DUCKWEED: A MODEL FOR PHYTOREMEDIATION TECHNOLOGY

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ABSTRACT

The Lemnaceae or duckweed family comprises 37 species of smallest and simplest flowering plants. Duckweeds have a fast growth rate, can survive under a wide range of temperature and pH conditions and are easy to maintain and harvest which makes them an excellent candidate for bioremediation of wastewaters. The main objective of the present review is to extend an appreciation for the potential of living and non-living biomass of duckweed in remediating waters contaminated with heavy metals. Along with showing the detailed mechanism of phytoremediation by duckweed, the paper also discusses the enhancement of duckweed phytoremediation by the integration of transgenic technology. Furthermore, the paper explores other applications of duckweed specifically as fuel, animal feed, in human nutrition, in medicine and as a life support system. Apart from this, various disposal mechanisms for harvested duckweed have been analysed. Current understanding of removal efficiencies of several contaminants by employing duckweed is limited mainly to laboratory experiments. More concentrated and persistent efforts to develop efficient approaches for the genetic transformation of duckweeds can expand the development and utilization of duckweeds.

Keywords: *duckweed, phytoremediation, heavy metals, transgenic plants, non-living biomass*

INTRODUCTION

At the present time, contamination of soil, surface water and groundwater with organic and inorganic pollutants and their restitution has become a paramount concern for environmentalists. Among such pollutants, increasing concentrations of heavy metals and their prolonged persistence in soil and water have created an alarming situation for human life and aquatic biota. Several physical and chemical techniques, inclusive of chemical precipitation, oxidation or reduction, filtration, ion exchange, reverse osmosis, membrane technology, evaporation and electrochemical treatment have been employed as remediation strategies. These techniques suffer from various limitations, namely high costs, low efficiency, generation of secondary pollutants, etc. and are not eco-friendly [1, 2]. Hence it is a requisite to employ low cost and eco-friendly means of remediating media contaminated with heavy metals. Phytoremediation or use of plants as a tool for bioremediation of soil and water by extracting, sequestering or detoxifying contaminants has emerged as a more suitable alternative [3, 4]. This environmental clean-up technology, initially proposed by Utsumamiya (1980) and Chaney (1983) [5], is solar-driven and in average 10-fold cheaper than engineering based heavy metal remediation methods, like ion exchange, filtration and absorption. Ideally, plants with fast growth rate, high biomass, easily harvestable, having a wide root system and that can tolerate and accumulate different types of heavy metals are considered suitable for phytoremediation [6, 7]. The technology has five subsets applicable to toxic metal remediation from soil and water. These are:

- Phytoextraction - plant biomass induces contaminants into shoots after they are taken by the plant roots [8],
- Rhizofilteration - remediation of contaminated water by plant roots through absorption, concentration and precipitation [9],
- Phytostabilization - mobility of contaminants reduced by plant adsorption or precipitation [10],
- Phytovolatalization - uptake of pollutants and releasing them into the atmosphere after conversion into a volatile form [10].

Around 400 species of plants are hyperaccumulators that can absorb high concentrations of metal contaminants through their roots and are being used in phytoremediation. These plants have been found to accumulate metals at a rate 50 - 100 times higher than normal plants [11].

Aquatic plants play an important role in the uptake, storage and recycling of metals from wastewaters [12]. Aquatic plants like *Eichhornia crassipes, Azolla filiculoides, Pistia stratiotes, Hydrilla verticillata, Typha domingensis, Salvinia cucullata, A. caroliniana, A. pinnata, Lemna minor, L. aequinoctialis, L. gibba* and *Spirodela polyrhiza* are suitable for removal of heavy metals, as reported by several researchers [13 - 19]. Duckweed (known as a monocotyledon of the Lemnaceae family, recently classified as subfamily Lemnoideae among aroid family Araceae [20]) is widely and efficiently used for phytoremediation of contaminated water due to its ability to grow in a wide range of temperature, pH and nutrient levels [21]. It is a small group of free-floating aquatic plants with only five genera: *Spirodela, Landoltia, Lemna, Wolffia* and *Wolffiella* and 37 species [22 - 24]. Different duckweed species exhibit variable sensitivity to heavy metals.

This paper provides a review on phytoremediation potential of duckweed for heavy metals, like chromium (Cr), nickel (Ni), lead (Pb), zinc (Zn), cadmium (Cd), copper (Cu), etc. along with a detailed mechanism of their removal from wastewaters. Furthermore, the paper discusses the approach of genetic engineering to enhance the duckweed phytoremediation ability. Besides, the paper explores other fields of duckweed application and alternatives for its disposal.

