Chapter 17

Plant Nutrient Phytoremediation Using Duckweed

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Abstract Over the last 40 years a great deal of research has been published on the use of duckweed to treat wastewater both from point sources (feedlots, food processing plants) and from non-point sources. These plants can recover nutrients such as nitrogen and phosphorus from contaminated waters in those agricultural practices. They can also remove or accumulate metals, radionuclides, and other pollutants in their tissues. In addition, the duckweed can be used as a feed source for livestock and poultry as well as an energy source for biofuel production. A summary of some of the published work done using duckweed species to phytoremediate natural, domestic, industrial, and agricultural wastewaters is presented.

Keywords Duckweed · Plant nutrients · Phytoremediation · Lemnaceae · *Lemna* · *Wolffia*

17.1 Introduction and Background of Duckweed

Duckweeds belong to the arum family Araceae, subfamily Lemnoideae, a family of floating, aquatic plants. This family consists of five genera with at least 40 species identified as of 1997 (Les et al. 2002). Duckweeds are among the smallest and simplest flowering plants, consisting of an ovoid frond a few millimeters in diameter and a short root usually less than 1 – cm long (Fig. 17.1). The frond represents a fusion of leaves and stems and represents the maximum reduction of an entire vascular plant (Landolt 1986). Some species of the genus *Wolffia* are only 2 mm or less in diameter; other *Lemna* spp. have frond diameters of about 5 – 8 mm. The largest species of Lemnaceae have fronds measuring up to 20 mm in diameter (*Spirodela* sp.). The minute flowers are rarely found in most species. Under adverse conditions such as low temperatures or desiccation, modified fronds called turions appear which sink to the bottom of the water body. These turions can resurface at the onset of favorable conditions of light, moisture and temperature to start new generations of duckweed plants (Hillman 1961, Perry 1968). Because flowering in Lemnaceae is rare, reproduction normally occurs by budding from mature fronds. The tolerance of Lemnaceae fronds and turions to desiccation allows a wide dispersal of Lemnaceae species. This low level of gene flow and infrequent sexual reproduction has produced substantial levels of genetic divergence among populations, despite an absence of morphological differentiation (Cole and Voskuil 1996). However, asexual reproduction in Lemnaceae allows for rapid reproduction in this family. Occasionally extreme weather events, such as unusually high summer temperatures, can cause mass flowering (Bramley 1996). Usually flowering has to be induced with plant hormones or photoperiod manipulation (Cleland and Tanaka 1979). All Lemnaceae flowers are minute and barely discernable without magnification (Landolt 1986).

Due to its ease of culture and worldwide distribution, a tremendous literature exists on duckweed ecology, physiology, production, and systematics. Landolt and Kandeler's two monographs on Lemnaceae are the

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A.A. Ansari et al. (eds.), *Eutrophication: Causes, Consequences and Control*,
DOI 10.1007/978-90-481-9625-8_17, © Springer Science+Business Media B.V. 2011

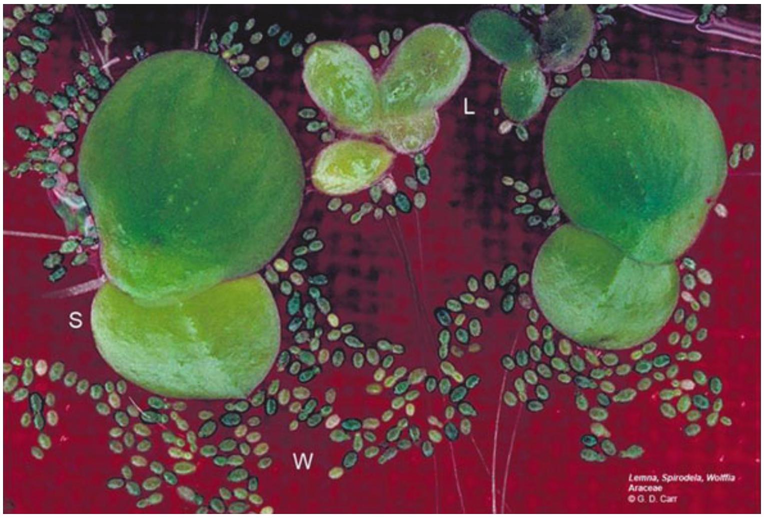

Fig. 17.1 *Spirodela* (*large*), *Wolffia* (*small*), and *Lemna* (*intermediate*)

most comprehensive works on Lemnaceae and list virtually all published works up to 1986 (Landolt 1986, Landolt and Kandeler 1987). In addition there are several web sites that have more updated information on duckweed biology and applications (Cross 2007, Landesman 2008).

