistance to adverse environmental factors, capable of adsorbing pollutants, including heavy metals. Species of the genus *Salvinia* and *Azolla* are used to assess the ecological state of water and study ecotoxicological effects of pollutants.

Aim. Analysis and generalization of the latest scientific results on the use of species of the *Salviniaceae* family for phytoremediation and phytoindication of contaminated water.

Results. In this review, we have highlighted key information on emerging phytotechnologies, including phytodegradation, phytostabilization, rhizofiltration, rhizodegradation, and phytovolatization. The growth and distribution features of species of the genus *Salvinia* and *Azolla* were described and current information on the use of water ferns for cleaning polluted water from heavy metals, inorganic and organic pollutants was presented. Data on the physiological and molecular mechanisms of the genus *Salvinia* and *Azolla* species adaptation to the toxic effect of pollutants of various origins were discussed. We focused special attention on the use of water ferns of the Salviniaceae family to control water pollution.

Key words: *Salviniaceae*, aquatic ecosystems, phytoremediation, bioindication, organic and inorganic pollutants.

Anthropogenic impact, intensive industrial production and unbalanced environmental policy have led to serious environmental pollution. Waters are particularly adversely affected, as large, industrially developed regions are located on the banks of reservoirs and rivers. At the same time, within cities, even small closed reservoirs are subject to pollution, as they are sewage reservoirs for natural precipitation and absorbers of vehicle exhaust gases.

The United Nations Environment Program has defined phytoremediation as an effective eco-technology that involves the use of plants to remove, detoxify and immobilize environmental pollutants [1]. To diagnose the degree of anthropogenic pollution of water ecosystems, the method of phytoindication is used, which suggests detection of the dependence between the state of waters and the biological indicators of both individual plants and plant groups, including phytocenoses. Today, the achievements, tasks and prospects of phytoremediation and phytoindication are actively discussed by scientists of many countries of the world [2–5].

Vascular cryptogamous plants are one of the most ancient higher plants that appeared on the planet more than 300 million years ago. The most widespread among them are representatives of the Polypodiophyta division, which grow in all climatic zones, are distinguished by a significant diversity of life forms, have a wide range of adaptive features, which allows them to exist in any environmental conditions [6]. Since these plants have a high potential in the accumulation of pollutants and water detoxification, significant stress tolerance and a high rate of biomass formation [7], they deserve attention as promising biological objects for the development of the latest biotechnologies for phytoremediation and phytoindication.

In our review, we focused on water ferns of the Salviniaceae family, analyzed and summarized the latest information on the use of these plants for cleaning and biotesting of polluted waters, discussed the mechanisms of resistance to the action of pollutants.

Phytoremediation: eco-technology of cleaning using plants

Phytoremediation is an ecological method of purification of a contaminated environment with the participation of plants, the mechanism of which involves the absorbtion of pollutants by plants, the accumulation them in tissues, decomposition and transformations into harmless forms [1, 4, 5, 8, 9]. The use of plants for wastewater treatment began about 300 years ago [10]. Today, an effective accumulators of the inorganic and organic pollutants from reservoirs have been recognized the species of water macrophytes from families *Ranunculaceae, Lemnaceae, Cyperaceae, Salviniaceae, Haloragaceae, Hydrocharitaceae, Potamogetonaceae, Typhaceae, Najadaceae, Pontederiaceae* and *Juncaceae* [3].

Phytoremediation technology is a successful tool for reducing contamination of the aquatic environment. The initial stage is screening of plants able to store the heavy metals and other pollutants. For phytoremediation, the fast-growing species, that are easy to collect and handle, are selected [11]. The ontogenetic, physiological and biochemical traits as well as photosynthetic activity should be taken into account. The success of phytoremediation also depends on the intensity of pollution [12].

Different phytotechnologies are used to purify contaminated ecosystems, including phytodegradation, phytostabilization, rhizofiltration, rhizodegradation and phytovolatization [3]. The reduction of the content of pollutants in the soil occurs due to their uptake and binding by plant root system. In the process of immobilization, the roots accumulate, adsorb and precipitate pollutants, which is important for the removal of organic and inorganic contaminants from the soil, sludge and silt media [13-15]. During phytoextraction, pollutants are absorbed and hyperacumulated in different parts of the plant [16]. Absorption from soil, groundwater, residues and sludge with subsequent evaporation of pollutants into the atmosphere occurs during phytovolatilization [17]. Plants metabolize contaminants by means of compounds formed in their tissues [18, 19]. Rhizofiltration includes adsorption and precipitation of pollution into the substrate surrounding the root area [20]. Plants secrete various organic compounds that attract microbial communities present in the soil, which contributes to the decomposition of pollutants. This technology is called biosorption [21] and is used to remove heavy metals (HM) from wastewater [2, 22].

HM, such as cadmium (CD), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn), nickel (Ni), vanadium (V) as well as metalloid selenium (SE) are the most common pollutants of aquatic ecosystems. When exceeding the threshold concentration, they become toxic to plants, induce the formation of active forms of oxygen, inhibit photosynthesis and respiration, can cause plant death [23].

Hydrophyte ferns of the Salviniaceae family in phytoremediation of contaminated waters

Hydrophyte ferns, in particular representatives of the *Salviniaceae* family, which includes two genera *Salvinia* and *Azolla* [24], belong to promising plant species for water purification. Water ferns of the genus *Salvinia*, which includes 12 species [24], are characterized by high growth rates, adaptability and tolerance to adverse environmental factors, they are able to adsorb pollutants.