DUCKWEED SPECIES IN PHYTOREMEDIATION

Duckweeds are the smallest and simplest flowering plants that currently exist. They represent a highly modified structural organization, including the simplification and loss of many anatomical features [25]. Their reduced body structure is organized as “thalloid” or “frond” and stem is absent. Roots are simple in *Lemna* and are entirely lacking in two genera (*Wolffia* and *Wolffiella*). Though duckweeds are angiosperms, they dominantly reproduce by vegetative propagation. The utility of duckweed species for bioremediation is sustainable because they recycle the nutrient from the wastewater and recover the aquatic ecosystem efficiently [26]. An overview of studies regarding phytoremediation potential of different species of duckweed is presented hereinafter.

Lemna

Lemna minor (common duckweed) is commonly found in temperate regions, except Eastern Asia and Australia. Singh et al. [27] in his study has revealed that the duckweed (*L. minor*) showed better lead removal than other aquatic plants, such as *Pistia stratiotes* (Water Lettuce), *E. crassipes* (Water Hyacinth), *Hydrila verticillata* (Hydrilla) from polluted water and can be used in phytoremedial approaches. *L. minor* is not suitable for accumulation of Ni^{2+} from contaminated wastewaters [28]. *L. minor* can grow well in pH range 6 - 9 making it a suitable plant for phytoremediation. However, nitrate has few inhibitory effects on the plant growth [29]. Uysal [30] and Thayaparan et al. [31] showed that *L. minor* could efficiently reduce chromium in water at low concentration. Uysal and Taner [32] reported that lead accumulation in *L. minor* was highest at pH 4.5 and then it decreased at pH 6 which was later confirmed by Kaur et al. [33]. *L. minor* also showed great potential for the removal of chromium, zinc, lead and cadmium from textile wastewaters [34]. A comparative study conducted on *L. minor* and *E. crassipes* distinctly stated that *L. minor* can remove nickel metal more thoroughly as compared to *E. crassipes* [35]. *L. gibba* was found to be an appropriate remedy for boron at low concentrations (2 mg/l) [36]. It can also accumulate uranium (120 %), boron (40 %), and arsenic (133 %) [37]. Jafari and Akhavan [38] investigated the capacity of 3 duckweeds, *L. minuta, L. minor, L. trisulca*, to purify zinc polluted water, during which *L. trisulca* (97 %) was found to have highest percentage of removal. In another study, *L. polyrhiza* / *Spirodela polyrhiza* when exposed to 10 mg/l of zinc, lead and nickel for 4 days accumulated 27.0 µg/mg of zinc, 10.0 µg/mg of lead and 5.5 µg/mg of nickel [39]. Azeez and Sabbar [40] in their study on phytoremediation of oil refinery by *L. minor* showed that it can successfully be used for wastewater pollutants removal. Daud et al. [41] in their study proved that *L. minor* significantly reduced the concentration of heavy metals in a landfill leachate. Removal efficiency of *L. minor* for all the metals from landfill leachate was more than 70 %, with the maximum value for copper (91 %). Studies have shown that *L. minor* has the potential to grow, develop and bioremediate iron rich mine effluent [42]. Both *L. minor* and *Spirodela polyrhiza* are potential cadmium accumulators [43].

Spirodela

Spirodela polyrhiza was found to be an extractor and accumulator of arsenic, nickel and cadmium [44, 45]. *S. polyrhiza* was seen to have accumulated more than 1000 mg/kg of lead and nickel in its dry biomass [46]. Islam et al. [47], while evaluating the performance of *S. polyrhiza* for treatment of Cr(VI) water, submitted that the high bioconcentration factor (4558) proved the appropriateness of the plant for extracting chromium metal from water. *Spirodela* exhibited symptoms of toxicity for zinc at high exposure concentrations (40 - 50 ppm) [48]. S. *polyrhiza* has been capable of removing cadmium and lead from media efficiently under laboratory conditions [49]. Loveson et al. [50] apprised that constructed wetlands with *Spirodela* mat may help prevent the spread of heavy metal contamination from land to the aquatic environment. High metal removal rates, close to 100 %, were reported in wetlands in the study, which is quite promising.

Landoltia

It consists of one species, namely *Landoltia punctata*. Its major application is in the field of starch production. Shi et al. [51] in their study compared the toxic effects of copper oxide nanoparticles (CuO-NP) and soluble copper salt ($CuCl_2$) on *L. punctata*. The results stated that copper was easily absorbed from CuO-NP suspension. Therefore, copper content was four times higher in CuO-NP exposed fronds than in fronds exposed to equivalent dose of soluble copper.