The genera *Lemna*, *Spirodela*, and *Wolffia* of the family Lemnaceae play an important ecological role in lakes, ponds, and wetlands. They often are an important source of food for waterfowl (Krull 1970) and aquatic invertebrates. The outer margins of duckweed fronds (phyllosphere) support dense populations of diatoms, green algae, rotifers, and bacteria (Coler and Gunner 1969). Associated with this epiphytic community is an assortment of insects, including beetles, flies, weevils, aphids, and water striders (Scotland 1940). Some of these insects may become abundant enough to affect the duckweed population. Together with the frond biomass this microfauna enhances the nutritive value of duckweed to grazing animals such as ducks, geese, nutria, turtles, coots, fish, and snails, all of which have been recorded as feeding on duckweed.

Duckweed populations are limited mostly by light, nutrients, and temperature (Hillman 1961). Duckweed populations can grow very densely in nutrient-rich environments, so much so that layers of fronds grow one on top of another to form a mat that can be up to 6 – cm thick. This thick mat creates an anaerobic environment in the water body on which this mat floats, thus promoting anaerobic digestion and denitrification of the water body in which the duckweed grows. Since duckweed floats freely on water surfaces, strong winds can sweep fronds from the water surface.

The presence of duckweed in an aquatic environment has both direct and indirect effects on that environment. When duckweed is abundant enough to completely cover a pond, ditch, or canal, this layer of opaque fronds can shade out rooted aquatic macrophytes (Janes et al. 1996) as well as reduce phytoplankton abundance. In eutrophic environments such as the polders of Holland, *Lemna* sp. can form a climax community that prevents *Chara* and other submerged macrophytes from getting established (Portielje and Roijackers 1994). A complete cover of duckweed on the water surface can lead to the creation of an anaerobic environment in the water column, which in turn can make that water body inhospitable to fish and aquatic insects (Pokorny and Rejmankova 1983, Leng et al. 2004).

The presence of duckweed can contribute to the organic matter present in a water body. Layers of *Lemna minor* L. excrete amino acids and humic substances into the aquatic environment which can provide nutrients to other organisms such as bacteria, epiphytic algae, and indirectly to snails, springtails, isopods (*Asellus* sp.), and other microdetrivores (Thomas and Eaton 1996). Dead and dying duckweed fronds fall to the bottom of the water column where their decay contributes organic matter, nitrogen, phosphorus, and other minerals to the benthos (Laube and Wohler 1973). In addition cyanobacteria residing in the phyllosphere of duckweed fronds can fix atmospheric nitrogen, providing a nitrogen input in oligotrophic environments (Tran and Tiedje 1985). This can be an important source of nutrients in aquatic environments.

Duckweeds are among the fastest growing aquatic angiosperms in the world, frequently doubling their biomass under optimum conditions in 2 days or less (Culley et al. 1981). Based on growth rates recorded in the literature, duckweeds can grow at least twice as fast as other higher plants (Hillman 1978). Depending on the genus, duckweed daughter fronds are produced vegetatively in pairs (*Lemna* and *Spirodela*) or as a daughter frond from the basal end of the mother frond (*Wolffia*). Each daughter frond repeats the budding history of its clonal parents, resulting in exponential growth (Landolt 1987). *Lemna*, *Spirodela*, and *Wolffia*, three important genera of Lemnaceae, are all subject to self-shading (intra-specific competition) and reach a steady – state condition where frond death equals frond multiplication. Hence Lemnaceae is subject to density-dependent growth (Ikusima 1955, Ikusima et al. 1955). Once essential nutrients are depleted or waste products build up, the growth rate declines.

When duckweed was cultured in axenic (sterile) conditions using chemically defined media under artificial lights, growth rates were recorded that far exceeded growth rates measured under natural conditions (Hillman 1961). Excessively high light levels (more than 200 Wm^{-2}), nutrient shortages, and the presence of herbivores, parasites, and commensal organisms antagonistic to duckweed populations greatly reduce the growth rates of duckweeds in natural environments (Landesman 2000). Duckweed growing in wastewater treatment plants, however, is under less pressure from herbivores because the high ammonia and low dissolved oxygen levels prevalent in wastewater may exclude potential grazers such as fish and turtles. Wastewater environments also have abundant supplies of nitrogen and phosphorus as compared to natural aquatic environments.