Salvinia natans (L.) All. is an annual hydrophyte fern widespread in Ukraine with a summer-green phenoritmotype [25]. It grows on the border of air and water environments, is characterized by a different structure of floating and submerged in the water layer photosynthetic organs — fronds. Submerged fronds are morphologically similar to roots (Fig. 1). The species occurs sporadically within its range in the temperate climate zone. It is

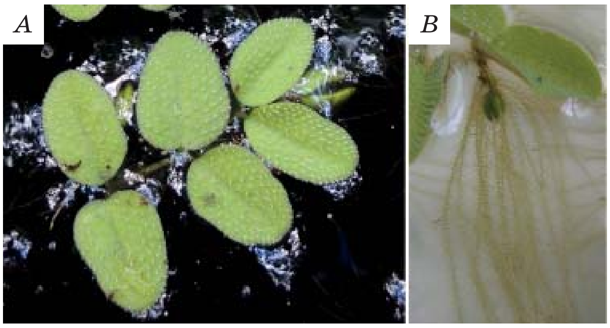

***Fig. 1. A* — *Salvinia natans* in wild nature on the surface of the pond (Mizhrichynskyi Regional Landscape Park); *B* — the single submerged frond of Salvinia natans and stem apical bud with the young floating fronds**

widespread in mesoeutrophic and eutrophic freshwater closed or low-flow reservoirs and irrigation canals with a silty-sandy bottom.

In Ukraine, it occurs in the reservoirs of the Dnipro, Desna, Siversky Donets, Southern Bug, Dniester, Danube, Uzh, Latoritsa, Borzhava valleys, as well as in the ponds of the Forest Steppe and Steppe (Fig. 2) [26]. Due to the softening of the climate, the range of the fern has recently expanded. Significant populations of *S. natans* were reported in northern Europe in the Vistula River Delta [27].

The life cycle of Salvinia is represented by two independent generations: an asexual sporophyte and a sexual gametophyte. An adult Salvinia sporophyte is characterized by a clonal structure, formed during the formation of new modules that develop radially around the central (oldest) part of the plant. The complexity of the clone structure is determined by its age. The older the clone, the more complex its structure. The growth of clone modules proceeds similarly to the growth of side branches in other higher plants [28].

Modern climatic conditions have led to an extension of the growth period of the sporophyte, resulting in an increase in the number of vegetative generations from five or more, instead of two or three, which contributes to the spread of this fern and its occupation of new territories [29].

We have investigated the microstructure of the surface and the cell ultrastructure of floating and submerged fronds [30, 31], determined biometric indicators [28] and phytohormonal balance in the organs of the *S. natans* sporophyte at different stages of ontogenesis [6], analyzed the features of the photosynthetic apparatus functioning [32]. According to our observations, the species settled naturally and successfully develops on the surface of closed reservoirs on the left bank of the Dnieper within the city of Kyiv. These reservoirs are largely eutrified and have been subject to long-term lead pollution due to emissions of exhaust gases from automobile transport and waste of the now defunct Radykal enterprise — one of the most problematic in terms of environmental impact of industrial facilities within Kyiv. Significant pollution does not prevent the water fern *S. natans* from successful growth and reproduction, covering large surface areas (up to 20%, according to our estimates). Since the increase in biomass of *S. natans* is extremely fast, it is possible to periodically collect it and take it away for further burial in landfills, thus contributing to the gradual purification of these waters. In addition, *S. natans* can be easily spread into uninhabited waters by the transfer of green, free-floating sporophytes that proliferate rapidly.

It was reported previously that many species of terrestrial ferns are able to tolerate such concentrations of heavy metals that are toxic to other plants [33] and, accordingly, about the possibility of effective soil restoration using these plants. At the same time, a significant amount of pollutants adsorbed from the soil accumulated in plant tissues. Plant species considered to be weeds, including aquatic weeds, also show a high ability to hyperaccumulate some pollutants, such as herbicides, metalloids, and synthetic dyes [34, 35]. Macrophytes, particularly the free-floating ferns of the genus *Salvinia*, are well known for their physiological properties that allow them to minimize the cellular toxicity of hazardous chemicals when these pollutants hyperaccumulate [36, 37]. Along with macrophytes *Eicchornia* spp., *Pistia stratiotes*, *Lemna* spp. representatives of the genus Salvinia have the highest potential for hyperaccumulation of pollutants, and, therefore, for phytoremediation of contaminated waters [38]. *S. natans* can be used as an effective agent for phytoremediation of polluted waters.

Thus, the expediency of using *S. natans* for phytoremediation of wastewater contaminated with chromium and zinc salts was shown [39]. Also, *S. natans* was successfully used in experimental work on the purification of untreated complexly contaminated wastewater containing, in addition to other pollutants, dissolved ammonia in the form of NH_4^+ ions and NO_2^- nitrites [40]. *S. natans* neutralizes significant concentrations of auxin herbicides, in particular 2,4-D in culture medium [41],