Wolffia

Wolffia globosa is one of the smallest flowering plants consisting of small rootless spherical fronds. Zhang et al. [52] investigated potential of *W. globosa* for arsenic accumulation and found that it was able to accumulate ≥ 1000 mg As/kg in frond dry weight (DW) and tolerate up to 400 mg As/kg DW. Arsenite efflux appears to be the limiting factor in phytofilteration of arsenic using *W. globosa*. Moreover, *W. globosa* have shown a high level of tolerance to both chromium and cadmium [53]. Among five species of *Wolffia* (*W. globosa*, *W. australiana*, *W. cylindracea*, *W. columbiana W. arrhiza*). *W. columbiana* accumulated highest concentration of cadmium [54].

Wolffiella

Wolffiella is a genus of small rootless duckweed of the *Lemnaceae* family. No secondary research data was available on phytoremediation capacity of *Wolffiella*.

A summary of removal efficiencies of duckweed species for different heavy metals is presented in Table 1.

Table 1. Removal efficiency of duckweed for different heavy metals

S. N.	Species used	Heavy metal	Concentration in medium	Removal efficiency (%)	Accumulated concentration in plant	Remarks	Reference
1.	*L. minor*	Chromium	5 mg/L		4.423 mg Cr/g	pH 4.0	[30]
2.	*L. minor*	Zinc	1 - 20 mg/L	40 - 83			[38]
3.	*L. minuta*	Zinc	1 - 20 mg/L	35 - 89			[38]
4.	*L. trisulca*	Zinc	1 - 20 mg/L	49 - 97			[38]
5.	*L. minor*	Lead	10 mg/L	99.99		pH 5.0 - 6.0	[33]
6.	*L. minor*	Nickel	10 mg/L	99.30		pH 6.0	[33]
7.	*L. minor*	Lead	16 μg/L	98.70		Water sample - wastewater from Basra Oil Refinery, Iraq.	[40]
8.	*L. minor*	Copper	12 μg/L	99.80			[40]
9.	*L. minor*	Zinc	43 μg/L	72.00			[40]
10.	*L. minor*	Cadmium	5.1 μg/L	99.60			[40]
11.	*L. minor*	Zinc	1.47 mg/L	83.00		Water sample - Mahmood Booti landfill site, Iraq.	[41]
12.	*L. minor*	Lead	0.83 mg/L	78.00			[41]
13.	*L. minor*	Iron	1.17 mg/L	77.00			[41]
14.	*L. minor*	Copper	0.69 mg/L	91.00			[41]
15.	*L. minor*	Nickel	1.21 mg/L	76.00			[41]
16.	*L. minor*	Cadmium	2 mg/L		4734.56 mg/kg		[43]
17.	*S. polyrhiza*	Cadmium	3 mg/L		7711.00 mg/kg		[43]
18.	*S. polyrhiza*	Lead	0.91 mg/L	93.19			[46]
19.	*S. polyrhiza*	Nickel	2.92 mg/L	70 - 80			[46]
20.	*S. polyrhiza*	Chromium	4.5 mg/L		855.56 mg/kg		[47]
21.	*S. polyrhiza*	Lead	1 mg/L	53			[49]
22.	*S. polyrhiza*	Cadmium	1 mg/L	53			[49]
23.	*S. polyrhiza*	Copper	65 μg/L	79		Water sample - a wetland near Kuzhikundam Thodu creek at a location near HIL site boundary, Eloor industrial area, Ernakulam, Kerala, India.	[50]
24.	*S polyrhiza*	Lead	26 μg/L	95			[50]
25.	*S. polyrhiza*	Zinc	212 μg/L	66			[50]
26.	*S. polyrhiza*	Chromium	118 μg/l	53			[50]
27.	*S. polyrhiza*	Cobalt	7.2 μg/L	28			[50]
28.	*S. polyrhiza*	Manganese	8 μg/L	20			[50]
29.	*S. polyrhiza*	Mercury	23 μg/L	45			[50]
30.	*S. polyrhiza*	Nickel	19.3 μg/L	9			[50]
31.	*S. polyrhiza*	Copper	63 μg/L	74		Water sample - wetlands southwest of the "Amanthuruthu" wetland area, approx. 150 m west of the HIL, Eloor industrial area, Ernakulam, Kerala.	[50]
32.	*S. polyrhiza*	Lead	34.4 μg/L	91			[50]
33.	*S. polyrhiza*	Zinc	301 μg/L	62.4			[50]
34.	*S. polyrhiza*	Chromium	121 μg/L	49.0			[50]
35.	*S. polyrhiza*	Cobalt	8 μg/L	40			[50]
36.	*S. polyrhiza*	Manganese	7.3 μg/L	30.1			[50]
37.	*S. polyrhiza*	Mercury	3.4 μg/L	53.0			[50]
38.	*S. polyrhiza*	Nickel	22.3 μg/L	22.0			[50]
39.	*S. polyrhiza*	Iron	5.3 μg/L	98.1			[50]
40.	*S. polyrhiza*	Cadmium	3 μg/L	100			[50]
41.	*L. punctata*	Cu salt ($CuCl_2$)	0.6 mg/L		Roots: 550 - 600 μg/g DW; Fronds: 400 - 450 μg/g DW		[51]
42.	*L. punctata*	CuO-NP	1.0 mg/L		Roots: 800 μg/g DW aprox.; Fronds: 650 - 700 μg/g DW		[51]
43.	*W. globosa*	Cadmium	5 μM		143.12 mg/kg FW	pH 6.0	[54]