17.2 Duckweed for Phytoremediation of Contaminated Waters

Phytoremediation is defined as the method to utilize higher plants to alter contaminated environments. It is a cost-effective, low-impact, and environmentally sound remediation technology (Cunningham and Ow 1996). And phytoremediation includes five different mechanisms, which are rhizofiltration, phytostabilization, phytoextraction, phytovolatilization, and phytotransformation (Ghosh and Singh 2005). Rhizofiltration is that plants are used to absorb, concentrate, and precipitate contaminants from polluted aquatic environment by their roots; phytostabilization involves the stabilization of contaminated soils by sorption, precipitation, complexation, or metal valence reduction rather than the removal of contaminants; phytoextraction, also referred as phytoaccumulation, is the process that plants absorb, concentrate, and precipitate the contaminants in the biomass; phytovolatilization is the mechanism that plants extract certain contaminants in nearby roots and then transpire them into the atmosphere; phytotransformation, also referred as phytodegradation, is the process that plants remove contaminants from environment by their metabolism. More detailed information on these five different mechanisms is listed into Table 17.1.

17.2.1 As an Alternative Means of Wastewater Treatment

Duckweed has been utilized in the treatment of municipal and industrial wastewaters for more than two decades, which can be traced back to before 1990 (Oron et al. 1988). Duckweed is widely and effectively used for phytoremediation of contaminated water due to its ability to grow at wide ranges of temperature, pH, and nutrient level (Landolt and Kandeler 1987) in areas where land is available for its application (Krishna and Polprasert 2008). Considerable work was done in the 1970s and 1980s on the use of duckweed genera, especially *Lemna*, as a means of treating wastewater of both agricultural and domestic origin. When *Lemna* is grown in wastewater treatment ponds the floating mat of fronds is held in place by partitions and baffles that prevent wind from blowing fronds to one side off or completely off the surface of the treatment pond. These partitions and baffles are usually made of polyethylene in industrialized countries but may be made of bamboo or other natural materials in developing countries.

Table 17.1 Contaminant removal processes and mechanisms by phytoremediation

	Rhizofiltration	Phytostabilization	Phytoextraction	Phytovolatilization	Phytotransformation
Mechanism	Rhizosphere accumulation, absorption, concentration, precipitation	Complexation, sorption, precipitation, metal valence reduction	Hyper-accumulation, absorption, concentration, precipitation	Volatilization	Degradation by plant metabolism
Contaminant	Organics/inorganics, Pb, Cd, Cu, Zn, Cr, Ni	Inorganics, heavy metals	Inorganics, heavy metals	Organics/inorganics, Hg, Se	Organics, ammunition wastes, chlorinated solvents, herbicides
Environment	Industrial discharge, agricultural runoff, acid mine drainage	Soil, sediment, sludge	Diffusely polluted areas	Soil, water, sediment	Soil, water, groundwater
Reference	Chaudhry et al. (1998), USEPA (2000), Ghosh and Singh (2005)	Mueller et al. (1999), USEPA (2000), Ghosh and Singh (2005)	Rulkens et al. (1998), USEPA (2000), Ghosh and Singh (2005)	Bañuelos (2000), Henry (2000), Ghosh and Singh (2005)	Black (1995), Ghosh and Singh (2005)

As part of a facultative treatment system, duckweed can cover treatment ponds and reduce the growth of algae in these ponds as well as reduce nitrogen in the effluent from these ponds through ammonia uptake and denitrification (Alaerts et al. 1996; Hammouda et al. 1995). Duckweed can also be part of constructed wetland systems, either as a component of a wetland receiving wastewater or as plants that polish nutrients from wetland-treated effluents (Ancell 1998, Fedler et al. 1999, WEF 2001).

Harvesting wastewater-grown duckweed helps to remove surplus nutrients, which might otherwise be released into aquatic environments by wastewater treatment plants (Harvey and Fox 1973, Oron et al. 1988). Duckweeds, like other plants, take up nutrients from their surrounding environment (Landesman 2000). This ability has been exploited to remove surplus nutrients from swine lagoon effluents (Cheng et al. 2002b). The growing plants can then be harvested to remove surplus nitrogen and phosphorus. However, the application of duckweed in recovery (Cheng et al. 2002a) and removal of nitrogen and phosphorus in swine lagoon water was found to be subject to the water concentrations and seasonal climate since the primary mechanism is assimilation of those nutrients in environment; therefore, the appropriate light intensity and preferable temperature are key parameters for duckweed in removal of surplus nutrients (Cheng et al. 2002b), and duckweed prefers to take up NH_4^+ than NO_3^- by both roots and fronds (Fang et al. 2007).