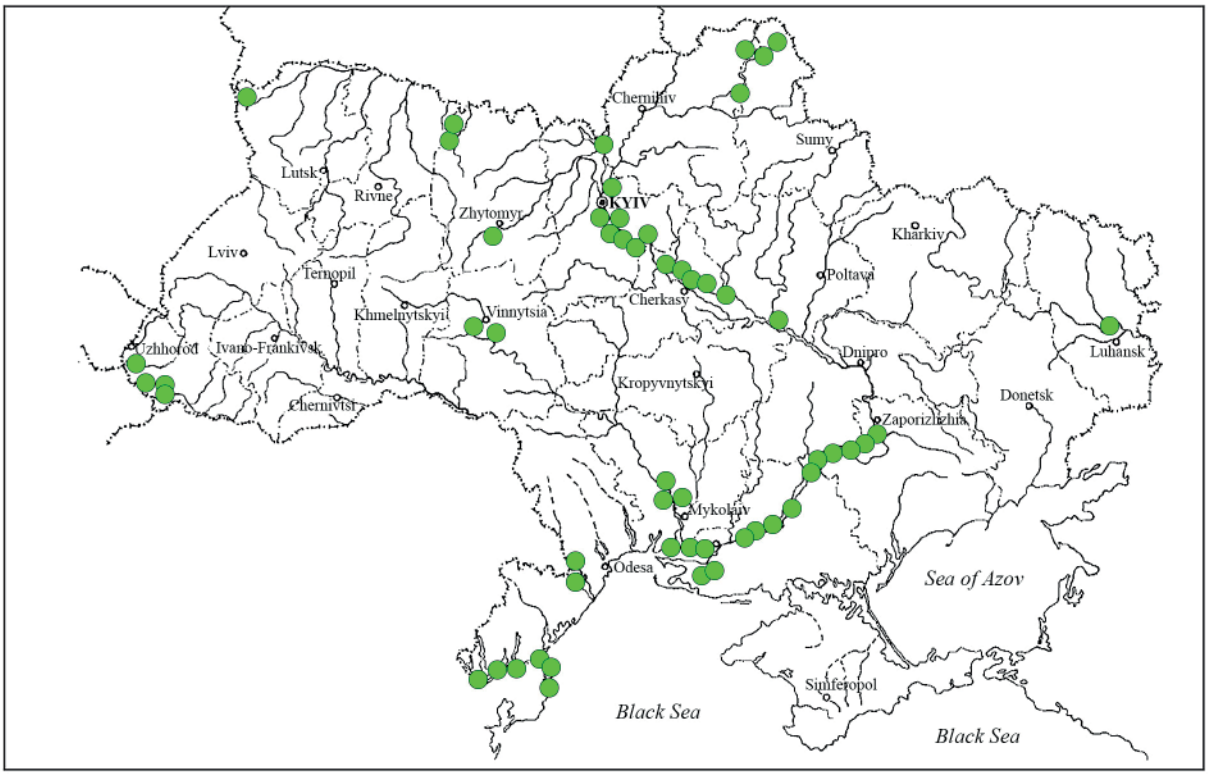

Fig. 2. **Map of distribution of the macrophyte *Salvinia natans* in Ukraine [26]**

actively binds complex aluminum compounds [42] and nitrogen-containing dyes dissolved in water [43]. This species of free-floating fern is characterized by high efficiency of the antioxidant system and osmotic stability of cells, which minimizes intracellular damage from herbicides [44]. In addition, it was reported that exogenous priming of *S. natans* with a solution of 500 μM 2,4-D for eight hours improves the tolerance of vegetative sporophytes to the impact of sodium (Na) and metalloid arsenic (As) [35]. In this study, primed sporophytes accumulated dissolved pollutants more actively than control ones.

Although *S. natans* is currently not used for mass industrial purification of wastewater and polluted waters, a set of experimental data convincingly proves that this free-floating macrophyte can be successfully used for phytoremediation (Table 1). First of all, due to their high tolerance to general water pollution and the ability to accumulate pollutants in significant concentrations, *S. natans* sporophytes effectively reduce the level of heavy metals and metalloids in contaminated water.

Salvinia molesta D.S. Mitchell also known as giant Salvinia is one of the most common aquatic weeds with a natural habitat in Brazil, from where it has spread to many tropical and subtropical regions of Africa, Asia, North and South America, Oceania, Australia, India, Indonesia since the beginning of the last century [45–47]. Colonies of *S. molesta* are formed from a tangled network about 100 free-floating plants. Each plant is 2.5 to 4.0 cm long, has two floating fronds, a submerged “root-like” frond and internodes. Floating fronds are bilobed, oval in shape with short petioles 1–5 mm long, the abaxial and adaxial surfaces are covered with trichomes. Immerged fronds up to 24 cm long, with short or long petioles, 0.2–1 cm long. Three stages in the ontogeny of *S. molesta* are distinguished: the first, when the plants have small floating fronds, which lie on the surface of the water; the second, when groups of plants with shuttle-shaped fronds are formed, and the third, when plants have vertically stacked fronds and form dense mats [48, 49]. *S. molesta* is found in slow-moving waters, including lakes, ponds, ditches, streams, rivers, and marshes. Under favorable conditions, mass groups of ferns form dense carpets up to 1 m thick, which double in size in 2–3 days [50]. Spores of *S. molesta* are sterile and non-viable. This fern is pentaploid, the number of chromosomes is

Table 1. **Summarized information on the phytoremediation of contaminated reservoirs by the aquatic ferns of the genus *Salvinia***

Salvinia spp.	Indicator of contamination	Indicator of phytoremediation	Source
Salvinia natans	15 mg/L	Zn — 84.8%; Cu — 73.8%; Ni — 56.8%; Cr — 41.4%	[91]
Salvinia natans	Cd — 80 mg/L; Pb — 50 mg/L; Ni — 20 mg/L	Cd — 23550 μg/g DW Pb — 9570 μg/g DW Ni — 42363 μg/g DW	[92]
Salvinia minima	Zn –1.00 mg/L Ni — 0.40 mg/L Cd — 0.03 mg/L Pb — 1.00 mg/L	Zn — 0.4046 mg/m^2 Ni — 0.0595 mg/m^2 Cd — 0.0045 mg/m^2 Pb — 0.1423 mg/m^2	[71]
Salvinia minima	0, 20, 40, 80, 160 M $NiCl_2$	16.3 mg/g	[76]
Salvinia minima	Pb (II) — 20–40 μM AsO_4^{3-} — 200 μM	Pb — 34 mg/g As — 0.5 mg/g DW	[78]
Salvinia minima	Cr (VI) in the form $K_2Cr_2O_7$ 1 and 2 mg/L	302.61 mg/g DW 451.39 mg/g DW	[79]
Salvinia minima	Cd (II) — 4 mg/L Pb (II) — 3 mg/L Cr (VI) — 4 mg/L	Cd (II) — 82.59%, Pb (II) — 97.44%, Cr (VI) — 80.31%	[73]
Salvinia minima	Wastewater	PO_4 — 59% NO_3 — 67.4%	[80]
Salvinia minima	$CuSO_4$ and $ZnSO_4$ — 80 μM/L	Cu — 6.96 mg/g DW Zn 19.6 mg/g DW	[93]
Salvinia minima	Wastewater Pb^{2+}, Zn^{2+}, Ni^{2+} 10 mg/L; Dyes methylene blue (MB), crystal violet (CV), Bismarck brown (BB) — 10 mg/L	Pb^{2+}, Zn^{2+} and Ni^{2+} 98.56, 95.69 and 92.99%; CV, MB and BB 99.4, 99.1 and 96.5 5	[77]
Salvinia molesta	Waste of coal mines	Pb — 96.96%; Ni — 97.01%; Cu — 96.77%; Zn — 96.38%; Mn — 96.22%; Fe — 94.12%; Cr — 92.85%; Cd — 80.99%	[94]
Salvinia molesta	Cu — 1.092 mg/L; Cr– 2.201 mg/L; Pb — 2.974 mg/L; Cd — 0.251 mg/L	Cu — 2.035 mg/L Cr — 1.05 mg/L Pb — 1.924 mg/L Cd — 0.018 mg/L	[95]
Salvinia molesta	Cu — 0,01 ppm Fe — 0,775 ppm Ni — 0,009 ppm Zn — 0,135 ppm	Cu –20% Fe — 4,5% Ni — 50% Zn — 10,3%	[56]
Salvinia molesta	Aqueous solutions of mercury chloride and lead chloride — 25, 50, 75, 100 mg/L	Pb — 85% Hg — 74%	[57]
Salvinia molesta	Wastewater Ni^{2+}, Cr^{3+}, Cd^{2+}, Pb^{2+},10 μg/L	Ni, Cr, Cd, Pb — 85–90%	[58]
Salvinia molesta	Industrial wastewater	Na — 30%	[59]
Salvinia molesta	Textile wastewater	BOD and COD — 99%,	[60]
Salvinia molesta	Household wastewate	Turbidity — 97.7 % phosphates — 97.7% ammonia nitrogen — 99 % nitrates — 90.6%	[61]
Salvinia biloba	Cu 5 μg/mL	11861 μg/L	[65]