TYPES OF PHYTOREMEDIATION IN DUCKWEED

After careful analysis of previous studies [17, 44, 54, 55 - 57] it can be established that phytofiltration/rhizofiltration is the dominant mechanism of phytoremediation in duckweed. The phytoremediation potential of the duckweeds can be further enhanced by the application of innovative approaches in phytoremediation.

Transgenic phytoremediation

Duckweeds genome sizes vary enormously, ranging from 158 megabase pairs (Mbp) in *Spirodela* to 1881 Mbp in *Wolffia*, a total 13-fold change [58 - 61], indicating duckweeds as an interesting model for studying genome size evolution. Knowledge of molecular taxonomy has led to significant progress for exploitation of duckweed in toxic metal and metalloid phytoremediation. Li and Xiong [62] and Vunsh et al. [63] used polyploidization as a tool for genetic modification of duckweeds. Many attempts have been made to develop a technology of genetic engineering of exogenous genes into nuclear genome through agrobacterium-mediated transformation and regeneration from tissue culture [64, 65]. This technology not only allows expressing recombinant protein, polymer, small molecules in duckweed system [66, 67], but facilitates functional gene studies in duckweeds as well [67]. The first stable transformed duckweed was obtained by Frey et al., (1980) through incubating intact plant of *Lemna perpusilla* with the *Escherichia coli* plasmids pMB9 and plasmid Bolivar Rodriguez 325 (pBR325) under optimized conditions [68]. Efficient genetic transformation protocols were developed in *L. gibba* and *L. minor* with a binary vector containing beta-glucuronidase and Neomycin phosphotransferase II (nptII) expression cassettes [67]. Transgenic duckweed could be regenerated after three months of agrobacterium-mediated transformation. The addition of the poorly assimilated carbohydrates of galactose or sorbitol yielded high levels of callus [64]. The stable and transgenic *S. oligorrhiza* showed a high protein yield, that is the transgene protein of Green fluorescent protein (GFP) expression, reached more than 25 % of total soluble proteins [65]. Canto-Pastor et al. [69] engineered an artificial microRNA (amiRNA) gene silencing system in *L. gibba*. An Arabidopsis photorespiratory pathway gene serine glyoxylate aminotransferase (SGAT), named as AtAGT1, was successfully overexpressed in *L. minor* [70]. The gene expression response to cadmium stress in *L. punctata* 6001 was analysed via RNA-Seq technique by Xu et al. [71]. A summary of this and other stress responses of duckweed is available in Table 2. The transcriptomic study using RNA Seq to determine toxicity and tolerance of ammonium (NH_4^+) was reported in *L. minor*. Bioinformatical analysis identified 70,728 unigenes and 14,207 differentially expressed genes (DEGs), most of which were down-regulated under NH_4^+ toxicity [72]. The gene expression data for ionizing radiation (IR) indicated that *L. minor* plants can shift from acclimation responses toward survival responses at increasing dose rates of ionising radiation [73]. Wang et al. [74] discovered that 3 days of exposure to 10 µM abscisic acid (ABA) induced irreversible turion development in *Spirodela*. Similar to a desiccating seed of a terrestrial plant, developing turions upregulated five and expressed two previously silent genes of the Late Embryogenesis Abundant (LEA) protein family. These LEA family proteins protect other proteins and confer resistance to dehydration, salinity and cold stress. Upregulation of seven ABA-responsive, three ethylene-responsive, and two heat shock responsive transcription factors was also observed. There were also ABA transcription factor binding sites in 30 of the upregulated genes, while 119 had a bind site for ethylene-responsive transcription factors. This pathway matches the ABA or environment triggered, calcium-dependent signal pathway observed in maturing seeds, reinforcing the similarity of turions and seeds on a molecular, invisible level.