Duckweed populations can remove nutrients from stormwater ponds. A monoculture of *L. minor* consistently removed a large amount of ammonia from stormwater while a mixture of *L. minor* and *Spirodela polyrhiza* removed the largest amount of phosphorus from stormwater within 8 weeks of treatment (Perniel et al. 1998). Recently, Drenner et al. (1997) have described a system for culturing periphyton on eutrophic effluents and raising fish that graze on this wastewater-grown periphyton. In this way, surplus nutrients are concentrated in fish flesh. A similar system could be designed using duckweed as the nutrient stripping plant (van der Steen et al. 1998).

Duckweed systems can remove 50–60% of nitrogen and phosphorus (Vatta et al. 1994) from domestic wastewater or even 73 – 97% of total Kjeldahl nitrogen and 63–99% of total phosphorus in duckweed-covered domestic wastewater (Körner and Vermaat 1998). The removal of chemical oxygen demand (COD) is faster in duckweed-covered domestic wastewater than uncovered wastewater, and organic degradation can be improved by additional oxygen supply and additional surface in duckweed-covered domestic wastewater (Körner et al. 1998). The removal efficiencies can be reached at high to 84, 88, 68, 58, and 87%

for COD, BOD5, NH_3^-N, TN, and TSS, respectively, in duckweed-based wastewater treatment system under optimum operating and environmental conditions (Krishna and Polprasert 2008). Furthermore duckweed systems evaporate 20% less water compared to other open water wastewater treatment systems (Oron et al. 1986, Borrelli et al. 1998). The reduced evaporation of duckweed-covered surfaces in wastewater treatment is an asset in arid climates.

Guidelines for the use of duckweed to remove ammonia and phosphorus from effluent from an algae culture system were given by Koles et al. (1987). Researchers at the Politecnico di Milano, Italy, have developed models for duckweed-based wastewater treatment plants (Boniardi et al. 1994, Rota et al. 1995). These models will greatly assist in the design and management of duckweed-based wastewater treatment systems (Landesman et al. 2005). Duckweed-based treatment systems have their limitations. They require large areas of land that may not be available near urban areas. In temperate climates duckweed growth slows in the winter. This may restrict the use of such treatment systems in cooler climates unless a greenhouse system is utilized. Duckweedbased treatment systems may be most useful in treating secondary effluents from small communities where land costs are low (Bonomo et al. 1997).

A series of investigations on duckweed application in restoration of eutrophic water were done in the past decades. Eutrophic water is associated with excessive nitrogen and phosphorus in water input by discharge from agricultural wastewater, industrial water, and domestic water. Eutrophic water had the risk of eutrophication defined as the negative effects of the excessive growth of phytoplanktons (Khan and Ansari 2005), degradation of water ecosystems, or even disappearance of the water body involved in. Duckweed was used to remove the targeted nutrients in eutrophic water due to its ability to survive in nutrient-laden environments and its rapid growth (Li et al. 2009) so that those nutrients can be removed by harvesting duckweed biomass (Li et al. 2007) and eutrophic water can be recovered by combining other technologies. The duckweed *L. minor* is suitable for phytoremediation of eutrophic waters at acidic pH and at temperature from 20 to 30°C (Ansari and Khan 2008); however, the duckweed *S. polyrhiza* cannot be used to recover the eutrophic waters at low temperature of 10–12°C (Song et al. 2006). Many mathematical models have been developed for duckweed systems to describe its phytoremediation of eutrophic waters (Frédéric et al. 2006); those models incorporated duckweed growth parameters including temperature, photoperiod, nitrogen concentration, phosphorus concentration, and mat density (Lasfar et al. 2007).

17.2.2 As a Means of Removing Heavy Metals and Other Toxic Elements in Waters

Heavy metals are readily accumulated and transported in aquatic environment in the form of dissolved or solid wastes from domestic, industrial, and agricultural runoff (Megateli et al. 2009). Heavy metal contamination in environment can be cost-effectively removed by phytoremediation. Such a technology is most suitable for developing countries (Ghosh and Singh 2005). Generally, heavy metal cannot degrade or decompose as other contaminants; therefore, their removal by phytoremediation mainly depends on phytoextraction mechanism. In recent years, there were many findings reported on the removal of heavy metals by duckweed phytoremediation.