Table 1. End

Salvinia spp.	Indicator of contamination	Indicator of phytoremediation	Source
Salvinia biloba	River water Pb — 30,57 mg/L	Pb — 86.7%	[37]
Salvinia biloba	Pb^{2+} 4.8 mg/L 9.1 mg/L 19.6 mg/L	Pb^{2+} 97.7% 96.6% 91.6%	[66]
Salvinia biloba	Contaminated water 100 μM Cd	Floating fronds — 23 mg/g DW Submerged fronds — 12 mg/g DW	[96]
Salvinia biloba	Contaminated water Cd, Cu, Pb and Zn 50 and 100 μM	Cu and Pb ≥96% , Cd — 79% and 56% under 50 and 100 μM, Zn — 77 and 70% under 50 and 100 μM	[69]
Salvinia biloba	Pb^{2+} 5.9, 8.2 and 22 ppm	5–10%	[67]
Salvinia biloba	Hg 0.05, 0.1 and 0.2 μg/mL	277.9 μg/g	[68]
Salvinia cucullata	NH_4^+ — 0.5, 1, 5, 10 and 15 mM	The content of nitrogen increased, potassium absorption decreased	[81]
Salvinia cucullata	Wastewater	BOD — 43.02% , COD — 31.04% , nitrates — 20.00% , ammonium — 5.26% , total phosphorus — 81.25%	[82]
Salvinia rotundifolia	Industrial wastewater Pb(II) 0.651 ppm	50 g of fresh biomass removed 85-95% Pb(II) from 1.5 l of wastewater	[89]
Salvinia auriculata	River water Ti, Fe, Mn, Cu, Zn, Sr	Ti — 3303, Fe — 4344, Mn — 2882, Cu — 1366, Zn — 34, Sr — 66 μL/μg	[88]
Salvinia auriculata	Artificial reservoirs Hg — 0.2 ng/L	Floating fronds — 85–246 ng/g DW Submerged fronds — 88–265 ng/g DW	[87]
Salvinia herzogii	Water solutions Cr, Cd, Pb	Submerged fronds – Pb > Cd > Cr, Floating fronds — Cd > Pb > Cr	[84]
Salvinia herzogii	River water Cr < 1 g/L; Ni < 3 g/L; Zn < 25 g/L	Zn — 35–42% , Ni — 47–52% , Cr — 99–100	[85]

45, which makes it genetically incapable of sexual reproduction and the completion of meiosis. Asexual vegetative reproduction at a fast pace is ensured by rhizome fragmentation and bud growth [49, 51]. Morphological and molecular studies have shown that *S. molesta* often mutates, which allows this species to quickly adapt to new environments [52]. The success of the growth of *S. molesta* depends on the temperature, illumination, pH, electrical conductivity, salinity and availability of nutrients in the aquatic environment [53]. *S. molesta* biomass is used for ethanol production. Monosaccharides from fern hydrolyzate were obtained by thermal acid hydrolysis, ultrasonic treatment, and enzymatic saccharification [54]. It has been established that *S. molesta* can absorb a significant amount of nutrients, in particular up to 8 mg of nitrogen per g of dry biomass per day [55].

Floating and submerged fronds of *S. molesta* accumulate and remove pollutants, including lead, copper, mercury, arsenic, zinc, and cadmium, from industrial and municipal wastewater [56, 57]. Fern removes up to 85% of lead and 74% of mercury within ten days [57]. *S. molesta* effectively purifies wastewater from phenolic compounds and dyes [58], sodium compounds [59] and [60]. In domestic wastewater, *S. molesta* plants reduced turbidity, phosphate, ammonia nitrogen, and nitrate content by 97.7, 99.7, 99%, and 90.6%, respectively. Plants with greater biomass were more effective in removing excess nutrients [61]. *S. molesta* counteracts the harmful effects of HM due to the high activity of antioxidant defense enzymes: superoxide dismutase, catalase, peroxidase and ascorbate peroxidase. In floating fronds, enzyme activity was higher and less arsenic accumulated than in submerged ones [62]. In general, the fern *S. molesta* has proven itself as an effective phytoremediant of harmful substances of various nature from polluted waters and wastewater (Table 1).

Salvinia biloba Raddi is an autochthonous free-floating macrophyte originating from South America. This species is common in Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay. As a decorative culture, ferns can be found in various regions of the globe. The floating bilobed fronds of *S. biloba* have short petioles 1–9 mm long, a heart-shaped base with a system of hairs, long papillae, and heterogeneous areolar veins. Submerged fronds up to 45 cm long, with short or long petioles up to 2 cm long [63]. Floating and submerged fronds under favorable conditions form a huge vegetative mass on the surface of the water [64].