Table 2. Response of duckweed to exposure to different conditions

S. N.	Exposure	Response	Duckweed species	Reference
1.	Silencing of CH42, a magnesium chelatase subunit, using amiRNA platform	Reduction of chlorophyll pigmentation.	*L. minor*	[69]
2.	Overexpression of Arabidopsis photorespiratory pathway gene serine glyoxylate aminotransferase (SGAT), named as AtAGT1	Promote salt tolerance in duckweeds and solve the freshwater salinity problems.	*L. minor*	[70]
3.	Cadmium stress	Genes involved in DNA repair acted as an early response, RNA and protein metabolism would likely respond, genes involved in sulphur and reactive oxygen species (ROS) metabolism were upregulated, Vacuolar sequestration.	*L. punctata 6001*	[71]
4.	NH_4^+ toxicity	Lignin biosynthesis related genes in the phenylpropanoid biosynthesis pathway were up-regulated, accumulation of ROS which can cause oxidative damage leading to cell death, antioxidant enzyme system was also activated.	*L. minor*	[72]
5.	Ionizing radiation (IR)	Lower dose rates - trigger acclimation responses. Higher dose rates - genes related to antioxidative defence systems in terms of DNA repair and cell cycle were highly expressed.	*L. minor*	[73]
6.	Abscisic acid	Induced irreversible turion development and an increase of two enzymes involved in starch and cell wall production in Spirodela fronds.	*Spirodela sp.*	[74]

Non- living/dried duckweed biomass

Successive use of dried and dead plant biomass (as simple biosorbent substance) to remove the metals from water has gained popularity over the past few years, because it has high efficiency in detoxifying dilute effluents, not effected by toxic wastes, minimize the volume of chemical and/or biological sludge to be disposed off, it has no nutrient requirements, and it is cost-effective, natural and easy to transport and handle [75 - 81]. The dried duckweed biomass shows a porous structure with free spaces. In addition, duckweed possess diverse functional groups, namely carboxyl, amide, thiol and hydroxyl, which can be a potential binder for heavy metals, like arsenic and lead [82, 83]. The adsorption capacity of dried duckweed biomasses is listed in Table 3. The dried *S. polyrhiza* was examined and found out to be an efficient adsorbent to eliminate the basic dye of methylene blue from aqueous solution [84]. In the evaluation by Romero-Guzmán et al. [82], dead biomass of *L. minor* retained As(V) more strongly than *E. crassipes*. Dried biomass of *S. intermedia*, *L. minor* and *P. stratiotes* were investigated for simultaneous removal of metals (Cd^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+}) from wastewater derived from industrial activities. The studied biomasses removed lead and cadmium efficiently and *L. minor* biomass presented the highest mean removal percentage, whereas *P. stratiotes* had the lowest results for all metals tested [85]. Tang et al. [83] indicated dried biomass of *L. punctata* and *S. polyrhiza* to be promising adsorbents that may be used as alternative approaches for Pb^{2+} removal from contaminated water. Further studies have suggested that dried powder of *L. aequinoctialis* and *L. perpusilla* effectively removes lead from aqueous solution [86, 87]. Untreated dry powder of *L. aequinoctialis* has also proved to be a convincing adsorbent for cadmium [88]. Besides, *L. minor* powder has also exhibited excellent removal capability for both inorganic and organic mercury [89]. Methyl parathion and cadmium were successfully removed by *L. gibba* powder [90]. Younis et al. [91] evaluated that duckweed *L. gibba* L. could be used as low-cost biosorbent for removal of phenol ions from industrial wastewater. Upatham et al. [92] examined the effects of concentration and pH of solution on the biosorption of cadmium and chromium by using dry *W. globosa* biomass. The maximum adsorption of cadmium was observed at an initial pH of 7 which diminished with decreasing pH as cadmium and hydrogen (H^+) ions compete for active sites of *W. globosa* at lower pH. Adsorption of chromium decreased with increasing pH, because increase in pH would favour the formation of chromate (CrO_4^{2}) ions that are not readily adsorbed by

W. globosa. In a study on dried powder of the *Landoltia punctata* duckweed to remove iodate (IO_3^-) from aqueous solutions it was discerned that IO_3^- is reduced to iodine (I_2) and I^- by hydroxyl groups, thereby demonstrating duckweed (*Landoltia punctata*) as a promising biosorbent for remediation of radioactive iodine pollution [93]. Nie et al. [94] used *Landoltia punctata* as a biosorbent to remove uranium dioxide (UO_2^{2+}) from aqueous solutions. The maximum sorption capacity was 131.8 mg/g dry matter. Moreover, studies have conveyed that melamine treated *L. minor* has higher adsorption capacity than untreated *L. minor* for thorium (IV) biosorption under the condition of optimization [95].