Khellaf and Zerdaoui (2009) addressed that the duckweed *Lemna gibba* L. can be successfully employed to remove Zn from contaminated water by 61 – 71%. Another research found that the duckweed *L. gibba* could remove Zn and Cu rapidly in the first 2 days with concentration reduction higher than 60% and then slowly in the following 8 days with reduction of 10 – 20%; however, the removal of Cd was linear and determined by initial Cd concentration and the removal was about 90% after 6 or 8 days with initial concentrations of 0.1 or 0.001 mgL^{-1} (Megateli et al. 2009). Duckweed phytoremediation has its limitation in heavy metal removal due to heavy metal's toxicity. Hou et al. (2007) stated that Cd^{2+} was more toxic than Cu^{2+} for the duckweed *L. minor*; the tolerance levels of Cd and Cu were smaller than 0.5 and 10 mgL^{-1}, respectively, and *L. minor* was recommended to phytoremediate low-level contaminated waterbody by Cu and Cd.

S. polyrhiza was found to have a large capability for the uptake and accumulation of heavy metals, surpassing that of algae and other angiosperms. For example, the zinc concentration in frond tissue was 2,700 times higher than that of its medium (Sharma and Gaur 1995). Under experimental conditions *L. minor* proved to be a good accumulator of cadmium and copper and a moderately good accumulator of chromium. Duckweed can accumulate other toxic elements such as selenium (Ornes et al. 1991), technetium (Hattink 2000), lead (Jain et al. 1990, Kruatrachue et al. 2002), uranium, and arsenic (Mkandawire et al. 2004). The growth rates and ease of harvest make duckweed species useful for phytoremediation of certain heavy elements as compared to many algal species that require much more extensive harvesting equipment (Zayed et al. 1998). Duckweed can therefore prove useful in treating effluents from mining operations. However, heavy metal concentrations can depress duckweed growth reducing its effectiveness in removing toxic elements from the water body in which it grows (Boniardi et al. 1999). The duckweeds *L. minor* (Alvarado et al. 2008), *L. gibba* (Marín and Oron 2007, Sasmaz and Obek 2009), and *S. polyrhiza* L. (Rahman et al. 2007) investigated for their phytoremediation ability to remove arsenic, boron, and uranium in water; *L. gibba* was found to be a suitable candidate used for the treatment of water containing boron with concentration lower than 2 mgL^{-1} (Marín and Oron 2007) and to accumulated arsenic (133%), uranium (122%), and boron (40%) (Sasmaz and Obek 2009); *L. minor* had good treatment of water with arsenic lower than 0.15 mgL^{-1} (Alvarado et al. 2008); *Spirodela polyrhiza* L. was identified as a good arsenic phytofiltrator by physico-chemical adsorption mechanism (Rahman et al. 2007).

17.2.3 As a Means of Removing Toxic Organic Compounds from Wastewater

Duckweed species can accumulate toxic organic compounds such as phenols, chlorinated phenols, pharmaceuticals, and surfactants. Duckweed species can do this directly or indirectly through microbiota living on frond surfaces. For example, surfactants like alkylbenzene sulfonate and alcohol ethoxylate are mineralized by duckweed microbiota (Federle et al. 1989). Duckweed can take up fluorinated agricultural chemicals (Reinhold 2006) and detoxify chlorinated phenols (Barber et al. 1995). The duckweed *S. oligorrhiza* L. wash proven to have the ability to uptake and transform DDT and organophosphorus pesticides (Gao et al. 2000a,b). The ability of duckweed to perform reductive dechlorination can be used in phytoremediation of industrial wastewaters (Ensley et al. 1997). Duckweed species definitely have the potential to contribute to natural systems of bioremediation.

17.3 Duckweed's Other Practical Application

In addition to the application for phytoremediation of contaminated waters, duckweed has been developed for other applications. Duckweed can be used as livestock food, toxicity testing, and raw material for biofuel production.

17.3.1 As a Source of Livestock Feed

The value of duckweed as a source of feed for fish and poultry has been promoted by the World Bank, especially in developing countries (Skillicorn et al. 1993). Research at Louisiana State University demonstrated the value of using dried duckweed fronds as a feed source for dairy cattle and poultry (Culley et al. 1981). Research at Texas Tech University has shown that duckweed species have potential as a feed ingredient for cattle, sheep, and pigs (Johnson 1998, Moss 1999). Duckweed also has potential as a feed ingredient in fish farming (Gaigher et al. 1984).