The use of *S. biloba* to treat water contaminated with cadmium, chromium, zinc, nickel, copper, and lead has been reported [37, 65, 66]. *S. biloba* proved to be tolerant to long-term (for 30 days) exposure to high concentrations of lead, which made it possible to use the fern to purify waters polluted by this heavy metal [67]. *S. biloba* is tolerant to mercury, which allows the fern to be used as a phytoremediant in waters contaminated with this metal [68]. Floating *S. biloba* fronds adsorbed HM ions from artificially polluted water in different ways. Removal of copper and lead ions (≥96%) was more active, cadmium (79 ± 4%) and zinc (77 ± 5%) ions were less active [69]. Pollutants are removed from contaminated waters by their adsorption and subsequent accumulation in *S. biloba* cells [70].

Therefore, the fast-growing and capable of hyperaccumulation of pollutants water fern *S. biloba* is suitable for phytoremediation of contaminated waters (Table 1).

Salvinia minima (Willd) is a floating fern, which, due to its high productivity and tolerance to a wide range of temperatures, is classified as a weed in tropical and subtropical regions. It is widespread in North, Central and South America. The floating fronds of *S. minima* are rounded in shape, contain hairs and heterogeneous areolar veins; have short petioles, 1–2 mm long. Underwater submerged fronds up to 4.5 cm long with short 1–2 mm petioles. The upper side of the floating fronds folds to the axis of the stem and is morphologically abaxial [63].

S. minima grows normally at low concentrations of cadmium (0.03 mg/l), nickel (0.40 mg/l), lead (1.00 mg/l), zinc (1.00 mg/l) and is able to adsorb HM at higher concentrations in the culture medium [71]. Hyperaccumulation of lead after exposure of ferns to a solution of 40 μM $Pb(NO_3)_2$ depended on the chelation and biosequestration of metals mediated by phytochelatins. Submerged fronds accumulated significantly more lead (II) than floating fronds, which was correlated with increased phytochelatin synthase (PCS) activity. Lead (II) accumulation occurred in the floating fronds due to a marked increase in the expression of the *SmPCS* gene [36]. Lead accumulates in ferns in the form of quasi-spherical and elongated nanoparticles (PbNP), which are localized on the cell membranes of floating and submerged fronds. Cellulose, lignin and pectin act as lead ion reducers [72]. The removal of lead from waters occurs by bioadsorption and subsequent accumulation in the cells of fronds. The distribution of lead between different compartments of the fern depends on the availability of nutrients, chelating agents and environmental conditions [73, 74]. In lead-contaminated waters, the rate of photosynthesis in *S. minima* was reduced by 44%, membrane damage was observed in the cells of submerged fronds, stomata were closed in floating fronds, and as a result, CO_2 intake decreased [75]. A concentration of nackel above 80 μM changed the integrity of cell membranes, affected photosynthesis, the efficiency of photosystem II, and reduced the content of photosynthetic pigments [76]. The fern is a hyperaccumulator of nickel, which is stored mainly in submerged fronds (16.3 mg/g of dry biomass). Quick absorption of nickel occurs in the first 6-12 hours of contact with the metal, and slows down over time [76].

S. minima is an effective biosorbent of methylene blue (MB), crystal violet (CV) and Bismarck brown (BB) dyes and HM ions lead (II), zinc (II) and nickel (II). The removal efficiency of dyes and metal ions exceeded 90% (99.4, 99.1 and 96.5% for CV, MB, BB and 98.56, 95.69 and 92.99% for lead, zinc and nickel respectively) under an initial dye concentration of 10 mg/l and a high concentration of metal ions. The maximum adsorption capacity for CV, MB, BB dyes was 94.13, 150.98 and 228.81 mg/g, and for lead, zinc and nickel ions — 174.32, 232.43, 171.40 mg/g. It was established that the accumulation of dyes and HM ions occurred mainly due to chemisorption. In general, the fern was found to be an effective ecological means of purification of wastewater containing dyes and heavy metal ions [77]. *S. minima* accumulates in large quantities and removes from aqueous solutions HM cadmium, lead, chromium and metalloid arsenic [78, 79]. It was reported that the intensity of pollutant accumulation is affected by the intensity of lighting and the pH of aqueous solution [73]. *S. minima* also adsorbs numerous nutrients from eutrophied waters. This fern grows rapidly in municipal wastewater and can effectively remove excess NO_3 and PO_4, as well as reduce biological and chemical oxygen consumption (BOD and COD) by 67.4% and 72.4% and conductivity by 89%, 59% and 59% respectively during 28 days [80]. So, *S. minima* was an effective purifier of inorganic and organic pollutants (Table 1).

Salvinia cucullata Roxb. ex Bory comes from India. Its floating fronds have short petioles 0.5–1 mm long; the plates are rounded at the top, truncated at the base, 0.5–1 × 1,1–1.5 cm. Hairs are only on the abaxial surface, the papillae are short or absent, the areolar veins are heterogeneous. Submerged fronds are 3.5 cm long, with short 0.2–1 mm petioles [63].

Fern grows well in a nitrate-contaminated environment (0.5–1 mM). With an increase in pollutant concentrations up to 5 mM, growth is suppressed, potassium absorption was inhibited, but the amount of nitrogen in fronds increased, which became the basis for the use of *S. cucullata* as fertilizers, animal feed and in waters purification [81]. A significant decrease in biological and chemical oxygen consumption, nitrates and phosphates content (Table 1) was observed in wastewater after cultivation of ferns during 45 days [82].

Salvinia herzogii de la Sota is common in Argentina and Brazil. Floating two-bladed fronds with a high-cut top of up to 1/3 of the length of the plate and a heart-shaped base 1.5–2.5 × 2.7–3.8 cm are attached to short petioles 0.4–1.0 mm. The frond surface is covered with hairs that are divided into four segments at the apex and connected to the tips. It has long papillae and heterogeneous areolar veins. Submerged fronds are up to 10 cm long, with long 0.5–1 cm petioles [63]. *S. herzogii* is capable to change the morphology of fronds depending on the population density, which allows to compete for resources [83]. Submerged fronds of *S. herzogii* accumulate more chromium, cadmium, lead, zinc and nickel than floating fronds, and the removal of chromium from wastewater occurred faster than zinc and nickel [84, 85]. The absorption of chromium and cadmium by floating fronds of *S. herzogii* occurred due to the bioadsorption, helating and ion exchange [86].