Table 3. Adsorption capacity of different duckweed adsorbents

S. N.	Duckweed species	pH	Temp. (K)	Metal	Q_m (adsorption capacity) mg/g DW	Ref.
1.	*L. punctate*	4.6	298.15	Lead	250	[82]
2.	*S. polyrhiza*	4.6	298.15	Lead	200	[82]
3.	*L. perpusilla Torr.*	4.6	298.15	Lead	87	[86]
4.	*L. aequinoctialis*	4	298.15	Lead	57	[85]
5.	*W. globosa*	7	298.15	Cadmium	80.65	[92]
6.	*W. globosa*	1.5	298.15	Chromium	73.53	[92]
7.	Melamine treated *L. minor*	5.5	-	Thorium	129.88	[95]

MECHANISM OF DUCKWEED PHYTOREMEDIATION

Heavy metals are highly pernicious and cannot be chemically degraded. Certain plant species have the ability to accumulate heavy metal in roots and then in above ground plant biomass. Plants may exude organic acids and protons making ionic species present in media biologically available for biosurfactants and chelators [96 - 100]. These ionic species bind with chelators and then pass through cellular membranes more easily [101, 102]. These contaminants are transported through the plant via apoplastic and symplastic and/or transmembrane pathways [96, 98, 103, 104]. After transportation, metals are sequestered in the cell walls, vacuoles and/or Golgi complexes [104]. This mechanism of contaminant removal is called direct phytoremediation. Although plants do not remove the contaminants in explanta phytoremediation, they stabilize them with the association of selective microorganisms and decrease the risk of potential receptors (plants as well as animals) [96, 103, 105 - 107]. Duckweeds are well recognized for their capability to eliminate the metals from the contaminated environment [41, 108, 109]. Active transport of heavy metals in free-floating aquatic plants occur through roots from where metals are transferred to other plant organs. Passive transport is associated with the direct contact of the plant body with the pollution medium [110]. A comparative analysis of uptake and detoxification mechanisms in different species of duckweed is summarized in Table 4.

MAJOR APPLICATIONS OF DUCKWEED

Due to duckweed's remarkable capability to quickly absorb nitrogen, phosphorus and other nutrients, scientists are currently exploring ways that duckweed can convert agricultural and municipal wastewater into clean water. Subsequently, biomass produced can be used for feed applications or biofuel if it was used to treat harmful industrial wastewater.

Water treatment

Global distribution, tolerance of ammonia, heavy metals, other stresses, high yield of biomass (especially at 20 – 30 °C), ease of harvest, high protein and starch content, and a wide range of uses make duckweeds suitable for treating agricultural, municipal, and even industrial wastewater. The classic example of a duckweed treatment system and feed application would be the Mirzapur Bangladesh hospital wastewater facility, which was designed by the PRISM group, monitored from 1989 to 1991 [113]. Professor Zhao Hai's group from Chengdu Institute of Biology, Chinese Academy of Sciences, also has extensive records from their pilot plant at Dianchi Lake, in subtropical Yunnan, China [114] (Figure 1).

Table 4. Uptake and detoxification mechanism of different duckweed species

S. N.	Duckweed species	Metal	Uptake mechanism	Toxic effects	Detoxification mechanism	Reference
1.	*L. minor*	Iron	Biosorption & bioadsorption.	Hydroxyl radicals generated through Fenton reaction caused membrane disintegration and cell death. Roots gained an orange - brownish colour due to formation of iron plague.	Subset of genes related to Fe homeostasis & those coding for ferritin (protein involved in Fe storage) are activated. Vacuoles store Fe to avoid cytotoxicity.	[42]
2.	*S. polyrhiza*	Chromium	CrO_4^{-2} transported by phosphate - sulphate carrier (active transport).	-	Accumulated and translocated through symplast in a manner that does not disrupt cytoplasmic function.	[47]
3.	*W. globosa*	Arsenic	Phosphate transporters participate in As(V) uptake - active transport & some aquaporin channels might participate in As (III) uptake - passive transport.	Arsenate uptake, but concurrent production of arsenite.	-	[52]
4.	*W. globosa*	Cadmium	Mainly passive adsorption via apoplast component (due to cell binding).	Homeostasis interference due to high amounts of cadmium actively taken up by the plant cells when cell binding capacity was saturated.	-	[54]
5.	*L. minor*	Lead and zinc	-	Interference with the photosystem resultantly inducing chlorosis, decrease in soluble protein content, decrease in chlorophyll a and carotenoid content.	Catalase (CAT) activity increased, CAT acts on hydrogen peroxide (H_2O_2) and converts it to water and oxygen. Increase in enzyme activity.	[111]
6.	*L. minor*	Cadmium	-	Root elongation & frond no. decreased, Hormesis response seen. Influences antioxidant system (mainly CAT).	High Cd tolerance can be attributed to an increase in Cd inactive forms. At lower concentrations Cd stress activate peroxidases (POD), superoxide dismutase (SOD), total antioxidant capacity (T-AOC) and malondialdehyde (MDA).	[112]