A great deal of work has been done on the nutritional value (Table 17.2) of species of Lemnaceae, especially *Lemna*, *Spirodela*, and *Wolffia* (Rusoff et al. 1980, Landesman et al. 2004). Duckweed has

Table 17.2 Chemical composition of *L. gibba* meal (% dry matter)

Chemical composition	Dry matter (%)
Dry matter	3.5
Crude protein	41.7
Crude fat	4.4
Acid detergent fiber	15.6
Non-fiber carbohydrate	17.6
Ash	16.2

Table 17.3 Amino acid composition of dried *L. gibba* (g amino acid/100 g dry *L. gibba*)

Amino acid	g amino acid/100 g dry *L. gibba*	Amino acid	g amino acid per/100 g dry *L. gibba*
Taurine	0.03	Methionine	0.64
Aspartic acid	3.51	Isoleucine	1.66
Threonine	1.68	Leucine	2.89
Serine	1.39	Tyrosine	1.27
Glutamic acid	3.67	Phenylalanine	1.75
Proline	1.42	Histidine	0.73
Glycine	1.93	Ornithine	0.05
Alanine	2.30	Lysine	1.85
Cysteine	0.44	Arginine	2.14
Valine	2.12	Tryptophan	0.40

been fed to pigs, cattle, sheep, chickens, ducks, and fish and can substitute for soybean meal in animal feed rations (Robinette et al. 1980, Haustein et al. 1994, Bell 1998, Moss 1999, Johnson 1999, Leng 2004). *Wolffia arrhiza* is collected for human food in Thailand and Laos and is sold at local markets in these countries (Bhanthumnavin and McGarry 1971). Its amino acid composition (Tables 17.3 and 17.4) is more like that of animal protein than plant protein having a high lysine and methionine content, two amino acids normally deficient in plant products (Dewanji 1993). Finally, dried duckweed can provide vitamins, minerals, and pigments such as beta-carotene in livestock diets, reducing the need to add these compounds to rations and thus reducing the cost of producing feed.

Research was conducted at Texas Tech University to utilize duckweed species as part of a system for recycling cattle wastes from feedlots (Fedler and Parker 1998). Duckweed growing in a series of ponds receiving wastewater from a cattle feedlot concentrated nitrogen, phosphorus, and other elements, both purifying this wastewater and providing an ingredient for cattle feed. Since the protein content of duckweed was found to be almost as high as that of soybean meal, duckweed production provided both a means of water purification and a source of livestock feed as well (Allen 1997, Johnson 1998, Moss 1999). It was found that a level of up to 11% of the protein requirements for cattle could be supplied by duckweed and provide added growth benefits as compared to soybean meal as the protein source (Johnson 1998).

Mature poultry can utilize dried duckweed as a partial substitute for vegetable protein such as soybean meal in cereal grain-based diets (Islam et al. 1997). Duckweed used at a level of up to 15% in broiler diets can represent an important alternative source of protein for poultry feeds in countries where soybean or fish meal is unavailable (Haustein 1994). When dried *Lemna* spp. Nex fed to crossbred meat ducks as a substitute for soybean meal there was no significant difference in the carcass traits between treatments (Bui et al. 1995). The protein from duckweed has a biological value equivalent to that of soya beans in diets formulated for ducklings (Nguyen et al. 1997). Duckweed has a high organic matter and protein content but has a low digestibility for ducks. When duckweed was used to replace half the ration in diets for ducks resulted in a reduced feeding costs by up to half (Khanum et al. 2005).

Diets formulated for pigs can substitute duckweed for soybean meal (Leng et al. 1995). Duckweed has

Table 17.4 Essential amino acid composition of dried *L. gibba* meal (g amino acid/100 g dry *L. gibba*)

Essential amino acid	g amino acid/100 g dry *L. gibba*
Leucine	2.89
Arginine	2.14
Valine	2.12
Lysine	1.85
Phenylalanine	1.75
Threonine	1.68
Isoleucine	1.66
Tyrosine	1.27
Histidine	0.73
Methionine	0.64
Cysteine	0.44
Tryptophan	0.40

been ensiled with other feed crops such as corn or cassava leaves to produce an alternative diet for pigs raised on small farms in Vietnam and that fresh duckweed (providing 5% of the diet dry matter) has a stimulating effect on weight gain (Du 1998). The addition of duckweed (*Spirodela* sp.) to corn significantly increased both the pre-ensiled and the postensiled protein content of the silage (Eversull 1982).

What has not been found are articles published on the effect of incorporating duckweed meal into penaeid shrimp diets. Fresh and decomposed duckweed (*Spirodela* sp.) has been used as feed for the Australian red claw crayfish (*Cherax quadricarinatus*) (Fletcher and Warburton 1997). They found that decomposed *Spirodela* species supported crayfish growth as well as commercial pellets did. The abundance of carotenoids and pigments can stimulate crustacean growth (Hertampf and Piedad-Pascual 2000).