Salvinia auriculata together with other macrophytes *Elodea densa, Sagittaria montevidensis, Pistia stratiotes* and *Eichhornia crassipes* in two artificial reservoirs actively accumulated mercury. The concentration of HM in the organs of plants ranged from 46–246 ng/g to 37–314 ng/g of fresh weigh. Negative correlation between mercury content and plant biomass has emphasized the importance of juvenile plants using to absorb mercury [87]. *S. auriculata* accumulated potassium, calcium, titanium, iron, manganese, chromium, cuprum, zinc and strontium, the content of which increased over time (Table 1). Coefficients of concentrations for all metals except strontium reached the highest value in 46 days of cultivation of ferns in contaminated river water [88].

It was reported that *S. rotundifolia* removed 85–95% of lead from contaminated industrial wastewater [89]. Ferns *S. auriculata, S. biloba, S. herzogii, S. minima, S. molesta, S. natans* and *S. rotundifolia* accumulated HM gold, cadmium, chromium, cesium, cooper, iron, manganese, nickel, lead, strontium and zinc up to 6000–18000 mg/kg of dry biomass, which allows to use them for effective purification of industrial and wastewater (Table 1) [90].

Hydrophyte ferns of the *Azolla* genus in phytoremediation of polluted waters

The genus *Azolla* unites aquatic ferns, which are characterized by small fronds and bright colors from green to burgundy. Due to the significant water repellency of the scaly fronds, the fern floats on the surface of stagnant water in tropical, subtropical, and temperate regions around the world [97, 98]. Reproduction occurs mainly through rapid vegetative segmentation, the biomass of the fern doubles in two to four days [99]. The degree of sporulation is quite low and requires certain conditions for instance, it can be induced by far-red light [100]. A unique feature of ferns of this genus is the formation of symbiosis with the nitrogen-fixing cyanobacterium *Anabaena azollae* [101]. Due to the significant rate of nitrogen assimilation and extremely high productivity, ferns of the genus Azolla are used in agricultural production as biofertilizers [102, 103] and feed bioadditive for animals in aquaculture [104]. Recently, *Azolla* ferns have attracted attention as potential phytoremediants. The ability of *Azolla* ferns to absorb heavy metals attracts special attention of researchers (Table 2).

Azolla filiculoides Lam. is an invasive plant that is widespread in tropical and temperate regions throughout the world. An adult plant is 1–2 cm in size, colored at the edges in pink, orange or red. It differs from other Azolla species by the presence of single-cell trichomes on the surface, which provide water-repellent properties of plant. The ability to successful absorb nitrogen and phosphorus compounds from wastewater was found in *A. filiculoides* [105]. Growth stimulation of *A. filiculoides* under the addition of nitrogen and phosphorus had a limiting concentration of 50 μM/L, at higher concentrations chlorosis due to iron deficiency was observed [106]. The fern *A. filiculoides* is known as a hyperaccumulator of lead, cadmium, chromium, nickel, silver and gold [107]. When *A. filiculoides* was grown for 15 days in solutions containing 5, 10, and 25 mg/L of lead, nickel, and cadmium, the cleaning efficiency reached a maximum at day 10 at a metal concentration of 5 mg/L and decreased at a higher level of pollution [108]. Accumulation of cadmium by A. *filiculoides* was an order of magnitude lower than that of copper when these compounds were added in a complex with EDTA, while cadmium alone caused significant damage to photosystem II [109]. Water pollution with iron, chromium and aluminum did not prevent the growth of the fern *A. filiculoides*, instead, aluminum had even a small stimulating effect. Fern removed 92% of iron, 96% of aluminum and more than 80% of chromium [110]. Water pollution with iron, chromium and aluminum did not prevent the growth of the fern *A. filiculoides*, instead, aluminum had even a small stimulating effect. *A. filiculoides* effectively absorb nickel from aqueous and galvanic solutions, even at extreme pH values [111]. Ferns of the genus Azolla are also able to accumulate and remove organic compounds from the water environment. Thus, *A. filiculoides* removed up to 50% of diclofenac and 60% of levofloxacin from the water [110]. *A. filiculoides* absorbs up to 90% of the phenolic substance pyrocatechol, which is a precursor of pesticides and flavorings and one of the most famous water pollutants [112]. Phenanthrene, a tricyclic aromatic hydrocarbon, one of the most common environmental pollutants from vehicle exhaust and asphalt heating, is absorbed by *A. filiculoides* by 88, 69, and 60% at contamination levels of 1, 5, and 10 mg/L, respectively [113].

Azolla pinnata R. Brown, the smallest species from the genus Azolla, endemic to the coastal areas of Africa, Asia and Australia. Triangular stems 2.5 cm in length that bears many rounded or angular green, blue-green, or dark red leaves each 1-2 mm long, coated in tiny hairs, giving them a velvety appearance. The growth of this fern did not depend on the presence of nitrogen in the environment, apparently, the fern supplied itself with this macroelement due to symbiotic nitrogen fixation [114]. *A. pinnata* plants absorbed 86.97% of iron sulfate and 81.14% of zinc sulfate at an initial concentration of 100 ppm for 20 days [115]. *A. pinnata* actively accumulated the herbicide 2,4-D and converted it into less toxic compounds that were deposited in the cell walls [116].

A. caroliniana Willd. is native for North and South America, the Caribbean. Scaly leaves 5–10 mm long are green or red, they are coated with two-cell trichomes. Plants purified water from mercury and chromium compounds by almost 100% in 12 days of the experiment. At the same time, the content of metals in fern tissues increased from 71 to 964 mg/kg of biomass, chromium absorption was more effective [117]. This fern also accumulated up to 5 mg of lead per 1 kg of dry matter at a concentration of 20 mg/L of lead acetate, but the toxicity of the metal had a significant negative effect on the photosynthetic apparatus and plant metabolism [118].

A. microphylla Kaulf. occurs in North America. Floating fronds 0.6–2 mm long are green or red, submerged fronds reach a length of 5 cm. Cultivation of *A. microphylla* significantly improved the quality of wastewater from fish breeding ponds (temperature, turbidity, pH, ammonium content) [119]. The accumulation of aluminum in the body of *A. microphylla* occurred in proportion to the increase in the concentration of $AlCl_3$ in the water, while significant activation of the antioxidant system contributed to detoxification and maintenance of metabolic and growth processes in floating fern fronds [120].