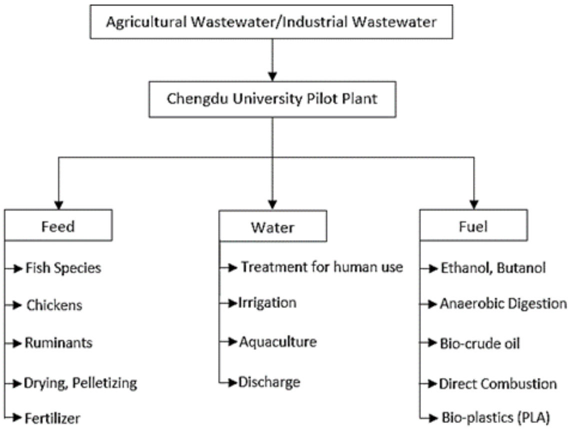

Figure 1. Flowchart of duckweed wastewater treatment and biomass application [114]

Bioenergy

Bioenergy applications of duckweed are discussed in Figure 2. Duckweed biomass exhibits good characteristics for bioethanol production due to its relatively high starch and low lignin percentage. The first commercially viable example of ethanol fermentation, the Andrew Young Foundation conducted a private research trial using the ecosystem technology, produced by resource recovery experts Greenbelt Resources Corporation, which was presented in a feasibility study report conducted by an independent agency and submitted to the United States Department of Agriculture (USDA) in 2017. With successful feasibility determined, the foundation created a corporation called Duckweed Days LLC (Limited Liability Company), which partnered with Greenbelt Resources to conduct a pilot system development project in Paso Robles, California, USA, in 2018. Leveraging its farming and agricultural expertise as well as its engineering prowess, Greenbelt has developed a species agnostic prototype cultivation, harvesting and processing system.

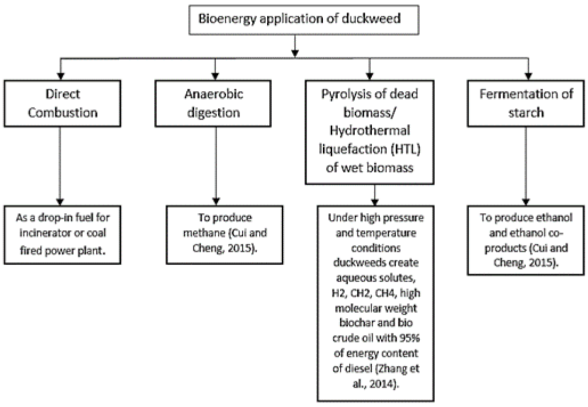

Figure 2. Bioenergy applications of duckweed [115]

Animal feed

Duckweed can recycle nutrients back into the food supply, provided it is monitored for heavy metals and other hazards, and legally approved. Agriquatics wastewater treatment proposed blueprint for a municipal treatment facility designed by Agriquatics. The systems start with solids removal through laminar flow separators and hydrocyclones, and transport solids to an array of bacterial digesters. A series of duckweed ponds remove solutes and their circular shape facilitates central harvesting. Water is then filtered and disinfected with conventional methods. Duckweed biomass can be tested, sterilized, and converted to Tilapia fish feed [115]. Duckweeds have been a traditional feed for fish and poultry in South-East Asia for centuries. Duckweed was found to be beneficial in replacing ~15 % of the soybean meal in the feed for chickens or broilers, and 40 % in the case of laying hens [113, 116]. Finally, ruminants have shown promising results with high nitrogen digestibility in merino sheep, and cattle consuming and effectively digesting up to 10 % of their weight in dried duckweed per day [116].

Human nutrition

The *Wolffia* genus of the duckweed family has been traditional cuisine in Thailand, Burma and Laos for centuries, since *Wolffia* in its plant tissue do not produce calcium oxalate crystals, a causative agent of human kidney stones. At present, there are three large companies producing *Wolffia* or *Lemna* for human consumption, namely Hinoman, Parabel and Green Onyx (Table 5).

Table 5. Companies producing duckweed for human consumption [115]

S. N.	Company	Strategy	Product
1.	Hinoman	Greenhouse precision agriculture cultivation.	*Wolffia* with 25 % carbohydrate content, 45 % protein content and a complete and bioavailable amino acid profile, such as egg or soy, with a higher Protein digestibility-corrected amino acid score (PDCAAS) than soy.
2.	Parabel	Open pond *Lemna* cultivation and protein extraction.	Protein powder.
3.	Green Onyx	Developed robotic farming systems.	Dispense *Wolffia* on demand.
4.	Plantible Foods	Developing a gentle protein isolation process using *Lemna*.	Colourless, tasteless protein isolate with physical properties of egg whites to create a vegan product.