Perhaps the most promising use of duckweed is as a feed for pond fish such as carp and tilapia (Landesman et al. 2002). Ponds for duckweed production can be located next to fish culture ponds, eliminating the need for expensive drying to produce a dried feed. Nile tilapia and a polyculture of Chinese carps fed readily on fresh duckweed added to their ponds, and the nutritional requirements of tilapia appear to be met by duckweed (Saber 2004). *W. arrhiza* L. alone supported the growth of two species of Indian carp and four species of Chinese carp as well as one species of barb *Puntius javanicus* (Bikr.) (Naskar 1986). The herbivorous grass carp (*Ctenopharyngodon idella*) digests duckweed species such as *Lemna* and *Wolffia* quite well and it could, by itself, support production of this fish (Cassani et al. 1982, Van Dyke and Sutton 1977). Duckweed has also been tested as a component in the diet of catfish (Robinette et al. 1980), silver barb (Azim et al. 2003), and tilapia (Hassan and Edwards 1992; Fasakin et al. 1999) where it was also able to be substituted for soybean meal. A system for combining duckweed and fish culture was developed in Bangladesh for use by small farmers in developing countries by the non-governmental organization PRISM (Skillicorn et al. 1993). This system could sustain a dry weight production of duckweed in excess of 20 – 35 metric tons a year, (Leng 1999). Hence, duckweed can become a competitive source of plant protein especially in tropical countries.

17.3.2 As an Inexpensive and Accurate Way of Toxicity Testing

Due to its small size and ease of growth, duckweed species make useful organisms for toxicity testing (Lakatos et al. 1993). Duckweed species offer many advantages for the testing of toxic compounds. Duckweed fronds assimilate chemicals directly from their aquatic media into their leaf tissue, allowing for toxicant application in a controlled manner. The growth assay for toxicant assessment is rapid and can be performed without special equipment by counting leaves. Since *Lemna* and *Spirodela* are inexpensive to maintain and the fronds are small, multiple treatments are easy to do simultaneously (Greenberg et al. 1992). Duckweed species have been used to test the toxicity of oils (King and Coley 1985), herbicides (Nitschke et al. 1999), phenol (Barber et al. 1995), and polycyclic aromatic hydrocarbons (Huang et al. 1992), among other toxicants.

A new company in Germany has devised a *Lemna* toxicity test that has been approved by the European Commission (*Lemna*Tec 1999), and the use of duckweed for toxicity testing is mentioned in Standard Methods (1995). Duckweed can be used in both static and the dynamic test procedures (Davis 1981, Wang 1990, Taraldsen and Norberg-King 1990).

17.3.3 Miscellaneous Uses

The ease and convenience of culturing duckweed species under both natural and artificial lights make this species an ideal teaching tool, both at the university and at the primary school level. An example of an experiment using duckweed that can be performed by elementary school students was published in the *Journal of Biological Education* by a Japanese teacher and two research workers (Kawakami et al. 1997). Since duckweed is so quick and easy to grow, students can learn how to study concepts of exponential growth, heavy metal toxicity, photosynthesis, and asexual reproduction. The effect of environmental variables like light and temperature can also be studied using duckweed (Robinson 1988).

An allelopathic effect of duckweed on mosquito larvae may have public health significance. Extracts of *L. minor* caused significant mortality in the larvae of

Aedes aegypti L., a known vector of human diseases such as malaria. The presence of *L. minor* interfered with egg oviposition by *Culex pipiens pipiens* L. and was lethal to *C. p. pipiens* larvae at the first instar stage (Eid et al. 1992). Duckweed may provide a source of mosquito anti-larval compounds that could have commercial significance. Another use for duckweed is as fertilizer. In developing countries like India and Bangladesh where fertilizer is scarce and expensive for the small farmer, duckweed collected from local ponds and wetlands can provide a cheap and effective fertilizer for rice and other crops (Ahmad et al. 1990). It also makes an excellent compost and much of the duckweed harvested from Louisiana wastewater treatment ponds is used for this purpose. Finally a new use for duckweed biomass as a cell-structured support material has emerged as a new technology for yeast fermentation. *W. arrhiza* biomass was extracted with ethanol and loaded with yeast cells. This yeast-impregnated *W. arrhiza* was placed in a semicontinuous fluid-bed fermenter for the production of beer (Richter et al. 1995). New uses for duckweed species will doubtless arise as more researchers learn to appreciate the versatility and potential of Lemnaceae.