Table 2. **Summarized information on the phytoaccumulation of heavy metals by aquatic ferns of the genus *Azolla* from polluted waters (adapted from [107])**

***Azolla* spp.**	**Heavy metal (HM)**	**Initial concentration of HM**	**Duration of the experiment (d)**	**Accumulation of HM (DW) or absorption efficiency (%)**	**Source**
Azolla pinnata	Hg	3.0 mg/L	13	667 µg/g	[125]
	Hg	10.0 µg/L	21	450 µg/g	[126]
	Hg	3.0 mg/L	6	940 µg/g	[127]
	Cd	3.0 mg/L	13	740 µg/g	[125]
	Cd	10.0 mg/L	7	2759 µg/g	[128]
	Cr(III)	3.0 mg/L	13	1095 µg/g	[129]
	Cr(VI)	20.0 µg/L	14	9125 µg/g	[130]
	Ni	500 mg/L	7	16252 µg/g	[128]
	Fe	100 ppm	20	87 %	[118]
	Zn	100 ppm	20	81 %	[118]
Azolla caroliniana	As	80.0 µg/L	7	>120 µg/g	[131]
	Pb	1.0 mg/L	12	416 µg/g	[132]
	Cd	1.0 mg/L	12	259 µg/g	[132]
	Cr(VI)	1.0 mg/L	12	356 µg/g	[110]
	Cr(III)	1.0 mg/L	12	964 µg/g	[110]
	Hg	1.0 mg/L	12	578 µg/g	[110]
	Pb	20 mg/L	10	5 mg/g	[111]
Azolla filiculoides	As	80.0 µg/L	7	>60 µg/g	[131]
	Cr(VI)	20.0 µg/L	14	12 383 µg/g	[130]
	Cr(III)	9.0 mg/L (ppm)	4	1904 ppm	[133]
	Cd	9.0 mg/L (ppm)	4	10441 ppm	[133]
	Cd	10.0 mg/L	7	2608 µg/g	[128]
	Ni	9.0 mg/L (ppm)	4	8814 ppm	[133]
	Ni	500 mg/L	7	28 443 µg/g	[128]
	Cu	9.0 mg/L (ppm)	4	9224 ppm	[133]
	Zn	9.0 mg/L (ppm)	4	6408 ppm	[133]
	Fe	5.0 mg/L	8	92%	[112]
	Al	5.0 mg/L	8	96%	[112]
	Cr	5.0 mg/L	8	10%	[112]
	Pb	5 mg/L 10 mg/L 25 mg/L	15	95% 97% 79%	[115]
	Ni	5 mg/L 10 mg/L 25 mg/L	15	71% 69% 77%	[115]
	Cd	5 mg/L 10 mg/L 25 mg/L	15	93% 89% 66%	[115]
	Cd	1 mg/L 2.5 mg/L 2.7 mg/L		188.7 mg/kg 673.5 mg/kg 93.11 mg/kg	[117]
	Cu	2.6 mg/L		1169.45 mg/kg	[117]
A. microphylla	Cr(VI)	20.0 µg/L	14	14 931 µg/g	[130]
	Ni	500 mg/L	7	21 785 µg/g	[128]
	Cd	10.0 mg/L	7	1805 µg/g	[128]
	Al	100, 250, 500 and 750 µM	6	195.8 µg/g FW	[119]
A. imbricata	Cd	0.5 µg/L	9	183 µg/g	[134]

It was shown also, that in *A. imbricata*, excess cadmium induced the expression of genes encoding anthocyanin biosynthesis [121]. Cultivation of *A. japonica* reduced the nitrogen content in the medium by half in less than a week [122]. It was determined that purification of environment from antibiotics by ferns Azolla spp. occurred in three stages: absorption of the substance by the fern with the formation of reactive oxygen species, which were partially neutralized, conjugation of the substance with the participation of glutathione transferase and glutathione, and deposition of the assimilated substance in the apoplast, vacuoles, and cell wall [123].

The ability to adsorb an excess of macronutrients has been established for all species of ferns of this genus, although they differ in their tolerance to pollutants. Thus, *A. microphylla* showed greater tolerance to supraoptimal nitrogen concentrations than *A. caroliniana*, *A. imbricata*, and *A. mexicana*. In addition, this species had the highest nitrogen absorption efficiency [124].

Hydrophyte ferns of the *Salviniaceae* family in phytoindication of water pollution

To determine the pollution of waters, bioindication methods are used. Special signs that allow us to assess changes in mineralization and purity of the environment are distinguished in indicator plants. These include physiological (level of transpiration, pigmentation, salt content), morphological (size, branching), phenological (anomalies of the development rhythm, growing season) indicator signs. The most sensitive according to these indicators are aquatic macrophytes, the species composition and productivity of which reflect the nature of water pollution with organic substances, heavy metals, pesticides, etc. Due to a closer connection with the aquatic environment, aquatic macrophytes are the most convenient object for phytoindication of waters [135].

Thus, it was shown that the growth rate of the fern *S. natans* increased by 20% when the water was polluted with nickel at a concentration of 0.25 mg/l, while it was significantly inhibited at metal concentrations of 0.5, 0.75, 1 and 2 mg/L [136]. Phenotypic changes of the floating fronds of *S. biloba* were detected on the fifth day of cultivation in artificially contaminated water with cadmium (100 µM), which were manifested in the form of chlorosis and necrosis. In the floating fronds, the metal content on the third day was 3 mg/g DW, and on the tenth — 23 mg/g DW, while in the submerged fronds, it was 3 and 12 mg/g DW, respectively [96]. Prolonged exposure to lead and cadmium (for 10 days) induced changes in the content of photosynthetic pigments (carotenoids, chlorophylls *a* and *b*), secondary metabolites (anthocyanins and flavonoids), soluble carbohydrates, changed the stability of cell membranes of floating and submerged fronds. Such adverde effects were correlated with qualitative changes in the fern phenotype. The plants showed typical signs of toxicity, such as chlorosis and necrosis of floating fronds, the appearance of a brownish-red color on the surface of plants, and a decrease in total plant biomass [37, 96]. *S. biloba* is a bioindicator of cuprum contamination in aquatic ecosystems. At the high concentrations of metal, symptoms of plant intoxication and death were observed [65]. When studying the phytoextraction capacity of *S. natans*, it was shown that the fern actively accumulated lead and copper (>3.328±0.032 and >2.641±0.014 mg/kg DW, respectively). High concentrations of HM negatively affected the growth and habit of the fern, which allows the use of *S. natans* for biotesting [137].