Phosphorous reclamation

Economically mineable, organically available phosphorous is expected to be scarce by 2050 or 2100, and production might decline by 2030, raising its price possibly beyond the reach of poorer farmers [117]. Fortunately, phosphorous can be recycled by better farming practices or by using more aquatic plants and other methods to recapture more than the current rate of 50 % from human wastes.

As a life support system

Duckweed was additionally described as one of the most attractive higher plants for longduration supporting human life in space [118]. National Aeronautics and Space Administration (NASA) is interested in developing closed-loop life support systems for long-term missions. *L. aequinoctialis* was found to have a 32 % increase in growth rate in simulated microgravity [118]. Therefore, Space Lab Technologies, LLC is currently collaborating with the University of Colorado at Boulder on a Phase 2 grant from NASA to develop the μG-LilyPond^{TM} growth chamber as part of a life support system [115]. Presently the system is designed to provide fresh food and oxygen, with the eventual goal of converting urine to clean water.

Medicine

There have been academic papers reporting over 20 transgenic therapeutic proteins in duckweed reaching as high as 7 % of total soluble protein [119]. Given the lower cost of production and lower risk of transmissible pathogens compared to mammalian cell lines, duckweed may provide genetically engineered proteins for medical or other applications. *L. minor* can be used for synthesis of recombinant proteins [120]. *L. punctata* is rich in flavonoids [121] and used in traditional Chinese medicine. It has also potential for pharmaceutical drugs.

DUCKWEED DISPOSAL

Appropriate phytoremediation technology needs intervallic harvesting of the plant biomass in order to assimilate and confiscate heavy metals and nutrients from water bodies. Conversion of biomass into superior material is a significant factor in promoting this technique. Many studies have reported that aquatic plants like duckweed biomass after phytoremediation can be used as animal feed and in biogas production [122] (Figure 3). Rolli et al. [48] recommend that harvested biomass may be used for composting and as a supplement to fertilizers. Dry biomass of *L. minor* generated during phytoremediation of iron, without other toxic metals, could be an important fertilizer for iron-deficient soils, which comprise one-third of the world's soils [42]. Dushenkov et al. [123] have pointed out that the high water content of aquatic plants impedes the drying, composting, or incineration process. Dried duckweed can be used as a drop-in fuel for a trash incinerator or coal-fired power plant. This would concentrate heavy metals in the smoke, which could be scrubbed, and ash can be properly disposed or encapsulated for reuse in concrete or gypsum [115].

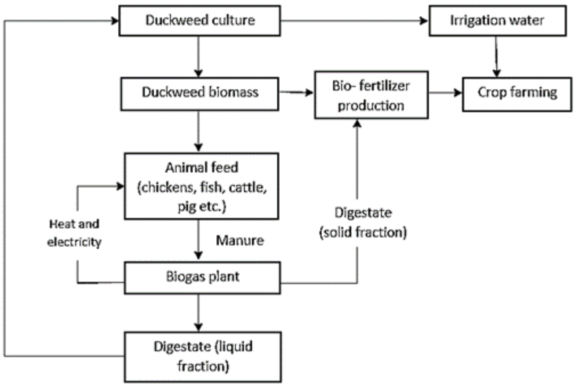

Figure 3. Schematic presentation of duckweed disposal [124]

CONCLUSION

Heavy metal pollution is a major environmental concern for which conventional remediation approaches prove unfulfilling. Utilization of phytoremediation seems to be less destructive, economical, and environmentally sound clean-up technology. Duckweed has been reported to be very useful in phytoremediation of organic matter, suspended solids, heavy metals and soluble salt from wastewater. Application of duckweed both in bioaccumulation (with living plant biomass) and bio-sorption (with dead plant biomass) can be done successfully for the elimination of heavy metals. Comprehensive interaction, transport and chelating activities regulate the storage and accumulation of heavy metals by the duckweed. Plant biomass can be used later on for production of biogas, as fertilizer and as animal feed. Genetic engineering of duckweed to enhance its heavy metal uptake capacity is in its preliminary phases. Due to their high biomass, accumulation rate and nutrient content duckweeds have increasingly been considered as bioenergy and food source, as response to global resources exploitation and environmental crisis. The present review highlights the benefits of using duckweed to treat water contaminated with heavy metals, which are currently limited to laboratory experiments and batch systems and are rarely on microcosm and mesocosm scale. The novel abilities of duckweed in various fields can be enriched by the use of genetic engineering. Thus, duckweed is a promising plant resource which deserves further research and development.

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