From an energy standpoint, most terrestrial plants vary from about 14.8 to 18.4 kJ/g while aquatic plants vary from 10.0 to 21.5 kJ/g. Duckweeds average about 13.5 kJ/g. When you consider the production levels of the various plants, duckweeds can produce from 122 $\times$ 10^6 to 539 $\times$ 10^6 kJ/ha annually, yet the range for many species of aquatic plants considered varies from a low of 12 $\times$ 10^6 to a high of 2,900 $\times$ 10^6 kJ/ha annually (Fedler et al. 2007). Table 17.5 shows the production level of duckweeds at various locations around the world.

17.4 Summary

Duckweeds of the family Lemnaceae are small, floating, aquatic plants with a worldwide distribution. They are one of the fastest growing angiosperms and can double their biomass within 2 days under optimal conditions. They have a high protein content (10 – 40% protein on a dry weight basis) although the moisture content (95%) of fresh duckweed biomass is quite high as well. Potentially, members of the Lemnaceae (of the genera *Lemna*, *Spirodela*, and *Wolffia*) can produce edible protein six to ten times as fast as an equivalent area planted with soybeans. Therefore species of Lemnaceae potentially have a great value in agriculture.

A great deal of work has been done on the nutritional value of species of Lemnaceae, especially *Lemna*, *Spirodela*, and *Wolffia*. Duckweed has been fed to pigs, cattle, sheep, chickens, ducks, and fish and can substitute for soybean meal in animal feed rations. Its amino acid composition is similar to that of other plant proteins except for having a higher lysine and methionine content, two amino acids normally deficient in plant products. Finally, dried duckweed can provide vitamins, minerals, and pigments, such as beta-carotene in livestock diets, reducing the need to add these compounds to rations and thus saving the producer money while having a higher quality feed as compared to the normal basal diet usually fed.

Much research has been done on the use of duckweed in wastewater treatment systems. As part of a facultative treatment system, duckweed can cover treatment ponds and reduce the growth of algae in these ponds as well as reduce nitrogen in the effluent

Table 17.5 Annual worldwide duckweed growth rates (Leng et al. 1995)

Location	Yield (tons/acre)	Yield (metric ton/ha)	Source
Thailand	4.5 – 4.9	10 – 11	Hassan and Edwards (1992), Landolt and Kandeler (1987)
Israel	5 – 8	10 – 17	Porath et al. (1979)
Russia	3.1 – 3.6	7 – 8	Landolt and Kandeler (1987)
Uzbekistan	3 – 7	7 – 15	Landolt and Kandeler (1987)
Germany	7– 10	16 – 22	Landolt and Kandeler (1987)
India	10	22	Landolt and Kandeler (1987)
Egypt	5	10	Landolt and Kandeler (1987)
Louisiana	1 – 10	2 – 23	Culley and Epps (1973), Rusoff et al. (1980),
	12 – 35	27 – 79	Mestayer et al. (1984)
Israel	16 – 23	36 – 51	Oron et al. (1984)

from these ponds through nitrogen uptake and denitrification. Duckweed can also be a part of constructed wetland systems, either as a component of a wetland receiving wastewater or as plants that polish nutrients from wetland-treated effluents.

Due to their small size and ease of growth, duckweed species make ideal organisms for toxicity testing. A new company in Germany has devised a *Lemna* toxicity test that has been approved by the European Commission, and the use of duckweed for toxicity testing is mentioned in Standard Methods. Duckweed can be used in both the static and the dynamic test procedures.

Duckweed plays an important role in the ecology of wetland environments by providing a substrate for the growth of diatoms, protozoa, and bacteria. This phyllosphere (microorganisms living on the outer frond surface) in turn supports insect life as well as enhancing the nutritive value of duckweed for waterfowl and wetland animals such as nutria and turtles. In addition cyanophytes residing in the phyllosphere of duckweed fronds can fix atmospheric nitrogen, providing nitrogen input in oligotrophic aquatic environments.

The three dominant duckweed genera (*Lemna*, *Wolffia*, and *Spirodela*) will all grow on organic (for example, wastewater) as well as an inorganic media (for example, Hoagland's medium). All three species grow faster on organic as opposed to inorganic media with equivalent amounts of nitrogen and phosphorus. This may be due to the ability of duckweed species to take up organic molecules directly from the media in which they grow. Even inorganic media supplemented with glucose will support faster duckweed growth than media without glucose.

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