A number of investigations are devoted to the elucidation of the impact of HM pollution on the physiological state of aquatic macrophytes in natural and experimental conditions. It has been reported that *S. natans* is able to accumulate high levels of HM. Thus, the accumulation of chromium, iron, nickel, copper, lead, and cadmium ranged from 6 to 9 mg/g DW, while the accumulation of cobalt, zinc, and manganese was ~4 mg/g DW. The accumulation of HM affected the photosynthetic activity of fern, in particular, the efficiency of carbon assimilation, photochemical activity, and photophosphorylation [91]. Significant growth rate, simplicity of cultivation, distribution and sensitivity to various harmful substances as well as the ability to hyperaccumulate pollutants contribute to the successful use of *S. natans* for biotesting and purification of contaminated waters [138].

Chlorosis was detected in the fern *S. cucullata*, which was grown in a medium containing 0.5, 1, 2, and 4 mg/L cadmium and 5, 10, and 40 mg/L lead. With an increase in the duration of the negative impact and a raise in the concentration of HM, the growth of plants slowed down, the accumulation of biomass decreased, and the content of chlorophyll reduced. Submerged fronds accumulated more cadmium and lead than floating fronds [139]. The fern grew well in a medium containing 0.5–1 mM NH_4^+. On the other hand, the concentration of the pollutant above 5 mM inhibited the growth rate, the number and length of the submerged branches decreased, signs of chlorosis appeared [81]. It was reported that with increasing concentrations of cadmium, copper, chromium, mercury, lead, nickel, and zinc in the aquatic environment, growth and raw biomass accumulation by macrophytes *S. natans*, *S. molesta*, and *S. auriculata* were inhibited [140].

In *A. microphylla* plants under high concentrations of aluminum in water (up to 750 μM), the size of the submerged fronds and the content of phenols and flavonoids significantly decreased, but the level of chlorophylls, sucrose, starch, photosynthesis efficiency and nitrogen-fixing capacity remained almost normal [119]. The growth of *A. filiculoides* was inhibited by 42% under the contamination with phenathrene at a concentration of 10 mg/L, simultaneously the content of photosynthetic pigments was significantly reduced [123]. The presence of 1 mM phenol in the environment also negatively affected the morpho-biochemical parameters of this fern: numerous necrosis was observed, the malondialdehyde content increased significantly [141].

Therefore, aquatic ferns of the Salviniacea family can be used as a valuable tool for biotesting water contaminated with pollutants of various nature.

Conclusion

Aquatic macrophytes of the Salviniaceae family play an important role in the phytoremediation of contaminated waters, improve water quality, promote the circulation of nutrients, stabilize and optimize the habitat of other species of flora and fauna. They are characterized by rapid growth, accumulation of significant biomass, are able to absorb heavy metals and other hazardous waste, possess physiological and molecular mechanisms of adaptation to the toxic effects of pollutants. Aquatic macrophytes of the Salviniaceae family remove pollutants from waters by surface adsorption and incorporate them into their own system or store them in bound form. Species of the genera Salvinia and Azolla are successfully used to assess the ecological state of waters, the ecotoxicological effects of pollutants are studied on them, and biotechnological approaches for biotesting are developed. The properties and characteristics summarized in the review reveal the enormous potential of water ferns for the creation of ecologically acceptable and economically profitable modern biotechnologies for the purification of large volumes of polluted waters from substances harmful to the environment.

This review was prepared as a part of research work 0121U114018 “Exogenous phytohormones in the regulation of growth and development of aquatic macrophytes of the genus *Salvinia* — perspective for phytoindication and phytoremediation of contaminated water” supported by the National Academy of Sciences of Ukraine.

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ВОДНІ ПАПОРОТІ РОДИНИ *Salviniaceae* У ФІТОРЕМЕДІАЦІЇ ТА ФІТОІНДИКАЦІЇ ЗАБРУДНЕНИХ ВОДОЙМ

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Проблематика. Водні екосистеми зазнають значних стресових навантажень та виснаження через надходження забруднюючих речовин неорганічного та органічного походження, що створює серйозну загрозу для здоров'я людей. Програма ООН з навколишнього середовища визначила фіторемедіацію як ефективну екотехнологію видалення, детоксикації та іммобілізації полютантів за допомогою рослин. Папороті гідрофіти родини *Salviniaceae* належать до перспективних фіторемедіантів. Вони характеризуються високими темпами росту, стійкістю до несприятливих екологічних чинників, здатні адсорбувати полютанти, серед яких важкі метали. Види родів *Salvinia* та *Azolla* використовують для оцінки екологічно стану водойм та дослідження екотоксикологічних ефектів забруднюючих речовин

Мета. Аналіз та узагальнення новітніх наукових результатів з використання видів родини *Salviniaceae* для фіторемедіації та фітоіндикації забруднених водойм.

Результати. У цьому огляді ми навели ключову інформацію про новітні фітотехнології, серед яких фітодеградація, фітостабілізація, ризофільтрація, ризодеградація та фітоволатизація. Охарактеризували особливості росту і розповсюдження видів родів *Salvinia* та *Azolla* та представили актуальну інформацію щодо використання водних папоротей для очистки забруднених водойм від важких металів, неорганічних та органічних забруднювачів. Обговорили відомості щодо фізіологічних та молекулярних механізмів адаптації видів родів *Salvinia* та *Azolla* до токсичної дії полютантів різного походження. Окрему увагу ми зосередили на використанні водних папоротей родини *Salviniaceae* для контролю забруднення водойм.

Ключові слова: *Salviniaceae*, водні екосистеми, фіторемедіація, біоіндикація, органічні та неорганічні забруднювачі.